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Supplemental information

Phase 1 clinical trial to assess safety and efficacy of NY-ESO-1-specific TCR T cells in HLA-A*02:01 patients with advanced soft tissue sarcoma

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Supplemental information

Table S1. Comparison of efficacy and adverse events of different NY-ESO-1-specific TCR-T cell clinical trials. Related to Figures 1 and Table 2.

TCR-T sponsor (NCT number)	Lymphodepletion regimen	IL-2 usage	ORR	PFS (m)	OS (m)	Adverse events	Reference
GlaxoSmithKline (NCT01343043)	Flu 30mg/m^2/d × 4d Cy 1800 mg/m^2/d × 2d	unused	50% (6/12)	3.59	24.3	5/12 CRS Grades 1: n = 2 Grades 2: n = 1 Grades 3: n = 2	D'Angelo et al.[1], 2018; Gyurdieva et al.[2], 2022; Ramachandran et al.[3], 2019
	Flu 30mg/m^2/d × 4d Cy 1800 mg/m^2/d × 2d		31% (4/13)	3.05	9.9		
	Cy 1800 mg/m^2/d × 2d		20% (1/5)	2	19.9		
	Flu 25 mg/m^2/d × 5d Cy 600 mg/m^2/d × 3d		27% (4/15)	5.2	26.2		
National Cancer Institute (NCT00670748)	Flu 30 mg/m^2/d × 4d Cy 60mg/kg/d × 2d	720,000 IU/kg, q8h maximum 5 days	61% (11/18)	~ 5	> 24	No cell-related toxicities were occurred, but one patient experienced a treatment-related death.	Robbins et al.[4], 2015; Robbins et al.[5], 2011
Mie University (NCT02366546)	Cy 750 mg/m^2/d × 2d	unused	3/7 > 30% tumor regression	NA	NA	3/9 CRS (Grades 2) 1/9 lung injury (Grades 3)	Ishihara et al.[6], 2022
	Flu 20 mg/m^2/d × 5d Cy 750 mg/m^2/d × 2d						

Abbreviations: TCR-T, T-cell receptor T cell. NCT, National Clinical Trial. Flu, fludarabine. Cy, cyclophosphamide. ORR, objective response rate. PFS, progression-free survival. OS, overall survival. m, months. NA, not applicable. CRS, cytokine release syndrome.

Table S2. Characteristics of administered TCR-T cells. Related to Table 1.

Patient No.	Cell viability (%)	Cell yield ($\times 10^9$)	Vβ8 Positive (% of CD3)	CD8+ (% of CD3)	CD4+ (% of CD3)	Tetramer+ CD8+ (% of CD3)	Tetramer+ CD4+ (% of CD3)	Tumor-reactive IFN-γ ELISPOTS 2,000 PBMC
T01	91.61	29.00	83.46	29.97	67.46	26.08	60.91	122
T02	94.03	49.75	88.80	70.68	27.50	62.25	22.61	209
T03	87.11	22.75	84.68	36.21	53.91	34.39	43.51	206
T04	91.49	51.62	91.68	52.13	45.95	48.14	41.07	173
T05	90.94	30.60	93.96	83.98	13.23	74.45	11.16	175
T06	78.41	25.95	94.08	91.56	4.42	88.94	4.23	165
T07	96.57	15.05	80.10	61.46	25.87	47.59	20.71	239
T08	85.45	8.14	84.92	72.14	21.40	60.34	17.41	189
T09	83.48	30.85	87.84	78.81	12.17	71.24	10.20	226
T10	89.77	28.05	90.32	80.98	14.99	78.37	14.19	310
T11	89.11	23.60	89.18	46.80	39.95	45.24	37.01	235
T12	92.54	28.50	70.16	65.33	26.50	40.06	17.03	149

Footnotes: Tumor-reactive ELISPOT responses were evaluated by stimulating with the HLA-A 02:01+ and NY-ESO-1+ multiple myeloma cell line IM9.

Table S3. Clinical response. Related to Figures 1.

Parameter	Level 1 (n = 3)	Level 2 (n = 3)	Level 3 (n = 3)	Level 4 (n = 3)	Total (n = 12)
CR	0	0	0	0	0
PR	2	1	0	2	5
SD	1	2	1	1	5
PD	0	0	2	0	2
ORR (%)	66.7	33.3	0	66.7	41.7
DCR (%)	100	100	33.3	100	83.3

Abbreviations: CR, complete response. PR, partial response. SD, stable disease. PD, progressive disease. ORR, objective response rate. DCR, disease control rate.

Supplementary figures

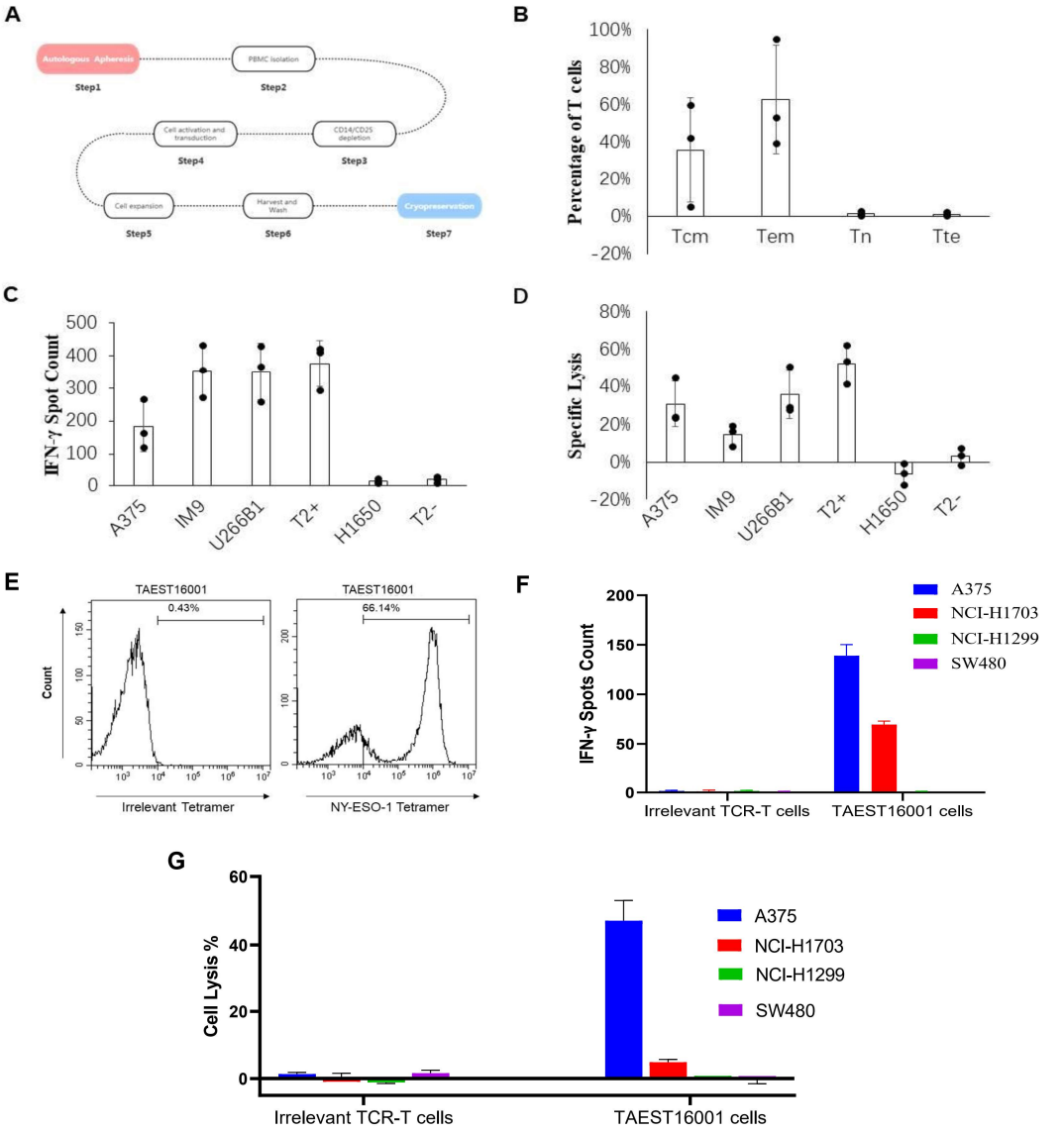

Figure S1. Phenotypic and functional characterization of GMP-Grade Manufacturing of TAEST6001 cells. Related to Table 1 and STAR Methods. (A) Manufacturing schema of TAEST16001 cells. Clinical sites collect and ship patient apheresis products to the manufacturing site within 24 hours. Cells are depleted of CD14 and CD25 positive cells, and activated with αCD3/αCD28 beads and transduced with a lentiviral vector encoding the TAEST16001 TCR gene. Cells are expanded for 9-14 days and then harvested, washed, and frozen for release testing. (B) T cell subset composition. The expression of surface antigens was determined by flow cytometry. Tn (Naïve, $CD45RO^{-}CCR7^{+}$), Tcm (central memory, $CD45RO^{+}CCR7^{+}$), Tem (effector memory, $CD45RO^{+}CCR7^{-}$), Tte (terminally differentiated effector, $CD45RO^{-}CCR7^{-}$). IFN-γ release (C) and cytotoxicity (D) measurements of TAEST16001 cells after co-culturing with tumor cell lines. A375, IM9 and U266B1 are both HLA-A*0201 and NY-ESO-1 positive. H1650 is HLA-A*0201 positive but NY-ESO-1 negative. T2+ (T2 cells loaded with the NY-ESO-$1_{157-165}$ peptide), T2- (T2 cells loaded with an irrelevant peptide). IFN-γ release was determined using the IFN-γ ELISpot assay and cytotoxicity was assessed using the lactate dehydrogenase (LDH) release assay with an effector: target ratio of 5:1. (E) The expression of

TAEST16001 cells was evaluated by flow cytometry using NY-ESO-1 tetramer or irrelevant tetramer staining. (F) IFN-γ release of TAEST16001 cells after co-culturing with tumor cell lines. T cells expressing an irrelevant TCR served as a negative control. (G) TAEST16001 TCR, or irrelevant TCR (as a negative control) transduced T cells were co-cultured with tumor cell lines for 24 h and the specific killing of tumor cells was assessed using the LDH release assay. A375 (HLA-A2^{+}, NY-ESO-1^{+}), NCI-H1703 (HLA-A2^{+}, NY-ESO-1^{+}), NCI-H1299 (HLA-A2^{-}, NY-ESO-1^{+}), SW480 (HLA-A2^{+}, NY-ESO-1^{-}).

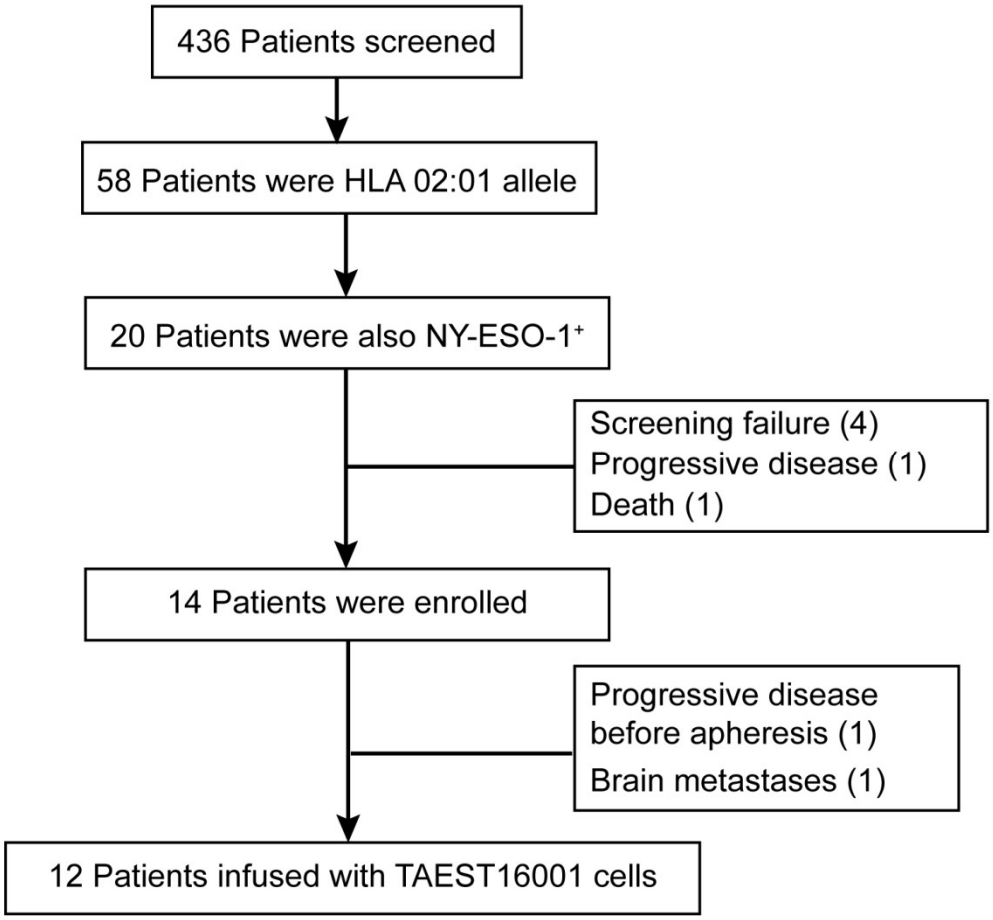

Figure S2. Trial profile. Related to Figure 1.

