A phase I clinical study of PD-1 knockout engineered T cells treating patients with advanced Non-Small Cell Lung Cancer

Protocol No.: MHC-001

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List of abbreviations

AE	Adverse event
A/G	Albumin/ globulin
AKP	Alkaline phosphatase
ALB	Albumin
ALK	Anaplastic lymphoma kinase
ALT	Alanine transaminase / glutamic-pyruvic transaminase
ANC	Absolute neutrophil count
APTT	Activated partial thrombplastin time
AST	Aspartate aminotransferase / glutamic oxalacetic transaminase
bTMB	Blood tumor mutation burden
BUN	Blood urea nitrogen
CABG	Coronary artery bypass grafting
CAR-T	Chimeric antigen receptor T cell therapy
CEA	Carcinoma embryonic antigen
CDR3	Complementary determining region 3
CHF	Congestive heart failure
CIK	Cytokine-induced killer cells
Cl	Chlorine
CK-MB	Creatine kinase MB
CNS	Central nervous system
CR	Complete response
Cr	Creatinine
CRF	Case report form
CRP	C-reactive protein
CRS	Cytokine release syndrome
CSR	Clinical study report
CT	Computed tomography
CTCAE4.03	Common terminology criteria for adverse events, version 4.03
CtDNA	Circulating tumor DNA
CTLA-4	Cytotoxic T-lymphocyte antigen-4
CYFRA21-1	Cytokeratin fragment antigen 21-1
DCR	Disease control rate
DFS	Disease free survival
DLT	Dose-limiting toxicity
DOR	Duration of response
EC	Ethics committee
ECG	Electrocardiograph

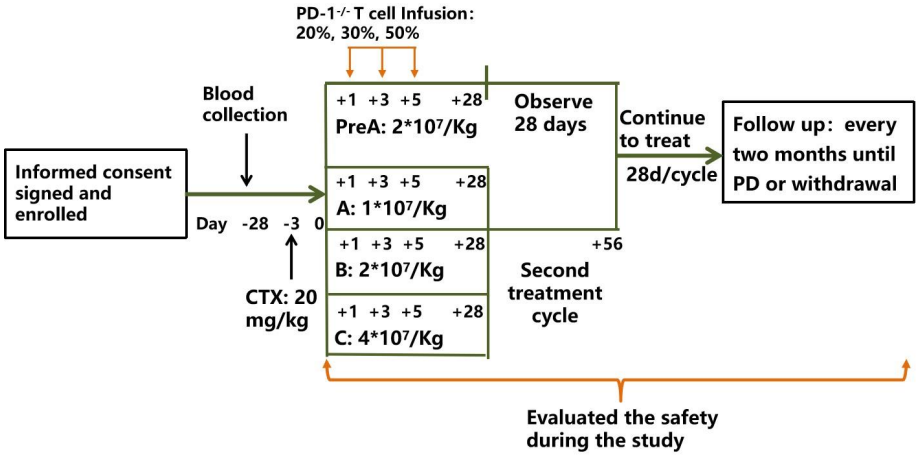
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDC	Electronic data capture
LVEF	Left ventricular ejection fraction
EGFR	Epidermal growth factor receptor
EOT	End of treatment
FDG	F-deoxyglucose
GCP	Good Clinical Practice
G-CSF	Granulocyte colony-stimulating factor
GVHD	Graft-versus-host disease
HBV	Hepatitis B virus
HBV sAg	Hepatitis B virus surface antigen
HCV Ab	Hepatitis C virus Antibody
HCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Council for Harmonization (drug registration)
IFN-α	Interferon-α
IFN-γ	Interferon-λ
INR	International normalized ratio
IrRC	Immune-related response criteria
irCR	Immune-related Complete Response
irPR	Immune-related Partial Response
irSD	Immune-related Stable Disease
irPD	Immue-related Progressive Disease
IUD	Intrauterine device
IUS	Intrauterine system
Ka	Potassium
LAHB	Left anterior hemi-block
LDH	Lactic dehydrogenase
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
Na	Sodium
NCI	National Cancer Institute
NE	Not evaluable
NS	Normal saline
NSCLC	Non-small cell lung cancer
NSE	Neuronspecific enolase

NY-ESO-1	New York - esophageal squamous cell carcinoma-1
OS	Overall survival
PD	Progressive disease
PFS	Progression free survival
PD-1	Programmed cell death -1
PD-L1	Programmed death - ligand 1
PET-CT	Positron emission tomography Computed tomography
PPF	Production possibility frontier
PR	Partial response
PTT	Partial thromboplastin time
QTc	QT correction
RECIST	Response evaluation criteria in solid tumors
RR	Response rate
SAE	Serious adverse event
SD	Stable disease
sgRNA	Single-guide RNA
TBL	Total bilirubin
TCR	T cell receptor
TKI	Tyrosine kinase inhibitor
TNF-α	Tumor necrosis factor-α
TSH	Thyroid-stimulating hormone
TTP	Time to progress
ULN	Upper limit of the normal
WBC	White blood cell
WOCBP	Women of child-bearing period

Protocol synoposis

Protocol No.	MHC-001
Title	A phase I clinical study of PD-1 knockout engineered T cells treating patients with advanced Non-Small Cell Lung Cancer
Sponsor	West China Hospital, Sichuan University
Clinical Phase	Phase I
Study type	Interventional treatment
Objective and principle	To investigate the safety and tolerability of CRISPR-Cas9 edited autologous PD-1 T cells (PD-1 edited T cell) in patients with PD-L1 postive advanced non-small cell lung cancer (NSCLC) after failure of multiple lines of prior standard treatments.
Primary objective	To investigate the safety and tolerability of CRISPR-Cas9 PD-1 edited T cell in patients with advanced NSCLC.
Secondary objective	1) To investigate the antitumor activity of CRISPR-Cas9 edited PD-1 T cells in patients with advanced NSCLC. 2) To investigate the changes in the potential immunological indexes and biomarkers. 3) To track the PD-1 edited T cells in patients and investigate the mechanism of the clinical antitumor activity.
Primary endpoint	The dose-limiting toxicity (DLT) rate of the cell therapy during the first treatment cycle.

Secondary endpoint	1) To evaluate the objective response rate (ORR) and 8-week disease control rate (DCR) of the cell therapy. 2) To evaluate time to first response, defined as the time from the first cell infusion to the first observed complete response (CR) or partial response (PR). 3) To evaluate the duration of response (DOR), defined as the time from the first observed CR or PR to the first observed PD or death from any cause. 4) To evaluate the progression free survival (PFS), defined as the time from the first cell infusion to the first observed PD or death from any cause. 5) To evaluate the overall survival (OS), defined as the time from the first cell infusion to death. 6) To evaluate the activation status and duration of PD-1 edited T cell after infusion. 7) To evaluate the change of *in vivo* cytokine levels and their correlation to the tumor response and adverse events of after PD-1 edited T cell infusion.
Study design	This phase I clinical study aims to investigate the safety and tolerability of PD-1 edited T cell therapy in patients with advanced NSCLC. About 20 patients with PD-L1 positive advancedNSCLC who have progressed after the multiple lines of prior standard therapeutic regimens are planned to be enrolled in this study. The safety and tolerability of the therapy will be investigated by using intergroup dose escalation method. This clinical study was a first-in-human trial using CRISPR technology knockouting PD-1 gene in T cell to treat lung cancer patients. Although there were no exact dosage/regimen and safty data, starting dose was selected referring to cell therapy or CAR-T treatment for considering benefit-risk of cancer patients (see section 5.3.1 Starting dose rationale). Pre-A: $2x10^7$ PD-1 edited T cells per kilogram of body weight（percentage of PD-1 knockout T cell may vary depending on

	technology efficiency. The study defines the Pre-A group as a preliminary exploratory dose group. The Pre-A group has a 28 days-observation after the first treatment cycle. If no DLT was observed in Pre-A group during the defined observation period, the study will continue other treat groups according to provisional dosage; If DLT or other unexpected toxicity occurs in the Pre-A group, the investigator will adjust the cell dose and interval of sequential cell therapy based on data from Pre-A group. There was three treatment groups and 28 days/cycle. Group A: $1x10^7$ PD-1 edited T cells per kilogram of body weight, 2 treatment cycles; Group B: $2x10^7$ PD-1 edited T cells per kilogram of body weight, 2 treatment cycles; Group C: $4x10^7$ PD-1 edited T cells per kilogram of body weight, 2 treatment cycles. (Treatment may continue after 2 cycles if considered to be benefitial to patient) 
Inclusion criteria	1. Informed consent form (ICF) is signed: (1) The enrolled patients must sign and date the EC-approved written ICFs according to the guidelines of the competent authority and study institution. The ICF should be signed before implementing any protocol-related procedure (not including the routine medical care of the patients). (2) The enrolled patients must be willing and able to comply with the visits, therapeutic regimen, and laboratory examinations specified in the

	schedule as well as the other requirements in the study. 2. Age: 18-70 years. 3. ECOG performance status 0-2. 4. NSCLC, stage IIIB/IV confirmed by histology or cytology. All patients must be screened for EGFR gene mutation and ALK gene fusion; patients with positive EGFR mutation or ALK gene fusion treatment failure of EGFR-TKI or ALK-TKI must have been experienced. 5. Patients must fail from the standard third-line or above therapy scheme. 6. Patients must be positive for PD-L1staining, as tested through tumor biopsy (TPS ≥ 1%). 7. Patients must have a measurable lesion (tumor lesion with a long diameter of more than 10 mm through CT scan; lymph node with a short diameter of more than 15 mm through CT scan) in accordance with the RECIST 1.1. 8. Life expectency of ≥ 3 months. 9. Adequate bone marrow function: • WBC ≥ 3.0×10^9/L. • ANC ≥ 1.5×10^9/L. • PLT ≥ 90×10^9/L, without transfusions within 14 days before the first cell therapy. • Hemoglobin ≥ 10.0 g/dL. 10. Adequate hepatic and renal function: Total bilirubin ≤ 1.5×ULN. • AST and ALT < 2.5×ULN; AST and ALT < 5×ULNif metastasis to liver. • Serum creatinine ≤1.5×ULN, or creatinine clearance rate ≥ 50 ml/min (based on the Cockcroft/Gault formula). 11. Coagulation functions: • INR ≤ 1.5×ULN. • PTT ≤ 1.5×ULN. 12.Adequate cardiovascular function: • Left ventricular ejection fraction ≥ 50%.

	• QTcF interval ≤ 450 ms. 13. ALP ≤ 2.5×ULN. 14. Recovery from all adverse events of previous systemic anti-cancertherapies to baseline or Grade ≤ 1, except forAlopecia. 15. Washout period for the previous anti-tumor treatment is not less than four weeks and washout period for the molecular targeting drug is not less than five half-life periods. Palliative radiotherapy must have been completed for at least two weeks; thoracic radiation therapy must have been completed for at least three months, and major surgery must have been completed for at least four weeks with recovery. 16. The patients must take contraception measures strictly. • Within the 72-hour period prior to the therapy, the serum or urine pregnancy test (HCG sensitivity is 25 IU/L at least or equivalent) of the women of childbearing potential (WOCBP) must be negative. • WOCBP must agree to take contraceptive measures strictly during the treatment period and for at least 18 months after the final cell infusion until no knockout T cell is detected in the blood. • The males who have sexual contact with WOCBP must agree to take contraceptive measures strictly during the treatment period and for at least 18 months after the final cell infusion until no knockout T cell is detected in the blood.
Exclusion criteria	1. Patients with active central nervous system (CNS) metastasis (including but not limited to carcinomatous meningitis and spinal cord compression) must be excluded. However,patients with metastatic CNS tumors may participate in this study if thepatients can be recoved to the baseline level at least two weeks before inclusion (without the residual signs or symptoms related to the CNS treatment). In addition, the patients must have stopped using corticosteroids for four weeks before inclusion. 2. Patients with weight loss in the recent six months ≥ 10%. 3. Patients accompanied by emergent symptoms caused by tumor that need immediate intervention. 4. Patients with medical history of interstitial lung disease (ILD) or immune related pneumonia.

	5. Patients with peripheral neuropathy. 6. Patients with clinically significant coagulation abnormalities, or patients who are receiving thrombolytic or anticoagulant therapy. 7. Patients with a history of human immunodeficiency virus (HIV) positivity or with the acquired immunodeficiency syndrome (AIDS), or with active hepatitis B or hepatitis C. 8. Patients that have other malignancy. 9. Patients with active, known or suspected autoimmune diseases. 10. Patients who have received systemic treatment by corticosteroids (the equivalent dose >10 mg prednisone/day) or other immunomodulators (interleukin-2, IFN-α, IFN-γ, cyclosporine, G-CSF, mTOR inhibitor) within the 28 days prior to the study. Patients who receive inhaled or topical corticosteroids and adrenal corticosteroids (the equivalent treating dose <10 mg prednisone/ day) can participate in the study if they are lack of active autoimmune diseases. 11. Clinically significant cardiac disease or impaired cardiac function, such as: • Clinically significant heart disease such as CHF requiring treatment, or uncontrolled hypertension defined by blood pressure > 140/100 mmHg at rest (taking mean of 3 consecutive readings). • History (< 6 months prior to screening) or current evidence ofclinically significant cardiac arrhythmias, atrial fibrillation and/orconduction abnormality, e.g. congenital long QT syndrome, Grade ≥2/complete AV-blockage or hypokaliemia CTCAE Grade ≥ 3. • History/evidence of acute coronary syndromes (including myocardialinfarction, unstable angina, coronary artery bypass graft (CABG),coronary angioplasty, or stenting), < 6 months prior to screening. • Complete left bundle branch block (CLBBB). • Right bundle branch block (RBBB) + left anterior hemiblock (LAHB - bifascicular block). 12. Patients with a history of allergies to cyclophosphamide and other agents involved in this study. 13. Patients that do not have enough venous access.

	14. Pregnant or lactating women, where pregnancy is defined as the state of a female from conception until the termination of gestation. 15. Patients with mental disorders are mandatorily detained for treatment or are judged as inappropriate for inclusion by the investigator. 16. Patients who are difficult to communicate with or difficult to be followed up for a long time. 17. Other inappropriate conditions in discretion of the investigator's judgment.
Safety evaluation	The incidence rate and severity of AE (CTCAE4.03) and SAE, including laboratory tests, vital signs, ECG changes, etc.
Effectiveness evaluation	Tumors are assessed at baseline, the 8^{th} week, the 12^{th} week and once every 8 weeks during the treatment and follow-up period per RECIST 1.1.
Schedule	2016/08-2018/05: Recruitment and treatment periord. 2018/06-2018/12: Follow-up, data analysis and reporting.

1. Study background

1.1 Overview of tumor immunotherapy background

Immunotherapy has been considered as an effective treatment for cancer, following surgery, chemoradiotherapy and targeted therapy. In the battle against tumor cells, the activity, survival, and migration of T cells are being inhibited by the evolved tumor cells through different ways. Therefore, the anti-tumor effect of T cells decreases or dysfunctions significantly, resulting in immune tolerance and immune escape. The immune checkpoints, such as PD-1/PD-L1 axis, are vital factors for immune escape.

Antibodies targeting the immune checkpoints of PD-1/PD-L1 and cytotoxic T cell antigen-antibody 4 (CTLA-4) are the two most attractive immunotherapy targets, which have achieved a breakthrough in caner therapeutic field [1-3]. The clinical application of a CTLA-4 inhibitor for melanoma was approved by the U.S. Food and Drug Administration (FDA) in 2011, and a PD-1 inhibitor was approved for NSCLC in 2015. The CheckMate 017 study [4] indicated that Nivolumab (a PD-1 inhibitor), as a second line treatment for patients with squamous cell cancer, could prolong the overall survival (OS) by 3.2 months and acheived a higher one-year OS rate than the standard chemotherapy therapy (42% vs 24%; NCT01642004). The CheckMate 057 study also revealed that Nivolumab could extend the OS by 2.8 months as a second-line treatment for non-squamous cell lung cancer compared with standard chemotherapy (NCT01673867) [5]. The KEYNOTE 001 reported that Pembrolizumab (a PD-1 inhibitor) treatment extended the OS to 12 months in NSCLC patients (NCT01295827) [6]. These inspiring results make immunotherapy to target T cells by blocking immune checkpoints a research hotspot.

However, monoclone antibody to blocking immune checkpoint has inherent disadvantages: (1) there are different immune checkpoints functioning on specific sites, and it is difficult to apply antibodies in combination; (2) antibodies only act on membrane proteins; (3) the action of antibodies is temporary and their effect will disappear rapidly due to degradation; (4) resistance to immunotherapy is common with neutralized antibody.

Transfusion of specific PD-1 knockout T cells back into the body might not only overcome the above disadvantages, but also avoid susceptibility to tuberculosis and viral hepatitis, and reduce the incidence of dilated cardiomyopathy and lupus-like autoimmune disease after complete-PD-1 knock therapy [7-10]. It has been reported that the transfusion of PD-1 knockout CD8+T cells from PD-1 knockout mouse spleen and peripheral blood into wild-type mice decreases the functional cytokine secretion of individual cells for a short period of time. However, accelerated proliferation of the PD-1 knockout CD8+T cells produced more cells and finally increased the overall functional cytokine secretion. It has also been reported that apoptosis of the PD-1 knockout CD8+T cells

increases late after 42 days of transfusion, which indirectly suggests that PD-1 knockout CD8+T cell transfusion is safe [11].

1.2 Application prospect of gene editing in immunotherapy study

Conventional gene knockout technology is very complex, expensive, inefficient, easily off-target and time-consuming, limiting its application in cell immunotherapy. The CRISPR-Cas9-OPT gene editing system that will be applied in this study is the third generation of gene editing system, following ZFN system and TALEN system. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat), in this system, consists of the exogenous DNA that is induced by approximately 20 spacers and repeated sequences at both ends, which is transcribed into guide RNAs and then forms a complex with CAS9 protein. The complex will recognize and bind to the specific sequences of DNA through guide RNAs, and then CAS9 will target and remove the DNA in the binding region [12]. Comparing with other gene editing technologies, the CRISPR-Cas9 is simple to manipulate and highly efficient in knocking out target genes, and can edit multiple genomic regions in the same cell [13]. These advantages have promoted the application of CRISPR-Cas9 in clinical translational studies in recent years.

In a cancer gene therapy study, MCL-1, which is a crucial gene for Burkitt lymphoma, can be specifically edited by the CRISPR-Cas9 system, leading to the inhibition of tumor growth [14]. Similar studies have also demonstrated that using CRISPR-Cas9 to edit E-cadherin in a bladder carcinoma cell line can inhibit the growth of the cancer cells [15].

As for treating virus-related tumors, many *in vitro* studies have reported that the CRISPR-Cas9 system can successfully and specifically edit the genome of human hepatitis B virus (HBV) and reduce the generation of the HBV antigens [16]. Human papilloma virus (HPV) can integrate its own DNA into the host genome, highly express of the virus proto-oncogenes E6 and E7, and then induce cervical cancer. Several studies have shown that an editing system that targets E6 and E7 genes can be established using CRISPR-Cas9 and that this can be used to inhibit the proliferation and tumorigenic ability of HPV16-infected SiHa cells [17].

Basic research in cancer immunotherapy has demonstrated that using CRISPR-Cas9 to knock out the PDCD1 gene (encoding the PD-1 protein) can decrease the PD-1 expression on T cells and increase the secretion of IFN-γ [18]. The anti-tumor ability of chimeric antigen receptor T cell immunotherapy (CAR-T) cells is significantly improved after using CRISPR-Cas9 technology, which combines immune checkpoint blockade therapy with CAR-T, to further knock out the expression of PD-1 in CD19 CAR-T cells in *in vitro* experiments. Additionally, this has been demonstrated in experimental animals with PD-1 positive expression [19]. In June 2016, the recombinant DNA advisory committee (RAC) of the national institutes of health (NIH) first

approved a phase I clinical trial led by Professor Carl June from the University of Pennsylvania. Initially, the investigators plan to transfect NY-ESO-1 into T cells that have been isolated from patients with myeloma, sarcoma, and melanoma. Secondly, CRISPR will be used to knock out the PDCD1 gene in these T cells. They hypothesize that it will bring a beneficial effect by combining these two gene-editing approaches.

Cell therapy in cancer hasalso evoluted rapidly in recent years. Clinical studies using CAR-T for treatment of malignant hematological tumors, such as acute lymphoblatic leukemia, and lymphoma such as diffuse large B cell lymphoma, indolent B cell lymphoma and acute lymphoblastic lymphoma, have demonstrated the safety and efficacy of this type of therapy [20, 21]. For solid tumors, clinical trials for the application of CAR-T in neuroblastoma, metastatic renal cell carcinoma, and ovarian cancer have been reported and demonstrated its safety and feasibility in clinical application [22-25].

Since September 2013, we have used the CRISPR-Cas9-OPT optimized platform to specifically knock out the PD-1 gene and the immune checkpoint gene CTLA-4 in T cells, to improve the function of T cells. Our preliminary data from *in vivo* and *in vitro* experiments suggest that: (1) the sgRNAs can efficiently knock out PD-1 and CTLA-4; (2) it is feasible to knock-out PD-1 in T cells; (3) there is a significant improvement in the secretion of tumor killing cytokine from T cells after the knock-out of PD-1. These results suggest applying this optimized system to target PD-1 on T cells in cancer immunotherapy. Due to the ease of manipulation and development, and the low cost, this therapy shows the potential for broad application.

1.3 The study technology and work basis

1.3.1 Study-related technology

This novel project has been co-developed by the Thoracic Oncology Department of the West China Hospital at the Sichuan University and MedGenCell Biotech Co., Ltd. MedGenCell Biotech Co., Ltd. is a biotech company located in the Tianfu Life Science Park (Chengdu, Sichuan) and provides biotech development, technology consultation, technology promotion, clinical trials, and technical support.

The CRISP-Case9 vector has been established, which knocks out PD-1 in healthy human T cells with a high efficiency (ranging from 40%-90%). The cytokine secretion was increased in the PD-1 knock-out T cells in vitro. The enyme-linked immunoSpot (ELISPOT) test showed that the function of the specifically activated T cells was enhanced following PD-1 knock-out.

There were two major challenges of genome editing technology: (1) the low efficiency of gene knockout; (2) the potential off-target toxicity caused by CRISPR-Cas. The team collaborating on this project has been working on the optimization of the Cas9 system since July 2012. The

works including not limiting are as followed:

1. Optimization of the CRISPR-Cas9 system for the knockout of mammal genes. This system that uses CRISPR-Cas9 to establish a gene knockout mouse model through pronuclear injection. An application for the patent has been filed ('A eukaryotic gene targeting method and helical structural DNA sequence without species limit or bio-safety problem', Application No.201310028668.2), and the related article was published in *Cell Research*[26]. This optimization laid the foundation for all genome manipulation in different animals through the use of the Cas9 system via pronuclear injection.

2. Multiple gene knock-outs have been accomplished in mice and rats through pronuclear injection. We performed gene manipulation in mice at 10 points of five target genes by injecting 10 sgRNAs at the same time. These 10 gene regions were all manipulated and detected in the founders. This operation enables the knock-out of 10 genes in rats or mice simultaneously. This has several advantages: first, it reduces the working time and cost of experiments; second, it simplifies the process of knocking out multiple genes, which has previously been hard to perform. As human diseases are usually caused by multiple gene alterations, the simultaneous manipulation of multiple genes will be more attractive. Based on the above-mentioned research, we performed the conditional gene knock-out in rat by using the CRISPR-Cas9 system. After the conditional knock-outloxP template was introduced, the homologous recombination was mediated by CRISPR-Cas9. The articles reporting this research were published in*Cell Research*[27].