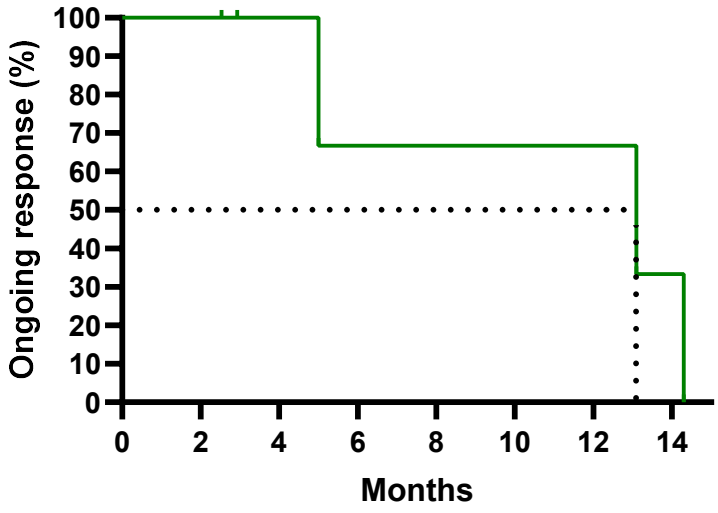

Figure S3. Kaplan–Meier estimates of the duration of response. Related to Figure 1. Tick marks indicate the time of data censoring at their last date of contact.

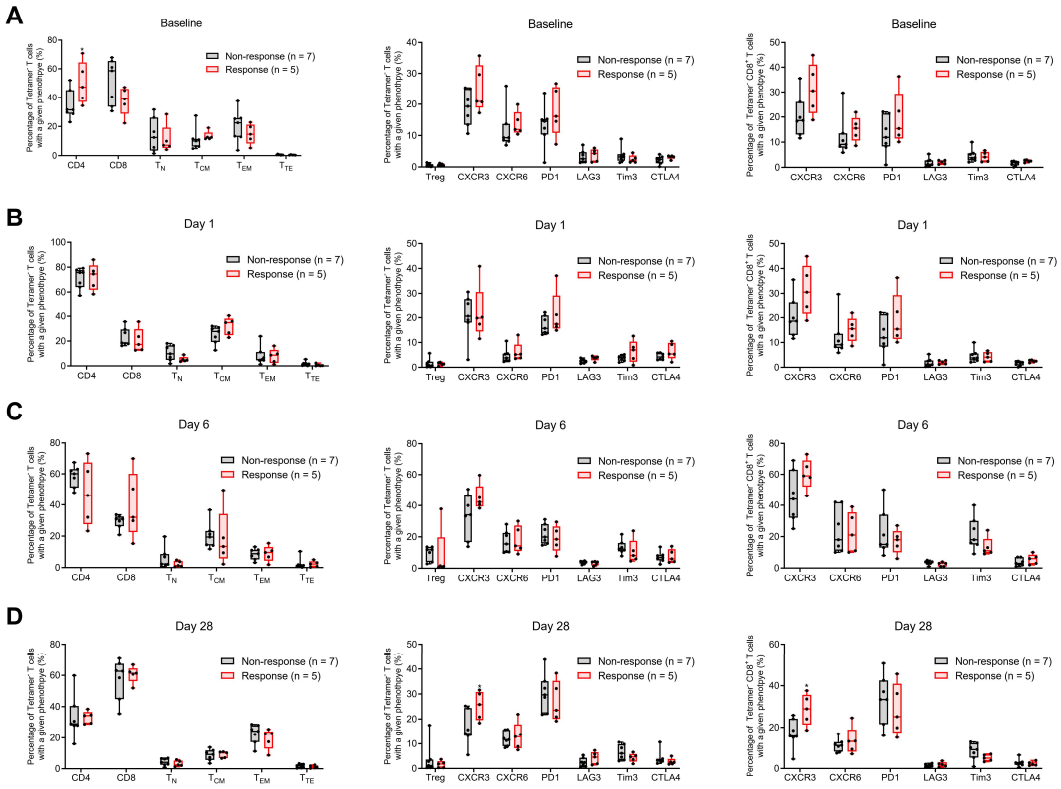

Figure S4. Associations between tumor response and T cell subsets in peripheral blood detected at the predefined time. Related to Figure 5. T cell subsets, such as $CD3^+CD4^+$, $CD3^+CD8^+$, naïve T cells(T_N), central memory T cells (T_{CM}), effector memory T cells (T_{EM}), terminally differentiated effector T cells (T_{TE}), $CD3^+CD25^+Foxp3^+$ (regulatory T cells, Treg), $CD3^+CXCR3^+$, $CD3^+CXCR6^+$, $CD3^+PD\text{-}1^+$, $CD3^+LAG\text{-}3^+$, $CD3^+TIM\text{-}3^+$, $CD3^+CTLA4^+$, $CD3^+CD8^+CXCR3^+$, $CD3^+CD8^+CXCR6^+$, $CD3^+CD8^+PD\text{-}1^+$, $CD3^+CD8^+LAG\text{-}3^+$, $CD3^+CD8^+TIM\text{-}3^+$, and $CD3^+CD8^+CTLA4^+$, was detected at baseline (A) and day 1 (B), day 6 (C), and day 28 (D) after cell infusion. The horizontal lines within each box represents the median, the lower and upper borders of each box represent the inter-quartile range, and the bars show the range. We calculated the *P* values using the two-sided Wilcoxon rank sum test. $*P < 0.05$.

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Methods S1: Clinical trial protocol of the study, related to the STAR Methods.

TAEST16001 Early phase clinical trial
Xiangxue Life Science Technology (Guangdong) Co., Ltd

TAEST16001 in patients with soft tissue sarcoma-predominant advanced malignant solid tumors expressing positive NY-ESO-1 (genotype: HLA-A*02:01): an open-label, single-arm, and early phase clinical study

Organization:	**Sun Yat-sen University Cancer Center**
Chief physician:	**Xing Zhang**
Sponsor：	**Xiangxue Life Science Technology (Guangdong) Co., Ltd**
Contract research Organization	**Kun Tuo Xincheng Pharmaceutical Research and Development (Beijing) Co., Ltd.**
Biometric company	**Kun Tuo Xincheng Pharmaceutical Research and Development (Beijing) Co., Ltd.**
Version number	**2.0**
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Abbreviation list

Abbreviations	Full name
ADL	Activities of Daily Living
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALB	Albumin
ALC	Absolute Lymphocyte Count
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Transaminase
$AUC_{0\sim inf}$	Curve from Zero up to infinity with Xtrapolation of the Terminal Phase
$AUC_{0\sim 28}$	Area under the Concentration-time Curve from Zero up to a Definite Time Day 28
AUCss	Area under the concentration-time curve during a dosing interval at steady state
BOR	Best Overall Response
BUN	Blood Urea Nitrogen
Cmax	Maximum Concentration
CR	Complete Response
CRE/Cr	Creatinine
CRO	Contract Research Organization
CRS	Cytokine Release Syndrome
CSR	Clinical Study Report
CT	Cancer-Testis Antigen
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Cell
DBIL	Direct Bilirubin
DLT	Dose Limiting Toxicity
DCR	Disease Control Rate
DOR	Duration Of Response
EC	Ethics Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group

eCRF	electronic Case Report Form
EOS	End of Study
Fib	Fibrinogen
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GLOB	Globulin
GLU	Glucose
HBsAg	Hepatitis B surface antigen
HBcAb	Hepatitis B core antibody
HBV	Hepatitis B Virus
HGB	Hemoglobin
HIV	Human Immunodeficiency Virus
HIVAb	Human Immunodeficiency Virus antibody
HLA	Human Leukocyte Antigens
ICF	Informed Consent Form
IL-2	Interleukin-2
INR	International Normalized Ratio
IRC	Independent Review Committee
LYMPH	Lymphocyte Count
MONO#	Monocyte Count
MRI	Magnetic Resonance Imaging
MTD	Maximal Tolerance Dose
Mtor	Mammalian Target of Rapamycin
NCI	National Cancer Institute
NEUT#	Neutrophile Granulocyte Count
NMPA	National Medical Products Administration
ORR	Objective Response Rate
PD	Progressive Disease
PFS	Progression-free Survival
PK	Pharmacokinetic
PLT	Platelet
PBMC	Peripheral Blood Momonuclear Cell
PR	Partial Response
PT	Prothrombin Time
qPCR	quantitative PCR
QTc	QT correct
RBC	Red Blood Cell
RCL	Replication Competent Lentiviruses

RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Statistical Analysis Report
SBP	Systolic Blood Pressure
SD	Stable Disease
SDV	Source Data Verification
SOC	System Organ Classes
SOP	Standard Operation Procedures
SRC	Safety Review Committe
TBIL	Total Bilirubin
TCR-T	T Cell Receptor-T
TP	Total Protein
UA	Uric Acid
ULN	Upper Limit of Normal
URE	Urea
WBC	White Blood Cell

Program abstract

Project	XLS16001
Title	TAEST16001 in patients with soft tissue sarcoma-predominant advanced malignant solid tumors expressing positive NY-ESO-1 (genotype: HLA-A*02:01): an open-label, single center, early phase clinical study
Phase	Phase I
Sponsor	Xiangxue Life Science Technology (Guangdong) Co., Ltd
Drug	TAEST16001 injection (hereinafter referred to as TAEST16001 cells)
Organization	1~2 centers, and the leader is Sun Yat-sen University Cancer Center
Planned number of	12-30 cases
Study duration	Expected to last for 18-27 months
Study population	The dose escalation study is planned to include patients with advanced malignant solid tumors with pathological diagnosis and positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01), including soft tissue sarcoma, primary liver malignancy, ovarian malignancy, non-small cell lung cancer (NSCLC), breast cancer, etc., mainly in patients with soft tissue sarcoma. Expanded enrollment study is planned to include patients with advanced soft tissue sarcomas with definite pathological diagnosis and positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01).
Objectives	**Primary objective：** ● To evaluate the safety and tolerability of TAEST16001 cells in the treatment of advanced malignant solid tumors such as soft tissue sarcomas with positive expression of tumor antigen NY-ESO-1 (HLA-A*02:01). **Secondary objective:** ● To describe the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of TAEST16001 cells after infusion into humans, observe their proliferation and persistence in vivo, and their effects on human immunological activity; ● To preliminary evaluation of the efficacy of TAEST16001 cells in the treatment of patients with advanced malignant solid tumors such as soft tissue sarcoma expressing positive tumor antigen NY-ESO-1 (genotype: HLA-A*02:01) according to RECIST1.1 criteria.

	Exploratory objective: ● To preliminary evaluate the efficacy of TAEST16001 cells in the treatment of patients with advanced malignant solid tumors such as soft tissue sarcoma with positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01) according to the iRECIST criteria. ● To evaluate the effect of TAEST16001 cell therapy on the quality of life of patients.
Study endpoint	**Primary endpoint:** ● Maximum tolerable dose (MTD) and dose limiting toxicity (DLT); ● Incidence of adverse events (AE), serious adverse events (SAE), adverse event of special interest (AESI) (including CRS, neurotoxicity), laboratory tests (type, frequency and severity), and abnormal ECG and vital signs.
	Secondary endpoint: ● After infusion of TAEST16001 cells, the peak value (Cmax), peak time (Tmax) and $AUC_{0\text{-}28}$ of TAEST16001 cells in peripheral blood. If possible, $AUC_{0\text{-}inf}$, terminal phase elimination rate constant (λ_z), and elimination half-life ($t_{1/2}$) will be evaluated. ● Immunological activity: T cell subsets, peripheral blood antigen-specific CTL, effector cell activity. ● Objective response rate (ORR), disease control rate (DCR) and progression free survival (PFS) assessed according to RECIST1.1
	Exploratory endpoint: ● Objective response rate (ORR), disease control rate (DCR) and progression free survival (PFS) evaluated according to iRECIST criteria; ● EORTC QLQ-C30 scale score.

Study design	**1. Overall design** This study is an open-label, single arm, dose increasing early clinical study, which is divided into two parts: "3+3" designed dose escalation study and expanded enrollment study. This study is to evaluate the safety, tolerance, PK and PD characteristics, and preliminary effectiveness of TAEST16001 cells in treating patients with advanced malignant solid tumors, mainly soft tissue sarcomas, with positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01). This study is planned to recruit 12-30 patients with advanced malignant solid tumors with positive expression of tumor antigen NY-ESO-1 (genotype: HLA-A*02:01). The dose escalation study is planned to include patients with advanced malignant solid tumors with positive expression of tumor antigen NY-ESO-1 (genotype: HLA-A*02:01), including soft tissue sarcoma, primary liver malignancy, ovarian malignancy, non-small cell lung cancer (NSCLC), breast cancer, etc., mainly patients with soft tissue sarcoma. The expanded enrollment study is planned to include patients with advanced soft tissue sarcoma with positive expression of tumor antigen NY-ESO-1 (HLA-A*02:01). Patients eligible for screening (including genotype, tumor antigen screening, and primary screening) will go through 3 study phases: **Screening period** (pre-screening to study day -1): patients who are qualified for genotype and tumor antigen screening (pre-screening) were subjected to leukocyte apheresis for the preparation of TAEST16001 cells, while patients enter the main screening period and lymphodepleting chemotherapy. Patients in the dose escalation and expansion study received lymphodepleting chemotherapy 7 days before the first time of TAEST16001 cell infusion (study day -7), specifically: cyclophosphamide (15mg/kg/d) and fludarabine (20 mg/m2/d), for 3 days. **Patients who received lymphodepleting chemotherapy were defined as enrolled patients.** **Treatment and observation period** (study day 1-28): intravenous infusion of TAEST16001 cells at a single or divided times of the total dose, DLT observation (the dose escalation study phase) or treatment observation (the expansion study phase), and the first efficacy evaluation; Patients in the dose escalation and expansion study received intravenous infusion of TAEST16001 cells on day 5 after lymphodepleting chemotherapy (i.e. 4 days between the end of lymphodepleting chemotherapy and the start of cell infsuion): if the cell infusion dose level was 1 and 2, the total amount of TAEST16001 cells (calculated as TCR-T positive cells) was infused on the first day of the study; If the cell infusion dose level was 3 and 4, the total amount of TAEST16001 cells (calculated as

	TCR-T positive cells) was planned to be infused at the ratio of 60% and 40% on the first and second days of the study. After the first TAEST16001 cell infusion, patients will be subcutaneously injected with low-dose IL-2 (from the 1st day to the 14th day of the study), 500,000u/time. The first IL-2 injection will be carried out within 30 minutes after cell infusion, twice daily (with an interval of 10-12h) for 14 days. The safety and tolerance were observed before, during and after intravenous infusion (study day 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28) until 28 days after the first cell infusion. Among them, the DLT observation period is from the first cell infusion to 28 days after cell infusion for the patients enrolled in the dose escalation phase. The first efficacy evaluation will be conducted on day 28 after the first cell infusion (study day 28). **Follow up period** (study day 29-270 [9 months]): safety and efficacy observation. Safety: All AEs and SAEs will continue to be collected, unless the patient starts subsequent anti-tumor therapy after 90 days (3 months) of TAEST16001 cell infusion until 270 days (9 months) of cell infusion due to ineffectiveness or disease progression. From initiation new antitumor to the end of the study, only those AEs related to treatment and SAEs will be collected. Efficacy: After entering the follow-up period, patients will be underwent tumor evaluation at 2 months, 3 months, 6 months and 9 months after the firstTAEST16001 cell infusion, unless the patient started subsequent antitumor therapy due to treatment failure/disease progression, withdrawal from the study, death or loss to follow-up, whichever occurred first. **The end of study (EOS)** is defined as completion of protocol-specified follow-up (within 270 days [9 months] after TAEST16001 cell infusion) for all subjects, early withdrawal from the study for any reason other than DLT, death or loss to follow-up, whichever occurs first. Thereafter, patients will enter a long-term safety follow-up of up to 15 years for lentiviral replication and secondary tumor. The frequency is once a year from study day 270 to 5 years after cell infusion, and once every 3 years from 5 years to 15 years after cell infusion. Details can be found at the overall study design diagram. **2. Dose escalation study** The dose increase is carried out according to the "3+3" increasing principle. A total of 4 dose levels are set (calculated as TCR-T positive cells): dose level 1: $5\times10^8 \pm 30\%$; dose level 2: $2\times10^9 \pm 30\%$; dose level 3: $5\times10^9 \pm 30\%$;

	dose level 4: 1.2×10^{10} ± 30%。 Three patients will be enrolled first. If no DLT occurs, the next higher dose group will be enrolled; if 1 out of 3 patients in a dose group has DLT, 3 additional patients in this group will be administered with the same dose. Dose escalation was discontinued if greater than or equal to 1 of the 3 supplemented patients had DLT. The previous dose of this dose is defined as the MTD; if no DLT occurred in the additional 3 patients, the dose is escalated to the next cohort. Dose escalation for the same patient is not allowed. In the dose level 1 and dose 2 groups, each patient needs to be observed for at least 14 days after TAEST16001 cell infusion. If there is no DLT, the next patient in the same dose group is allowed to undergo cell infusion. In the dose level 3 and 4 groups, each patient needs to be observed for 14-28 days after receiving TAEST16001 cell infusion (the specific interval can be evaluated and adjusted by the researcher according to the cumulative safety results of patients in the dose level 1 and 2 groups). If there is no DLT, the next patient in the same dose group is allowed to undergo cell infusion. After receiving the first infusion of TAEST16001 cells, the last patient in the previous dose group was observed for at least 28 days, If there is no DLT, the first patient in the next dose group was allowed to undergo cell infusion after discussion and decision by the safety review committee (SRC). **MTD was defined as** the previous lower dose of the dose group in which ≥2/6 patients experienced DLT. If no MTD was detected at dose level 4 (1.2×10^{10} ± 30%) in this study, no further dose escalation will be performed. In the course of dose increase, during the 28-day DLT observation period, if the patient withdraws from the DLT observation period due to reasons other than DLT and fails to complete the DLT observation period, the patient will be replaced and one additional case needs to be included. **3. Expanded enrollment study** Under the premise of dose escalation study, with the consent of the investigator and the sponsor, and based on the cumulative safety, PK/PD, preliminary efficacy and other relevant data, after communication and approval with the regulatory authority (NMPA), about 6-18 patients with advanced soft tissue sarcoma with positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01) were enrolled at MTD dose level or dose level 4 (if MTD was not reached), to further evaluate the safety, tolerability, PK/PD characteristics and efficacy of TAEST16001 cell infusion. **4. Pharmacokinetic (PK) and Pharmacodynamic (PD) studies** Pharmacokinetic (PK) and pharmacodynamic (PD) studies

	will be conducted simultaneously during the dose escalation and expanded enrollment study. Blood samples of the enrolled patients will be collected at the designated time point (see the research flow chart for details) for PK and PD evaluation after single or fractional TAEST16001 cell infusion in total doses. PK/PD assessment of TAEST16001 cells after infusion into humans will be performed from the starting of the first cell infusion to 270 days (9 months) after the first cell infusion, or to the time that no cells detected by flow cytometry or DNA copy number is lower than the detection limit of qPCR for two consecutive times, whichever occurs first. The number of patients with complete PK and PD parameters collected in each dose group should be greater than or equal to 3. If the number of subjects whose complete PK parameters are collected is less than the number required by the protocol, it will be decided whether additional patients need to be enrolled after discussion between the sponsor and the investigator.
Inclusion criteria:	Patients must meet the inclusion criteria with * before genotype and tumor antigen detection, and all inclusion criteria must be met for enrollment: Patients must meet all of the following criteria to be enrolled in this study: 1. An informed consent form (ICF) should be signed before any research-related operations (genotype and tumor antigen screening and primary screening); 2. *Age ≥18 years old and ≤70 years old; 3. * Advanced malignant solid tumors with definite pathological diagnosis; *Note: The dose escalation research part intends to include patients with solid tumors such as soft tissue sarcoma, primary liver malignancy, ovarian malignancy, non-small cell lung cancer (NSCLC), breast cancer, etc., mainly patients with soft tissue sarcoma. Patients with soft tissue sarcoma will be included in the expanded study.* 4. Unresectable advanced solid tumors that have failed to standard treatment (disease progression or recurrence or intolerance, such as chemotherapy, radiotherapy, targeted therapy, etc.) or lack effective treatment, including but not limited to the following tumor types: **1) Soft tissue sarcoma:** a) Soft tissue sarcomas failed to be treated with chemotherapy containing doxorubicin and ifosfamide; **2) Primary liver malignancies:**

	a) Child-Pugh liver function score grade A within 7 days before cell infusion; **3) Ovarian malignant tumor:** a) Failed to platinum based chemotherapy (such as paclitaxel combined with carboplatin). **4) Non-small cell lung cancer (NSCLC):** a) Failed (disease progression or toxicity intolerance) to previous standard therapy (platinum-containing chemotherapy regimen or driver gene-targeted therapy) or lack of effective treatment; **5) Breast cancer:** a) Patients who failed to standard treatment or did not suitable for standard treatment. 5. At least 1 measurable lesion (according to RECIST1.1 criteria [see Appendix 4 for details]); 6. Genotype and tumor antigen screening must meet the following two criteria: 1) HLA-A*02:01 positive; 2) Positive expression of NY-ESO-1: immunohistochemical staining positive cells ≥ 20%; 7. *ECOG score 0-1 and estimated survival time greater than 3 months; 8. Color-Doppler-Echocardiography indicates that the left ventricular ejection fraction is greater than or equal to 50%; 9. Laboratory inspection results should at least meet the following specified indicators: - White blood cell count ≥3.0×10^9/L; - Absolute neutrophil count (ANC)≥1.5×10^9/L (without G-CSF and GM-CSF support, at least 14 days before lymphodepleting chemotherapy); - Absolute lymphocyte count (ALC) ≥ 0.7×10^9/L; - Platelet (PLT) ≥ 75×10^9/L (no blood transfusion therapy 14 days before lymphodepleting chemotherapy); - Hemoglobin ≥ 9g/dL (no blood transfusion therapy 14 days before lymphodepleting chemotherapy); - International normalized ratio (INR) ≤1.5×ULN, unless receiving anticoagulant therapy; - Activated partial thromboplastin time (APTT)≤1.5×ULN, unless receiving anticoagulant therapy; - Serum creatinine≤1.5mg/dL (or 132.6μmol/L) - Creatinine clearance rate ≥60mL/min; - Aspartate aminotransferase (AST/SGOT)≤2.5×ULN; - Alanine aminotransferase (ALT/SGPT)≤2.5×ULN; - Total bilirubin (TBIL)≤1.5×ULN;

	Note: For patients with liver metastases or patients with primary liver tumor lesions, aspartate aminotransferase and alanine aminotransferase should be ≤5×ULN. 10. *Women of childbearing age who have not undergone sterilization before menopause must agree to take effective contraceptive measures from the beginning of study treatment (lymphodepleting chemotherapy) to one year after the last cell transfusion, and the serum pregnancy test is negative within 14 days before the first time of cell infusion. 11. *Men who have not undergone sterilization surgery must agree to use effective contraceptive measures from the beginning of the study treatment (lymphodepleting chemotherapy) until one year after the last cell infusion.
Exclusion criteria:	The exclusion criteria with * need to be verified before the patients are tested for genotype and tumor antigen, and all exclusion criteria need to be verified for enrollment. Patients who met any of the following criteria were not eligible for this study: 1. Received the last dose of anti-tumor therapy (chemotherapy, endocrine therapy, targeted therapy, immunotherapy, tumor embolization or Chinese medicine/Chinese herbal medicine treatment with anti-tumor indications, etc.) within 4 weeks before cell infusion; 2. Received live attenuated vaccine within 4 weeks before cell infusion; 3. *Any ingredient used in the treatment of this study is known to cause allergic reactions; 4. No recovered to < grade 2 (CTCAEv5.0) from previous surgery or treatment-related adverse reactions; 5. *Patients with a history of meningeal metastases or central nervous system metastases in the past, or patients with clear underlying diseases of the central nervous system and left significant symptoms within 6 months before cell infusion; 6. Uncontrolled Hypertension with medication (systolic blood pressure>160mmHg and/or diastolic blood pressure>90mmHg) or cardiovascular and cerebrovascular diseases with clinical significance (such as active disease), such as cerebrovascular accident (within 6 months before signing the master informed consent),