3. Due to its high efficiency, CRISPR-Cas9 causes an off-target effect, which limits its application. Through collaboration with the Sanger institute, we successfully reduced the off-target effect to an undetectable level by combinating mutant Cas9 with double sgRNA technology. This was published in*Nature Methods*[28].

4. By cooperating with domestic investigators, we performed monkey gene knock-out through pronuclear injection. This research work was published in *Cell*[29].

1.3.2 Pre-clinical data[18]**:**

(1) The identification of sgRNAs that efficiently knockout PD-1.

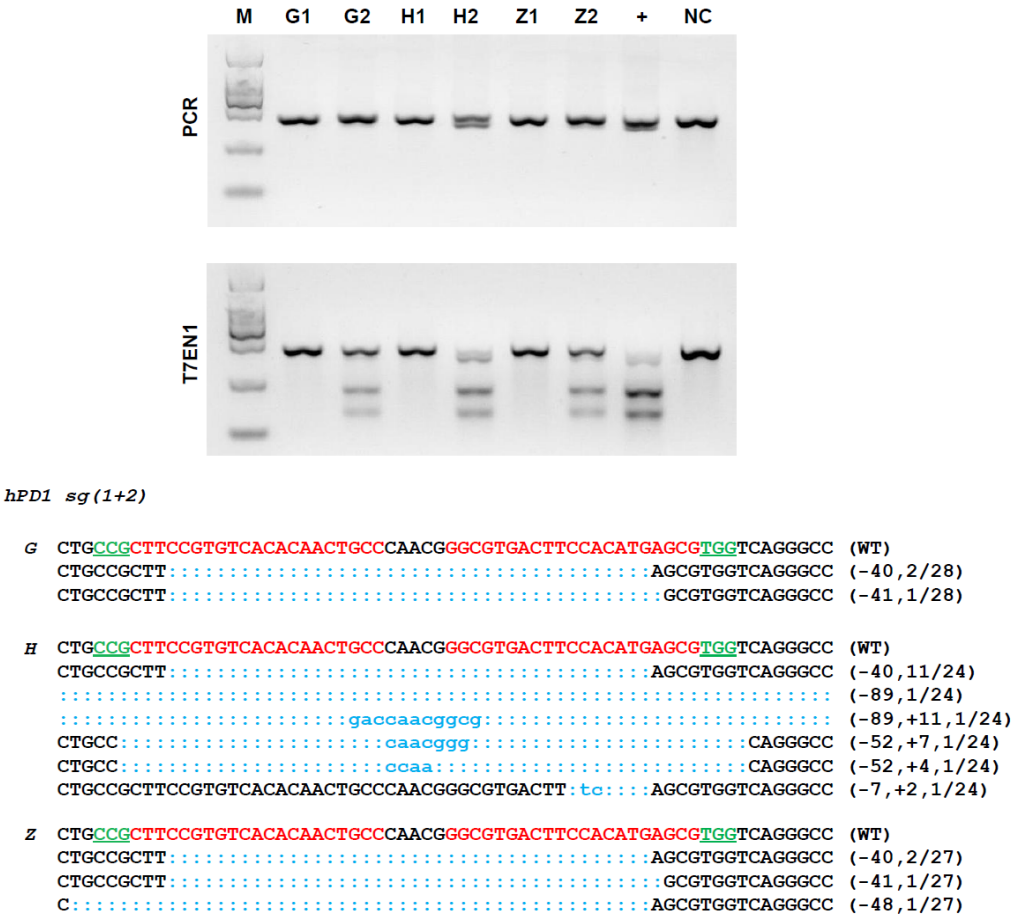


Figure 1. The knockout efficiency of different sgRNAs targeting PD-1 was tested in HeLa cell line.

(2) Electro-transfer efficiency of T cells.

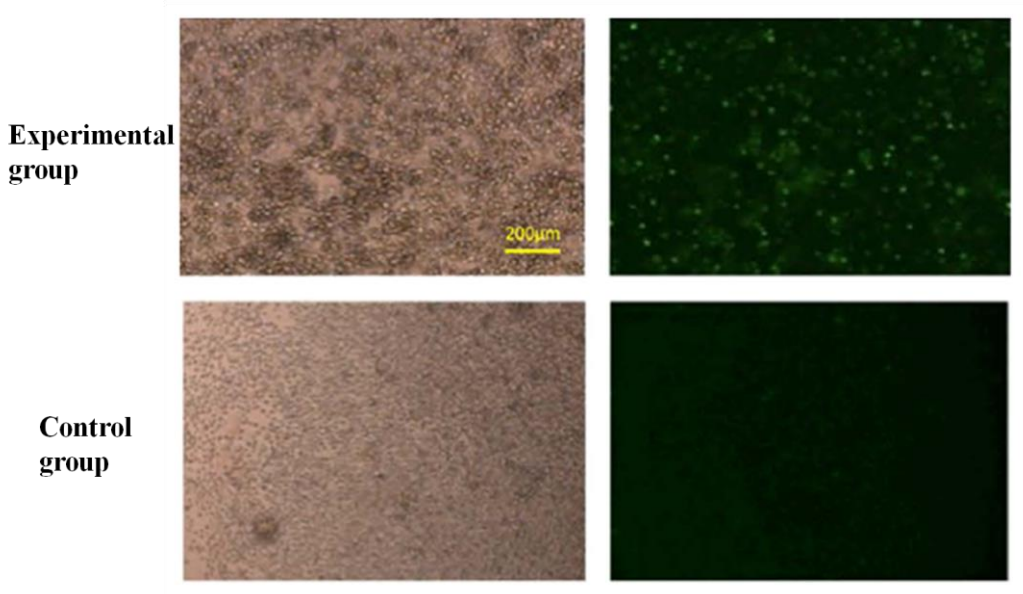


Figure 2. Representative images of the transfected cell by light microscope and fluorescence microscope. The positive infected cells are observed under fluorescence microscope. Results: Qualitative detection of GFP by fluorescence microscope72 h after electrotransfection was performed to evaluatetransfection efficiency.The outcome from flow cytometry revealed that the transfection efficiency was 35-40%.

(3) Electrotransfection of the T cells from three unscreened donors.

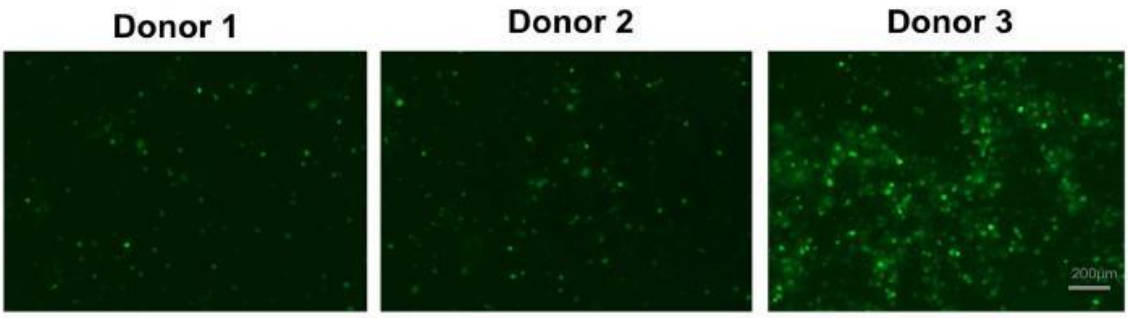


Figure 3. Electrotransfection of the T cells from three unscreened donors. There was discrepancy in the electrotransfection efficiency among different individuals. Without screening, the electrotransfection efficiency was 30-50%; under external screening, the efficiency was 70-95%; the QC standard for the transfusion product is above 10%.

(4) The knock-out of PD-1 has been performed in T cells, and the capability of cytokine secretion of T cells has been significantly improved.

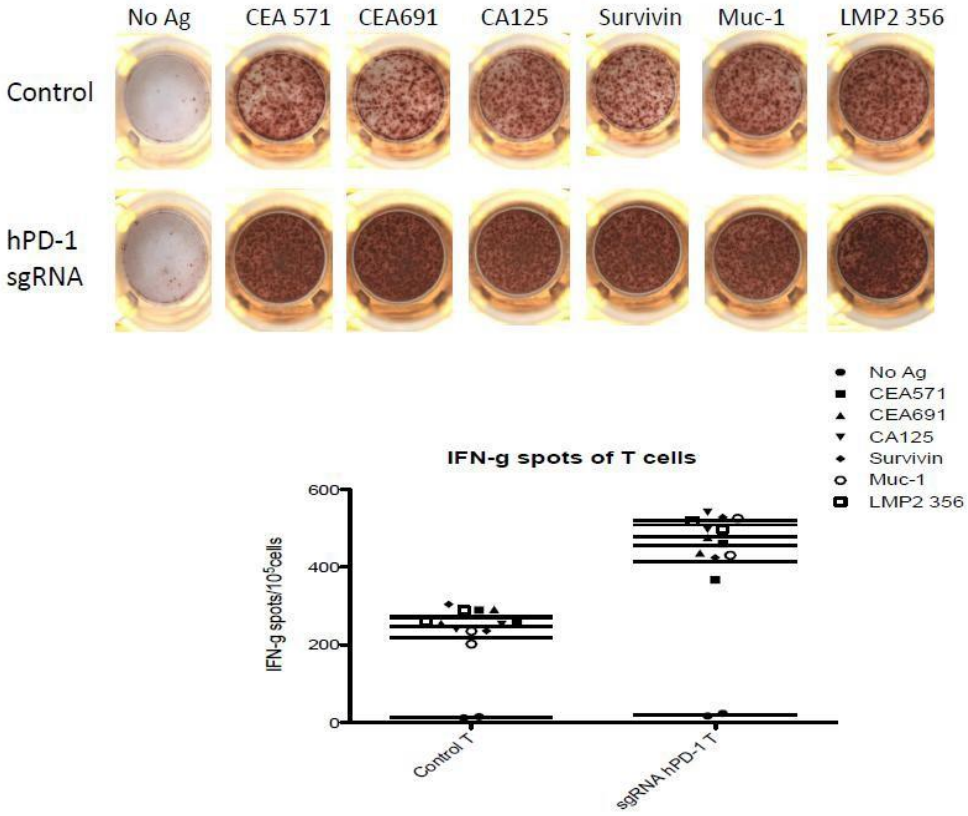


Figure 4. The knock-out of PD-1 in T cells, and the capability of cytokine secretion of T cells has been significantly improved. The changes in the cytokine secretion of the T cells that were isolated from patients' ascites and electrotransfected with Cas9/sgRNA were detected by Ellispot.

(5) The cytotoxicity test proved that PD-1 KO T cells had higher efficiency in targeted tumor cell killing than the common CIK cells.

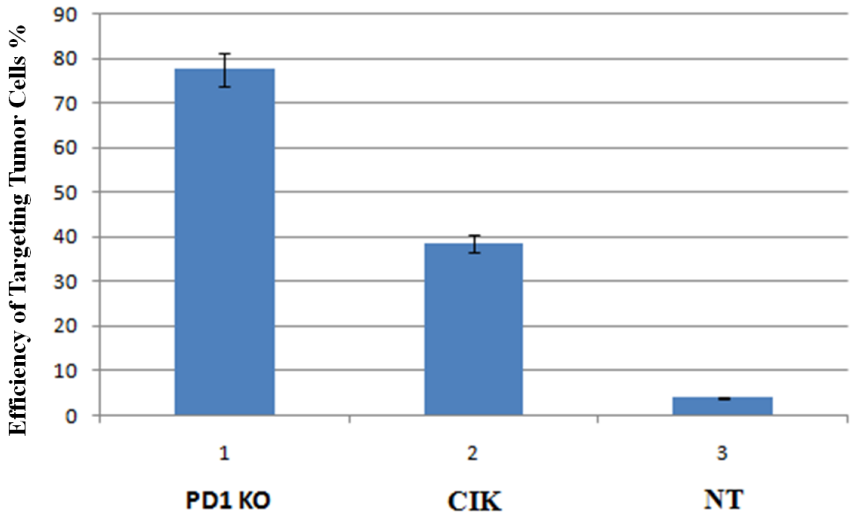


Figure 5. The cytotoxicity tests. PD-1 KO T cells showed higher efficiency in the targeting of tumor cells than the common CIK cells and the NT cells.

(6) The *in vivo* toxicity of PD-1 KO T cells in NSG mice.

Group	N	Accidental deaths	Pathologic change [a]	GVHD [b]
WT T cells	5	0/5	0/5	0/5
PD-1 knockout T cells	5	0/5	0/5	0/5

a: On the 30th day after cell transfusion, the mice were sacrificed. H&E staining of the heart, liver, kidney, and lung tissues were performed. The Giemsa-Wright staining of bone marrow smear was also performed to check the infiltration of the T cells.

b: The wrinkled skin, body weight changes, and activity changes of the mice were observed.

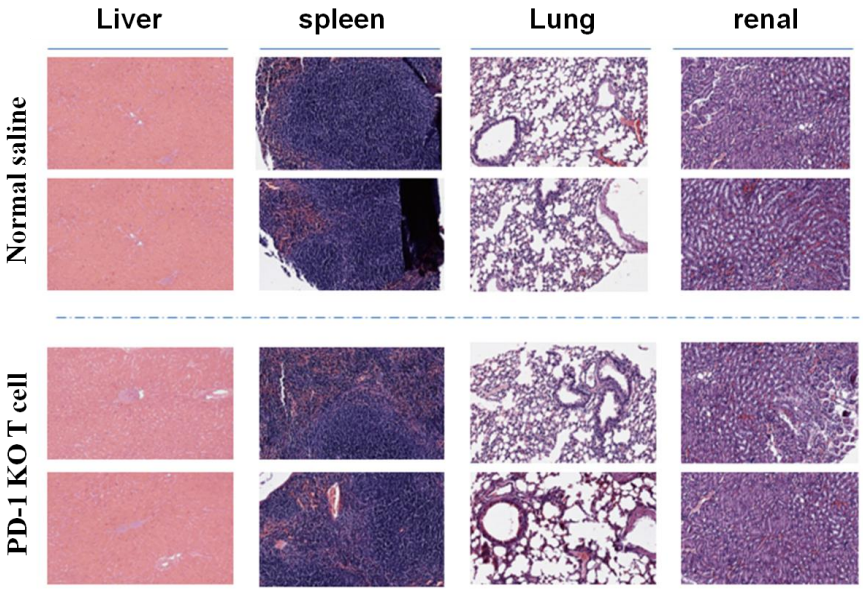


Figure 7. The *in vivo* toxicity test results. Images were captured using a 10X magnification.

Since September 2013, we have utilized the CRISPR-Cas9 system and completed the following research: 1. sgRNAs that can highly efficiently knock out PD-1 and CTLA-4 have been

identified; 2. The PD-1 knock-out has been performed on T cells, leading to a significant improvement in the cytokine secretion of the engineered T cells. These results demonstrate the feasibility of the use of the CRISPR-Cas9 system to target T cell PD-1 in cancer immunotherapy for patients. Considering the simple operation and reasonable development expenses, this therapeutic method shows the potential for broad application.

2 Study objectives

2.1 Primary objective

To investigate the safety and tolerability of CRISPR-Cas9 edited PD-1 T cell in patients with advanced NSCLC.

2.2Secondary objectives

1) To investigate the antitumor activity of CRISPR-Cas9 edited PD-1 T cells in patients with advanced NSCLC.

2) To investigate the changes in the potential immunological indexes and biomarkers.

3) To track the PD-1 edited T cells in patients and investigate the mechanism of the clinical antitumor activity.

2.3 Primary endpoint

The dose-limiting toxicity (DLT) rate of the cell therapy during the first treatment cycle.

2.4 Secondary endpoint

1) To evaluate the response rate (RR) and 8-week disease control rate (DCR) of the cell therapy.

2) To evaluate time to first response, defined as the time from the first cell infusion to the first observed complete response (CR) or partial response (PR).

3) To evaluate the duration of response (DOR), defined as the time from the first observed CR or PR to the first observed PD or death from any cause.

4) To evaluate the progression free survival (PFS), defined as the time from the first cell infusion to the first observed PD or death from any cause.

5) To evaluate the overall survival (OS), defined as the time from the first cell infusion to death.

6) To evaluate the activation status and duration of PD-1 edited T cell after infusion.

7) To evaluate the change of *in vivo* cytokine levels and their relation to the tumor response and adverse events of after PD-1 edited T cell infusion.

3Study design

3.1 Description of the study design

This phase I clinical study aims to investigate the safety and tolerability of PD-1 edited T cell therapy in patients with advanced NSCLC.

About 20 patients with PD-L1 positive advancedNSCLC who have progressed after the multiple lines of prior standard therapeutic regimens are planned to be enrolled in this study. The safety and tolerability of the therapy will be investigated by using intergroup dose escalation method.

This clinical study was a first-in-human trial using CRISPR technology knockouting PD-1 gene in T cell to treat lung cancer patients. Although there were no exact dosage/regimen and safty data, starting dose was selected referring to cell therapy or CAR-T treatment for considering benefit-risk of cancer patients (see section 5.3.1 Starting dose rationale). Pre-A: $2x10^7$ PD-1 edited T cells per kilogram of body weight (percentage of PD-1 knockout T cell may vary depending on technology efficiency). The study defines the Pre-A group as a preliminary exploratory dose group. The Pre-A group has a 28 days-observation after the first treatment cycle. If no DLT was observed in Pre-A group during the defined observation period, the study will continue other treat groups according to provisional dosage; If DLT or other unexpected toxicity occurs in the Pre-A group, the investigator will adjust the cell dose and interval of sequential cell therapy based on data from Pre-A group.

There was three treatment groups and 28 days/cycle.

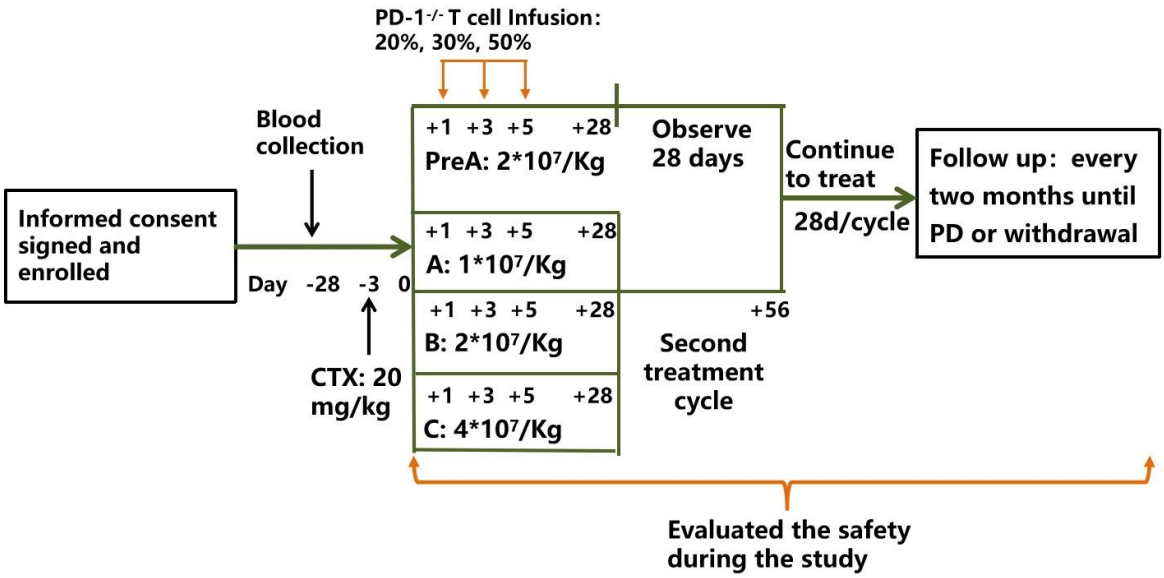
Group A: A dose of 1×10^7 PD-1 edited T cells per kilogram of body weight splited into three doses of 20%, 30%, and 50% will be conducted on the 1st, 3rd, and 5th day of each cycle, 2 treatment cycles;

Group B: A dose of 2×10^7 PD-1 edited T cells per kilogram of body weight splited into three doses of 20%, 30%, and 50% will be conducted on the 1st, 3rd, and 5th day of each cycle, 2 treatment cycles;

Group C: A dose of 4×10^7 PD-1 edited T cells per kilogram of body weight splited into three doses of 20%, 30%, and 50% will be conducted on the 1st, 3rd, and 5th day of each cycle, 2 treatment cycles.

(Treatment may continue after 2 cycles if considered to be benefitial)

Summary schema of the study design:

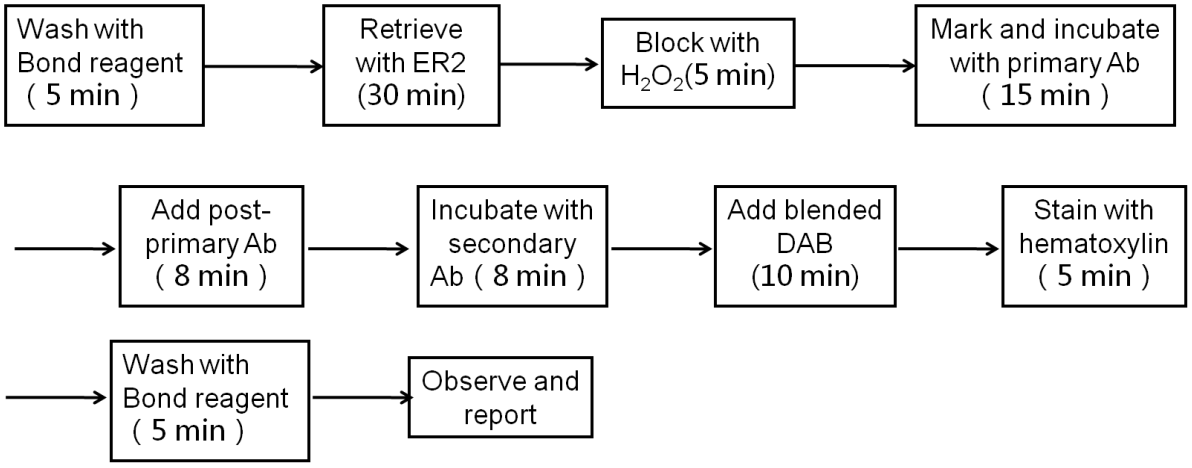


3.1.1 Molecular screening

All of the patients must sign the ICFs for molecular screening. Please find more information in section 7.1.1.

All of the patients enrolled in this study should be confirmed as PD-L1 positive (TPS ≥ 1%) by immunohistochemical staining.

The main standard procedure for detecting PD-L1 is as following:



After the tumor being confirmed as PD-L1positive, the patient can sign the study main ICF, and start the screening/baseline visit.

Remarks: If the patients have already been confirmed to bePD-L1positive through staining with Roche Ventana PD-L1 (SP-142, SP-263) and Dako PD-L1 (Dako28-8, Dako22c3) regent prior to this clinic study, they can directly sign the main study ICFs, and the screening/baseline visit can be initiated [30].

3.1.2 Screening period

The screening period is composed of the screening phase one and phase two. Molecular pre-screening should be completed in the screening phase one, the manufactured T-cell expansion and

baseline evaluation will be performed in the screening phase two. The patients must sign the ICFs before blood collection and screening evaluation. The baseline evaluation must be performed within the four weeks before the first treatment cycle. Refer to 7.1.2 for additional information.

3.1.3 Treatment period

The enrolled patients will be divided into three treatment groups. The treatment will be discontinued if the treatment is finished or if any of the following conditions occur: progressive disease (PD) confirmed by the investigators (according to RECIST 1.1), unacceptable toxicities, pregnancy, end of the treatment bythe investigator or the patients.

The safety will be monitored according to the scheduled follow-up plan (Table 7-1).

For the imaging assessments of the tumor (such as CT and MRI scan), all of the image scans will be scheduled according to the study scheme from the beginning of this study. The scan after the first treatment cycle will be performed at weeks 8 (+1) and 12 (±1). Afterward, the tumor should be assessed every 8 (±1) weeks until the disease progresses, the patient dies, the patient is lost to follow-up, or the patient withdraws the ICF.

The evaluation of tumor responseshould follow the RECIST 1.1guideline in Appendix 12.2; refer to the irRC in Section 12.3.

3.1.4 End of treatment

The end of treatment (EOT) visit will be arranged for patients within the seven days following the confirmation of PD or the discontinuation of treatment due to any other reasons (table 7-1).

3.1.5Follow-upperiod

All patients will be followed up every two months within the 24-month period following the last treatment cycle, to assess the safety (AE or SAE).

3.1.6 Survival follow-upperiod

Once a patient completes the treatment and/or the following tumor assessment, he/she will be followed up every two months to check the survival until the patient is dead, lost to follow-up, or withdraws the consent for survival follow-ups, or the study is completed (refer to the definition of EOT in 3.2). The survival follow-up will be conducted through phone calls.

3.2 Definition of the end of study

The end of study (EOS) was defined as the sixth month after the last enrolled patient finishes the last treatment.

3.3 Early termination of the study

The investigators can terminate the study at any time. If the study has to be terminated early, a patient visit should be arranged as soon as possible, and the assessment for early study termination that is listed in Section 7.1.6 should be performed. To protect the benefit of the patients,

the investigators should inform the patients of other procedures which they should comply with. The investigators are responsible for reporting to the ethics committee and the relevant departments regarding the early termination of this study.

4Population

Advanced NSCLC patients with positive PD-L1 expression (TPS ≥ 1%) who previously failed at least three-lines of treatments were enrolled.

4.1 Inclusion criteria

1. Informed consent form (ICF) is signed:

(1) The enrolled patients must sign and date the EC-approved written ICFs.

according to the guidelines of the competent authority and study institution. The ICFs should be signed before implementing any protocol-related procedure (not including the routine medical care of the patients).

(2) The enrolled patients must be willing and able to comply with the visits, therapeutic regimen, and laboratory examinations specified in the schedule as well as the other requirements in the study.

2. Age: 18-70 years.

3. ECOG performance status 0-2.

4. NSCLC, stage IIIB/IV confirmed by histology or cytology.

All patients must be screened for EGFR gene mutation and ALK gene fusion; patients with positive EGFR mutation or ALK gene fusion treatment failure of EGFR-TKI or ALK-TKI must have been experienced.

5. Patients must fail from the standard third-line or above therapy scheme.

6. Patients must be positive for PD-L1staining, as tested through tumor biopsy (TPS ≥ 1%).

7. Patients must have a measurable lesion (tumor lesion with a long diameter of more than 10 mm through CT scan; lymph node with a short diameter of more than 15 mm through CT scan) in accordance with the RECIST 1.1.

8. Life expectency of ≥ 3 months.

9. Adequate bone marrow function:

• WBC ≥ 3.0×109/L.

• ANC ≥ 1.5 ×109/L.

• PLT ≥ 90×109/L, without transfusions within 14 days before the first cell therapy.

• Hemoglobin ≥ 10.0 g/dL.

10. Adequate hepatic and renal function:

Total bilirubin ≤ 1.5×ULN.

• AST and ALT < 2.5×ULN; AST and ALT < 5×ULN if metastasis to liver.

• Serum creatinine ≤1.5×ULN, or creatinine clearance rate ≥ 50 ml/min (based on the Cockcroft/Gault formula) .

11. Coagulation functions:

• INR ≤ 1.5×ULN.

• PTT ≤ 1.5×ULN.

12. Adequate cardiovascular function:

• Left ventricular ejection fraction ≥ 50%.

• QTcF interval ≤ 450 ms.

13. ALP ≤ 2.5×ULN.

14.Recovery from all adverse events of previous systemic anti-cancer therapies to baseline or Grade ≤ 1, except for Alopecia.

15. Washout period for the previous anti-tumor treatment is not less than four weeks and washout period for the molecular targeting drug is not less than five half-life periods. Palliative radiotherapy must have been completed for at least two weeks; thoracic radiation therapy must have been completed for at least three months, and major surgery must have been completed for at least four weeks with recovery.

16. The patients must take contraception measures strictly.

• Within the 72-hour period prior to the therapy, the serum or urine pregnancy test (HCG sensitivity is 25 IU/L at least or equivalent) of the women of childbearing potential (WOCBP) must be negative.

• WOCBP must agree to take contraceptive measures strictly during the treatment period and for at least 18 months after the final cell infusion until no knockout T cell is detected in the blood.

• The males who have sexual contact with WOCBP must agree to take contraceptive measures strictly during the treatment period and for at least 18 months after the final cell infusion until no knockout T cell is detected in the blood.

4.2 Exclusion criteria

1. Patients with active central nervous system (CNS) metastasis (including but not limited to carcinomatous meningitis and spinal cord compression) must be excluded. However,patients with metastatic CNS tumors may participate in this study if thepatients can be recoved to the baseline level at least two weeks before inclusion (without the residual signs or symptoms related to the CNS treatment). In addition, the patients must have stopped using corticosteroids for four weeks before inclusion.

2. Patients with weight loss in the recent six months ≥ 10%.

3. Patients accompanied by emergent symptoms caused by tumor that need immediate intervention.

4. Patients with medical history of interstitial lung disease (ILD) or immune related pneumonia.

5. Patients with peripheral neuropathy.

6. Patients with clinically significant coagulation abnormalities, or patients who are receiving thrombolytic or anticoagulant therapy.

7. Patients with a history of human immunodeficiency virus (HIV) positivity or with the acquired immunodeficiency syndrome (AIDS), or with active hepatitis B or hepatitis C.

8. Patients that have other malignancy.

9. Patients with active, known or suspected autoimmune diseases.

10. Patients who have received systemic treatment by corticosteroids (the equivalent dose >10 mg prednisone/day) or other immunomodulators (interleukin-2, IFN-α, IFN-γ, cyclosporine, G-CSF, mTOR inhibitor) within the 28 days prior to the study. Patients who receive inhaled or topical corticosteroids and adrenal corticosteroids (the equivalent treating dose <10 mg prednisone/ day) can participate in the study if they are lack of active autoimmune diseases.

11. Clinically significant cardiac disease or impaired cardiac function, such as:

- Clinically significant heart disease such as CHF requiring treatment, or uncontrolled hypertension defined by blood pressure > 140/100 mmHg at rest (taking mean of 3 consecutive readings).
- History (< 6 months prior to screening) or current evidence ofclinically significant cardiac arrhythmias, atrial fibrillation and/orconduction abnormality, e.g. congenital long QT syndrome, Grade ≥2/complete AV-blockage or hypokaliemia CTCAE Grade ≥ 3.
- History/evidence of acute coronary syndromes (including myocardialinfarction, unstable angina, coronary artery bypass graft (CABG),coronary angioplasty, or stenting), < 6 months prior to screening.
- Complete left bundle branch block (CLBBB).
- Right bundle branch block (RBBB) + left anterior hemiblock (LAHB - bifascicular block).

12. Patients with a history of allergies to cyclophosphamide and other agents involved in this study.

13. Patients that do not have enough venous access.

14. Pregnant or lactating women, where pregnancy is defined as the state of a female from conception until the termination of gestation.

15. Patients with mental disorders are mandatorily detained for treatment or are judged as inappropriate for inclusion by the investigator.

16. Patients who are difficult to communicate with or difficult to be followed up for a long time.

17. Other inappropriate conditions in discretion of the investigator’s judgment.

5. Treatment

5.1 Cell therapy protocol

5.1.1 Pre-treatment

A single dose (20 mg/kg) of cyclophosphamide will be administrated as a pre-treatment for lymphocytes depletion on the third day before the cell transfusion of the first treatment cycle. Cyclophosphamide will be diluted with 20-30 ml of normal saline (NS) and intravenously injected for more than 10 min.

5.1.2 Cell infusion

5.1.2.1 Treatment assignment

Pre-A: $2x10^7$ PD-1 edited T cells per kilogram of body weight (percentage of PD-1 knockout T cell may vary depending on technology efficiency). The study defines the Pre-A group as a preliminary exploratory dose group. The Pre-A group has a 28 days-observation after the first treatment cycle. If no DLT was observed in Pre-A group during the defined observation period, the study will continue other treat groups according to provisional dosage; If DLT or other unexpected toxicity occurs in the Pre-A group, the investigator will adjust the cell dose and interval of sequential cell therapy based on data from Pre-A group;

Group A: A dose of 1×10^7 PD-1 edited T cells per kilogram of body weight splited into three doses of 20%, 30%, and 50% will be conducted on the 1st, 3rd, and 5th day of each cycle, 2 treatment cycles, 28 days/cycle;

Group B: A dose of 1×10^7 PD-1 edited T cells per kilogram of body weight splited into three doses of 20%, 30%, and 50% will be conducted on the 1st, 3rd, and 5th day of each cycle, 2 treatment cycles, 28 days/cycle;

Group C: A dose of 1×10^7 PD-1 edited T cells per kilogram of body weight splited into three doses of 20%, 30%, and 50% will be conducted on the 1st, 3rd, and 5th day of each cycle, 2 treatment cycles, 28 days/cycle.

In this study, each treatment group has two treatment cycles. The safety and tumor progressionwill be evaluated when the patients have received two treatment cycles. If no DLT or PD is observed, the investigators willdiscuss with the patientthe further therapeutic regimens based on the potential benefitial to the patient who is willing to continueto receive treatment:

1) If patient decides not to continue treatment, the patient will be followed upaccording to the study workflow (7 visits and evaluation) until end of study. Additionally, the patient has the right to choose other cancer therapeutic strategies.

2) If the patient agrees to continuecell therapy, she/he can receive treatment until end of study. According to the irRC criteria, if PD is identified through tumor imaging, another evaluation should be conducted after at least four weeks to confirm the PD status. During the period to

confirmation of the PD, if the patient's performance status does not deviate from the baseline (ECOG: 0-2 scores), or no intolerable therapeutic side effects/other conditions that are inappropraite for further cell infusion therapy occur, and the patient agrees to continue treatment, the patient may continue to receive the cell infusion treatment. If the next imaging test is scheduled within four weeks, it is not necessary for the patient to take the next appointed imaging test. If the clinical conditions of the patient are stable, the tumor imaging test can be re-initiated at an appointed time point and continued until PD is confirmed through imaging assessment. Given that tumors may pseudoprogress within the first several months after the initial cell infusion therapy and then shrink in some patients, patients are allowed to continue the cell infusion therapy after the first presence of PD until confirmed. For the patients with unstable clinical conditions, it is not necessary to repeat the imaging examination to confirm PD. Tumor pseudoprogression includes:

- Worsening of the original target lesion
- Worsening of the original non-target lesion
- Identification of new lesions

Cells for each infusion treatment cycle should be derived from the newly collected blood. The total number of cells for three treatment groups is: $1x10^7$, $2x10^7$, and $4x10^7$ PD-1 edited T cells per kilogram of body weight, respectively, and the total volume are 100 ml/time.

5.1.2.2 Cell infusion procedure

1. The vital signs, temperature and blood pressure should be tested and recorded within 30 min before the cell infusion. If any fever occurs (> 38.5°C), the cell infusion shall not be performed.

2. Diphenhydramine will be intramuscularly injected within approximately 30 min before the cell infusionto prevent the cell infusion reaction.

3. Use 0.9% normal saline (NS) to establish the intravenous line.

4. Gently shake the cell bag 3-4 times to suspend the cells.

5. Start infusion, shake the cell bag gently every 20 min during the infusion and slightly pinch the bottom of the cell bag several times to prevent cell aggregation.

6. Cell infusion speed: during the first cell infusion to a patient, the initial speed should be adjusted to about 15-20 drops/min. The patient's tolerability will be evaluated 10-15 min after the infusion. If no adverse event is observed in the patient, the infusion speedwill be adjusted to be reasonably faster, around 60 drops/min. For elderly patients and patients with cardio-pulmonary insufficiency, the infusion speed should be slowed downand close attention should be paidto the patient's tolerability to the infusion speed. The speed of infusion can be adjusted according to the condition of the individual.

7. The NS will be administratedafter the cell transfusion.

Remarks: The vital signs and symptoms of patient should be monitored within 30 min before and after the cell infusion and at the time when the cell infusion is finished.

5.2 Concomitant treatment

5.2.1 Acceptable concomitant treatment

Recordall of the medicine and non-medicine treatment (includingphysical therapy, natural drugs, and blood transfusion) that is administrated to the patients within the four weeks before the treatment and during the treatment period.

During the study, the patients will be allowed to use external, ophthalmic, intraarticular, intranasal and inhaled corticosteroids (at extremely low systemic inhaled doses).

Patientes will be allowed to take corticosteroids to prevent (for instance, the allergy to contrast agent) or cure (for instance, delayed allergic reaction caused by contact allergen) non-autoimmune diseases in a short term (< 1 week).

Combined palliative and supportive treatments are allowed to be used to treat the disease-related symptoms (e.g. diphosphonate). During the cell infusion treatment, the use of RANK-L inhibitors (including, but not limited to, Xgeva) will not be allowed. The previous palliative radiotherapy should be finished at least two weeks before the cell therapy is commenced.

5.2.2 Local palliative treatment

Patients who have no evidence of clinical PD or imaging PD (according to RECIST 1.1) are allowed to receive local palliative treatments (including palliative radiotherapy or palliative surgical resection to the symptomatic non-target bone lesions, skin lesions or CNS lesions) before the treatment ends. Patients who have no evidence of clinical or imaging progression (according to RECIST 1.1), and for whom the lesion that causes hemoptysis is not the only measurable target lesion, will be allowed to receive the local palliative treatment to that target lesion after discussing with the principle investigator and getting permission.

Before starting palliative therapies, the objective evidences of the PD of the patients should be evaluated, especially if the latest tumor response evaluation is conducted more than four weeksbefore the local palliativetreatment. If the second tumor progression is confirmedaccording to irRECIST before the local palliative treatment, the treatment of the patients will be stopped.

It is currently unclear whether the toxicity that is induced by radiotherapy overlaps with the toxicity that is induced by the cell therapy. Because no data regarding the effects of combined radiotherapy and cell therapy has been reported, if the patient requires palliative radiotherapy(radiotherapy is not allowed in thoracic lesions) then the cell therapy should be stopped for at least one week before the radiotherapy. Cell therapy could be resumed at least one

week after the radiotherapy is completed. Close monitoring should be paid to the patients during and after the radiotherapy. Once the toxicities occur, the cell therapy should be continued only after AEs recover to Grade 1 and below, or to the baseline level or stable.

5.2.3 Restricted treatment

During the study, the investigators will judge whether the patients will benefit from the concomitant therapy and they will decide whether to apply it or not.The investigators will record this in the corresponding sections of the case report form.

During the study, the following drugs are contraindicated (unless they are used to treat the drug-related AEs):

1. Immunosuppressors.

2. During the study, the prophylactic usage of recombinant human growth factors (such as G-CSF and its analogues and hemopoietin-α and its analogues) is contraindicated. The use of prophylactic usage of recombinant human growth factors will be acceptable only if it really medically benefits the patients and the patient's eligbility for further treatment will not be affected, as judged by the investigators. GM-CSF is contraindicated throughout the study process.

3. High doses of systemic corticosteroid that will lead to immunosuppression (except for those specified in Section 5.1.5.1).

4. Any antitumor medicine is contraindicated, including, but not limited to, chemotherapy, targeted drugs (Gefitinib, Tarceva, AZD9291 active ingredient, antitumor Chinese medicine, etc), hormonotherapy, immunotherapy (including but not limited to treatment of thymus gland), extensive nonpalliative radiotherapy, and investigated treatment for NSCLC).

Except for the local palliative treatment specified in Section 5.2.2, any other radiotherapies or surgerical treatment of any tumor lesions are not allowed during this study. Patients who receive other non-palliative therapy will have to discontinue the study treatment.

5.2.4 Treatment duration

For proper planning and evaluation in this study, a treatment cycle is defined as 28 days. Patients will receive treatment following the protocol until PD (RECIST 1.1) or intolerable toxicity occurs, the termination decision is made by the investigator, the consent forms are withdrawed by the patient, or the patient dies.

5.3. Dose escalation guidelines

5.3.1 Starting dose rationale

In 2010, the FDA approved the first therapeutic vaccine PROVENGE which is infusedat least 5×10^7 cellsinto the patients per two weeks for three times. In addition, some clinical studies indicated that no treatment related adverse events above Grade 3 were observed and no related

lethal effect was observed after multiple myeloma patients received the receptor-engineered T cell therapy at an average dose of 2.4×10^9 ($0.45\text{-}3.9\times10^9$) [31]. Single infusion is mainly used in the chimeric antigen receptor engineered T cell (CAR-T) therapy with a commonly used dose of 1×10^6/kg. A phase I clinical study indicated that no treatment related adverse events of Grade 3 or above were observed after the patients received the escalated CAR-T dosing (1×10^8, 1×10^9, 1×10^{10} cells) [32]. Anotherrecent study in NSCLC patients indicated that no serious adverse events were observed after the patients received the CAR-T infusion at an average dose of 0.97×10^7/kg (0.45 to 1.09×10^7/kg) [33]. Therefore, accordingly to thedose levels of DC-T cell therapy approved by the FDA in 2010 and the CAR-T cell therapy in the existing clinical studies, and considering the balance of potential treatment safety and benefits to decide the initial dose of this study.

5.3.2 Provisional dose levels

The initial dose of the exploratory group Pre-A was $2x10^7$ PD-1 edited T cells per kilogram of body weight. This dose will be split into three applications (20%, 30%, and 50%, respectively).A cycle is 28-days, and one treatment cycles. Besides, the cell dose of the treatment group A/B/C will sequentially increase $1x10^7$ to $2x10^7$ and $4x10^7$ PD-1 edited T cells per kilogram of body weight.

5.3.3 Guidelines for dose escalation and determination

If DLT occurs in more than 1/3 of cases in the treatment group, another three patients will be enrolled into this group. Further enrollement for this group will be stopped if DLT occurs in one more case, and the data will be updated and re-evaluated.

To apply the rule of dose escalation, the investigators and related staff (including study physicians and statisticians) will evaluate the toxicity (including the AEs unrelated to DLT and laboratory test abnormality) and safety profile of the tested group. Only when the safety evulation of the tested group has been completed, another treatment group will be started.

5.3.4 Intrapatient dose escalation

The escalation dose is from $1x10^7$ to $2x10^7$ and $4x10^7$ PD-1 edited T cells per kilogram of body weight in the three treatment groups A, B, and C. Engineered T-cells spliting into three applications (20%, 30% and 50%) will be conducted on the 1st, 3rd, and 5th day of each cycle. The patient must be able to tolerate the lowest dose, and the effect of the prior dose has beenassessed, the patient can receive the sequential treatments.

5.3.5 Other dosing regimens

N/A

5.3.6 Definition of Dose Limiting Toxicity

Unless otherwise specified, NCI CTCAE 4.03 will be applied to assess the toxicity. DLT is

defined as an AE or a laboratory abnormal value that is probabaly attributable to the T cell infusion within first 28 days after cell transfusion. An abnormal laboratory value can be attributed to the T cell infusion if it happens within one cycle after the first cell infusion (≤ 28 days) or occurs in thefirst cycle but is later confirmed in the second cycle, and meets any criterion of the following conditions:

- Any adverse event that is ≥ Grade 3 and is confirmed to be or probably attributable to the cell therapy.
- Any toxicity related to the cell therapy that requires systemic glucocorticoid treatment.
- Autoimmune toxicity that is ≥ Grade 2.

Treatment will be discontinued when a patient experiences DLT, and the follow-up will be initiated according to the descriptions in Table 7-1.

Before patients enroll in a higher dose group, all of the AEs ≥ Grade 2 that have occurred in the the current dose level must be analyzed.

5.4 Dose modification

5.4.1 Criteria for cell therapy delay, reinitiation and discontinuation

5.4.1.1 Criteria for delayed cell infusion

1. Any AEs are ≥Grade 2 that are related to the cell therapy, except the following events:
   - For any Grade 2 fatigue or non-clinically significant laboratory abnormality that is related to the cell therapy, it is not neccessary to delay the treatment;
   - If the baseline level of AST, ALT or total bilirubin is within Grade 1, and the patient presents Grade 2 after the treatment while does not receive the hormonotherapy. In this case, it is not neccessary to delay the treatment;
   - Any Grade 3 laboratory abnormality that is related to the cell therapy, except the following conditions:
   1) Recovery from Grade 3 lymphopenia or leukopenia.
   2) Any amylase or lipase abnormality ≥ Grade 3, which is related to the cell therapy, without pancreatitis symptoms or clinical manifestations, do not require the therapy to be delayed but to require consultion of the major investigators.
2. Any AE or laboratory abnormality that investigators consider it necessary to delay the therapy.
3. If there are any clinical manifestations, the patient should be re-evaluated every week or more frequently, and therapy should be reinitiated when the patient conforms to resuming treatment criteria.
4. For other conditions that may deteriorate the safety of the patient, the cell infusion can be delayed or stopped according to the evaluation of safety by investigators based on the actual

conditions.

If the cell fusion therapy is postponed for more than 14 days, the patients will not reinitiate the treatment, unless otherwise specificied. If the reason for a delay of more than two weeks of therapy is not related to the cell therapy, continuation is acceptable upon the approval of the principle investigator. Before the treatment is resumed in the patients with more than two weeks of delay, it is necessary to consult the principle investigator and perform tumor evaluation according to the study protocol.

5.4.1.2 Criteria of reinitiation of cell therapy

After the cell therapy, related AEs are controlled to below Grade 1 level or baseline level, the patient can reinitiate cell therapy. The following conditions are listed:

- Patients with Grade 2 fatigue can resume the therapy.
- Patients with Grade 2 but not Grade 3 cell therapy related skin AE can undergo the therapy again.
- Patients, whom with an abnormal baseline level of AST/ALT or total bilirubin ≤ Grade 1 toxicity, has Grade 2 AST/ALT or total bilirubin right now, while the previous treatment delay was not caused by the Grade 2 AST/ALT or total bilirubin. In this case, the patients can resume the therapy.
- Patients with continuous Grade 1 pneumonia can undergo further therapy after being approved through discussion.

5.4.1.3 Criteria of discontinuation of cell therapy

The cell therapy should be discontinued permanently in cases with the following conditions:

- ≥ Grade 3 Cytokine release syndrome;
- Any cell therapy-related condition ≥ Grade 2 that requires systemic glucocorticoid therapy, such as pneumonia, nervous system damage, diarrhea/colitis, liver damage, nervous system damage, impaired kidney function, symptomatic endocrinopathy, uveitis, ophthalmodynia or blurred vision;
- Patients with cell treatment-related Grade 3-4 lung toxicity, diarrhea or colitis and ≥ Grade 2 cell therapy related endocrinopathy will stop the treatment;
- Any AE, laboratory abnormality or intercurrent illness that, the investigators judge, would lead to great clinical risk in the patient if the cell therapy was continued.