	myocardial infarction (within 6 months before signing the master informed consent), unstable angina, congestive heart failure with New York Heart Association (NYHA) class II or above, or severe arrhythmia that can not be controlled with drugs or have potential impact on study treatment; ECG results show clinically significant abnormality or average QTcF ≥ 450ms in 3 consecutive times (at least 5 minutes between each time) (see Appendix 2 for the formula); 7. * Complicated with other serious organic diseases or mental diseases; 8. Patients with systemic active infections requiring treatment, including but not limited to active tuberculosis, known HIV positive patients or patients with clinically active hepatitis A, B and C, including virus carriers, shall be excluded; 9. * Suffering from autoimmune diseases: Patients with a history of inflammatory bowel disease and autoimmune diseases determined by the researcher to be unsuitable for this study, such as systemic lupus erythematosus, vasculitis and invasive lung disease, should be excluded (except for vitiligo subjects); 10. Within 4 weeks before cell infusion and during the study period, it is planned to use (if there is long-term use) systemic pinesterols, hydroxyurea and immunomodulatory drugs (such as: α or γ interferon, GM-CSF, mTOR inhibitor, cyclosporin, thymosin, etc.); 11. * History of organ allotransplantation, allogeneic stem cell transplantation and renal replacement therapy; 12. * Known uncontrolled diabetes, pulmonary fibrosis, interstitial lung disease, acute lung disease or liver failure; 13. * Known alcohol and/or drug abuser; 14. * Pregnant or lactating women; 15. Subjects with any co-existing medical conditions or diseases that the investigator judges may affect the development of this trial; 16. *Subjects without legal capacity/restricted capacity; 17. Patients who have received similar gene therapy products before cell infusion, and the researchers believe that they are not suitable for enrollment; 18. Patients who are judged by the investigator to be difficult to complete all visits or operations required by the research

	protocol (including the follow-up period), or who have insufficient compliance to participate in this study; or patients who are deemed unsuitable for inclusion by the investigator.
Screening of genotype and antigen markers	After the patient signs the ICF for gene and tumor antigen detection, the patient will be tested for HLA genotype. 2ml whole blood samples of the patient need to be collected and sent to a third-party central laboratory for detection. After the HLA genotype is determined as A*02:01, the tumor antigen markers are detected, and the patient is required to provide 3-5 unstained sections or agree to biopsy. The test was performed by the local laboratory of the clinical research center. Patients whose NY-ESO-1 immunohistochemical staining shows positive cells ≥ 20% can sign the main ICF and continue the follow-up clinical study procedures / steps.
Leucocyte apheresis and preparation of TCR-T cells	Identify subjects who are genotyped for HLA-A*02:01 positivity and NY-ESO-1 tumor antigen positivity (positive cells ≥20% determined by immunohistochemistry) can sign the main ICF, and the patient's leukocytes will be collected on the study day -25 for the preparation of TCR-T cells. Peripheral blood mononuclear cells (PBMC) (approximately 1×10^9 cells) will be collected using an apheresis machine, and activated with CD3/28 magnetic beads. CD4+ and CD8+ T cells were transduced with lentivirus containing NY-ESO-1 TCR, and amplified to the required number; the harvested TCR-T cells were frozen in liquid nitrogen, and released after the required indicators meet the QC standards
Lymphodepleting chemotherapy	The lymphodepleting chemotherapy regimen was FC regimen: cyclophosphamide (15 mg/kg/d) and fludarabine (20 $mg/m^2/d$) for 3 consecutive days. If serum creatinine or creatinine clearance or glomerular filtration rate is abnormal on the day of lymphodepleting chemotherapy (before administration), or if serum creatinine >1.5 times ULN, or creatinine clearance (Cockcroft-Gault formula) or radioisotope glomerular filtration rate (GFR)≤30mL/min/1.73m^2, chemotherapy should be suspended. If serum creatinine >2 mg/dL for more than 3 days, patients will be excluded from the study and regarded as dropout cases, and then screened and supplemented new cases.

Standard for delayed cell infusion after lymphodepleting chemotherapy	If serious adverse reactions (such as heart, respiration, liver and kidney dysfunction) occur in patients receiving lymphodepleting chemotherapy, the infusion of TAEST16001 cells should be delayed, and the investigator should determine whether to delay the infusion time; if the delay exceeds 14 days, the investigator shall determine whether it is necessary to carry out lymphodepleting chemotherapy again. If the patient has symptoms such as uncontrollable infection and severe deterioration, the infusion of TAEST16001 cells should also be delayed. In case of any of the above conditions, at the discretion of the investigator, the patient can be withdrawn from the study, and only all AEs reported within 28 days after the study-related procedure or treatment (such as leukapheresis, lymphocyte depletion chemotherapy) or starting new anti-tumor therapy are collected. Data related to efficacy assessments are no longer collected.
Dose and method of cell infusion	**1. Dose escalation study:** Patients will receive 5×10^8 ±30% 、 2×10^9±30% 、 5×10^9±30%, or 1.2×10^{10}±30% (calculated as TCR-T positive cells) TAEST16001 cells infusion, depending on the dose level (1-4). **2. Expanded enrolment study:** Patients will receive infusion of TAEST16001 cells (calculated as TCR-T positive cells) at the MTD dose level or at dose level 4: 1.2×10^{10}±30% (if no MTD is detected). **3. Infusion times:** – If the infusion dose levels are 1 and 2 (5×10^8±30% or 2×10^9±30%), the planned total amount of TAEST16001 cells (calculated as TCR-T positive cells) was infusion for a single time on the first day of the study; – If the infusion dose levels are 3 and 4 (5×10^9±30% or 1.2×10^{10}±30%), the total amount of TAEST16001 cells (calculated as TCR-T positive cells) was planned to be infusion at the ratio of 60% and 40% on the first and second days of the study. **4. Application of IL-2:** After the first infusion of TAEST16001 cells, patients will be subcutaneously injected with low-dose IL-2 (from the 1st day to the 14th day of the study), 500,000u/time twice daily (with an interval of 10-12h), for 14 days. The first injection will be carried out within 30 minutes after the first cell infusion.

Sample size estimation	This study is divided into two parts: dose escalation study and expanded enrollment study. The dose escalation study part is designed according to the principle of 3+3, with a total of 4 dose levels. It is estimated that about 2-24 patients will be enrolled in the study. The final sample size depends on the number of DLT, the number of dose groups escalated before DLT is observed, and the determination of MTD. The expanded enrollment study is planned to enroll 6-18 patients with indications. The total sample size of this study is expected to include 12-30 patients with the target indications.
Permitted concomitant medication and prohibited/cautious medication	**1. Permitted concomitant medications** - Antiemetic and hemorrhagic cystitis prevention drugs, including granisetron or ondansetron, and mesna, are permitted before lymphodepleting chemotherapy. - The researcher is allowed to use (but not limited to) antibiotics, red blood cell infusion and platelet infusion, colony stimulating factors, bronchodilators, epinephrine or transfusions, colony-stimulating factors, bronchodilators, epinephrine, anti-inflammatory drugs, and other supportive treatments **after patient is enrolled**, according to the medical guidelines of the clinical research center. - The researcher is allowed to use, but not limited to, such as tocilizumab, and glucocorticoid, in the treatment of drug-related AEs, such as CRS, after TAEST16001 cell infusion. **2. Prohibited/cautious medication** -Pharmacological doses of corticosteroids (prednisone ≥ 20 mg/day or equivalent doses of other corticosteroids) and other immunosuppressive agents must be avoided within 7 days before leukocyte apheresis and within 5 days before the first infusion of TAEST16001 cells. - Corticosteroids and other immunosuppressive agents should be avoided within 3 months after the first infusion of TAEST16001 cells, unless they are used to control toxicity associated with TAEST16001 cell therapy. Other drugs that may affect the evaluation of the study, such as non-steroidal anti-inflammatory drugs, should also be avoided during this period unless necessary. -It is forbidden to receive other anti-tumor therapy (except lymphodepleting chemotherapy) beginning from leukocyte

	apheresis until disease progression or study completion, including but not limited to chemotherapy, immunotherapy, targeted drugs, radiotherapy, autologous/allogeneic hematopoietic stem cell transplantation, and other clinical trial drugs. Traditional Chinese medicine / Chinese herbal medicine with anti-tumor indications cannot also be used.
Safety and tolerability evaluation	**<u>Safety evaluation:</u>** Cytokine release syndrome (CRS) and neurotoxicity will be graded using ASTCT 2018 criteria, and remaining adverse events (AEs) will be graded using CTCAE version 5.0 criteria. Unless otherwise specified in the protocol, AEs will be assessed throughout the study period, and dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) will also be observed in the dose-escalation study. The observation period of DLT was from the first time of cell infusion to 28 days after cell infusion. *NOTE: Only all SAEs related to study operations were collected from the time the patient signed the genotype and tumor antigen screening ICF to the primary ICF.* *All AEs and SAEs will be collected for patients who received TAEST16001 cell infusion (either partial or complete) from the time the patient signed the primary ICF until the end of the study. Unless subsequent anti-tumor treatment is started due to ineffectiveness or disease progression from 90 days (3 months) to 270 days (9 months) after TAEST16001 cells infusion, all AEs will not be collected, only those related to treatment and all SAEs will be collected from the beginning of replacement of anti-tumor treatment to the end of the study.* *For patients who signed the main ICF but did not undergo any TAEST16001 cell infusion for any reason, all AE and SAE within 28 days after the study related operation or treatment (such as leucocyte apheresis, lymphocyte clearance chemotherapy) or to the time of starting new anti-tumor treatment (whichever occurs first) were collected.* Adverse events, including vital signs, physical examination, 12-lead electrocardiogram, clinical laboratory test indicators, ECOG score, EORTC-QLQ-C30 scale, AE and serious adverse events (SAE), and adverse events of special interest (AESI), were graded. **<u>Definition of dose limiting toxicity (DLT):</u>** DLT is defined as the following adverse events that occur within 28 days after the completion of the first cell infusion in the dose escalation study and are determined to be related to cell therapy by the Safety Review Committee (SRC) (CRS and neurotoxicity are graded according to ASTCT 2018

	standard, and the remaining AES will be evaluated according to CTCAE version 5.0 standard): 1) Grade 4 hematologic toxicity (except lymphopenia) not caused by underlying disease persists for more than 28 days from the date of cell infusion; 2) All Grade 3 CRS lasting >7 days and all Grade 4 CRS; 3) All grade 3 non-hematological toxicities lasting >7 days and all grade 4 non-hematological toxicities regardless of duration, except for the following: - Grade 3 nausea and/or decreased appetite; - Aphasia/language impairment or blurred consciousness / cognitive impairment recovering to grade 1 or below within 2 weeks, or to baseline within 4 weeks; - Airway protection requires intubation ≤7 days; - Nephrotoxicity requiring dialysis ≤7 days; -Grade 3 alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, elevated bilirubin, or other abnormal liver function, recovering to grade 2 or below within 14 days; -Grade 4 transient liver function abnormalities, recovering to Grade 3 or below within 72 hours; -Grade 3 or 4 fever; -Immediate hypersensitivity (related to cell infusion) occurred within two hours of cell infusion. After standard treatment, the hypersensitivity was reversed to grade 2 or below within 24 hours after TAEST16001 cell infusion. 4) Considering to be other AEs of DLT after SRC discussion **Maximum tolerated dose (MTD):** In the dose escalation study, the previous lower dose of the dose group in which ≥2/6 patients experienced DLT.
Pharmacokinetic (PK) and pharmacodynamic (PD) evaluation	**1. Blood collection for PK:** Collect 6ml of venous blood per test item at each time point and send it to the central laboratory for testing. Collection time points: within 2 hours before lymphodepleting chemotherapy, within 2 hours before cell infusion, 1h ±10min after the first infusion of cells (study day 1), and on days 2, 4, 7, 14, 21, 28, 60, 90, 180 and 270. Note: blood samples for PK should be collected according to the study plan. For details, please refer to the laboratory manual. However, on the day when the subject develops AESI, SAE or withdraws from the study, unscheduled PK blood samples should be collected as soon as possible. **2. PK evaluation parameters:** The detection of TAEST16001 cells in peripheral blood by flow cytometry and the detection of TCR-T DNA copy number by qPCR were simultaneously detected, and the following parameters were calculated:

	Peak value (Cmax), time to peak (Tmax) and AUC_{0-28} of TAEST16001 cells in peripheral blood. If possible, AUC_{0-inf}, terminal phase elimination rate constant (λz) and elimination half-life (t1/2) will be evaluated. **3. <u>Blood collection for PD:</u>** Collect 6mL or 3mL of venous blood per test item at each time point and send it to the central laboratory for testing. Collection time points: within 2 h before leukocyte apheresis, 1 h ± 10 min after the first infusion of cells (study day 1), and on study days 7, 28, 60, 90, 180, and 270. **4. <u>PD evaluation parameters:</u>** T cell subsets, peripheral blood antigen-specific CTL, effector cell activity assay. *Note: blood samples for PD should be collected according to the study plan. For details, please refer to the laboratory manual. However, on the day when the subject develops AESI, SAE or withdraws from the study, unscheduled PK blood samples should be collected as soon as possible.*
Efficacy evaluation	**<u>Efficacy evaluation criteria:</u>** Efficacy evaluation according to solid tumor evaluation criteria (RECIST 1.1), subjects who are evaluated as disease progression (PD) by RECIST1.1 can be re-evaluated as iUPD according to iRECIST criteria. After 4-6 weeks, imaging reexamination was conducted to determine whether they were iCPD according to iRECIST; observe until disease progression (iCPD) or carry out other anti-tumor therapy, whichever occurs first. **<u>Efficacy evaluation method:</u>** The tumor lesion size of the patients will be evaluated by imaging, and the tumor remission will be evaluated. The imaging methods used by patients at all follow-up points for efficacy evaluation must be the same. The first efficacy evaluation was conducted 28 days after the first infusion of TAEST16001 cells, and then the efficacy evaluation was conducted at the 2nd, 3rd, 6th and 9th months after the infusion. 1) Objective response rate (ORR): the proportion of patients whose best overall response (BOR) is PR or CR (RECIST 1.1 standard); 2) Disease control rate (DCR): the proportion of patients whose best overall remission (BOR) is PR, CR or SD (RECIST 1.1 standard); 3) Progression free survival (PFS): the time interval from patient enrollment (lymphodepleting chemotherapy) to disease progression (PD) (RECIST 1.1 criteria) or death from any cause. In this study, an Independent Review Committee (IRC) was established. The IRC was composed of three doctors with independent reading qualifications. When the reading results between the two IRC members were inconsistent, the third IRC

<table>
<tr><td></td><td>member had to review the results. The tumor imaging assessment is arbitrated, and the result of the arbitration is the final tumor assessment result of the IRC. The results of the IRC readings will not be fed back to the research center.</td></tr>
<tr><td>Quality of life</td><td>Evaluation will be performed using the EORTC QLQ-C30 scale.</td></tr>
<tr><td>Statistical analysis</td><td>1. Statistical analysis set
For statistical analysis, the following analysis sets are defined:
Analysis sets and descriptions
<table>
<tr><th>Population</th><th>Descriptions</th></tr>
<tr><td>DLT analysis set</td><td>In the dose-escalation study, patients who developed DLT after complete infusion of the target scalar TAEST16001 cells and patients who did not develop DLT and completed the 28-day follow-up after the first cell infusion.</td></tr>
<tr><td>Security analysis set</td><td>In the dose escalation study and expanded enrollment study, patients who received partial or planned total dose of TAEST16001 infusion.</td></tr>
<tr><td>Pharmacokinetics (PK) analysis set</td><td>In the dose-escalation and the expanded enrollment study, patients who received partial or planned total dose of TAEST16001 infusion with no protocol violations or events that impacted the pharmacokinetics analysis.</td></tr>
<tr><td>Pharmacodynamics (PD) analysis set</td><td>In the dose-escalation and the expanded enrollment study, patients who received partial or planned total dose of TAEST16001 cell infusion with baseline and at least one post-baseline evaluable pharmacodynamic parameter.</td></tr>
<tr><td>Efficacy evaluable set</td><td>Patients receiving partial or planned total dose of TAEST16001 cell infusion therapy with baseline tumor assessment and at least one post-baseline tumor assessment.</td></tr>
</table>
2. General analysis
After the research plan is determined, the statistical professionals are responsible for formulating the Statistical Analysis Plan (SAP), and the detailed statistical analysis method will be described in detail in the SAP. For measurement data, use the number of cases, mean, standard deviation, median, maximum value, and minimum value for statistical description; for count data or grade data, use frequency for statistical description. The data from the most recent lymphodepleting chemotherapy will be used as the baseline data.</td></tr>
</table>

	3. Safety and Tolerability Analysis Security analysis will be conducted in the security analysis set. Safety was assessed by summarizing AE/SAE/Adverse Events of Special Interest (AESI) (CRS and neurotoxicity), changes in laboratory findings, abnormal ECGs, vital signs, changes in ECOG scores. CRS and neurotoxicity will be graded using the ASTCT 2018 criteria, and the remaining AEs will be graded using the CTCAE 5.0 criteria. Adverse events were coded using the International Dictionary of Medical Terms (MedDRA). The number and frequency of AEs that occurred in patients after lymphodepleting chemotherapy and the first cell infusion until the end of the study were summarized using the appropriate term according to the human organ system classification. All AESI (CRS and neurotoxicity), SAEs (including death), and AEs leading to permanent discontinuation of cell infusion are summarized in separate list. Changes in laboratory findings will be summarized according to the CTCAE 5.0. For laboratory indicators, the highest grades that occurred during the trial were summarized in counts and percentages. The changes of ECG abnormalities, vital signs and ECOG scores will be compared with the baseline level and the level before cell infusion for descriptive statistics. Some vital signs and laboratory examination results will be summarized by drawing charts based on time for the data of each subject. In addition, all DLTs will be summarized and described (DLT set), and the occurrence details of DLTS will be listed. **4. Efficacy analysis** Efficacy analyses will be performed in the efficacy-evaluable sets. Tumor evaluation will be based on RECIST1.1 criteria, and exploratory tumor evaluation will also be based on iRECIST. Tumor assessments were performed by investigators of the research center or designated researchers. At the same time, IRC was set up in this study to read the imaging of all subjects independently. During statistical analysis, the tumor evaluation results of researchers and IRC will be analyzed separately. Objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS) and their 95% confidence intervals were calculated after cell infusion. Median values and 95% confidence intervals for CR, PR, SD, and PFS were estimated using the Kaplan-Meier method. Subjects without disease progression or death on the analysis date, or subjects without disease progression when receiving any further anti-tumor treatment, will be censored at the last full tumor assessment before the data cutoff or the date

	of anti-tumor treatment. If disease progression or death was recorded after a single missing tumor assessment, as a prespecified value, the actual event date of disease progression/death was used for the PFS event date. If disease progression or death was documented after ≥2 missing tumor assessments, the PFS time for these subjects was censored at the date of the last adequate tumor assessment without disease progression. Kaplan Meier method and appropriate summary statistics were used to describe the PFS assessed by the investigators. **5. Pharmacokinetic (PK) analysis** PK analysis will be performed in the PK set. Descriptive statistical analysis was performed on the PK parameters at each visit time point, and the mean, standard deviation, median, minimum, maximum, geometric mean, and coefficient of variation of the geometric mean were reported. **6. Pharmacodynamic (PD) analysis** PD analysis will be performed in the PD set. Descriptive statistical analysis was performed on the PK parameters at each visit time point, and the mean, standard deviation, median, minimum, maximum, geometric mean, and coefficient of variation of the geometric mean were reported. **7. Life quality score analysis** The mean, standard deviation, median, minimum, maximum, geometric mean and geometric mean variation coefficient of the EORTC-QLQ-C30 scale results collected at different infusion dose groups at different measurement time points will be reported. If possible, appropriate methods will be used to plot the trend of statistical scores.
Safety Review Board	The safety review committee consists of the main researchers or their representatives, sponsor representatives, and CRO representatives. Clinical pharmacology experts, statisticians, and other experts are included when necessary. The safety review committee may decide dose escalation, increase the dose cohort by 3 evaluable patients, discontinue dose escalation, assess and/or supplement enrollment, or explore other study plans.
Analysis description	The primary statistical analysis of this study will be the safety analysis, PK, PD analysis, and preliminary efficacy analysis after the last subject in the dose escalation study portion completes the DLT evaluation cycle (28 days after the first cell infusion or on the day of the DLT).This statistical analysis mainly provides data support for the determination of MTD. After the study, the final safety, effectiveness analysis, PK and PD analysis will be carried out as required.

Observation / step	Screening period				Treatment and observation period										Follow-up period				Early Withdrawal /Unscheduled Visit	Long-term follow-up period
	Pre-screening period	Primary screening phase			Cell infusion	DLT observation (only for patients enrolled in the dose escalation portion of the study)														
		Apheresis	Lymphodepleting	Before cell infusion		Safety and efficacy evaluation (applicable to all enrolled patients)														
Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15**	**V16**	**V17**	**End of Study (EOS) Visit**		
Study time (days)		**-25 to -8**	**-7 to -5**	**-4 to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**21**	**28**	**60**	**90**	**180**	**270**		
Visit window (days)		**-14**		**+14**										**±1**	**±3**	**±3**	**±7**	**±7**		
Informed consent for genotype and tumor antigen screening	×																			
HLA-A*02:01 and NY-ESO-1 detection #	×																			
Informed consent of primary screening ✲		×																		
Include and exclusion criteria	×	×																		
Tumor disease history and past medical history[1]	×																			
Demographic data	×																			
Height and weight [2]		×										×	×	×	×	×	×	×	×	
Physical examination [3]		×			×						×	×		×	×	×	×	×	×	
Vital signs [4]		×	×	×	(×)	(×)	×	×	×	×	×	×	×	×	×	×	×	×	×	
ECOG performance status		×	×	×								×		×	×	×	×	×	×	
EORTC QLQ-C30 life quality score		×												×	×	×	×	×	×	
Imaging examination（CT/MRI）[5]		×	(×)											×	×	×	×	×	×	
Color Doppler echocardiography		×																		
12-Lead ECG [6]		×			(×)	(×)	×	×	×	×	×	×		×	×	×	×	×	×	
Fingertip oxygen saturation [7]		×	×	×	(×)	(×)	×	×	×	×	×	×		×	×	×	×	×	×	
Routine blood test [8]		×	×	×				×			×	×	×	×	×	×	×	×	×	

Observation / step	**Screening period**				**Treatment and observation period**										**Follow-up period**				**Early Withdrawal /Unscheduled Visit**	**Long-term follow-up period**
	Pre-screening period	**Primary screening phase**			**Cell infusion**	**DLT observation (only for patients enrolled in the dose escalation portion of the study)**														
		Apheresis	**Lymphodepleting**	**Before cell infusion**		**Safety and efficacy evaluation (applicable to all enrolled patients)**														
Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15**	**V16**	**V17**	**End of Study (EOS) Visit**		
Study time (days)		**-25 to -8**	**-7 to -5**	**-4 to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**21**	**28**	**60**	**90**	**180**	**270**		
window of visit (days)		**-14**		**+14**										**±1**	**±3**	**±3**	**±7**	**±7**		
Routine urine test		×		×				×			×	×	×	×	×	×	×	×	×	
Blood biochemistry [9]		×	(×)	(×)				×			×	×	×	×	×	×	×	×	×	
Coagulation function		×						×				×		×	×	×	×	×	×	
Myocardial enzyme		×						×				×		×	×	×	×	×	×	
Virological test (hepatitis B/C, HIV)		×																		
Serum pregnancy (females of childbearing potential)[10]		×		×														×	×	
Leukocyte apheresis [11]		×																		
Lymphodepleting chemotherapy			×																	
TAEST16001 cell infusion [12]					×	(×)														
Immunologic test*		×		×							×	×		×	×	×	×	×	×	
Cytokine detection (IL-6 、TNFα、IFNγ)		×	×	×				×			×			×						
Inflammatory factor detection（CRP、Ferritin）		×	×	×				×			×			×						
Tumor assessment		×												×	×	×	×	×	×	
Blood collection for pharmacokinetic (PK)			×		(×)†	×		×			×	×	×	×	×	×	×	×	×	
Blood collection for pharmacodynamic (PD)		×			(×)						×			×	×	×	×	×	×	

Observation / step	Screening period				Treatment and observation period										Follow-up period				Early Withdrawal/Unscheduled Visit	Long-term follow-up period
	Pre-screening period	Primary screening phase			Cell infusion	DLT observation (only for patients enrolled in the dose escalation portion of the study)														
		Apheresis	Lymphodepleting	Before cell infusion		Safety and efficacy evaluation (applicable to all enrolled patients)														
Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15**	**V16**	**V17**	**End of Study (EOS) Visit**		
Study time (days)		**-25 to -8**	**-7 to -5**	**-4 to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**21**	**28**	**60**	**90**	**180**	**270**		
window of visit (days)		**-14**		**+14**										**±1**	**±3**	**±3**	**±7**	**±7**		
DLT/MTD evaluation [13]						×---------->														
AE[14]		(×)---------->																	×	
SAE[15]	×	(×)---------->																	×	
Concomitant medication [16]		(×)---------->																	×	
RCL detection [17]			×		×									×				×	×	×
Biopsy of tumor [18]																			(×)	
Secondary tumor																				×

Note: × means it must be carried out. (×) means see footnotes for details. If cardiac adverse reactions occur during the infusion, ECG monitoring and symptomatic treatment are required,and routine blood test, blood biochemistry and myocardial enzymes shall be detected within 2h±5min after infusion; The latest data from V3 visit will be used as the baseline of the whole study.

#: Patients under standard treatment (not confirmed failure of standard treatment) can sign the genotype and tumor antigen screening ICF to enter the pre-screening. If the patient's genotype and tumor antigen meet the requirements of this trial, they can sign the main ICF to enter the main screening of this study when the current treatment fails or they are not suitable to continue the current treatment.