5.4.2 Follow-Up for DLT and other toxicities

If the treatment is stopped temporarily or permanently due to AE or laboratory abnormality, the patient should be followed up once a week for at least four weeks. After that, the patient should be followed up once every four weeks until the event disappears or becomes stable. If the cell

infusion is to be delayed more than 14 days beyond the next scheduled infusion day, patients should stop the treatment permanently and be followed up as described above. All AEs and SAEs related to the therapy should be followed up to 24 months after the end of study.

Follow-up for other toxicity: if discontinued treatment due to AE or laboratory abnormality, the patient must be followed up at least once a week for four weeks, and then once every four weeks until the event disappears or becomes stable.

5.4.3 Anticipated risk and safety considerations of the study treatment

This study protocol provides dose modification criteria, DLT definition and detailed guidance for delay, reinitiation and discontinuation of the study treatment. The recommended supportive treatment and cancer immunotherapy guidelines for anticipated toxicities are provided in Appendix 5.5, including the treatment for AEs (CRS, etc).

5.5 Supportive treatment

The cell therapy treated patients may, as recommended by the investigators, be treated with appropriate supportive therapies, based on the guidelines for immune-related advert events (irAE).

5.5.1 Cytokine release syndrome (CRS)

Cell therapy may cause the release of inflammatory cytokines into the blood as a cytokine release syndrome. The cytokines usually reach the peak level at 5-7 days after cell infusion. A serious CRS may result in acute respiratory distress syndrome, multiple organ failure, and even death.

In this study, the biomarkers of CRS such as IL-6, IL-10, CRP, TNF-α, etc will be routinely monitored in each cycle at the time before cell infusion, 24 hours after cell infusion, and on the 7th day after the cell infusion. In case of clinical suspicion or consideration of the CRS, the patient will be intensively clinically monitored for body temperature, blood pressure, oxygen saturation and neurological disorders. The blood sampling for cytokine detection is also recommended.

The patients with CRS will not present specific symptoms initially, but some non-specific symptoms of the respiratory, gastrointestinal, cardiovascular, renal, and neurological systems may be observed [34-36].

Main clinical signs and symptoms of CRS:

Organ system	Signs and Symptoms
Constitutional	Fever ± rigors, malaise, fatigue, anorexia, myalgia, arthralgia, nausea, emesis, headache
Skin	Rash
Gastrointestinal	Nausea, vomiting, diarrhea

Respiratory	Tachypnea, hypoxia
Cardiovascular	Tachycardia, widened pulse pressure, hypotension, increased cardiac output (early), possible diminished cardiac output (late)
Coagulation	Elevated D-dimer, hypofibrinogenemia ± bleeding
Renal	Azotemia
Hepatic	Transaminitis, hyperbilirubinernia
Neurologic	Headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, seizures

Supportive care and close monitoring without special treatment are recommened for Grade 1 CRS and Grade 2 CRS without systemic complications.

The main clinical symptoms of severe CRS are persistent fever, hypotension, hypoxia and neurological symptoms. Fever may start 24 hours after the cell infusion and last for about 10 days. CRP will change during the CRS, which usually significantly increases at 4-9 days following the cell infusion. CRP > 20mg/dl may indicate severe CRS. Severe CRS is life-threatening and must be identified and intervened as early as possible. The diagnostic criteria are as follows:

- Continuous fever lasting at least 3 days;
- The maximum fold increase of two cytokines is 75-fold or more, or the maximum fold increase of one cytokine is 250-fold or more;

At least one symptom from clinical toxicity, such as hypotension (requiring treatment with at least one kind of pressor) or hypoxia (PO2 < 90%) or neurological symptoms (such as mental status changes, unresponsiveness, seizures).

CTCAEclassification	Clinical manifestation	Treatment

Grade 1	Non-specific clinical symptoms. Fever, nausea, fatigue, headache, myalgias, malaise.	Supportive treatment: Close monitoring: Cytokine, CRP, ESR, TNF-α: blood routine, biochemical test. Pathogenic bacteria culture to evaluate infection. Treatment is necessary if a fever or granulocytopenia occurs.
Grade 2 Symptoms require and respond to moderate intervention	Oxygen requirement of <40% orhypotension responsive to fluids or a low dose of one vasopressor (such as dopamine, norepinephrine) or Grade 2 organ toxicity.	Evaluate whether there are extensive co-morbities or older age. If not: supporting treatment (pay close attention to the cardiac functions and function of other organsand the cytokine level) If yes: refer to grade 3-4 CRS
Grade 3 Symptoms require and respond to aggressive intervention	Oxygen requirement ≥ 40% or hypotension requiring a high dose or multiple vasopressors or Grade 3 organ toxicity or grade 4 transaminitis.	Viligant supporting treatment. Administration of tocilizumab* (4 mg/kg). If no improvement in the patients' condition is observed within 24-48 hours, the above dose can be administrated again ± a glucocorticoid (the initial dose of methylprednisolone is 2 mg/kg/day and can be regulated correspondingly). For patients with serious nervous system symptoms, dexamethasone can be considered (0.5 mg/kg/day, single dose ≤ 10 mg).
Grade 4 Life-threatening symptoms	Requirement for ventilator support orGrade 4 organ toxicity (excluding transaminitis).	

Vasopressors dose conversion (≥ 3 h infusion time is required for all doses)

Norepinephrine	Monotherapy	≥ 20 ug / min
Dopamine	Monotherapy	≥ 10 ug / kg / min
Phenylephrine	Monotherapy	≥ 200 ug /min
Epinephrine	Monotherapy	≥ 10 ug / min
Use of antidiuretic hormone		Antidiuretic hormone + norepinephrine corresponds to ≥ 10 ug / min

Use of combined vasopressors	Non-antidiuretic hormone	Norepinephrine equals to ≥ 20 ug / min

Norepinephrine equivalent dose = [norepinephrine equivalent dose (ug / min)] + [dopamine (ug / kg / min) ÷ 2] + [phenylephrine (ug / min)] + [epinephrine (Ug / min) ÷ 10]

5.5.2 Treatment transfusion/allergic reaction related to cell therapy

Transfusion reaction and allergic reaction may occur in the process of cell infusion, including chills and fever (usually about 38°C or over 40°C in serious cases), accompanied with headache, nausea, emesis, pruritus, urticaria, fast pulse, general malaise, arthralgia, hypotension, hypertension, bronchospasm and other allergic reactions. In serious cases, it may cause angioneurotic edema, laryngismus, asthma, anaphylactic shock, heart failure and pulmonary edema.

The transfusion/allergic reaction occur quickly in general and is potentially life threatening for critical ill patients. These patients shall be closely monitored clinically and treated promptly and properly.

Some patients may suffer from delayed allergic reactions, which occur within one week of the cell infusion. An allergic reaction is characterized by local or systemic pruritus, rash and other signs. The treatment for these symptomes includes oral antihistamine and/or glucocorticoid.

Measurements for treating transfusion/allergic reactions

Transfusion/allergic reaction classification	**Treatment**	**Follow-up**
Grade 1 symptoms: Slight, temporary reaction; discontinuation of infusion is not needed; no need for treatment.	Stay at the bedside. Slow down the infusion rate. Closely monitor the patient's symptoms, consciousness, blood pressure, breathing, pulse, urine output, etc., until the symptoms are remitted.	Medication prevention: It is recommended to use appropriate drugs 30 minutes prior to treatment for the prevention of the recurrence of the transfusion reaction when further cell infusions are conducted, such as: Diphenhydramine at 50 mg, intramuscular injection (or other similar drugs), acetaminophen at 325-1000 mg (or other similar drugs), etc.
Grade 2 symptoms: treatment or discontinuation of infusion is needed, but	**Stop the cell infusion immediately** Replace infusion fluid and	If the patient's symptoms remit within a short time: restart cell infusion at 50% of the original

symptomatic treatment (antihistamine NSAIDAS, narcotics, infusion treatment) has a rapid effect; prevention of medication ≤ 24 hr.	conduct intravenous infusion with normal saline. Use diphenhydramine 50 mg (or an equivalent dose of a similar drug) for intravenous infusion; acetaminophen 325-1000 mg; proper use of glucocorticoid and/or bronchodilator; stay at the bedside and closely observe the patient's symptoms, consciousness, blood pressure, breathing, pulse, urine output, etc. until symptom remission. If necessary, conduct on-site physical storage, blood sampling (blood routine test, blood culture, etc.).	infusion speed and if there is no notable discomfort after 30 minutes, speed up the infusion rate, and closely monitor, or resume the original speed Medication prevention: when another cell infusion is conducted, it is recommended to reventively use the following drugs 30 min in advance of treatment: diphenhydramine, acetaminophen, etc. Use glucocorticoids for prevention when necessary If ≥ Grade 2 transfusion/allergic reaction reoccur, terminate cell therapy.
Grade 3-4 symptoms Grade 3: delay in symptom remission (such as failure to respond quickly to symptomatic treatment and/or discontinuation of infusion); relapse of improved symptoms; hospitalization needed for sequelae. Grade 4: life-threatening; emergency treatment needed	**Stop the cell infusion immediately** Change infusion liquid and tubes; conduct intravenous infusion of normal saline; proper use of bronchodilators is recommended: including but not limited to: epinephrine 0.2-1 mg (diluted to 1:1000) through subcutaneous injection or epinephrine 0.1-0.25 mg (diluted to 1:10,000) through slow intravenous injection; diphenhydramine 50 mg + methylprednisolone 100 mg or an equivalent dose of other glucocorticoids through intravenous infusion.	**Cell therapy is permanently terminated** Stay at the bedside and closely monitor the patient until the symptoms completely remit and condition is stable. If necessary, conduct on-site physical therapy, check blood routine and biochemical indexes, etiological culturing (such as: the liquid being infused, blood, etc.). **Continue follow-up:** Some patients may suffer from a delayed allergic reaction within one week of the cell infusion, including expression local or systemic pruritus, rash and others symptoms. For these symptoms, such symptomatic treatment as oral antihistamine drug and/or glucocorticoid will be provided.

6. Patient numbering, treatment assignment or randomization

6.1 Patient numbering

In this study, the subject ID is used to identify each patient when the patient is first enrolled for molecular screening, and is used as the major identification of the patient throughout the entire study. The format of the subject ID is as follows: 00101, 00102, and 00110. Each patient has a unique subject ID in the database. Once a subject ID is assigned to one patient, it can not be used again.

6.2 Treatment assignment or randomization

According to the study design, the patients will be assigned into a specified treatment group without randomization.

6.3 Treatment blinding

N/A. This is an open-label, phase I study.

6.4 Preparations for cell therapy

The investigators or the responsible staff members at the study center must inform the patients that the study treatment needs to be performed according to the study protocol. Only the authorized staffs in the study center can perform the cell transfusion for the patients. The conditions of the patients must be recoded in detail throughout the whole study.

Table 6-4. Preparation for cell therapy

Therapy	Application	Preparations
Cell therapy	In this study, cell infusion must be performed by the authorized staff.	See Section 5.1.2.1 for detailed cell transfusion procedure.

6.4.1 Package and labeling of the study cells

The package label of the study cells is displayed as below. The label is produced in duplicate and one label should be taken from the cell storage bag and filed in the study document.

Patient No.: Cell type:
Cell quantity: Capacity:
Storage temperature: Storage condition:
Date of manufacture: MM/DD/YY 00:00
Expiration date:
Manufacturer:
Study ID: MHC-001

6.4.2 Cell supply and storage

The cells should be maintained between 2°C to 8°C during transportation and storage in the

laboratory and the study center. The study center must appoint an authorized staff member to receive, handle and store the cells for the study treatment. The cells must be stored safely and properly in a location that only the investigators and the appointed staff members at the study center have access to. On the reception of the cells, they should be transfused into the patients according to the cell therapy requirements (Section 5.1.2.1).

6.4.3 Cell product compliance and records

6.4.3.1 Compliance

During every patient visit, the investigators and/or the study staff members will evaluate the compliance of the patient, and record the information on the original document.

6.4.3.2 Record of the study cell therapy

In the study treatment, the investigators or the authorized representative will record the distribution and usage of the cells accurately after the cells arrive at the study center. The monitors will review all of the record forms of the cell therapy during the visit to the center and at the completion time of the cell therapy.

At the end of the study, or at any designated time during the study, the investigators will submit all unused records, used study cell therapy supplies, labels and copies of the completed cell therapy record forms to the monitoring party for review.

6.4.3.3 Disposal and destruction

The used cell packages should be destroyed as medical waste in the study center. If the cells are not completely infused for some special reason, the residual cells should be sealed, packed and frozen/refrigerated for storage strictly according to the operation standards, and further disposal conditions should be recorded clearly.

7 Visit schedule and assessments

7.1 Visit schedule

All of the visits are listed in the following Table (Table 7-1). If the corresponding evaluation is performed during the visit, it should be marked with an X. All data that are generated from these evaluations must be supported by the original documents of the patients. At any time during the study, additional assessment can be performed according to the clinical symptoms. Especially for the clinically significant changes, additional laboratory test should be performed during follow-ups, based on the investigators' judgement. The patients in each dose group should be hospitalized for observation during days 1 to 14. Before discharge, the patient should be evaluated and the safety of the patient should be confirmed.

If it is an unscheduled evaluation, these results must be recorded as “an unscheduled evaluation” in the eCRF.

Table 7-1 Flowchart of Study Visits:

Study procedure	Screening 1	Screening 2	Treatment				Cell therapy completion		Follow-up for safety (every 2 month) [j]	End of treatment	Follow-up
	Molecular screening	28 days before drug administration	C1D1	C1D8	≥ C2D1	≥ C2D8	Cell therapy completion	28 days after completion			
Sign the ICF	X	X									
PD-L1 status test [l]	X										
Collection of fresh tumor tissue samples[m]		X									
Blood culture		X									
Inclusion/ exclusion criteria		X									
Medical history/smoking history		X									
Demographic data		X									
Vital signs		X	X	X	X	X	X	X	X	X	
Physical examination, body weight		X	X	X	X	X	X	X	X	X	
Height		X	X [b]								
ECOG score		X	X	X	X	X	X	X	X	X	
12 leads ECG		X	X		X		X	X	X	X	
Heart Doppler ultrasound		X	X [c]								
Hematology/blood biochemistry/ coagulation profile/urine test		X	X [a]		X	X	X	X	X	X	
Cardiac markers		X	X [c]								
Thyroid function test		X	X [a]		X		X	X	X		

Tumor markers		X	X [a]		X		X	X	X		
HIV/HBV/HCV		X									
Stool routine test		X	X [a]		X		X	X	X	X	
Cytokine level, erythrocyte sedimentation rate (ESR)		X	X [a]	X [e]							
Lymphocyte subset distribution		X	X [a]	X	X	X	X	X	X	X	
Pregnancy test [f]		X	X		X		X			X	
Collection of blood samples		X	X [d] The details are seen in 7.2.3 Biomarkers.								
Tumor evaluation [i]		X			X		X			X	
blood collected[h]		X		X		X					
Cell therapy [g]			X		X						
AEs		Continuously monitored during the study									
Combination medication		Continuously monitored during the study									
Follow-up survival [k]											X

Note: Each evaluation should be completed within seven days before the treatment. In the major treatment period, one treatment course (cycle) is defined as 28 days. All safety evaluation should be completed before the treatment. D: day. C: cycle.

a. If no PD or clinical indication, which prompts further assessment, is found in a patient in the screening assessment within seven days before the study treatment, there is no need to perform further blood biochemistry testing, hematology testing, coagulation profiling, thyroid function tesing, tumor marker testing, cytokine level testing, urine routine testing, stool routine testing or ECG examinations.

b. Body weight measurement. The patient's body weight should be measured on the screening/baseline visit day, the first day of the first cycle and the first day of the following cycles. If the baseline evaluation is performed within 72 hours before the first cell therapy, the body weight will not be measured again on the first day of the first cycle.

c. The baseline visit evaluation should be performed. In case of any abnormality, additional examinations should be performed.

d. The detailed blood sampling is displayed in Section 7.2.3. Biomarkers, including:ctDNA andblood samplingfor diverse T lymphocyte receptors diversity.

e. Cytokine level detection at: 24h after each transfusion, the seventh day after the final infusion in each cycle; if cytokine storm is suspected in clinic, the number of tests can be increased according to the clinical guidelines. Refer to Section 7.2.2.5 for details.

f. For all WOCBPs, the result of serum pregnancy test obtained within 72 hours before the study treatment should be negative.

g. Cell therapy: the cell therapy is performed on the day 1, 3 and 5 of each cycle.

h. Blood collected: The blood for amplifying the cells for further treatment cycles should be collected within three weeks before the treatment and on the 8th day after the prior treatment cycle.

i. The assessment results that are determined by imaging during the screening period are valid within 28 days before the treatment. The RECIST 1.1 criteria will be used for assessmentduring the screening (by using chest + abdomen CT or MRI scans); meanwhile, bone scintigraphy/bone scan and brain scan/head CT/MRI are required. Further scans should be performed according to the schedule, calculated from the completion day of the treatment (± 7 days). If any brain metastasis occurs in the patient, a brain imaging examination must be performed in the following tumor evaluations. If any bone metastasis occurs at the time of baseline assessment, a bone scintigraphy/bone scan is further required in these patients every six months (± 7 days), to monitor new lesions. If the radiologic assessment can evaluate the bone metastasis lesion, then a further bone scintigraphy/bone scan is not required. For other conditions, corresponding examinations should be performed according to the clinical indications. All of the evaluations must be completed before the next treatment. If the imaging evaluation has been performed within four weeks of the treatment completion, there is no need for re-examination. The tumor evaluation should be performed in the eighth (or ninth), twelfth week and every eight weeks during the following period (refer to Section 13.2 for details).

j. After completion of the cell therapy, patients should be followed up for safety every two months.

k. After PD or withdrawal from the treatment, patients should be followed up every two months to collect the survival information, including anti-tumor therapy.

l. PD-L1 status test was used by fresh tissue or paraffin-embedded tissue.

m. The investigators could collect unscheduled tissue sample based on clinical situations.

7.1.1 Molecular screening

The study center is required to perform a molecular screen to the patients for the PD-L1 expression positivity. The molecular screening will be performed in the Pathology Department of the study center using the Leica bondmax automatic immunohistochemistry machine. PD-L1 status detection was used by fresh tissue or paraffin-embedded tissue. Before the molecular screening, the candidate patients are asked to sign the ICFs for molecular screening.

The details of the PD-L1 status are displayed in Section 7.2.3.3.

After confirmation that the tumor is PD-L1 positive (TPS ≥ 1%), the patient should sign the main ICF for the study before the screening/baseline visit.

Note: If the patient's tumor has previously been identified to be PD-L1 positive by Roche Ventana PD-L1 (SP-142, SP-263) and Dako PD-L1 (Dako28-8, Dako22c3), it is unnecessary to test the PD-L1 status again. The patient should sign the main ICF for the study before the screening/baseline visit.

7.1.2 Screening

All patients should sign the ICFs that has been approved by the ethics committee before any evaluation and operation of the clinical study is to be performed. The patient or the person signing the ICF must acquire one copy of the signed ICF (the clinical routine tests can be performed before signing the ICF). The investigator must record this process in the original medical record.

The baseline evaluation during the screening phase must be performed within the four weeks before the first treatment cycle. The screening examination should be performed within the 28 days before the first cell infusion. All the results should be obtained before the patients are confirmed to be eligible for the study. If the patient conforms to all inclusion criteria without conformation to any of the exclusion criteria, then he/she can be enrolled in the study.

The following procedures and evaluations should be conducted within the 28 days before the first cell infusion:

- Medical record collection: the records, such as past medical history, operation history, demographic data and baseline information.
- Evaluation based on the inclusion and exclusion criteria.
- Complete physical examination: examine all of the systems in the body, including body weight, height, body surface area and vital signs (blood pressure, heart rate, respiratory rate and pulse rate).
- ECOG performance score.

- Collection of fresh tumor tissue samples.
- HIV, HBVsAg and HCV Ab must be tested within the 28 days before the study treatment, and if the HBVeAb is positive, the HBV- DNA must be tested and confirmed to be negative.
- Blood culture.
- Hematology test (including hemoglobin, red blood cell, white blood cell count and classification, and platelet count).
- Blood biochemistry: liver function test (TBL, ALT, AST, AKP, ALB, A/G and LDH), kidney function test (BUN and Cr), and amylase, lipase, blood glucose, and blood electrolyte (K+, Na+ and Cl-) measurement.
- Thyroid function test: TSH, free T3, and free T4.
- Myocardial enzymes: troponin, myoglobin and CK-MB.
- Tumor markers (CEA, CYFRA21-1 and NSE).
- Lymphocyte subpopulation: CD3/CD4/CD8/NK.
- Urine routine test.
- Stool routine test.
- Coagulation test (aPTT and INR).
- Twelve lead ECG.
- Ultrasonic cardiogram.
- Pregnancy test: For all WOCBPs, the serum pregnancy test result obtained within 72 hours before the study treatmentmust be negative.
- Imaging study: mainly use chest plus abdomen enhanced CT, bone scan (or systemic PET-CT) and head enhanced MRI.
- Collection of blood for T cells expansion: patients in group A: 40 ml; groups B and C: 60-80 ml. The actual blood volume is confirmed according to the routine blood test result of the patient at that time (please see Section 7.2.3 for details).
- Cytokine level monitoring and erythrocyte sedimentation rate (ESR): IL-6, IL-10, CRP, TNF-α, ESR, IL-2 receptor, IL-2, IL-8, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF, and FLT3L. Please refer to Section 7.2.1.5 for details.

Remarks: if the peripheral blood of the patient that is collected during the screening period for editing and amplification does not generate enough edited T cells for the treatment transfusion within 28 days, the patient is disqualified from the screening. The patients who fail the screening can be screened again and may participate in the study.

7.1.3 Screening failure

If a patient signs the main ICF but does not meet the eligibility criteria, it will be considered a screening failure. The investigators should record the reason for the failure in the original document and in the eCRF during the screening phase. For the patients that failed in the screening, the evaluation date including the demographic data, ICF,and inclusion/exclusion criteria must be collected. Other data from these patients will not be recorded in the clinical database except for the SAEs occurred during the screening phase (refer to Section 8.1 for the SAE reporting process details).

7.1.4 Treatment phase

Each assessment should be completed within the seven days before the treatment. During the major treatment period, one treatment cycle is defined as 28 days. All of the safety assessments should be completed before the treatment. A three-day delay is allowed in each cycle, due to holidays, weekends, bad weather or other unpredictable conditions, and is not considered to be a protocol deviation.

7.1.4.1 Main treatment period

One cell therapy cycle is defined as 28 days. The following assessment procedures are required during the study.

7.1.4.1.1 Assessment within one week prior to each treatment cycle

- General physical examination: examine all of the systems/organs in the body, including body weight, height, body surface area and vital signs (blood pressure, heart rate, respiratory rate and pulse rate).
- ECOG performance status.
- Hematology test (including hemoglobin, red blood cell, white blood cell count and classification, and platelet count).
- Blood biochemistry: liver function test (TBL, ALT, AST, AKP, ALB, A/G and LDH), kidney function test (BUN and Cr), and amylase, lipase, blood glucose, blood electrolyte (K+, Na+ and Cl-) measurements.
- Thyroid function test: TSH, free T3, free T4.
- Tumor marker (CEA, CYFRA21-1 and NSE).
- Lymphocyte subpopulation: CD3/CD4/CD8/NK.
- Urine routine test, stool routine test, coagulation profile (aPTT and INR).
- Twelve lead ECG.
- Pregnancy test: for all WOCBPs, a negative result in serum or urine pregnancy test must be obtained within the 72 hours before the study treatment.
- Cytokine level monitoring and ESR: IL-6, IL-10, CRP, TNF-α, IL-2, IL-8, Fractalkine,

IFN-r, MCP-1, IL-5, GM-CSF, and FLT3L. Please refer to 7.2.2.5 for details.