＊: Informed consent for primary screening: Informed consent for primary screening signed within 14 days prior to apheresis.

*: Immune multi-factor detection: including IL-1, IL-8, IL-10, IL-12, MCP-1. Blood samples are sended to a third-party central laboratory for testing, and the estimated blood sample volume requires 2ml of whole blood.

†: Pharmacokinetic (PK) blood collection: on the day of cell infusion, blood was collected before and after the infusion;

ECOG: Eastern Cooperative Oncology Group; ECOG Score: Performance Status Score; EORTCQLQ-C30: quality of life scale for cancer patients;

CRP=c-reactive protein; DLT=dose-limiting toxicity; PK=pharmacokinetics; PD=pharmacodynamics; RCL = Replication-competent lentivirus; AE = adverse event; SAE = serious adverse event.

1. History: including but not limited to pathological diagnosis (soft tissue sarcoma, primary liver malignant tumor, ovarian malignant tumor, non-small cell lung cancer (NSCLC), breast cancer, etc.), previous tumor treatment history and previous diagnosis and treatment history of other diseases.

2. **Height and weight**: height is measured only during the screening period, and weight is measured continuously during the screening period and the 14th day of infusion and thereafter.

3. **Physical examination**: a complete physical examination is performed only during the screening period, including the head, eyes, ears, nose, throat, neck, heart, chest (including lungs), abdomen, limbs, skin, lymph nodes, nervous system and the general condition of the patient. In the follow-up visit (or when clinically necessary), limited physical examination can be carried out according to the symptoms.

4. **Vital signs**: including body temperature, respiration, blood pressure and pulse rate / heart rate; （×） If there is cell infusion on the same day, the above indicators of the subjects shall be measured repeatedly every 15 minutes (± 2 minutes) before and within 1h after cell infusion for at least one hour. After the first hour, the body temperature, respiration, blood pressure and pulse rate/heart rate were measured every hour (± 10 minutes) until the sixth hour.

5. **Imaging examinations (CT/MRI)**: The imaging examination methods used by patients at all efficacy evaluation follow-up points must be the same during the study period. Investigators can increase the frequency of imaging examinations according to the needs of the subjects' conditions. If additional imaging examinations are required during the main screening period, they should be completed within 7 days before lymphodepleting chemotherapy. The most recent imaging study before cell infusion was used as the baseline for tumor assessment.

6.**12-lead electrocardiogram**: (×) If cells are infused on the same day, it shall be conducted before cell infusion and 2h±15min after infusion.

7. **Fingertip blood oxygen saturation**: (×) If cells are infused on the same day, it should be carried out before cell infusion and 2h±15min after infusion.

8. **Routine blood test**: red blood cell count, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, platelet count, and hemoglobin.

9. **Blood biochemistry**: including blood sodium, blood potassium, chloride, blood urea nitrogen/blood urea, creatinine, creatinine clearance rate (Cockcroft-Gault formula) (can also be replaced by radioisotope glomerular filtration rate [GFR]), blood glucose, ALT, AST, alkaline phosphatase, total bilirubin and lactate dehydrogenase. (×) On the day before the administration of the first dose of lymphodepleting chemotherapy and before the cell infusion after lymphodepleting chemotherapy, only rapid blood biochemistry was performed: blood urea nitrogen/blood urea, creatinine, and creatinine clearance (Cockcroft-Gault formula) (can also be replaced by radioisotope glomerular filtration rate [GFR]), ALT, AST, and serum potassium.

10. **Serum pregnancy** (females of childbearing potential only): Serum β-hCG pregnancy test must be negative 1 day before infusion of TAEST16001. During the study period, women of childbearing potential also underwent pregnancy testing whenever clinically indicated.

11. **Leukocyte apheresis**: 25 days before cell infusion, with a window period of -14 days (subject to the actual apheresis date). Blood routine testing is required for apheresis to calculate the number of mononuclear cells and hematocrit.

12. **TAEST16001 cell infusion**: The patients in the dose escalation and the expanded enrollment studies will receive TAEST16001 cell intravenous infusion on the 5th day after lymphodepleting chemotherapy (at an interval of 4 days): if the infusion dose level is 1 and 2, the planned total amount of TAEST16001 cells (calculated as TCR-T positive cells) will be infused in a single time on the first day of the study; after the first infusion of TAEST16001 cells, patients will be subcutaneously injected with low-dose IL-2 (500000u/time, from the first day to the 14th day of the study). The first injection is carried out within 30min after the cell infusion, twice a day (with an interval of 10-12h) for 14 days. (X) If the infusion dose level was 3 and 4, the total amount of TAEST16001 cells (calculated as TCR-T positive cells) was planned to be infusion at the ratio of 60% and 40% on the first and second days of the study.

13. **DLT/MTD** evaluation: only patients enrolled in the dose escalation study.

14. **AE**: (×) All AEs are collected from signing the primary ICF to 3 months after the first cell infusion. Three months after the infusion to 9 months after the infusion, all AEs will continue to be collected. Unless subsequent anti-tumor treatment is started due to ineffectiveness or disease progression from 90 days (3 months) to 270 days (9 months) after TAEST16001 cells infusion, from the beginning of replacement of anti-tumor treatment to the end of the study, all AEs will not be collected, only those related to treatment and all SAEs will be collected.

For patients who signed the main ICF but did not undergo any taest16001 cell infusion for any reason, only AE within 28 days after the study related operation or treatment (such as leucocyte apheresis, lymphocyte clearance chemotherapy) or until the start of new anti-tumor treatment (whichever occurs first) were collected. During the out of hospital period, the subjects need to record the AE in the subjects' diary card and return it to the Research Center at the next visit.

15. **SAE**: (×) From the time of signing the genotype and tumor antigen screening ICF to signing the main ICF, only all SAEs related to the research operation are collected; from signing the main ICF to 3 months after the first cell infusion, all SAEs are collected and reported; from 3 months to 9 months after infusion, all SAE will continue to be collected. For patients who signed the main ICF but did not have any TAEST16001 cell infusion for any reason, collect all SAEs during the study period. During the out of hospital period, the subjects need to record the SAE information in the subjects' diary card and return it to the Research Center at the next visit.

16. **Concomitant medication**: During the out-of-hospital period, subjects need to record the concomitant medication on the subject's diary card and return it to the research center at the next visit.

17. **RCL detection**: monitor lentivirus replication, and it is estimated that 4ml of blood is required. Blood collection on the day of V5 should be completed before cell infusion.

18. **Tumor lesion biopsy**: Investigators can perform tumor lesion biopsy according to the needs of the subject's condition. The inspection contents include: NY-ESO-1 detection, tumor immune microenvironment, and gene mutation analysis.

1. Study background

1.1 Summary

Immunotherapy is currently recognized as one of the most promising methods，in addition to surgery, chemotherapy and radiotherapy，to cure cancer。The effective immunotherapy methods mainly include antibodies and cellular immunotherapy. Antibodies have been used in the treatment of many cancers and have obtained the very good treatment effect, including: advanced melanoma, non-hodgkin's lymphoma, lung, kidney and bladder cancer. T cell therapy that belongs to the category of immune cell therapy mainly includes CAR-T (Chimeric Antigen Receptor, CAR) and TCR-T (T Cell Receptor-T). Immune cell therapy of CAR-T cells for acute and chronic lymphocytic leukemia has made it possible to cure and prolong the life of 80-90% of patients with advanced cancer who have failed conventional therapies,[1] including radiotherapy and chemotherapy. The CAR-T products of two companies have been approved by the U.S. FDA. However, the existing CAR-T treatment can only kill blood tumor cells with surface antigen expression, and has not been very effective in the treatment of tumors in solid tumors. Moreover, the adverse reactions caused by CAR-T treatment, such as cell factor storms, are sometimes difficult to control. Therefore, people need to explore new treatments. Herein, the sponsor now applies for the development of TCR-T cell therapy.

In view of the fact that tumor antigens cross-react with normal cell antigens and easily lead to adverse reactions,[2] this project mainly focuses on an antigen that is not expressed in normal cells but is expressed in testis, and is defined as cancer testis antigen. We prefer NY-ESO-1 antigen, which was first found in esophageal cancer, and later found to have 10-50% expression in melanoma, non-small cell lung cancer (NSCLC), liver cancer, breast cancer, prostate cancer, bladder cancer cancer, thyroid cancer and ovarian cancer, 60% expression in multiple myeloma, 70-80% expression in synovial cell sarcoma and 22.5% expression in osteosarcoma.

In 2015, the University of Pennsylvania, the University of Maryland and the UK Adaptimmune reported the breakthrough progress of TCR-T cell therapy in the journal Nature Medicine. The clinical trial showed that high-affinity anti-NY-ESO-1 and LAGE-1-specific TCR-T was effective in 16 of 20 patients with multiple myeloma (80%), with a mean progression-free survival of 19.1 months, and the side effects are mild, without serious side effects of CAR-T treatment.[3] Adaptimmune successfully received a $190 million investment. Another clinical study conducted by Dr. Rosenberg's team of the National Cancer Institute of the United States showed that anti-NY-ESO-1-specific TCR-T treatment of synovial cell sarcoma and melanoma, of which 61% synovial cell sarcoma and 55% of melanoma were clinically effective.[4,5] Due to the favorable clinical results of Adaptimmune's anti-NY-ESO-1-specific TCR-T in the treatment of synovial sarcoma, the US FDA approved this TCR-T product treatment for synovial sarcoma as breakthrough therapy, and on July 30, 2016, the European Medicines Agency approved this treatment into Priority Medicines. These clinical data suggest that TCR-T therapy can target a variety of tumors including soft tissue sarcomas.

1.2 Preclinical study results

1.2.1 Fundamentals of the project

The immune response of human T cells to tumor antigens depends on the

recognition of the TCR on the surface of T cells and the antigen complex of human Leukocyte Antigens (HLA). The TCR of most T cells in the body is composed of two polypeptide chains α and β, and the TCR lineage is selected to occur in the thymus of embryos. T cell clones with high affinity TCR have the potential of generating immune response to autologous cells However, in vivo clearance (negative selection), only T cell clones with low affinity TCRs are retained (positive selection). Therefore, under normal physiological conditions, the binding of TCRs to the antigen polypeptide-HLA complexes occurs in a low affinity form, Once the organism is found to have invasion of foreign pathogenic microorganisms or aberrant expression of normal cells (tumorigenesis) or proteins (antigens) in the body, the human body, in order to protect itself, from foreign-invaded pathogenic microorganisms or in vivo mutations / proteins Abnormal expression of tumor cells phagocytosis, the use of intracellular protein digestion system to swallow the cells into peptides, and then presented to T cells, naive T cells after antigen recognition, proliferation and differentiation, removal of foreign pathogenic microorganisms infected Cells, to maintain normal body. On the other hand, the affinity of T cell receptor for tumor-associated antigen is not as high as that of T cell in T cell receptor Thymus clones are selected to be in the affinity range. Although T cells can bind to tumor cells with low affinity, most tumors can escape the killing of T cells due to their downregulation of human lymphocyte antigens on the cell surface. Therefore, to effectively eliminate the tumor, it is necessary to increase the affinity of the T cells for the antigen and the binding ability to the tumor cells.

Under the leadership of Dr. Li Yi,[6] a leading expert in the State Thousand Talents Program, and Xiangxue Life Science Technology (Guangdong) Co., Ltd. has developed a high-affinity TCR technology with complete independent intellectual property rights. Tumor antigen peptides were found by proteomic profiling, and HLA antigen peptide tetramers (pHLA) were biochemically synthesized. The TCR proteins of T cells were identified by these pHLA antigen polypeptide tetramers. Tetramer-stained T cells were sorted by flow cytometry to clone the TCR gene. After rapid in vitro screening validation, molecular biology was used to induce mutations and phage display was used to obtain TCRs that were normally expressed. The affinity was determined precisely using a molecular interaction such as Biacore, and then, at the cell biology level, the resulting TCR was confirmed to be an antigen-specific high-affinity TCR. When this TCR gene is expressed on the surface of T cells, it can kill tumor cells without damage to normal cells. Finally, antigen-specific high-affinity TCR-T was shown to inhibit tumor growth by animal tumor models. After obtaining the high-affinity and non-specific TCR genes, we use lentivirus as a vector to transfer the high-affinity TCR gene that recognizes the tumor-associated antigen NY-ESO-1 into the patient's own T cells and back it to the patient to cure the tumor Patient or life-long effect.