• Collection of blood samples: please refer to Section 7.2.3 for details.

• The patient safety should be evaluated according to the NCI-CTCAE4.03.

If the patient undergoes the screening evaluation within the seven days prior to the study treatment and no PD or clinical indication prompts further evaluation, there is no need to perform the blood biochemistry, hematology test, thyroid function test, tumor marker measurement, lymphocyte subset measurement, cytokine measurement, coagulation profile, urine routine test, stool routine test and ECG examination again on the first day of the first cycle.

7.1.4.1.2 Assessment on day eight of each cycle

- General physical examination: examine all of the systems/organs in the body, including body weight, body surface area and vital signs (blood pressure, heart rate, respiratory rate and pulse rate).
- ECOG performance status.
- Collection of the blood for PBMC expansion: 60-80 ml based on the hematology examination results of the patient. If a treatment delay is caused by poor blood cells amplification of the patient, the cell therapy can be delayed up to one week. If the delay exceeds one week, the Investigator should discuss to decide whether the treatment should be continued.
- Hematology test (including hemoglobin, red blood cell and white blood cell count and classification, and platelet count).
- Blood biochemistry test: liver function test (TBL, ALT, AST, AKP, ALB, A/G and LDH), kidney function test (BUN and Cr), and amylase, lipase, blood glucose, blood electrolyte (K+, Na+ and Cl-) measurement.
- Lymphocyte subset distribution: CD3/CD4/CD8/NK.
- Cytokine level monitoring and erythrocyte sedimentation rate (ESR): IL-6, IL-10, CRP, TNF-α, ESR, IL-2 receptor, IL-2, IL-8, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF, and FLT3L. Please refer to Section 7.2.1.5 for details.
- Collection of blood samples: please refer to Section 7.2.3 for details.
- The tolerability of the prior cycle is evaluated according to the NCI-CTCAE4.03.

7.1.4.1.3 End of cell therapy

The visit after the completion of the cell therapy should be conducted within one week after the day of the last cell infusion. The time window (± 7 days) is allowed for all of the visits and evaluations. AEs and SAEs in all the patients should be followed up within the 28 days after the last cell infusion, until 24 months after the treatment ends.

- General physical examination: examine all of the systems/organs in the body, including body weight, body surface area and vital signs (blood pressure, heart rate, respiratory raterespiratory rate and pulse rate).
- ECOG performance status.
- Hematology test (including hemoglobin, red blood cell, white blood cell count and classification, and platelet count).
- Blood biochemistry: liver function test (TBL, ALT, AST, AKP, ALB, A/G and LDH), kidneyfunction test (BUN and Cr), and amylase, lipase, blood glucose, and blood electrolyte (K+, Na+ and Cl-) measurements.
- Thyroid function test: TSH, free T3, and free T4.
- Tumor markers (CEA, CYFRA21-1 and NSE).
- Lymphocyte subpopulation: CD3/CD4/CD8/NK.
- Urine routine test, stool routine test, coagulation profile (aPTT and INR).
- Twelve lead ECG.
- Imaging study: mainly use chest plus abdomen enhanced CT, bone scan (or systemic PET-CT) and head contrast-enhanced MRI.
- Cytokine level monitoring and erythrocyte sedimentation rate (ESR): IL-6, IL-10, CRP, TNF-α, ESR, IL-2 receptor, IL-2, IL-8, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF, and FLT3L. Please refer to Section 7.2.1.5 for details.
- Collection of blood samples: please refer to Section 7.2.3 for details.
- The tolerability of the prior cycle is evaluated according to the NCI-CTCAE4.03.

7.1.5 Follow-Up after end of cell therapy

The patients will go forward into the follow-up phase after cell therapy ends. The tumor evaluations of the patients will be followed up according to the scheduled visit in the visit form until PD occurs. The objective response of tumors to the cell therapy is evaluated according to RECIST 1.1 and referring to ir RC.

When the cell therapy is completed, the patient should visit the hospital, undergo safety examinations. The safety should be evaluated once a month during the first 12 months, and then once every two months during the next 12 months. The time window (± 7 days) is allowed for all the visits and evaluations.

- Complete physical examination: examine all of the systems in the body, including body weight, body surface area and vital signs (blood pressure, heart rate, respiratory rate and pulse rate).
- ECOG performance status.
- Hematology test (including hemoglobin, red blood cell, white blood cell count and

classification, and platelet count).

- Blood biochemistry: liver function test (TBL, ALT, AST, AKP, ALB, A/G and LDH), kidney function test (BUN and Cr), and amylase, lipase, blood glucose, and blood electrolyte (K+, Na+ and Cl-) measurements.
- Thyroid function test: TSH, free T3, and free T4.
- Tumor markers (CEA, CYFRA21-1 and NSE).
- Lymphocyte subpopulation: CD3/CD4/CD8/NK.
- Urine routine test, stool routine test, coagulation profiles (aPTT and INR).
- Twelve lead ECG.
- Pregnancy test: for all WOCBPs, the negative serum or urine result in pregnancy test must be obtained within the 48 hours before the study treatment.
- Imaging study: mainly chest plus abdomen enhanced CT, bone scan (or systemic PET-CT) and head contrast-enhanced MRI. Please refer to Section 7.2.2 for details.
- Cytokine level monitoring and erythrocyte sedimentation rate (ESR): IL-6, IL-10, CRP, TNF-α, ESR, IL-2 receptor, IL-2, IL-8, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF, and FLT3L. Please refer to Section 7.2.1.5 for details.
- Collection of blood samples: please refer to Section 7.2.3 for details.
- The tolerability of the prior cycle is evaluated according to the NCI-CTCAE4.03.

7.1.6 End of treatment (EOT)

When the patient discontinues the study treatment (including study completion and early termination), the visit evaluation should be completed within 14 days, and all the evaluations listed in the EOT should be performed. The eCRF should be filled in at the closing date and reasons for study discontinuation should be recorded. Follow-ups of the AEs and SAEs related to treatment should be completed until 24 months after the study ends. For patients with early withdrawal, the relevant information should be collected as much as possible.

- General physical examination: examine all of the systems in the body, including body weight, body surface area and vital signs (blood pressure, heart rate, respiratory rate and pulse).
- ECOG performance status.
- Hematology test (including hemoglobin, red blood cell, white blood cell count, differential and platelet count).
- Blood chemistry test: liver function tests (TBL, ALT, AST, AKP, ALB, A/G and LDH), renal function test (BUN and Cr), and amylase, lipase, blood glucose, and blood electrolyte (K+, Na+ and Cl-) measurements.
- Thyroid function test: TSH, free T3 and free T4.

- Tumor marker (CEA, CYFRA21-1 and NSE).
- Lymphocyte subpopulation: CD3/CD4/CD8/NK.
- Urine routine test, stool routine test, coagulation profiles (aPTT and INR).
- Twelve lead ECG.
- Cytokine level monitoring and erythrocyte sedimentation rate (ESR): IL-6, IL-10, CRP, TNF-α, ESR, IL-2 receptor, IL-2, IL-8, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF, and FLT3L. Please refer to Section 7.2.1.5 for details.
- Collection of blood samples: Please refer to Section 7.2.3 for details.
- The tolerability of the last cycle is evaluated according to the NCI-CTCAE 4.03.
- Imaging studies: contrast-enhanced CT imaging of the chest and abdomen, bone scanning (or systemic PET-CT) and contrast-enhanced brain MRI. Imaging studies shall only be conducted in cases where the discontinuation of the study is due to any reason other than PD. If a scanning test is performed within 28 days before the end of the study, it is not required to be repeated. Please refer to Section 7.2.2 for details.

7.1.7 Follow-ups

7.1.7.1 The safety of follow-up period.

All patients must undergo safety evaluation on the 30th day (± 7 days) after the EOT visit. Once treatment discontinues, all patients should be contacted once to evaluate the safety (AEs and/or SAEs), the following anti-tumor treatmentand the survival information. For the patients who permanently discontinue the study due to the AEs (including laboratory abmormalities), the follow-up must be continued until such events subside orstabiliz (the earlier should prevail). If the patients refuse to or fail to return for the safety evaluation visit, they should be contacted by telephone to exaluate their physical conditions. The attempts made to contact the patient should be recorded in documents (telephone contacts, date of registered mail, etc.). AEs and SAEs related to the study treatment should be recorded until 24 months after the termination of the study or begining new antitumor therapy.

- General physical examination: examine all of the systems in the body, including body weight, body surface area and vital signs (blood pressure, heart rate, respiratory rate and pulse rate).
- ECOG score.
- Hematology test (including: hemoglobin, red blood cell, white blood cell count and differential and blood platelet count).
- Blood chemistry test: liver function tests (TBL, ALT, AST, AKP, ALB, A/G and LDH), renal function test (BUN and Cr), and amylase, lipase, blood glucose, and blood electrolyte (K+, Na+ and Cl-) measurements.

- Thyroid function test: TSH, free T3, free T4.
- Tumor marker (CEA, CYFRA21-1 and NSE).
- Lymphocyte subpopulation: CD3/CD4/CD8/NK.
- Urine routine test, stool routine test, coagulation profile (aPTT and INR).
- Twelve lead ECG.
- Imaging studies:contrast-enhanced CT imaging of the chest and abdomen, bone scanning (or systemic PET-CT) and enhanced brain MRI. Please refer to Section 7.2.2 for details.
- Cytokine level monitoring and erythrocyte sedimentation rate (ESR): IL-6, IL-10, CRP, TNF-α, ESR, IL-2 receptor, IL-2, IL-8, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF, and FLT3L. Please refer to Section 7.2.1.5 for details.
- Blood sample collection: please refer to Section 7.2.3 for more details.
- The tolerability of the prior cycle is evaluated according to the NCI-CTCAE4.03.

7.1.7.2 PFS follow-up period

If a patient discontinues the treatment for any reason other than PD, a tumor evaluation will be continued asdescribed in Section 7.2.2, until PD, death, or initiation of a new anti-cancer treatment. For each patient that discontinues the treatment, the investigator or his representative will continue to collect the information about the initiation of other anti-cancer treatment until the cut-off date of the final data analysis. The patients that lost to be followed up should be recorded in the eCRF as censored and, if patients are lost in the follow-up period, the investigator should record the attempts to make contact with the patients in the original medical records, such as through telephone, registered mail, and so on.

7.1.7.3 Survival follow-up period

If the patient develops objective PD, survival follow-up should be performed once every two months. The survival information may be collected through several ways, including contacting the patients and/or their family by telephone. Information regarding the anti-tumor therapy, survival information and other ifformations should be collected.

7.2 Assessment types

7.2.1 Safety and tolerability assessments

The safety assessments include the monitoring and laboratory tests of all AEs and SAEs, such as the regular monitoring of hematology and urine routine test, regular assessment of vital signs, weight, performance status, physical examination, cardiac evaluation, ECG and other evaluations.

For more information about AE collection and report please refer to Section 8.

7.2.1.1 Physical examination

Physical examination should be performed according to the general medical practice, including medical history, inspection, palpation, percussion and auscultation. Physical examination will be processed at the screening/baseline visits, on day one and day eight of each cycle, and at the end of the treatment.

If a baseline examination has been performed within the 72 hours prior to the first cell therapy, repeated physical examination is not required on the first day of the first cycle, which is also applicable for the first day examination of the following cycles.

If clinically indicated, physical examination can be performed more frequently in the discretion of the judgment of the investigator. Any important findings recorded prior to signing of the ICF must be recorded on the medical history pages in the eCRF. Important new findings that are first observed or exacerbated after the signing of the ICF must be recorded on the AEs pages in the eCRF.

7.2.1.2 Vital signs

Vital signs include temperature, respiratory rate, blood pressure and heart rate. The blood pressure and heart rate should be measured in a sitting position and the patients should have rested for at least 10 minutes prior to the measurement. Any abnormality with clinical significance will be recorded as an AE in the eCRF.

7.2.1.3 Height and weight

Height will only be measured at screening and the patient’s weight will be measured at the screening/baseline visit, on the first day of the first cycle and the following cycles.

If a baseline evaluation has been performed within the 72 hours prior to the first cell therapy, there is no need to perform it again on the first day of the first cycle.

7.2.1.4 Performance status

The ECOG score will be evaluated during the process of screening before the first cycle (before cell therapy), on the first day of each following cycle and at the end of the treatment.

If a baseline evaluation has been performed within the 72 hours prior to the first cell therapy, it is not necessary to repeat the evaluation on the first day of the first cycle.

7.2.1.5 Laboratory assessments

The testing will be performed at the laboratory of the study center based on the visits listed in Table 7-1. Samples for laboratory tests will be collected and evaluated during the scheduled visit period, even if the study treatment has been suspended. Additional or repeated tests will be performed for safety and/or laboratory abnormalities based on the details. The priniciple investigator will take charge of the final evaluation of the laboratory findings, the completion of records and the filling in of the eCRF.

Abnormal laboratory values or findings can be deemed as AEs only when there are

clinical signs or symptoms that are meaningful or require treatment. These signs, symptoms or relevant diagnoses should be recorded as an AE in the eCRF. In addition, if an independent laboratory abnormality is of clinical significance (e.g. resulting in the discontinuation of the treatment, as a part of a SAE, or it is the SAE), it should also be recorded as an AE in the eCRF.

Monitoring of CRS:

The monitoring of CRS will be processed by collectingapproxmately 3 ml peripheral blood on the first day of the first cycle, 24 hours after the completion of the last cell infusion (day five) of each cycle, day twelve of each cycle (the 7^{th} day after last infusion of each cycle) during the study. If a CRS is suspected, additional sampling will be required based on the clinical signs.

The cytokines that are tested include: IL-6, IL-10, CRP, TNF-α, ESR, IL-8, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF and FLT3L. Plasma samples have been retained, and IL-8, IL-2, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF, FLT3L are all exploratory indexes for clinical study will be tested at the end of the study.

If the investigator suspects that a patient's clinical signs or symptoms are clinically significant, additional or repeated test can be conducted based on the conditions of the individual case. The principle investigator is responsible for the evaluation and confirmation of the lab test results with a detailed record.

7.2.1.6Tolerability evaluation

The tolerability will be evaluated based on the cell therapy dose, the treatment course, the incidence rate of AEs that cause disruption of the cell therapy (interruption of the treatment or dose reduction) and the early-discontinuation of the treatment.

7.2.2 Measurement of efficacy

The imaging evaluation results during the screening period are valid within the 28 day prior to the treatment. The RECIST1.1 assessment will be applied during the screening (by using chest and abdomen CT or MRI); meanwhile, it is necessary to carry out the bone scintigraphy/bone scanning and brain scanning/head and neck CT/MRI as clinically indicated. All of the scans should be performed according to the schedule from the completion day of the treatment (± 7 days). If the patients have brain metastases, the brain imaging must be performed during future tumor evaluations. If the patient is confirmed to have bone metastases at the time of the baseline assessment, then bone scintigraphy/bone scanning should be repeated at an interval of six months (± 7 days) to monitor the new lesions. If the imaging test can be used to evaluate the bone metastasis lesion, there is no need to repeat the bone scintigraphy/bone scanning.

All of the follow-up imaging scans will be processed based on the schedule from the first day of the first treatment cycle. The scan after the first treatment must be processed at weeks 8 (+1) and 12 (±1) ... After that, the tumor evaluation will be processed once every eight weeks (+/-7 days) until PD. Other corresponding examinations will be performed as clinically indicated. All of these evaluations must be completed before the next treatment. If the imaging study has been performed within the four weeks before the treatment completion, there is no need for the re-examination.

If the treatment for the patients is early discontinued for reasons other than PD, the tumor evaluation for those patients should be continued once every two months (according to the RECIST1.1 criteria) until the patients' death or the first presence of PD. Final tumor evaluation should be conducted between the fourth and sixth weeks after the first PD. All known and suspected lesions should be monitored through imaging and evaluated according to the RECIST1.1 criterion.

IrRC will allow re-evaluaton 4 weeks after the pgression as immnotherapy may cause short-time tumor flare (pseudoprogression) if general condition is stable.

7.2.3 Biomarkers

Table 7-2-3: Collection of Blood for biomarker tests.

Sample collected	Time points for collection	Blood sample volume
ctDNA (see 7.2.3.1 for details)	baseline, 28th day of second cycle, week 8, week 12, and then every eight weeks.	10 ml
Immune repertoire (see 7.2.3.2 for details)	Pre-A group: baseline, day six of first cycle, first day of second cycle. First and sixth days in each cycle if the patients continue with the cell infusion, or week 8 and 12 if the patients discontinue the cell infusion. Group A, B and C: baseline, day six of first cycle, first and sixth days of second cycle. First and sixth days in each cycle if the patients continue with the cell infusion, or week 8 and 12 if the patients discontinue the cell infusion.	5 ml or 10 ml*
T cell *in vivo*tracking	First and 12thday of each treatment cycle After treatment: the 28th day of the last treatment cycle, three months, six months, and one year after the last cell	10 ml

	infusion.	
Flow cytometry of regulatory T cells (Treg), memory T cells, activated T cells and PD-1	Before pre-treatment with cyclophosphamide (blank baseline), after pre-treatment with cyclophosphamide (medication baseline), 24h after the third cell transfusion of each cycle, the end of each cycle (day 28), week eight, three months, and six months.	5 ml

*Notes: A total of 15 ml peripheral blood is collected at the same time point for ctDNA and immune repertoire samples; 10 ml peripheral blood is collected at the time point for immune repertoire samples only.

7.2.3.1 ctDNA

Circulating tumor DNA (ctDNA) refers tothefree partially degradatedendogenous DNA in the circulating blood, which is mainly produced by tumor cell necrosis, apoptosis, and even the secretion of tumor cells [37]. With regards to the monitoring of anti-cancer treatment, the ctDNA level in plasma can reflect the blood tumor mutation burden (bTMB). The ctDNA level is highly correlated to the imaging tumor burden, and can be even more sensitive than imaging [38]. Tumors have temporal and spatial heterogeneity. Based on the mutation spectrum of ctDNA from the peripheral blood, the pedigree of the tumor cell clones can be analyzed [39], and the specific responsivity of the tumor clone to the treatment in this study can be further explored.

Ten microliters of peripheral blood will be collected each time at baseline, 28th day of second cycle, week 8, week 12, and then every eight weeks.

7.2.3.2 Immune repertoire

The diversity of T-lymphocyte and B-lymphocyte receptor repertoires is derived from the recombination of receptor genes. The recombination generates a huge diversity of receptors, of which, CDR3 has the highest diversity that best represents the specificity of receptor repertoires. The diversity of receptor repertoires also demonstrates a potential predictive role in the evaluation of the efficacy of immune checkpoint inhibitors. The exploration of the dynamic changes in T cell receptors (TCR) during and after the treatment and the exploration of the specific T cell clones that are possibly related to efficacy using this method have been proposed.

The peripheral blood will be collected at a volume of 5 ml per time at the baseline of overall treatment, 24 hours after the last cell infusion of each treatment cycle. In addition, 5 ml of peripheral blood will be collected from the patients in each group to analyze the

diversity of T cell receptors at each of the following time points: Pre-A group: baseline, day six of first cycle, first day of second cycle. First and sixth days in each cycle if the patients continue with the cell infusion, or week 8 and 12 if the patients discontinue the cell infusion.Group A, B and C: baseline, day six of first cycle, first and sixth days of second cycle. First and sixth days in each cycle if the patients continue with the cell infusion, or week 8 and 12 if the patients discontinue the cell infusion.

7.2.3.3 Screening of the tumor biomarkers (PD-L1)

It is required to evaluate the PD-L1 expression of the tumor. Although PD-L1 expression is not the best index to predict the efficacy of the PD-1/PD-L1 inhibitors, current data [40,41] still reveals that positive PD-L1 expression is related to the efficacy. Additionally, the NSCLC patients withpositive PD-L1 have a higher probability to recieve benefits from these inhibitors. Furthermore, among the current exploratory prediction indexes, PD-L1 could be tested simply and quickly using IHC. Therefore, this study will take PD-L1 positivity as one of the inclusion criteria to potentially enhance the therapeutic benefit to patients. PD-L1 positivity will be identified based on the types of tumor samples in the pathology laboratory of the study center. PD-L1 status detection was used by fresh tissue or paraffin-embedded tissue.

7.2.3.4 PDX model

Patient-derived xenografts (PDX) have been well established by inoculating the tumor cells that are derived from the patients to the mice with severe combined immunodeficiency, which can highly specifically simulate the *in vivo* condition of the patients [42, 43]. In addition, it has already been reported that the PDX model can be established by utilizing biopsies [44].

In this study, it has been proposed that fresh tumor specimens from the patients can be used to establish the PDX model, test the feasibility ofautologous infusion of the gene-edited cells and calculate whether the animal model can predict the clinical efficacy and allow analysis of the possible mechanisms. It is proposed that tumor samples from the patients are obtained by core needle biopsy before infusing the T cells, and inoculated into NOD SCID gamma (NSG) mice *in situ*, followed by the injection of 10^7 gene-edited T cells into the mice via the caudal vein to observe the effect on the mice. The peripheral blood, bone marrow and a tumor specimen will be collected from the mice to analyze the dynamic distribution and functional alteration of the cells.

7.2.3.5 *In vivo* T cell tracking

The edited T cells that will be infused in this study is not stem cells that can proliferate infinitely. The infused T cells will gradually be dismissed in patients over time. The dynamics of edited T cells *in vivo,* although not clear*,* may be associated wtih the efficacy and toxicity

of the treatment. The dynamics of this process is also critical for the determination of the optimal treatment period and time of cell infusion. In this program, digital PCR (ddPCR) and deep sequencing will be used to dynamically monitor the changes in the cell numbers in the peripheral blood of the patients over time, at the intended time points.

- Baseline and day 12 of each treatment cycle.
- The 28st day of last treatment cycle, three months, six months, and one year after the last cell infusion.
- Blood volume: 10 ml each time.

7.2.3.6 Flow cytometery for regulatory T (Treg) cells, memory T cells, activated T cells and PD-1.

Tumor-associated immuno-suppressive cells include Treg cells, myeloid derived suppressor cells, tumor-associated macrophages and immature dendritic cells. Treg isthe major immuno-suppressive cell type, which can negatively regulate other immune cells directly or indirectly, and can inhibit the anti-tumor immune response. Cyclophosphamide is a drug that is most commonly used in pre-treatment during tumor immunotherapy. Cyclophosphamide can reduce the number of Treg cells, inhibit the function of Treg, and enhance anti-tumor immunity function. Therefore, the number of Treg cells inthe peripheral blood will be dynamically monitored in this study.

T memory cells and activated T cells have anti-tumor immunity functions. T-memory cells are generated from the proliferation and differentiation of T cells following induction by a specific antigen. T memory cells maintain a long-term immune response activity to specific antigens. Activated T cells are derived from the activation of different T lymphocyte subset by antigens or cytokines. Activated T cells play a role in immune regulation. PD-1 is an important immuno-suppressive molecule. Overexpression of PD-1 on CD8+T cells indicates the effect of tumor immune suppression. In this study, the changes in PD-1 expression in T memory cells and activated T cells will be dynamically monitored after infusion by taking samples of the peripheral blood to evaluate the efficay of the cell therapy. The expresssion of PD-1 on pre-edited and post-edited T cells will also be detected.

Blood collection time points: before pre-treatment with cyclophosphamide (blank baseline), after pre-treatment with cyclophosphamide (medication baseline), 24 h after the third cell infusion of each cycle, the end of each cycle (day 28), Week eight, three months, and six months after the final treatment. Blood collection volume: 5 ml each time.