T cell cytotoxic T lymphocytes in vivo is the main anti-CD8 cytotoxic T cells (CTL), CTL mainly through the identification of antigens presented by HLA-I molecules to kill intracellular parasitic pathogens host cells and tumor cells And so on, this process requires the help of CD4 + T helper cells. CD4 + T cells also contain a portion of CTL that recognizes the antigen presented by the HLA class II molecule. CTLs kill target cells The immune synapses are formed by the TCR recognition of antigen polypeptide-HLA complexes and some adhesion molecules (LFA-1 and CD2, etc.) and secrete cytokines perperin and Granzyme, perforin monomer can penetrate the target cell membrane, with the help of calcium ions can form about 16nm of the channel, so that water, electrolyte into the target cells, leading to its dissolution, and granzyme can also enter

the target hole with the target Cells, which target cell death through the activation of apoptosis-related enzyme systems. CTL can kill target cells through the secretion of TNF , TNF and expression of membrane FasL; these molecules can be with the tumor cell surface TNF receptor and Fas binding, which activate Caspase signaling pathway, mediated tumor cell apoptosis. After killing tumor cells, CTL cells can continue to look for tumor cells that have the same specific tumor antigen expression and attack the tumor cells in the above-mentioned manner. One T cell can kill thousands of tumor cells continuously.

1.2.2 Study drug

The therapeutic used in this study was TCR-T cells against the NY-ESO-1 carcinoembryonic antigen. The patient's T cells were transfected in vitro with the genetically engineered lentivirus with the TCR gene (Figure 1) and these T cells expressing the anti-tumor antigen TCR are massively expanded to $1\times10^{9}\sim2\times10^{10}$. Patient are reinfused with TCR-T cells after lymphodepleting chemotherapy.

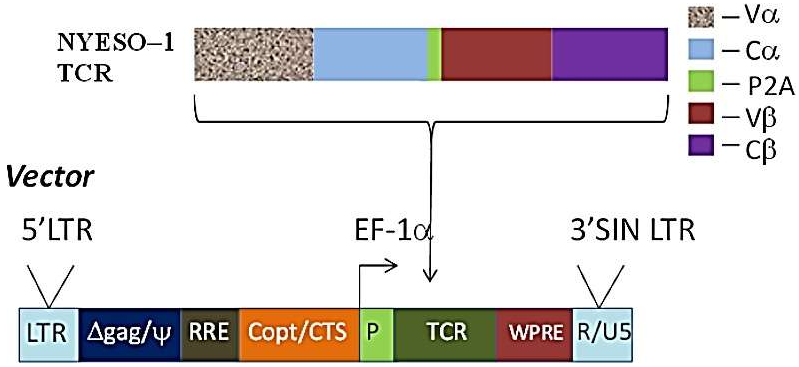


Figure 1: Structure diagram of TCR gene against NY-ESO-1 antigen cloned into lentiviral vector.

1.2.3 Preclinical study

Guangzhou Xiang Xue Pharmaceutical Co., Ltd. Life Science Research Center and Xiangxue Life Science Technology (Guangdong) Co., Ltd. has successfully developed with its own intellectual property against NY-ESO-1 antigen-specific high affinity TCR gene in vitro cell-specific detection , T cells expressing TACR against NY-ESO-1 antigen (TAEST16001) had a significant killing effect on tumor cells with corresponding tumor antigens, whereas no obvious tumor cells were detected on tumor cells without NY-ESO-1 antigen expression (Figure2).

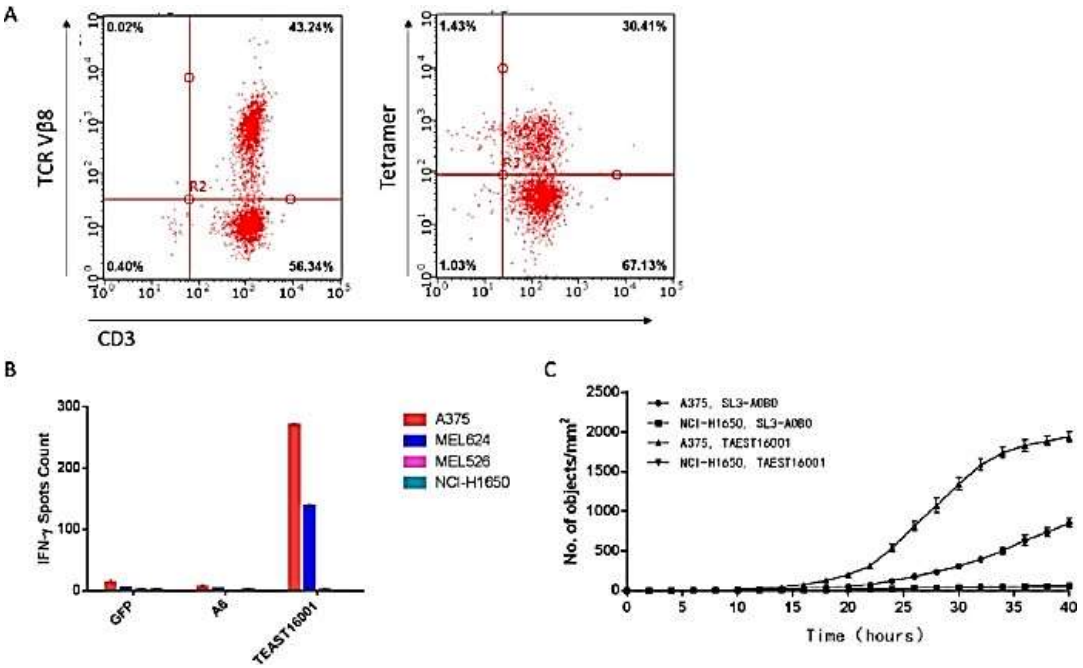


Figure 2: Functional characterization of virally transfected SL3-A10B0 TCR-T (TEAST16001).

*(A): The SL3-A10B0 TCR gene was transduced into CD3/CD28 magnetic beads-activated PBL cells using a third-generation lentivirus. Transfection efficiency was verified by flow cytometry using anti- TCR Vβ8 antibody and Tetramer. (B): TEAST16001 cells were co incubated with positive target cells A375, MEL624 (HLA-A*0201+/NY-ESO-1+), and negative target cells Mel526, NCI-H1650 (HLA-A*0201+/NY-ESO-1-), IFN-γ release was measured by Elispot. GFP-transduced cells and A6 TCR (non-HLA-A*0201+/NY-ESO-1+ specific) transduced cells served as negative controls. (C): Comparison of cytotoxicity of TEAST16001 and T cells transduced with wild-type SL3-A0B0 before optimization. The cytotoxicity assay utilizes the IncuCyte platform to detect caspase 3/7-dependent apoptosis by real-time imaging.*

In terms of specificity and toxicity, TAEST16001 inhibits cardiomyocytes, human astrocytes, human peripheral arterial endothelial cells, and prostate epithelium of normal human cells from HLA-A2-positive and HLA-A2-negative hearts Cells, human cardiac fibroblasts, human bronchial smooth muscle cells, human aortic smooth muscle cells, human liver astrocytes, human adrenal cortical cells, human mesangial cells, and human lung fibroblasts were unresponsive to ELISPOT experiments (Figure 3).

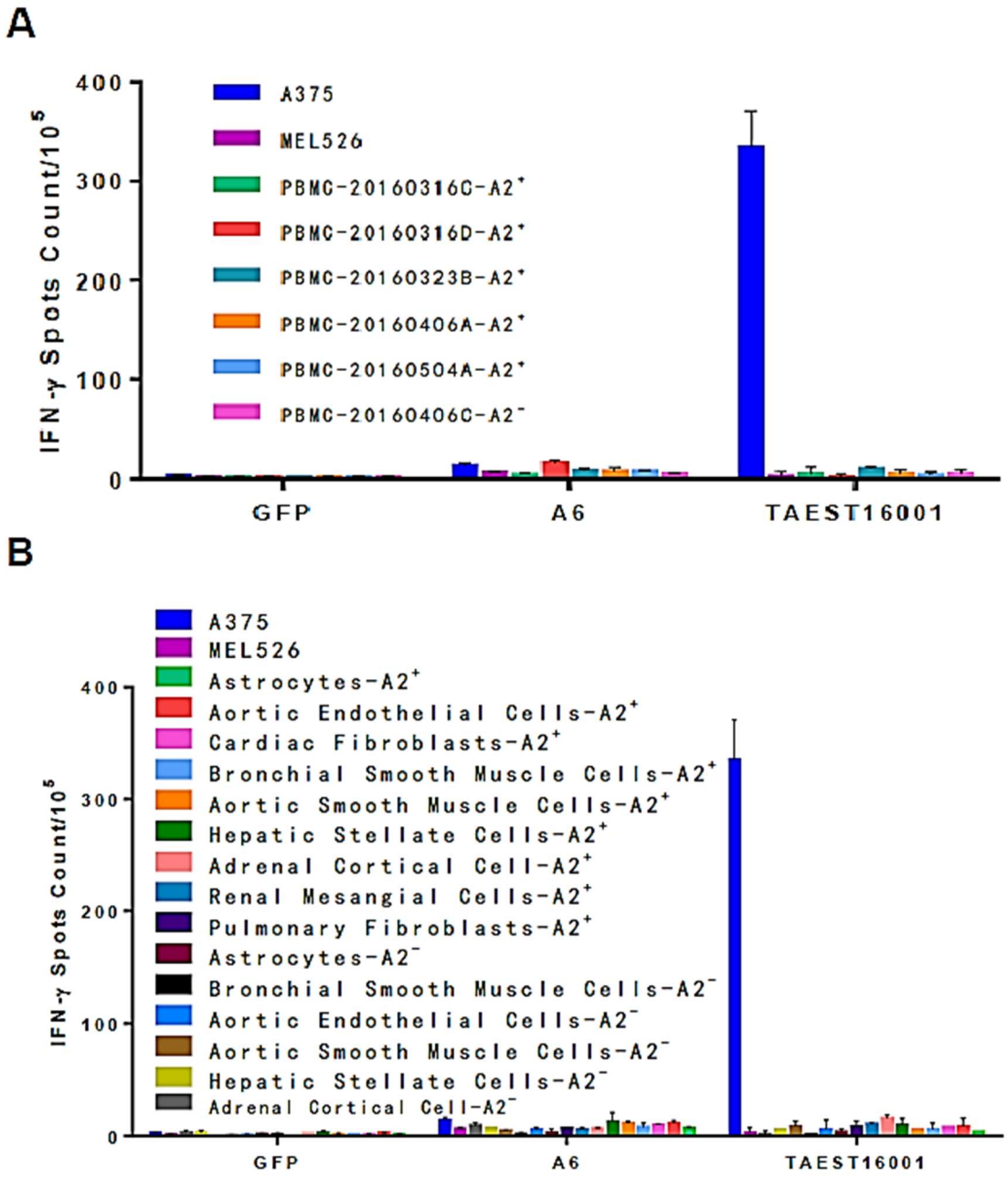
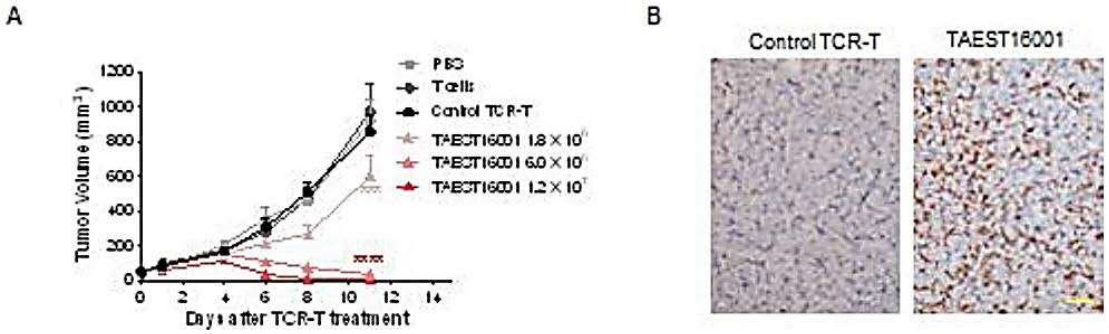


Figure 3: In vitro safety evaluation of TAEST116001.

TAEST116001 had no nonspecific response to a series of peripheral blood mononuclear cells from healthy people (a) and normal cells from different tissue (b). A375 as positive control target cells, MEL526 as negative control target cells, and A6 and GFP as negative control effector T cells.

Animal tumor models demonstrate that TAEST16001 can migrate to tumors and significantly inhibit tumor cell growth with the corresponding tumor antigen expression (Figures 4).



.Figure 4: TAEST16001 inhibits the growth of non-small cell non-small cell lung cancer (NSCLC).