7.2.4 Other descriptions

If the patient’s consent has been obtained, the remaining samples can be used for further analysis. The samples can be kept up to five years. These samples can be used to explore the

efficacy and/or safety (such as off-target analysis) of edited cells. At any phase of this study, patients can discontinue the study voluntarily; investigator can determine if patient continue or exit the study based on the judgment of the potential benefits to patients.

Patients will discontinue the study in the following cases:

- Patient withdraws the ICF
- Death
- AEs
- Laboratory abnormalities or other abnormal findings
- Misconduct in the study protocol
- Lost in follow-up
- PD
- New anti-cancer treatment
- Noncompliance of the patients

For those patients who do not agree to receive further treatment and evaluation, it is necessary to clarify whether they are willing to accept the survival follow-up (which can be made by telephone). If patients withdraw the ICFs and no longer participate in any study or follow-up, including the survival follow-up study, it shall be recorded in detail in the source documents and filed in the eCRF.

7.2.5 Withdrawal of ICF

Patients will be entitled to exit the study at any period of the study, which neither causes the discrimination of patients in following treatment, nor impacts on their decision to participate in other studies.

For patients who withdraw the ICFs, the reasons and whether there is any AE must be recorded clearly, and investigators will follow-up regarding the AE after the study.

7.2.6 Discontinuation of the study

If significant findings indicate enormous risks for patients, the investigators can terminate the study. No matter why the study is terminated, all available data from the subjects that terminate the follow-up shall be recorded clearly.

8. Adverse events

An adverse event (AE) refers to any new adverse medical event or any worsening disease after the patient signs the ICF. An AE may not have a causative relation to the treatment or drugs, thus, an AE includes, but is not limited to: the abnormal laboratory test; a symptom of clinical significance; a change in an examination result. AEs can be spontenously reported by the patients, recorded during open communication with the patients or discovered during the examination or evaluation of the patients.

For the existing cases before the ICF is signed, the relevant case history shall be recorded. For AEs, the investigators shall trace the details of the AEs to confirm whether the AE is related to the study treatment, assess the result of AE and evaluate whether it is a SAE.

AEs should be evaluated in accordance with the CTCAE version 4.03. If there is no grading for certain AEs in the CTCAE, the AEs shall be graded into grades 1-4, which corresponds to mild, moderate, severe and life-threatening.

An AE should be monitored from the time when the patient signs the prior consent and throughout the overall treatment period and safety follow-up period. The safety follow-up period is defined as the period from the time when the patients discontinue the cell therapy until death. Any SAE identified in the follow-up period should also be reported according to the regulation.

It should be judged by the investigators whether there is a causative relationship between any AE and the study treatment. The relationship between an AE and the study treatment could be: definitely related, probably related, probably unrelated or definitely unrelated. The incidence rate of the AEs that are definitely related and probably related to the study treatment will be listed.

Investigator should follow upthe patients that exit the treatment due to AEs, to follow up and record the outcomes of all AEs until such events are fully relieved or subside. Investigator must judge whether the AE is related to the study treatment, the measures taken during the study treatment (no action, discontinuation of the study treatment, temporary interruption for medicine, permanent discontinuation of the study treatment, unknown, etc.), and the application of concomitant treatment (with/without concomitant treatment), based on the supporting evidence. Investigators should also evaluate the outcomes of the AE (disappear/not disappear, recovered, in recovery, whether there are sequelae, death, unknown, etc.), and determine whether it is an SAE.

Abnormal values of laboratory tests:

The laboratory abnormalities with clinical significance (inducing clinical signs or symptoms, requiring concomitant treatment or adjustment of the trial, treatment) should be recorded as AEs, with an emphasis on the diagnosis results instead of the signs and symptoms (for example, anemia other than low hemoglobin). The laboratory abnormal values that recording as AEs should be followed up until they return to normal or can be reasonably explained. If an abnormal laboratory value or test result shows the same signs/symptoms as a reported AE, it will not be recorded as a different AE.

Investigator should follow up the patients that exit the study due to AEs, to follow up and record the outcomes of any AEs until such events are recovery or stable. Investigator

must judge whether the AE is related to the study treatment, the measures taken during the study treatment (no action, discontinuation of the study treatment, temporary interruption for medicine, permanent discontinuation of the study treatment, unknown, etc.), and the application of concomitant treatment (with/without concomitant treatment), based on the supporting evidence. Investigators should also evaluate the outcomes of the AE (disappear/not disappear, recovered, in recovery, whether there are sequelae, death, unknown, etc.), and determine whether it is an SAE.

8.1 Serious adverse events

8.1.1 Definition of serious adverse events

A severe adverse event (SAE) refers to any adverse medical events as follows:

- Cause death;
- Threaten life (risk of sudden death);
- Hospitalization or prolongation of current hospital stay;
- Cause a permanent or significant disability/incapacity (heavily damage the ability to perform daily activities);
- Cause a congenital abnormality/birth defect.

Hospitalization for the following reasons should not be reported as a SAE:

a) Hospitalization due to signs and symptoms of PD;
b) Regular treatment or monitoring for study indication, without exacerbation;
c) Social reasons and temporary hospitalization, without exacerbation;
d) Elective or advance treatment to pre-existing disease irrelevant to the indication in this study, without exacerbation after the ICF is signed.

If the patients receive treatment in outpatient or emergency departments without hospitalization, and the time involved does not meet the above definition of SAE, the involved events shall not be regarded as AEs.

8.1.2 Report

An SAE should be reported in the patients after signing the ICFs (on molecular screening). For those patients who have signed the main ICFs, no matter whether the screening fails or not, the reporting of an SAE will start from the time when the main ICF has been signed.

To ensure the safety, any SAE that occurs from the time when the ICF is signed to the time 28 days after the termination the treatment should be reported, no matter whether it is suspected to be related to the study treatment or not.

When an SAE that occurs after the EOT is suspected to have a causative relation with

the study treatment, information about the initiation, closure, complication and progression of the SAE must be reported to the relevant departments within 24h after the information was received. During different periods, an SAE should be reported as a new event if the SAE is completely unrelated to the previously reported SAE. All SAE information should be collected and recorded on the SAE report form; as a complete clinical report, all applicable sections on this form must be filled in.

In the follow-up section, the following items shall be described: if the event has subsided or continues; if any treatment has been given and how the treatment is applied; if the patient is continuing the study or has dropped off.

Any SAE shall be reported to the ethics committee and the relevant management department within 24 hours after it is identified (Appendix 2).

8.2 Pregnancy

In order to ensure the safety of patients, the pregnancy events during the study should be reported within 24h once recognized. The pregnancy should be followed up and the final outcomes should be recorded, including spontaneous termination, a detailed description during childbirth, birth defects, congenital abnormalities, and complications for the mother and/or the newborn.

The enrolled patients should take a strict and effective contraception measure. If the patients are pregnant during the study period, the cell therapy should be terminated immediately.

The female patients of bearing-age are required to use effective birth control during the cell therapy and for 18 months after stoping the cell therapy.

Efficient birth control includes:

- Female sterilization
- Male sterilization (at least six months before screening)
- The combination of any two of the following methods (a+b, a+c, or b+c):

a. Oral, injectable or implantable hormone, or other hormone with equivalent effect (failure rate < 1%), for contraception, for example, using hormone pessulum or transdermal hormone for contraception

b. Intrauterine devices (IUD) or intrauterine systems (IUS)

c. Barrier methods of contraception: condom or spermicidal foam/glue/film/cream/vaginal pessary (diaphragm or cervical/vaginal fornix cap)

9. Data management and analysis

9.1 Data confidentiality

Information about study subjects will be kept confidential and managed under the applicable laws and regulations.

9.2 Site monitoring

During the study, the monitor will visit the site regularly to check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the protocol to Good Clinical Practice, the progress of enrollment, and to ensure that study product is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits.

The investigators must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information recorded on CRFs must be traceable to source documents in the patient's file. The investigator must also keep the original signed informed consent form (a signed copy is given to the patient).

9.3 Data collection

For study using Electronic Data Capture (EDC), the designated investigator staff will enter the data required by the protocol into the Electronic Case Report Forms (eCRF).

The principal investigator is responsible for assuring that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner.

9.4 Statistical methods and data analysis

Analysis and reporting of study data will be based on the data from all patients. The following rules will be followed for reporting results unless stated otherwise:

For the categorical data, the frequency and percentage will be presented. For continuous data, mean, standard deviation, median, minimum, and maximum will be provided.

The treatment groups are specified based on the dose levels of the cell therapy. Each treatment group includes all of the patients who are assigned to receive the dose at the specific level. All summaries, tables, figures and analyses are displayed by treatment group.

Screening failure patients are referred to as those who signed the ICFs but did not meet the eligibility criteria. For these patients, the eCRF data collected according to Section 7.2 will not be included in the analysis, but will be reported as separate listings in the CSR.

9.4.1 Analysis data sets

Full analysis set:

The full analysis set (FAS) includes all of the patients who received at least one infuson of the cell therapy. The FAS will be the default analysis set unless specified otherwise.

Safety set:

The safety set includes all of the patients who received at least one infusion of the cell therapy and had safety evaluation. Patients will be analyzed according to the study treatment they actually received. The safety set will be used for safety summary of the study.

Pharmacokinetic analysis set:

The pharmacokinetic Analysis Set (PAS) consists of all of the patients in the FAS who have at least one blood sample that can provide evaluable pharmacokinetic data. The PAS is used for all pharmacokinetic analyses.

9.4.2 Patient demographics / other baseline characteristics

Demographic and other baseline data (including disease characteristics) will be listed and summarized descriptively by treatment group using FAS.

9.4.3 Treatments (study treatment, concomitant therapies, compliance)

Study treatment

The actual dose and the relative dose (the ratio of actual received dose to planned dose) will be listed and summarized with descriptive statistics by treatment group. The reasons for dose delay, reductions, interruption and discontinuation will be listed and summarized.

Concomitant therapies

Concomitant medications and significant non-drug therapies prior to and after the the treatment will be listed by patient and summarized by ATC (anatomical therapeutic chemical classification system) term and treatment group.

Compliance

Compliance to the protocol will be assessed by the number and proportion of patients with protocol deviations. Protocol deviations will be identified prior to database lock and will be listed and summarized by treatment group/disease group. Compliance to the study drug will be assessed by the number of dose delay, reductions and dose interruptions.

9.4.4 Primary objective

To evaluate the safety and tolerability after autologous transfusion of CRISPR-Cas9 knockout PD-1 T cells.

9.4.4.1 Variable

Safety analysis varibles include the incidence of dose limiting toxicities (DLTs) in the first cycle of treatment, incidence rate and severity of AEs and SAEs including changes in laboratory parameters, vital signs, and ECG results.

9.4.4.2 Safety analysis

The primary analysis will include the summary of observed DLTs by treatment group, and the summary of the frequency, severity of AEs, changes in laboratory findings, physical

examinations, vital signs, ECGs. The safety set will be used for all safety evaluation.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology. Severity of AE will be coded based on the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE 4.03). All AEs recorded during the study will be listed. The incidence of treatment-related AEs (definitely or probably related to the treatment) will be summarized by system organ class (SOC) and preferred term (PT), severity (based on CTCAE grades), relation to study treatment.

The number and percentage of patients with DLTs will be tabulated by SOC and PT severity. All observed DLTs will be listed.

Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by type of adverse event and treatment group.

All laboratory values will be graded by using CTCAE 4.03. For laboratory tests covered by CTCAE, a Grade 0 will be assigned for all non- missing values not graded as 1 or higher. Grade 5 will not be used. For laboratory tests where grades are not defined by CTCAE, results will be graded by the low/normal/high classifications based on laboratory normal ranges.

If the lower limits of normal ranges used in CTCAE definitions are missing, then they have to be replaced by a clinical meaningful limit.

The following summaries will be generated separately for hematology, biochemistry and urinary laboratory tests:

- frequency table for newly occurring on-treatment grades 3 or 4;
- shift tables using CTCAE grades to compare baseline to the worst on-treatment value; for laboratory tests where CTCAE grades are not defined, shift tables using the low/normal/high/(low and high) classifications to compare baseline to the worst on- treatment value;
- Listing of all laboratory data with values flagged to show the corresponding CTCAE grades and the classifications relative to the laboratory normal ranges.

The number and percentage of patients with ECG and vital signs abnormalities will be provided. All ECG and vital signs of patients with the abnormalities will also be listed.

9.4.4.3 Tolerability

Tolerability of the study treatment will be evaluated by dose interruptions, reductions and dose intensity.

9.4.5 Secondary objective analysis

9.4.5.1 Efficacy analysis

Efficacy analysis will use FAS.

The best objective response (BOR), objective response rate (ORR), 8-week disease control rate (DCR), time to response (TTR), duration of response (DOR), progression free survival (PFS) and overall survival (OS) will be analyzed toassess the efficacy of the study treatment. The tumor response will be evaluated by investigators based on the RECIST 1.1 and immune-related response criteria (irRC) (refer to Section 12.3).

The individual lesion and overall tumor response will be listed by patient and assessment date. BOR, TTR, DOR, PFS and OS will be listed by patients.

The BOR will be summarized as appropriate, and the estimates rate of each type of response will be provided along with 95% exact confidence interval (CI) by treatment group. The best change in tumor size from baseline will be graphically presented.

9.4.5.2 *In vivo* activity status and duration of PD-1 edited T cells

The number of PD-1 edited T cells in blood will be listed and summarized by treatment group and time point. Descriptive statistics will include the arithmetic mean and geometric mean, median, standard deviation, arithmetic coefficient of variation (CV), geometric coefficient of variation, minimum and maximum. In geometric mean calculations, concentration 0 is not included. Patient level and averaged concentration of PD-1 edited T cell measures will be displayed using longitudinal plots. Graphical presentation of arithmetic mean (±SD) concentrations at each scheduled time point will be provided by treatment group.

9.4.5.3 Immunological factors and biomarkers

Baseline level of PD-L1 will be listed, Soluble immune and inflammatory cytokines (e.g. IL-10, interferon gamma, IL-6, IL-6 receptor, CRP, and ferritin) will be listed by patient and may be summarized by treatment group and time point.

9.4.6 Exploratory analysis

The frequency of TCR, TCR diversity and bTMB among patients may be summarized and displayed in graphics by treatment group at each time point before and during the treatment. TCR diversity and bTMB were compared between patients with and without disease control benefit using the exact Wilcoxon test. Statistical significance was set at $p \leq 0.05$ (2-sided).

The relationship between the efficacy and safety variables and changes in the level of other molecules may be explored through plots. If any potential relationship is explicated in the graphics, Further analysis with statistical modeling may be performed. Additional exploratorty analysis may be performed as appropriate.

9.4.7 Interim analysis

No formal interim analysis is planned for the study. During the dose-escalation, decisions will be made based on the collected data following the study procedure. For each dose cohort,

when all patients complete the DLT observation, the dose level for the next cohort will be determined based on the observed data.

9.4.8 Sample size calculation

In this dose-escalation study, each dose cohort will enroll three to six patients. Each dose level is allowed to be tested in more than one cohort of patients sequentially. About 20 patients are planned to enroll for this study.

10 Ethical considerations

10.1 Regulatory and ethical compliance

The design, implementation and reporting of this clinical study will follow the ICH tripartite coordination guiding principles of good clinical practice, applicable to local laws and regulations and ethical principles derived from the Declaration of Helsinki.

10.2 Responsibilities of the investigators and IRB

Before the study initiation, the Declaration of Helsinki (2000 edition) must be followed and the relevant Chinese medical study norms and regulations must be implemented. The clinical study will be conducted only after the ethics committee of the study unit has approved the study protocol and the proposed ICF. Before each patient is chosen for the study, the investigator will be responsible for introducing the objective, procedures and possible risks completely and comprehensively to the patient or his representive in writing. Patients should be aware that they have the right to exit the study at any time. Each patient should be given a written patient ICF prior to screening and enrollment. It is the investigator's responsibility to obtain the ICF before the patient is screened and enrolled, and the ICF should be retained for future reference as a clinical study document. Before the study initiation, the investigator needs to sign on the signature page of the study protocol to confirm: He/she agrees to carry out the study in accordance with all of the guidelines and procedures in these documents and the study protocol, and permits the examination of related data and records by CRA, ethics and the competent authority as needed.

10.3 Informed consent procedures

Only eligible patients who have provided a written, ethically approved ICF can be enrolled in the study.

The ICF must be collected prior to the execution of any study procedure (i.e., all of the procedures described in the study protocol), especially the molecular screening. The process of collecting the ICF should be recorded in the patient's original medical record. The date of ICF should be recorded in the patient's eCRF.

The investigator will prepare ICF that is appropriate for the study and complies with the

ICH GCP guidelines and regulatory requirements.

Women of childbearing age should be informed that there is an unknown risk to the fetus if pregnancy occurs during the study, and the patient can only be enrolled in the study after she agrees to abide by the contraceptive requirements. The patient will not be enrolled in the study if she is unable to comply with the contraceptive requirements reliably.

10.4 Discontinuation of the study

The sponsor reserves the right to suspend the study under the conditions specified in the clinical study protocol.

10.5 Publication of study protocol and results

The sponsor ensures that the key design content of the study protocol will be published in a publicly accessible database (e.g. clinicaltrials.gov.). In addition, the study results will be submitted for publication and/or published in a publicly accessible clinical trial database upon the completion of the study and the conclusion of the study report.

10.6 Study documentation, record keeping and retention of documents

The study center will retain the appropriate medical records and study records of the study. The study center will permit the competent authority to conduct reviewing, auditing and assessing of study safety and progress for quality assurance, and to review the clinical record (copy the clinical record as applicable to legal regulations).

The original data are the information necessary for clinical studies to rebuild and evaluate the study, including original record of clinical findings, observation and other activities. Examples of these original documents and data records include, but are not limited to: hospital records, clinical and medical records, laboratory records, memos, patient diaries or assessment lists, drug relapse records, automated instrumental recordings, copies or transcripts with accuracy and completion validated, microfilms, negative films, microfilm strip and magnetic media records, X-ray recordings and patient documentation, and records kept by pharmacies, laboratories, and medical technological department participating in the study.

Data must be collected by the researchers that are authorized by the investigator. The primary data collection tool for this study is the experiment case report form (eCRF). The investigator should ensure the accuracy, completeness, clarity and timeliness of the data in the eCRF and all other required reports. The data in the eCRF that is derived from the original document should be consistent with the original document and the inconsistent data should be interpreted. All data required in the eCRF must be recorded and all missing data must be interpreted. All changes or corrections to the eCRF must be confirmed by signing the date

and providing initials, and the initial records must be reflected in the original medical records. An audit trail record should be maintained in the system for the eCRF. The investigator should retain records of changes and corrections in the eCRF.

The investigator/institution should maintain the study documentation in accordance with the requirements of the essential documents of the clinical study regulations and applicable legal regulations and/or guidelines. Investigators/institutions should take measures to prevent accidental damage or premature destruction of the documents.

10.7 Confidentiality of study documents and patient records

The investigator must ensure that the patients are anonymous and must not identify the patients by name in any of the documents submitted to other partners. The strict confidentiality of signing the ICFs and patient selection must be maintained so as to identify patients in the studycenter.

10.8 Compensation and insurance

10.8.1 Payment for transportation expenses, etc

The costs involved in the observations and inspections during the therapy will be borne by the investigator. The study institution should pay the patient for transportation and other expenses after approval from the EC.

10.8.2 Indemnity and insurance

If the patient has suffered health damage due to AEs as a result of participating in the study, the investigator will provide compensation for the damage unless the health damage is caused by the patient's himself. The investigator will take the necessary measures (such as insurance coverage) to undertake the relevant responsibilities.

11. Protocol compliance

The investigators declare that they will do their duty to avoid protocol deviations. The protocol should be amended if the investigator believes that deviating from the study protocol will improve the performance of the study. The protocol amendment can be implemented only after approving by EC. All major protocol deviations should be recorded and reported in the CSR.

11.1 Study protocol amendment

Any changes or additions to the study protocol should be documented in writing in the study protocol amendments, and must be approved by health authorities (if required) and the EC. Only amendments that are necessary for patient safety can be implemented prior to EC approval. Although approval is required for study protocol amendment, the investigator can take any required action immediately for the safety of the patients that are enrolled in the

study, even if the measure deviates from the study protocol. In this case, the investigator should inform the study center EC in time.

12. Appendix

12.1 ECOG performance status

0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able tocarry out light work or sedentary activity, eg, light house work, office work.
2	Ambulatory and capable of all self-care but unable to carry out any workactivities. Up and about more than 50% of waking hours.
3	Only limited self-care ability, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. No self-care ability. Totally confined to bed or chair.
5	Dead.

12.2 RECIST 1.1 Guideline[45]

Glossary

CR	Complete remission
CSR	Clinical study report
CT	Computed tomography
DFS	Disease free survival
eCRF	Electronic case report form
FPFV	The first visit of the first patient
LPLV	The last visit of the last patient
MRI	Magnetic resonance imaging
OS	Overall survival
PD	Progressive Disease
PFS	Progression free survival
PR	Partial remission
RAP	Report and analysis plan
RECIST	Remission evaluation criteria for solid tumor
SD	Stability of disease
SOD	Sum of diameters
TTF	Time to treatment failure
TTP	Time to progress
UNK	Unknown

1 **Background**

Omitted

2 **Objective**

Omitted

3 **Tumor measurability at baseline level**

3.1. Definitions

At baseline, tumour lesions/lymph nodes will be categorized measurable or non-measurable as follows:

3.1.1. Measurable

Tumour lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

• 10 mm by CT scan (CT scan slice thickness no greater than 5 mm).

• 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).

• 20 mm by chest X-ray.

• Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be P15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

3.1.2. Non-measurable

Other lesions include small lesions (longest diameter <10 mm or pathological lymph node short diameters ≥ 10 mm to <15 mm) and immeasurable lesions. Unmeasured lesions include: meningeal disease, ascites, pleural or pericardial effusion, inflammatory breast cancer, cancerous lymphangitis of the skin/lungs, abdominal mass that cannot be diagnosed and followed-up by imaging study, and cystic lesions.

3.1.3. Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:

• Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

• Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross. sectional imaging techniques such as CT or MRI can be considered as measurable lesions.

• Blastic bone lesions are non-measurable.

Cystic lesions:

• Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

• 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

• Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

3.2. Specifications by methods of measurements

3.2.1. Measurement of lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

3.2.2. Method of assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and P10 mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT

scan based on the assumption that CT slice thickness is 5 mm or less. As is described in Appendix II, when CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for bodyscans).

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next (described in greater detail in Appendix II). If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilization of these techniques for objective tumour evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in studys where recurrence following complete response or surgical resection is an endpoint.

Tumour markers: Tumour markers alone cannot be used to assess objective tumour response. If markers are initially above should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

4. Tumour response evaluation

4.1. Assessment of overall tumour burden and measurable disease

To assess objective response or future progression, it is necessary to estimate the overall tumour burden at baseline and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumour response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion. In studies where the primary end point is tumour progression (either time to progression or proportion with progression at a fixed date), the protocol must specify if entry is restricted to those with measurable disease or whether patients having non-measurable disease only are also eligible.

4.2. Baseline documentation of ‘target’ and ‘non-target’ lesions

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded).

Lymph nodes merit special mention since they are normal anatomical structures which

may be visible by imaging even if not involved by tumour. As noted in Section 3, pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of P15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumour. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20 mm · 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement All other pathological nodes (those with short axis P10 mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumour regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as ‘present’, ‘absent’, or in rare cases ‘unequivocal progression’ (more details to follow). In addition, it is possible to record multiple nontarget lesions involving the same organ as a single item on the case record form (e.g. ‘multiple enlarged pelvic lymph nodes’ or ‘multiple liver metastases’).

4.3. Response criteria

This section provides the definitions of the criteria used to determine objective tumour response for target lesions.

4.3.1. Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also

demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

4.3.2. Special notes on the assessment of target lesions

Lymph nodes. Lymph nodes identified as target lesions should always shave the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since abnormal lymphnodeis defined as having a short axis of <10 mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Target lesions that become 'too small to measure'. While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs, it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.

Lesions that split or coalesce on treatment. when non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that

would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the ‘coalesced lesion’.

4.3.3. Evaluation of non-target lesions

This section provides the definition soften criteria used to determine the tumour response for the group of non-target lesions. While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumour marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits.

Progressive Disease (PD): Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

4.3.4. Special notes on assessment of progression of non-target disease

The concept of progression of non-target disease requires additional explanation as follows: When the patient also has measurable disease. In this setting, to achieve ‘unequivocal progression’ on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumour burden has increased sufficiently to merit discontinuation of therapy. A modest ‘increase’ in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease. This circus stance arises in some phase III studys when it is not acroterion study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the inter predation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e. an increase in tumour burden representing an additional 73% increase in ’volume’ (which is equivalent to a 20%

increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from ‘trace’ to ‘large’, an increase in lymphangitic disease from localized to widespread, or may be described in protocols as ‘sufficient to require a change in therapy’. If ‘unequivocal progression’ is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

4.3.5. New lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour (for example, some ‘new’ bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient’s baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a ‘new’ cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient’s brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible ‘new’ disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive l FDG-PET at follow-up is a sign of PD based on a new lesion.

B. No FDG-PET at baseline and a positive FDG-PET at follow-up:

If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.

If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).

If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

4.4. Evaluation of best overall response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. On occasion a response may not be documented until after the end of therapy so protocols should be clear if post-treatment assessments are to be considered in determination of best overall response. Protocols must specify how any new therapy introduced before progression will affect best response designation. The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of newlesions. Furthermore, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement (see Section 4.6). Specifically, in non-randomized trials where response is the primary endpoint, confirmation of PR or CR is needed to deem either one the 'best overall response'. This is described further below.

4.4.1. Time point response

It is assumed that at each protocol specified time point, a response assessment occurs. Table 1 on the next page provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.

Table 1 Response of time point: Patients with Target Lesions (with/without non-target lesions)

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

When patients have non-measurable (therefore non-target) disease only, Table 2 is to be used.

Table 2 Response of Time point-the Patients with only Non-target Lesions

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease, and NE = inevaluable.

a 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

4.4.2. Missing assessments and unevaluable designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and

at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

4.4.3. Best overall response: all time points

The best overall response is determined once all the data for the patient is known.

Best response determination in trials where confirmation of complete or partial response IS NOT required: Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow-up after the first SD assessment would be considered unevaluable.

Best response determination in trials where confirmation of complete or partial response IS required: Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally 4 weeks later). In this circumstance, the best overall response can be interpreted as in Table 3.

Table 3. Optimal Total Remission with CR and PR Effects to Be Confirmed

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.
a If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

4.4.4. Special notes on response assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (<10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the case report form (CRF).

In trials where confirmation of response is required, repeated 'NE' time point assessments

may complicate best response determination. The analysis plan for the trials must address how missing data/assessments will be addressed in determination of response and progression. For example, in most trials it is reasonable to consider a patient with time point responses of PR-NE-PR as a confirmed response.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in Tables 1–3.

Conditions that define 'early progression, early death and in evaluability' are study specific and should be clearly described in each protocol (depending on treatment duration, treatment periodicity).

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

4.5. Frequency of tumour re-evaluation

Frequency of tumour re-evaluation while on treatment should be protocol specific and adapted to the type and schedule of treatment. However, in the context of phase II studies where the beneficial effect of therapy is not known, follow-up every 6–8 weeks (timed to coincide with the end of a cycle) is reasonable. Smaller or greater time intervals than these could be justified in specific regimens or circumstances. The protocol should specify which organ sites are to be evaluated at baseline (usually those most likely to be involved with metastatic disease for the tumour type under study) and how often evaluations are repeated.

Normally, all target and non-target sites are evaluated at each assessment. In selected circumstances certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

After the end of the treatment, the need for repetitive tumour evaluations depends on whether the trial has as a goal the response rate or the time to an event (progression/death). If 'time to an event' (e.g. time to progression, disease-free survival, progression-free survival) is the main endpoint of the study, then routine scheduled re-evaluation of protocol specified sites of disease is warranted. In randomized comparative trials in particular, the scheduled assessments should be performed as identified on a calendar schedule (for example: every 6–8 weeks on treatment or every 3–4 months after treatment) and should not be affected by delays in therapy, drug holidays or any other events that might lead to imbalance in a treatment arm in the timing of disease assessment.

4.6. Confirmatory measurement/duration of response

4.6.1. Confirmation

In non-randomized trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials (see the paper by Bogaerts et al. in this Special Issue 10). However, in all other circumstances, i.e. in randomized trials (phase II or III) or studies where stable disease or progression are the primary end points, confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.

4.6.2. Duration of overall response

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study). The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

4.6.3. Duration of stable disease

Stable disease is measured from the start of the treatment (in randomized trials, from date of randomization) until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD).

The clinical relevance of the duration of stable disease varies in different studies and diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease. Note: The duration of response and stable disease as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency. The frequency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be taken into account if comparisons between studys are to be made.

4.7. Progression-free survival/proportion progression-free

4.7.1. Phase II trials

This guideline is focused primarily on the use of objective response endpoints for phase II trials. In some circumstances, ‘response rate’ may not be the optimal method to assess the potential anticancer activity of new agents/regimens. In such cases ‘progression-free survival’ (PFS) or the ‘proportion progression-free’ at landmark time points, might be considered appropriate alternatives to provide an initial signal of biologic effect of new agents. It is clear, however, that in an uncontrolled trial, these measures are subject to criticism since an apparently promising observation may be related to biological factors such as patient selection and not the impact of the intervention. Thus, phase II screening trials utilizing these endpoints are best designed with a randomized control. Exceptions may exist where the behavior patterns of certain cancers are so consistent (and usually consistently poor), that a non-randomized trial is justifiable. However, in these cases it will be essential to document with care the basis for estimating the expected PFS or proportion progression-free in the absence of a treatment effect.

12.3 irRC evaluation criteria

Experts from the International Association of Immunotherapy have formally put forward the new immune-related response criteria in *Clinical Cancer Research* in 2009, which is

known as irRC.

SPD (the product of two maximum vertical diameters of the measurable lesion)is adopted in irRC to estimate the size of tumor. Different from the respective WHO assessment standard, which assess every lesion separately; irRC uses the total tumor burdens of all measurable lesions. Tumor burden is defined as the sum of SPD of all lesions, including SPD of new lesions.

Measurable lesions of irRC are defined as lesions greater than 5mm*5mm on CT scan, with maxium 5 lesions occuring on each visceral organ, no more than 10 lesions on all visceral organs, and no more than 5 lesions presenting on body surface.

Effect assessment of irRC: The curative effect evaluation in irRC is based on the increase or decrease in the overall tumor burden at the observation time as compared with the time of baseline assessment, confirmed by repeating the comparison between two consecutive operation time points intervened by at least 4 weeks, and classified.

Specifically, it is divided into four categories as follows:

irCR - all the lesions disappear completely;

irPR - in the continuous detections, tumor burden decreases by more than or equal to 50% compared with the baseline tumor burden;

irSD - inconformity to irCR and irPR standard, and no appearance of irPD;

irPD –tumor burden increases by more than or equal to 25% compared with the baseline tumor burden.

Comparison between irRC and Traditional WHO Standard

Item	WHO	irRC
Newly found measurable lesion (for example, ≥5mm*5mm)	Always indicates rogression disease (PD)	The overall tumor burden should be included to assess whether it is PD
Newly found ummeasurable lesion (for example, ≤5mm*5mm)	Always indicates rogression disease (PD)	Not defined as PD
CR	Disappearance of all the lesions is confirmed at two consecutive observation points with an interval of at least 4 weeks.	

PR	The diameters of all the measurable lesions decreasing more than 50% compared to the baseline, which are confirmed at two continuous observation points with an interval of at least 4 weeks. No new lesion or other disease development is observed.	The overall tumor burden decreasing more than 50% (irPR), compared to the baseline, is confirmed at two continuous observation points with an interval of at least 4 weeks.
SD	The lesion diameter increasing detected at two continuous observation points is less than 25%. No new lesion or other disease development is observed.	As confirmed at two continuous observation points, the overall tumor burden decreasing is less than 50% or increasing is less than 25% (irSD), compared to the baseline.
PD	The lesion diameter increasing ≥ 25% compared to baseline is detected at any observation point. And (or) new lesion and (or) other disease development are observed.	The overall tumor burden increasing ≥ 25% (irPD), compared to the baseline, is confirmed at any time between the two continuous observation points with an interval of at least 4 weeks.

In accordance to irRECIST, if a primary PD is identified by tumor imaging the tumor should be revaluated after at least four weeks to confirm the PD. During the period from identification to confirmation of PD, if the patient agrees to continue the cell infusion therapy, the patient may continue to receive the cell infusion treatment. If the next imaging examination is scheduled within 4 weeks, it is not necessary for the patients examined by confirmation tumor imaging to take the next appointed tumor imaging test. If the clinical conditions of the patient are stable, the tumor imaging examination can be re-initiated at an appointed time point and continued until PD is verified by imaging assessment. Given that tumors may burst within the first several months after the initial cell infusion therapy and then relieve in some patients, patients are allowed to continue the cell infusion therapy after the first presence of PD until the next PD presents. For the patients with unstable clinical conditions, it is not necessary to repeat the imaging examination to confirm PD. Tumor bursting includes:

- The original target lesion worsens
- The original non-target lesion worsens
- The origin of new lesions

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Amendments

Table of Changes (Version 1.5, Oct 10, 2016)

Section No.	Section Title	Description of Change (s)	Rationale
7.1.2; 7.1.4.2	Screening; Day 8 of each cycle	The following sentences were added: Collection of blood for PBMC expansion: 60-80 ml. The actual blood volume is confirmed according to the routine blood test result of the patient at that time.	According to protocol design for details.
7.2.3.5	*In vivo* T cell tracking	The following sentences were added: • Baseline and Day 12 of each treatment cycle • After end of treatment: the 28st day of last treatment cycle, three months, six months, and one year after the last cell infusion. • Blood sampling volume: 10 ml each time. Notes: the treatment of different groups may vary from two to four cycles; therefore, the number of blood sampling collection points are: eight (two cycles), 10 (three cycles), or 12 (four cycles).	In order to track the edited T cells in vivo.
13.5.17	Off-target effect	~~Off-target will be assessed for edited cells before the infusion of patient.~~ Updated to the following sentence: Blood sample with 10 ml was collected for assessment of off-targeton baseline, end of study and unscheduled timepoint based on investigator ajudgement.	A consideration of safety.

Table of Changes (Version 1.6, Jan 24, 2017)

Section NO. (s)	Section Title (s)	Description of Change (s)	Rationale
All sections	General	Replaced “21 Days/Cycle” with “28 Days/Cycle”	Updated protocol about the start of the time of T cell culture.
Protocol summary, 3.1; 5.1.2.1	Trial and design	The underlined words were added: Pre-A: 2x10^7 PD-1 edited T cells per kilogram of body weight, after the first treatment cycle, observe 28 Days and then evaluate the patient; if the patient does not have DLTs, one more treatment cycle will be allowed. Group A: 1x10^7 PD-1 edited T cells per kilogram of body weight 2 treatment cycles; Group B: 2x10^7 PD-1 edited T cells per kilogram of body weight 2 treatment cycles; Group C: 4x10^7 PD-1 edited T cells per kilogram of body weight 2 treatment cycles;	According to the national regulation of “Guidelines for Research and Assessment of Cell Product” published in Jan 2017.
2.3	Primary endpoint	The underlined wordswere added: The rate of dose-limiting toxicity (DLT) of the cell therapy during the period of the first treatment cycle.	To clarify the observed period of DLT.
3.1.1.1	Molecular pre-screening	~~PD-L1 detection will be further confirmed with the positive PD-L1 staining by Roche Ventana PD-L1 (SP142, SP263), which is performed in the pathology department of the trial center with Leica bondmax automatic immunohistochemistry machine.~~ The following text was added: After the tumor being confirmed to be PD-L1 positive, the patient can sign the ICF, and the screening/baseline visit can be initiated. Remarks: If the patients have been confirmed to have PD-L1 positive tumors through staining with Roche Ventana PD-L1 test (SP142, SP263) and Dako PD-L1 test (Dako28-8, Dako22c3) prior to their participation in the screening for this clinic trial, they candirectly sign the ICF for the trial, and the screening/baseline visit can be	To clarify that the enrolled patient was permitted to confirm by any PD-L1 test mentioned in protocol, because there are four standarded PD-L1 detest technology according to the literature.

		initiated.	
Protocol summary; 4.1	Inclusion criteria	4. NSCLC, stage IIIB/IV confirmed by histology or cytology; All patients must be screened for EGFR gene mutation and ALK gene fusion; For the patients with a positive EGFR mutation or ALK gene fusion, treatment failure of EGFR-TKI or ALK-TKI must have been experienced. 8. Life expectancy ≥ 3 months.	Updated the protocol
Protocol summary; 4.2	Exclusion criteria	~~12. Patients receivedprior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137 or anti-CTLA-4 antibodies.~~	Available to screen patients
5.1.2.1	Cell infusion procedure	Cell infusion procedure #6 was revised: 6. Cell infusion duration: At the first time of cells infusion to the patient, the drops at the beginning should be adjusted to about 15-20 drops/min for 10-15min to evaluate safety. If adverse events are not observed, the infusion speed will be adjusted to around 60 drops/min. For the elderly patients and the patients with cardio-pulmonary function insufficiency, the infusion speed should be slow down with close monitoring to the patient. The speed of infusion can be adjusted accordingly.	For more standardization.
5.3.2	Tentative dose	The initial dose of treatment group A: $1x10^7$ PD-1 edited T cells per kilogram of body weightin total. A dose of PD-1 edited T cells splited into three doses of 20%, 30%, and 50% will be conducted on the 1st, 3rd, and 5th day of each cycle, 28 days/cycle. Patients will be treated for a total of two cycles. Furthermore, the cell dose will gradually increase to $2.0x10^7$ and $4.0x10^7$ PD-1 edited T cells per kilogram of body weight per cycle in the next two treatment groups.	For details.
5.3.7	Definition of DLT	Unless otherwise specified, NCI CTCAE4 .03 will be applied to assess the toxicity. DLT is defined as the AE or a laboratory abnormal value that is probably attributable to the T cell infusion. An abnormal laboratory value can be attributed to the T cell infusion if it happens within one cycle after the first cell infusion (≤ 28 days) or occurs in thefirst cycle but is later confirmed in the second cycle, and meets any criterion of the following conditions	For details.
6.4.3.3	Disposal	The following text was added:	For details.

	and destruction	If the cells are not completely infused for some special reason, the residual cells should be sealed, packed and frozen/refrigerated for storage strictly according to the operation standards, and further disposal conditions should be documented clearly.	
7	Visit schedule and assessments	Visit schedule and assessments #1, #2, #4 and #5 were revised: 1.Deleted "a. Questionnaires for the patient - EORTC QLQ-C30 and EORTC QLQ-LC13" of the section screening; 2. The term of lymphocyte subpopulation was changed from "CD56" to "NK". 4. The total volume of the blood collection for PBMC expansion was revised: Group A: 40ml; Group B/C: 60-80ml. based on the hematology examination results of the patient at the time. 5. The cytokines that are tested were added as follow: IL-6, IL-10, CRP, TNF-α, ESR, IL-8, IL-2, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF and FLT3L.	This is phase I study with small size of patients, questionnaires had not meaning. The text was updated to further clarify and to add more cytokines as exploratoryindexes.
7.2.2.5	Laboratory assessments	The following text was added: Cytokine level monitoring: IL-6、IL-10、CRP、TNF-α、ESR、IL-8、Fractalkine、 IFN-r、MCP-1、IL-5、GM-CSF、FLT3L (plasma samples have been collected, and IL-8, IL-2, Fractalkine, IFN-r, MCP-1, IL-5, GM-CSF, FLT3L are all exploratory indexes which will be tested at the end of trial.)	According to protocol design for details.
7.2.3	Biomarker	The following text was added: *Notes: A total of 15 ml peripheral blood sample is collected at the same time point for ctDNA and immune repertoire; 10 ml peripheral blood sample is collected at the time point for immune repertoire only.	10ml blood could meet the detect of ctDNA and immune repertoire, when the detect of ctDNA and immune repertoire were at the same time point.
7.2.3	Biomarker	The following text was added: Flow cytometery analysis of regulatory T (Treg) cells, memory T cells, activated T cells and PD1 expression.	In order to explore the dynamic change of regulatory T (Treg)

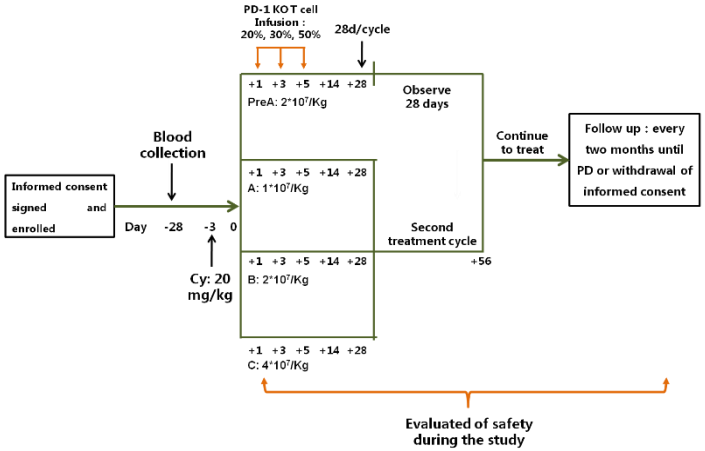
		Blood collection time points: before pretreatment with cyclophosphamide (blank baseline), after pretreatment with cyclophosphamide (medication baseline), 24 h after the third cell infusion of each cycle, the end of each cycle (day 21), week eight, three months, and six months after the last infusion. Blood sampleing collection volume: 5 ml each time.	cells, memory T cells, activated T cells and PD1 expression after T cell infusion.

Table of Changes (Version 1.7, June 05, 2017)

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
7; 7.1.2	Visit schedule and assessments: Table 7-1 Flowchart of Trial Visits; Screening	~~The blood collection for PBMC pre culture~~	According to protocol design.

Table of Changes (Version 1.8, Jan 30, 2018)

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
Home page	Protocol Title	A ~~single-arm, open, prospective~~phase I clinical trial of PD-1 knockout engineered T cells treating patients with advanced non-small cell lung cancer	
Protocol summary; 2.2	Secondary objective	2) ~~The potential immunological indexes and biomarkers.~~ Update to: 2) To investigate the changes of the potential immunological indexes and biomarkers. 3) To track the PD-1 KO T cells in patients and investigate the correlation of the clinical response.	Update to the protocol
Protocol summary; 2.4	Secondary endpoint	~~4) To evaluate the duration of the first confirmed stable disease (SD) (the time from the first confirmed SD to the first PD or death caused by any factor).~~	The PFS is secondary endpoint.
Protocol summary; 4.2	Exclusion criteria	~~13. The patients who accepted the anti-tumor vaccines or otherimmunostimulatory anti-tumor drugs.~~	The primary end point of the clinical trial was safety.
3.1	Description of the study design	Pre-A: Patients will receive A dose of 2×10^7PD-1 edited T cells per kilogram of body weight splited into three doses of 20%, 30%, and 50% will be conducted on the 1st, 3rd, and 5th day of each cycle. After finishing the first cycle and resting for 28 days, the patients will be allowed to receive another cycle of transfusion. Around 20 patients with advanced PD-L1 positive NSCLC that show progression after the multiline treatments will be enrolled in this dose escalation trial. Patients will be assigned into three groups, with a treatment cycle of 28 days.	The text was updated for more precise.

3.1; 5.0	Summary schema of the trial design	Summary schema of the trial design was updated: 	
5.1.1	Pretreatment	Add the underlined words in text: A single dose (20 mg/kg) of cyclophosphamide will be administrated as a pre-treatment intending intending for lymphocytes depletionon the third day before the cell transfusion<u>of the first treatment cycle</u>.	The clear definition of application of cyclophosphamide in study.
5.1.2.1	Treatment group	<u>1) If the patient decides to discontinue the cell infusion therapy, the patient will be followed upaccording to scheduled visits (7 visits and evaluation) until end of the study. Additionally, the patient has the right to choose other clinical therapeutic strategies to treat the cancer.</u> <u>If the patient agrees to continue the cell infusion therapy, she orhe can receive the cell infusion therapy until the end of the study. Based on the irRECIST criteria, if a primary PD is identified through tumor imaging, another evaluation should be conducted after at least four weeks to confirm the PD status. During the period from identification to confirmation of the PD, if the patient's general condition does not worsen from the baseline (ECOG: 0-2 scores), or no intolerable therapeutic side effects/other conditions that hold for further cell infusion therapy occur, and the patient agrees to continue the cell infusion therapy, the patient may continue to receive the cell infusion treatment. If the next imaging examination is scheduled around four</u>	The text was updated based on irRECIST.

		weeks, it is not necessary for the patient to take an additional imaging test. If the clinical conditions of the patient are stable, the tumor imaging examination can be re-initiated at an appointed time point and continued until PD is verified through imaging assessment. Given that tumors may flare within the first several months after the initial cell infusion therapy and then shrink in some patients, patients are allowed to continue the cell infusion therapy after the first presence of PD until the confirmation of next PD. For the patients with unstable clinical conditions, it is not necessary to repeat the imaging examination to confirm PD. Tumor flares includes: • Worsening of the original target lesion • Worsening of the original non-target lesion • Identification of new lesions	
5.3.5	Dose escalation of the patients	The escalation dose is from $1x10^7$ to $2x10^7$ and $4x10^7$ PD-1 edited T cells per kilogram of body weight in the three treatment groups A, B, and C. Engineered T-cells splitting into three applications (20%, 30% and 50%) will be conducted on the 1st, 3rd, and 5th day of each cycle. The patient must be able to tolerate the lowest dose, and the effect of the prior dose has been assessed, the patient can receive the sequential treatments.	To appropriately reflect the protocol.
5.5	Supportive therapy	The cell therapy treated patients may as recommended by the investigators, be treated with some appropriate supportive therapies, based on the guidelines for immune-related advert events (irAE).	To appropriately reflect the protocol.
6.4.1	Package and label of the trial cells	Package and label of the trial cells was updated as follow:	

		Patient No.: Cell type: Cell quantity: Capacity: Storage temperature: Storage condition: Date of manufacture: MM/DD/YY 00:00 Expiration date: Manufacturer: Trial ID: MHC-001	
8.	AEs	Abnormal values of laboratory tests: The laboratory abnormalities with significance (clinically significant, inducing clinical signs or symptoms, requiring concomitant treatment or adjustment of the trail, treatment) should be recorded as AEs, with an emphasis on the diagnosis results instead of the signs and symptoms (for example, anemia other than low hemoglobin). The laboratory abnormal values that conform the AE should be followed up until those AEs recove stable. If an abnormal laboratory value or test result shows the same signs/symptoms as a reported AE, it should not be recorded as aseperate AE. Investigator should follow-up the patients that exit the study due to AEs, to observe and record the outcomes of any AEs until such events are completely relieved or stable. Investigatormust judge whether the AE is related to the treatment, the measures taken after AE is observed (no action, discontinuation of the trial treatment, temporary interruption for medicine, permanent termination of the trial treatment, unknown, etc.), and the application of concomitant treatment (with/without concomitant treatment), based on the supporting evidence. Investigator should also evaluate the outcomes of the AE (disappear/not disappear, recovered, in recovery, whether there are sequelae, death, unknown, etc.), and determine whether it is an SAE.	Add the precisedescription about AEs.

9.	Data monitoring committee	~~The independent data monitoring committee will be set up, consisting of 3 independent medical consultants who are responsible for overseeing the safety of the trial. According to the analysis of available clinical and laboratory data, the committee may suggest the trial to be continued, revised, and/or suspended, based on the data. In the event of some new safety issues, especially a serious incident (such as death) during the trial or a safety notification regarding the trial treatment, a meeting shall be convened upon the request of the sponsors.~~	The independent data monitoring committee in our hospital did not set up at that time. This phase I study has been supervised by Clinical Research Administrative Board, West China Hospital.
9.4.4.1	Variable	Safety analysis varibles include the incidence of dose limiting toxicities (DLTs)~~ in 3 week and during the treatment~~ Safety analysis varibles include the incidence of dose limiting toxicities (DLTs) in the first cycle of treatment, incidence rate and severity of AEs and SAEs including changes in laboratory parameters, vital signs, and ECG results.	Definition of DLT was changed in protocol.
9.4.4.2	Safety analysis	~~The total observation period can be divided into three phases:~~ ~~1. Pre-treatment period: From the day that the patient signs the ICF to the day before the first trial treatment is given~~ ~~2. Treatment period period: From the day that first trial treatment is given to the day that 150 days after the last dose is given~~ ~~3. Post-treatment period: From the day that 151 days after the last dose is given.~~	The description of content was repeated.
10.4.6.2	Relationship between the clinical factors and therapeutic effect	~~Clinical factors include the pathogenesis, pre-therapeutic regimens, and patient performance status. Conduct a tentative trial by graphics on the relationship between efficacy indexes and clinical factors. Depending on specific circumstances, conduct further trial using statistical modeling if any potential relationship is identified. Specific analysis will be recorded in a separate report. Describe any additional data analysis depending on specific circumstances in a separate analysis planning document.~~	This is an evaluation of safty other than efficacy
9.4.8	Sample size	In the dose escalation trial, one dose group will enroll three to six evaluable patients receiving the	The text was updated in case of

	calculation	recommended dose	DLT.
13.4	Safe treatment process on tumor immunology drugs	~~13.4Safe treatment process on tumor immunology drugs~~	We figure out a brochure regarding AE treatment to replace the description.

Table of Changes (Version 1.9, Dec 04, 2018)

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
3.1	Description of the study design	The following text was added: This clinical study was a first-in-human trial using CRISPR technology knockouting PD-1 gene in T cell to treat lung cancer patients. Although there were no exact dosage/regimen and safety data, starting dose was selected referring to cell therapy or CART treatment for considering benefit-risk of cancer patients (see section 5.3.1 Starting dose rationale). Pre-A: $2x10^7$ PD-1 edited T cells per kilogram of body weight (percentage of PD-1 knockout T cell may vary depending on technology efficiency). The study defines the Pre-A group as a preliminary exploratory dose group. The Pre-A group has a 28 days-observation after the first treatment cycle. If no DLT was observed in Pre-A group during the defined observation period, the study will continue other treat groups according to provisional dosage; If DLT or other unexpected toxicity occurs in the Pre-A group, the investigator will adjust the cell dose and interval of sequential cell therapy based on data from Pre-A group.	More precise description about the dose of Pre-A group and give a clear interpret the choosing dose of $2x10^7$ PD-1 edited T cells per kilogram of body weight.
5.5.1	Cytokine release syndrome (CRS)	~~Cytokine release storm (CRS)~~	Corrected: Cytokine release syndrome.
7.1.1; 7.2.3.3	Molecular screening; Screening of the tumor biomarkers	The underlined is added: PD-L1 status detection was used by fresh tissue or paraffin-embedded tissue. Before the molecular screening, the candidate patients are asked to sign the ICF for molecular screening.	

8.1.2	Report	~~An SAE should be reported in the patients with an unknown condition of PD-L1 after the ICF (on molecular screening) is signed. Based on the evaluation by investigator, the event can be reported as an SAE only if it is suspected to have a causative relationship to the trial process (such as traumatic operation or biopsy). An SAE should be followed up until it subsides, or a clinically significant improvement or stabilization is observed.~~ ~~For those patients with a known PD-L1 condition who have signed the main ICF, no matter whether the screening fails or not, the reporting of an SAE will start from the time when the main ICF has been signed.~~ The deletion was replaced by underline content for concise: An SAE should be reported in the patients after the ICF (on molecular screening) is signed. For those patients who have signed the main ICF, no matter whether the screening fails or not, the reporting of an SAE will start from the time when the main ICF has been signed. To ensure the safety of patients, any SAE that occurs during the time when the ICF is signed to the 28th day after the patients terminate the treatment should be reported, no matter whether it is suspected to be related to the study treatment or not.	To accurately report the initiation and termination of SAE.
12.2	RECIST 1.1 Guideline 48	The following text was added: Eisenhauer EA. Et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47	

Gene Edited T-Cell Therapy for Advanced Lung Cancer.

Final statistical analysis plan

(Version 2, May 04, 2018)

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List of abbreviations

Abbreviation	Detail
AE	Adverse Event
ATC	Anatomic Therapeutic Chemical (Classification)
BMI	Body Mass Index
CI	Confidence Interval
CR	Complete Response
CTC	Common Terminology Criteria
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Disease Control Rate
DLT	Dose Limiting Toxicity
DOR	Duration of Response
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report/Record Form
FAS	Full Analysis Set
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
NSCLC	Non-Small Cell Lung Cancer
ORR	Overall Response Rate
OS	Overall Survival
PAS	Pharmacokinetic Analysis Set
PD	Progressive Disease
PFS	Progression Free Survival
PR	Partial Response
PT	Preferred Term
RAP	Report Analysis Plan
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SOC	System Organ Class
SS	Safety Set
TTR	Time to response

1 Introduction

This document provides detailed statistical methodology for the analysis of data from study MHC-001.

Data will be analyzed according to the data analysis section 9 of the study protocol version 1.8. Important information is given in the following sections.

2 Study objectives and study design

2.1 Primary objective

The primary objective is to investigate the safety and tolerability of autologous T cells with PD-1 knocked out by CRISPR-CAS9-OPT (PD-1 KO T cell) in patients with PD-L1 positive advanced non-small cell lung cancer (NSCLC) who have progressed after multiple-line standard treatment.

2.2 Secondary objectives

- To preliminarily investigate the anti-tumor activity of PD-1 KO T cell infusion therapy
- To investigate the changes in the potential immunological factors and biomarkers related to PD-1 KO T cell infusion therapy
- In vivo tracking of PD-1 KO T cells in patients and investigate the mechanism of the clinical response

2.3 Study design

This is an investigator-initiated Phase I open-label dose escalation study. Patients were those having PD-L1 positive NSCLC who have progressed after multiple-line standard treatment. Patients in each dose cohort would receive two cycles of treatment (28-day cycle) as planned, and could continue treatment in agreement with the investigator and patient if the treatment was deemed beneficial and tolerable, as long as there is no evidence of disease progression, intolerable toxicity, withdrawal of consent, or discontinuation for any other reason. Patients will be continually reassessed for evidence of toxicity.

Each dose cohort was planned to enroll and treat 3 patients. A clinical synthesis of the available toxicity information, including dose limiting toxicity (DLTs) and adverse events (AEs) other than DLTs in and post Cycle 1, pharmacokinetic (PK) data, efficacy information and other relevant data, would be reviewed by the investigator to determine whether to escalate, stay or deescalate for the next cohort.

3 Subjects and treatments

This study enrolled patients with histologically/cytologically confirmed advanced NSCLC, who were refractory to currently available therapies or had tumors for which no effective treatment was available, and had PD-L1 positive in tumor molecular test.

The study included the following planned dose cohorts:

- Pre-A testing cohort: $2x10^7$/kg (one cycle of interruption between the first treatment cycle and the second treatment cycle)
- Cohort A: $1x10^7$/kg
- Cohort B: $2x10^7$/kg
- Cohort C: $4x10^7$/kg

Each dose was split into 3 infusions: 20%, 30% and 50% of the total dose on D1, D3 and D5 of each cycle, respectively.

Statistical analyses results will be presented by treatment groups specified according to dose level unless otherwise specified.

3.1 Analysis sets

The analysis sets to be used are defined as below.

Screening Set: The Screened Set consists of all patients who have signed informed consent/assent and screened in the study.

Full Analysis Set(FAS): The FAS consists of all patients to whom study treatment has been assigned, and have received at least one infusion of PD-1 KO T cells.

Safety Set (SS): The Safety Set consists of all patients who received at least one infusion of PD-1 KO T cells and had at least one valid post-baseline safety assessment. The statement that a patient has no adverse events (on the Adverse Events eCRF) constitutes a safety assessment.

Patients will be classified according to the study treatment they actually received, where treatment received is defined as (i) the treatment assigned (dose-level and schedule planned) if it was received at least once, or (ii) the total dose received when starting therapy if assigned dose level is never received completely. Each patient will be classified into and analyzed consistently within one (and only one) treatment group.

Pharmacokinetic Analysis Set (PAS): The PAS consists of all patients in FAS who have at least one sample providing evaluable PK data.

3.2 Treatment groups

Dose levels investigated during the study will be considered to identify treatment groups. For example, the following may be considered: $1x10^7$/kg, $2x10^7$/kg, and $4x10^7$/kg.

4 Statistical analysis

Data will be summarized using descriptive statistics (continuous data) and/or contingency tables (categorical data) for demographic and baseline characteristics, efficacy measurements, safety measurements, and all relevant pharmacokinetic measurements. Unless otherwise described, all data will be listed appropriately by using FAS.

4.1 Patient disposition

The FAS will be used for the following summaries.

The number and percentage of patients who were still on-treatment at the date of data cut-off and who discontinued the study will be summarized by treatment group. Among patients who discontinued the treatment, primary reason for the discontinuation will also be summarized.

Number and percentage of patients in each analysis set will be summarized by treatment group.

4.2 Demographic and baseline characteristics

The FAS will be used for all baseline and demographic summaries demographic characteristics include age and sex. Baseline characteristics include weight, height, body mass index (BMI) and ECOG performance score at baseline. Disease history and characteristics include primary site of cancer, time from most recent relapse/progression to first PD-1 KO T-cells administration in month, histological grade, predominant histology/cytology, stage at initial diagnosis, types of lesions at baseline (target only/non-target only/both target and non-target/unknown).

In addition, the following category for age will be used to calculate the number and percentage of patients included in each category:

- Age: < 65 and ≥ 65 years.

4.3 Medical History

The FAS will be used for the following summaries and listings.

Relevant medical histories and current medical condition will be listed and summarized by primary system organ class, preferred term and treatment group. Medical history/current medical conditions will be coded using Medical Dictionary for Regulatory Activities (MedDRA) terminology.

4.4 Antineoplastic therapy

The FAS will be used for the following summaries and listings.

Prior antineoplastic therapy will be listed in three separate listings: medications, radiotherapy, and surgery.

The summary of prior antineoplastic medication will include the total number of regimens used, and setting, best response, and reason for discontinuation at last medication. All prior antineoplastic medications will be summarized by ATC class, preferred term and treatment group.

The summary of prior antineoplastic radiotherapies will include locations and setting at last radiotherapy.

The summary of prior antineoplastic surgeries will include the procedure and residual disease at last surgery.

Antineoplastic therapies since discontinuation of study treatment will be tabulated by treatment group, and will be listed by patient.

4.5 Concomitant therapy

The safety set will be used for the followingsummaries and listings.

Concomitant medicationswill be listed and summarized by ATC class and preferred term. These summaries will include medications starting on or after the start of study treatment or medications starting prior to the start of study treatment and continuing after the start of study treatment. Any prior medications starting and ending prior to the start of study treatment will be listedand summarized by ATC class and preferred term.

4.6 Study medication

The study drug dosing information will be listed by treatment group for the safety set.

4.7 Safety evaluation

The primary objective is to characterize the safety and tolerability of PD-1 KO T cell infusion in patients with PD-L1 positive NSCLC. Therefore, the primary analysis will include the summary of observed DLTs by treatment group, and the summary of the frequency, severity of AEs, changes in laboratory findings, physical examinations, vital signs, ECGs. The safety set will be used for all safety evaluation.

The number and percentage of patients with DLTs will be tabulated by system organ class (SOC), preferred term (PT) and treatment group. All observed DLTs will be listed.

The assessment of safety is based mainly on the type and frequency of adverse events (AEs) and on the number of laboratory values that fall outside of pre-determined ranges (Common Toxicity Criteria [CTC] grading limits or normal ranges as appropriate). Other safety data (e.g., electrocardiogram, vital signs, and special tests) will be considered as appropriate.

The safety summary tables will include assessments collected during on-treatment period starting from the date of the first study treatment administration until 28 days after the end of the last treatment cycle. Those collected during the pre-treatment and post-treatment periods are to be flagged.

4.7.1 Adverse events

Definition of adverse event and reporting

An AE is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) that occur after patient's signed informed consent for the main study (or for molecular screening for the document unavailable patient in the expansion part) has been obtained. Abnormal laboratory values or test results occurring after informed consent constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, require therapy (e.g., hematologic abnormality that required transfusion or hematological stem cell support), or require changes in study medication(s).

Analyses of AEs

All AEs recorded during the study will be listed. Treatment-related AEs will be summarized. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology. Severity of AE will be coded based on the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE 4.03). If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, corresponding to Grades 1 to 4, will be used. CTCAE Grade 5 (death) will not be used in this study; rather, information about deaths will be collected in the Death eCRF page.

The following AEs will be summarized:

- DLTs by SOC, PT and treatment group
- AEs regardless of study drug relationship by SOC, PT and treatment group
- AEs with suspected relationship to study drug by SOC, PT and treatment group
- SAEs regardless of study drug relationship by SOC, PT and treatment group
- SAEs with suspected relationship to study drug by SOC, PT and treatment group
- Grade 3 or 4 AEs regardless of study drug relationship by SOC, PT and treatment group
- Grade 3 or 4 AEs with suspected relationship to study drug by SOC, PT and treatment group
- AEs leading to study drug discontinuation regardless of study drug relationship by SOC, PT and treatment group
- AEs regardless of study drug relationship by PT and treatment group
- Deaths by SOC, PT and treatment group

All AEs, SAEs, AEs leading to study drug discontinuation, and deaths will be listed.

4.7.2Laboratory test abnormalities

All laboratory values will be graded by using CTCAE 4.03. Parameters for which a grading does not exist will be classified into low/normal/high group by means of laboratory normal ranges.

Analyses of laboratory values

The following summarieswill be produced for laboratory parameters in hematology, biochemistry, and urinalysis:

- Shift table using CTCAE grade to compare baseline to the worst post-baseline value
- For laboratory tests where CTCAE grades are not defined, shift table using the low/normal/high/(lowand high) classification to compare baseline to the worst post-baseline value

The following listings will be produced for all laboratory parameters:

- Listing of all laboratory data with values flagged to show the corresponding CTCAE grades and the classifications relative to the laboratory normal ranges

4.7.3Vital signs and weight

All vital signs including systolic blood pressure, diastolic blood pressure and pulse and weight will be listed.

The number and percentage of patients with abnormal vital signs and weight change will be provided. Patients with abnormal vital signs will also be listed.

4.7.4Electrocardiogram (ECG)

ECG data will be listed. Patients with abnormal ECG values will be listed.

4.7.5 Other safety data

Data from other tests will be listed, notable values will be flagged, and any other information collected will be listed as appropriate.

4.8 Efficacy evaluation

Tumor response will be defined as per RECIST v1.1, and refer to the irRC.

The FAS will be used for the following analyses.

4.8.1 Best overall response (BOR)

BOR will be summarized by treatment group. The waterfall plot will be used to depict anti-tumor activity. Details of waterfall plot will describe in section 5.3.8.

4.8.2 Overall response rate (ORR) and disease control rate (DCR)

Definitions of ORR and DCR are described in sections 5.3.1 and 5.3.2.

ORR and DCR will be presented as point estimate (at each treatment group) and corresponding 95% exact confidence interval according to Clopper-Pearson method.

4.8.3 Time to response (TTR) and duration of response (DOR)

Definitions of TTR and DCR are described in sections 5.3.3 and 5.3.4. Due to the small sample size, for patients who achieved best overall response of CR or PR, the TTR and DOR will be listed.

4.8.4 Progression free survival (PFS) and overall survival (OS)

Definitions of PFS and OS are described in sections 5.3.5 and 5.3.6. Due to the small sample size. The Kaplan-Meier method will be used to estimate overall median PFS and median OS. PFS and OS will be presented by patient in listings.

4.9 Pharmacokinetic (PK) evaluations

Patients in the PAS with at least one evaluable measurement of PD-1KO T cell concentration will be included in the PK analysis.

The concentration of PD-1KO T cells in blood will be listed and summarized by treatment group and time point. Descriptive statistics include arithmetic mean and geometric mean, median, standard deviation, arithmetic coefficient of variation (CV), geometric coefficient of variation, minimum and maximum. In geometric mean calculation, concentration 0 is not included.

Patient level and averaged concentration of PD-1KO T cell measures will be displayed using longitudinal plots. Graphical presentation of arithmetic mean (±SD) concentrations at each scheduled time point will be provided by treatment group.

4.10 Immunological factors and biomarkers

Baseline level of PD-L1 will be listed, and may also graphed against clinical response status using strip plots.

Soluble immune and inflammatory cytokines (e.g. IL-10, interferon gamma, IL-6, IL-6 receptor, CRP, and ferritin) will be listed by patient and summarized by treatment group and time point. Baseline and absolute and relative change (percent and or fold change) from baseline will be calculated for each treatment group and time point and summarized using sample size, mean, standard deviation, median, minimum and maximum. If both the baseline and post baseline values are below LLOQ, absolute, percent and fold change from baseline will not be imputed and reported as missing. The maximum change from baseline measure for each cytokine may also graphed against clinical response status and AEs of special interest using strip plots. Patient level and averaged cytokine measures and percent change from baseline may be displayed using longitudinal plots.

TCR diversity and bTMB were compared between patients with and without disease control benefit using the exact Wilcoxon test. Statistical significance was set at $p \leq 0.05$ (2-sided).

4.11 Interim analyses

Not applicable.

4.12 Determination of sample size

No formal statistical power calculations to determine sample size were performed for this study. Cohorts of at least 3 evaluable patients per dose level will be enrolled.

5 General definitions

5.1 Assessment windows, baseline and post baseline definitions, missing data handling

5.1.1 Date of first administration of study drug/treatment

The date of first administration of study drug/treatment will be derived as the first date when a non-zero dose of study drug is administered and recorded on the dose administration record eCRF.

The date of first administration of study treatment is also referred to as start of study treatment.

5.1.2 Date of last administration of study drug/treatment

The date of last administration of study drug/treatment is defined as the last date and time when a non-zero dose of study drug is administered and recorded on the dose administration record eCRF.

5.1.3 Study day, baseline, study visit and window

Study day

The study day for all assessments (both efficacy and safety) is calculated as the difference between the date of the event (visit date, onset date of an event, assessment date, etc.) and the start of study treatment. The first day of study treatment is study day 1.Note that for this study there is no study day 0, so the day immediately prior to study day 1 is study day -1.

For any events on or after the first dose of study drug, study day is calculated as: event date – date of first administration of study treatment + 1. As such, the first dose date was study day 1.

For any events before the first dose date, study day is calculated as: event date – date of first administration of study treatment. As such, one day before first dose date was study day -1.

Baseline

For comparison against baseline (e.g. for laboratory parameters), baseline is considered to be the last available assessment or value before or at the date of first study drug administration. Allowance for the baseline visit is within 28 days prior to first dosing.

Patients with no data on a particular parameter before first treatment will be deemed to have a missing baseline for that parameter.

5.2 Details of demographic and baseline characteristics analyses

BMI will be calculated as follows:

- BMI (kg/m^2) = Weight (kg) / [Height (m)]^2.

Time from [Date A] to [Date B] in month will be calculated as follows:

- ([Date B] – [Date A] + 1) / 30.4375.

Therefore, times such as time since initial diagnosis of primary cancer to first study drug administration in month will be calculated based on the method above. Demographic characteristics will be summarized by dose level.

5.3 Details of efficacy analyses

5.3.1 Overall response rate (ORR)

ORR is defined as the proportion of subjects with a best overall response of CR or PR.

5.3.2 Disease control rate (DCR)

DCR is defined as the proportion of subjects with a best overall response of CR, PR or SD.

5.3.3 Time to response (TTR)

TTR is defined as the duration from the date of first PD-1 KO T cell infusion to the date when the response criteria of CR or PR is first met.

5.3.4Duration of response (DOR)

DOR is defined as the duration from the date when the response criteria of CR or PR is first met to the date of relapse or death due to underlying cancer.

5.3.5Progression free survival (PFS)

PFS is the duration from date of first PD-1 KO T cell infusion to the date of relapse or death due to any reason.

5.3.6Overall survival (OS)

OS is the duration from date of first PD-1 KO T cell infusion to the date of death due to any reason.

5.3.7Date of last adequate tumor assessment

The last adequate tumor assessment is the last assessment with a result other than unknown. The last assessment is the assessment made before an event or a censoring occurred.

Note that tumor assessments made after commencement of a new antineoplastic therapy will not be used in the assessment of time related efficacy endpoints.

5.3.8Construction of waterfall plot

The waterfall plot will be used to depict anti-tumor activity. It displays the best percentage change from baseline in the sum of the longest diameter of all target lesions for each patient. To distinguish treatment group, different bar pattern will be used by treatment group.It will be based on the investigator reported overall lesion/radiological response.

Patient with missing/unknown best percentage change from baseline will be represented by a special symbol (e.g.,$^{\$}$) in the waterfall plot. Patient with unknown/missing best percentage change from baseline and with unknown overall response will be excluded.

The following display will be used (from left to right):

1. Bars under the horizontal axis representing tumor shrinkage
2. Bars above the horizontal axis representing tumor growth

3. “Zero” bars with stars ($) symbol for patient with missing best percentage change

5.3.9Clinical progression

Standard RECIST CRF pages do not allow for the capture of clinical progression. Clinical progression can only be reported in the treatment discontinuation form by using the “Disease progression” reason for drug discontinuation.

By default, the clinical progression will not be considered in the efficacy analyses based on RECIST.

5.4 Imputation rule of partial or missing dates

As a general rule, when a date will be recorded as a partial date, the missing day will be imputed to the 15th of the month (e.g., DEC2007 imputed to 15DEC2007), and if the day and month will be both missing then to July 1st of that year (e.g., 2007 imputed to 01JUL2007).

5.5 General presentation of descriptive summaries

Categorical data will be summarized by frequency count and percentages. Percentages will be calculated using the number of subjects in the relevant population as the denominator.

Continuous data will be summarized by appropriate descriptive statistics (i.e. mean, standard deviation, median, minimum and maximum).

All data will be listed appropriately.

The imputed dates are used only to allow the analysis of partial data, and are not displayed in data listings.