(A) H1299 A0201 non-small cell lung cancer (NSCLC) cells were inoculated into NOD/SCID/IL2gR-/- mice, and the mice were treated with the indicated doses of TAEST16001 after tumor formation. Vehicle control (PBS), T cells not transduced with TCR and T cells transduced with A6 TCR (control TCR-T) were used as controls, 5 mice per group (N=5). 1.2×10^{7}and 6 × 10^6 dose groups: TAEST16001 and the control TCR group was significantly different from day 6 to day 11 (P<0.0001, two-way ANOVA). 1.8 × 106 dose group: TAEST16001 and control TCR groups was significantly different from day 8 to day 11 (P < 0.001, two-way ANOVA). (B) NOD/SCID/IL2gR-/- mice engrafted with H1299 A0201 non-small cell lung cancer (NSCLC) cells were treated with 6×10^6 TAEST16001 or control TCR-T. Tumor sections were collected 48 hours later and stained for CD8 by immunohistochemistry. TAEST16001 cells, but not control TCR-T cells, were able to infiltrate the tumor environment. Scale bar

1.2.4 Indications

In order to meet the urgent clinical needs, according to the pharmacological characteristics of TAEST6001 TCR-T, the applicant believes that soft tissue sarcoma will be one of the preferred indications. Soft tissue sarcoma refers to malignant tumors that originate from any kind of extramal soft tissue tumors, including malignant tumors of fibrous tissue, synovial tissue, smooth muscle tissue, striated muscle, adipose tissue, vascular tissue and the like, and malignant tumors of neuroectodermal origin Tissue tumors are similar to soft tissue tumors and are therefore classified as soft tissue sarcomas. It is generally believed that the occurrence of soft tissue sarcoma may be related to radiation, genetic susceptibility and chemical carcinogens contact, but the vast majority of soft tissue sarcoma still cannot find a clear cause. Soft tissue sarcoma can occur in any part of the body, but occurs in the limbs of soft tissue sarcoma accounts for about 50%. According to the probability incidence of different parts of the body:lower extremity, trunk, upper extremity, head and neck, retroperitoneal. Soft tissue sarcoma accounted for only about 1% of all malignant tumors. According to the domestic statistics, the incidence of soft tissue sarcoma was 1.28-1.72 / 100000, which is slightly higher in men than women and mostly is adult young patients with middle-aged. The peak age is between 20 and 50 years old.

Soft tissue sarcoma has more than 50 tissue subtypes, the most important of which

include liposarcoma, synovial sarcoma, leiomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma and malignant schwannoma, Ewing family tumors (including Ewing sarcoma and peripheral primitive Neuroectodermal tumors), rhabdomyosarcoma and small round cell tumor. Surgical treatment of soft tissue sarcoma is still the most important means. So far, for resectable soft tissue sarcomas, extensive surgical excision combined with radiotherapy is the primary choice for first-line treatment. However, in spite of the improvement of the local control rate, more than 50% of the cases still have tumor progress and distant metastasis. Chemotherapy is the standard treatment for advanced soft tissue sarcomas in distant metastases, but the role of chemotherapy is controversial in patients with soft tissue sarcomas not distant metastases.

This is an open-label, single-arm, dose-escalation, early-stage study investigating safety and tolerability. The dose escalation study is planned to include patients with advanced malignant solid tumors with positive expression of tumor antigen NY-ESO-1 (genotype: HLA-A*02:01), including soft tissue sarcoma, primary liver malignancy, ovarian malignancy, non-small cell lung cancer (NSCLC), breast cancer, etc., mainly patients with soft tissue sarcoma. The expanded enrollment study is planned to include patients with advanced soft tissue sarcoma with positive expression of tumor antigen NY-ESO-1 (HLA-A*02:01).

1.3. Similar clinical studies

Dr. Morgan from Rosenberg Laboratories first published the results of a clinical trial of TCR gene therapy on cancer patients in Science in 2006. This trial is carried out in 15 patients with metastatic melanoma who were ineffective in standard treatment. In vitro, retroviral vectors are used to transduct TCRs that recognize MART-1 antigen into T cells of patients and are returned to the patient. The TCR-T cells expressing MART-1 persist in the patient for more than 90 days, Tumors in 2 patients were completely suppressed and disease-free survived for at least 18 months. The MART-1 TCR gene was detected in the patient's peripheral blood for over 1 year. This trial first demonstrated the feasibility of treating tumors with the TCR gene .

In 2009, Dr. Johnson at the same lab reported a clinically treated melanoma assay with a high avidity TCR recognizing MART-1 and a TCR of gp100 with a response rate of 30% for TCR-T in MART-1 / 20), whereas the response rate of gp100 TCR-T was 19% (3/16). However, the gp100 TCR-T developed a side reaction in which melanocytes of the skin, eyes and ears were destroyed.

Next, Dr. Robbins and colleagues from the same laboratory reported in Journal of Clinical Oncology (2011) and Clinical Cancer Research (2014) respectively that anti-NY-ESO-1 antigen specific TCR autologous T cells were treated with synovial sarcoma and melanoma with response rates of 61% (11/18) and 55% (11/20), respectively.

In 2015, TCR-T's cancer treatment achieved a breakthrough and gratifying effect. Clinical I / II with high-affinity anti-NY-ESO-1 and LAGE-1-specific TCR-T for multiple myeloma in the world-class journal Nature Medicine at the University of Pennsylvania,

University of Maryland and Adaptimmune, The phase-I/II trials (the high-affinity anti-NY-ESO-1-specific TCR was developed by Dr. Li Yi in 2005 using phage display technology) showed that 16 of 20 patients (80%) had a clinically effective treatment with an average of none Survival of the disease was 19.1 months, and the side effects of light, there is no serious side effects of CAR-T treatment. These clinical data indicate that TCR-T cells treat tumors that can target solid tumors in a variety of tumors.

However, the treatment of bone and soft tissue sarcoma with TCR-T has not been reported, especially for high-affinity anti-NY-ESO-1 specific TCR-T treatment of bone and soft tissue sarcoma should be the first time.

1.4. Risk-benefit assessment

1.4.1. Potential risk

The potential risks of this study include:

1) ≤grade 4 fever;

2) ≤Grade 2 shivering lasting less than 72 hours (in the case of intervention treatment);

3) Tachycardia, unresponsive to intervention therapy, lasting for 72 hours;

4) Hypotension, unresponsive to intervention therapy, lasting for 72 hours;

5) ≤grade 3 nausea and vomiting, lasting less than 48 hours;

6) ≤grade 3 neurotoxicity;

7) Cytokine release syndrome

a) Grade 2 CRS, lasting for 5 days (in the case of intervention therapy);

b) Grade 3 or 4 CRS manifesting as hypotension only, improving to below Grade 3 within 72 hours after treatment with a vasopressor (no mechanical ventilation required);

c) Grade 3 or 4 hypotension without other symptoms of CRS, which can be improved to below grade 3 within 72 hours after treatment with a vasopressor;

d) Grade 3 encephalopathy with duration ≤7 days and complete recovery within 28 days.

The above-mentioned expected adverse reactions caused by TAEST16001 treatment do not require treatment such as hormones that affect the proliferation and survival of TCR-T in vivo, and can be improved with appropriate treatment.

See Appendix 5, Appendix 6, Appendix 7 and Appendix 8 for safety risks.

1.4.2. Unknown risks

At present, it is unknown whether patient's own factors such as tumor burden, previous treatment, genetic susceptibility are related to the severity of CRS. In addition, it is unknown whether the infusion dose of TCR-T cells is related to the severity of CRS, and whether the duration and intensity of CRS are related to tumor response. However, there is evidence that CRS occurs in patients who are clinically responsive to TCR-T therapy, but it remains unclear whether hormone treatment of CRS/NT will weaken the therapeutic effect of TCR-T.

1.4.3. Potential benefits

According to the previous human studies of this product (see the Investigator's Manual for details) and the results of preclinical studies, this product is expected to have therapeutic effect in patients with advanced malignant solid tumors (including soft tissue sarcoma, primary liver malignancies, ovarian malignancies, non-small cell lung cancer (NSCLC), breast cancer, etc., mainly in patients with soft tissue sarcoma)with positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01). Patients with unresectable advanced solid tumors who have failed standard treatment (disease progression or recurrence or intolerable, such as chemotherapy, radiotherapy, targeted therapy) or lack effective treatment methods may benefit from the efficacy.

2. Study Objective and Study Endpoints

2.1. Study Objective

2.1.1 Primary objective

To evaluate the safety and tolerability of TAEST16001 cells in the treatment of advanced malignant solid tumors such as soft tissue sarcomas with positive expression of tumor antigen NY-ESO-1 (HLA-A*02:01).

2.1.2 Secondary objective

- To describe the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of TAEST16001 cells after infusion into humans, observe their proliferation and persistence in vivo, and their effects on human immunological activity;
- To preliminary evaluation of the efficacy of TAEST16001 cells in the treatment of patients with advanced malignant solid tumors such as soft tissue sarcoma with positive expression of tumor antigen NY-ESO-1 (genotype: HLA-A*02:01) according to RECIST1.1 criteria.

2.1.3 Exploratory objective

- To preliminary evaluate the efficacy of TAEST16001 cell in the treatment of patients with advanced malignant solid tumors such as soft tissue sarcoma with positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01) according to the iRECIST criteria.
- To evaluate the effect of TAEST16001 cell therapy on the quality of life of patients.

2.2. Study Endpoints

2.2.1 Primary endpoint

- Maximum tolerable dose (MTD) and dose limiting toxicity (DLT);
- Incidence of adverse events (AE), serious adverse events (SAE), adverse event of special interest (AESI) (including CRS, neurotoxicity), laboratory tests (type, frequency and severity), and abnormal ECG and vital signs.

2.2.2 Secondary endpoint:

- After infusion of TAEST16001 cells, the peak value (Cmax), peak time (Tmax) and AUC0-28 of TAEST16001 cells in peripheral blood. If possible, AUC0-inf, terminal phase elimination rate constant (λz), and elimination half-life (t1/2) will be evaluated.
- Immunological activity: T cell subsets, peripheral blood antigen-specific CTL,

effector cell activity.

- Objective response rate (ORR), disease control rate (DCR) and progression free survival (PFS) assessed according to RECIST1.1

2.2.3 Exploratory endpoint

- Objective response rate (ORR), disease control rate (DCR) and progression free survival (PFS) evaluated according to iRECIST standard;
- EORTC QLQ-C30 scale score.

3. Study design

3.1. Basis of study design

This is an study evaluating the safety and tolerance of TAEST16001 injection in the treatment of patients with advanced malignant solid tumors (including soft tissue sarcoma, primary liver cancer, ovarian cancer, non-small cell lung cancer (NSCLC), breast cancer, mainly soft tissue sarcoma) with positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01).

The design of this study is in accordance with the "Administrative Measures for Drug Registration" (2007), "Good practice for drug clinical research" (Draft for Comment) (2015), and “Technical Guiding Principles for Research and Evaluation of Cell Therapy Products (Trial)” (2017) issued by the National Medical Products Administration (NMPA). The results of this clinical study are the basis for additional studies in the future.

3.2 Overall design

This study is an open-label, single arm, dose increasing early clinical study, which is divided into two parts: "3+3" designed dose escalation study and expanded enrollment study. This study is to evaluate the safety, tolerance, PK and PD characteristics, and preliminary effectiveness of TAEST16001 cells in treating patients with advanced malignant solid tumors, mainly soft tissue sarcomas, with positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01).

This study is planned to recruit 12-30 patients with advanced malignant solid tumors with positive expression of tumor antigen NY-ESO-1 (genotype: HLA-A*02:01). The dose escalation study is planned to include patients with advanced malignant solid tumors with positive expression of tumor antigen NY-ESO-1 (genotype: HLA-A*02:01), including soft tissue sarcoma, primary liver malignancy, ovarian malignancy, non-small cell lung cancer (NSCLC), breast cancer, etc., mainly patients with soft tissue sarcoma. The expanded enrollment study is planned to include patients with advanced soft tissue sarcoma with positive expression of tumor antigen NY-ESO-1 (HLA-A*02:01).

Patients eligible for screening (including genotype, tumor antigen screening, and primary screening) will go through 3 study phases: screening period, treatment and observation period, and follow-up period.

(1) **Screening period** (pre-screening to study day -1): patients who are qualified for genotype and tumor antigen screening (pre-screening) were subjected to leukocyte apheresis for the preparation of TAEST16001 cells, while patients enter the main screening and lymphodepleting chemotherapy.

Patients in the dose escalation study and expanded enrollment study received lymphodepleting chemotherapy 7 days before the first time of TAEST16001 cell infusion (study day -7): cyclophosphamide (15mg/kg/d) and fludarabine (20

mg/m2/d), for 3 days. **Patients who received lymphodepleting chemotherapy were defined as enrolled patients.**

(2) **Treatment and observation period** (study day 1-28): intravenous infusion of TAEST16001 cells at a single or divided times of the total dose, DLT observation (the dose escalation study) or treatment observation (the expanded enrollment study), and the first efficacy evaluation. Patients in the dose escalation study and the expanded enrollment study received intravenous infusion of TAEST16001 cells on day 5 after lymphodepleting chemotherapy (at an interval of 4 days): if the infusion dose level was 1 and 2, the total amount of TAEST16001 cells (calculated as TCR-T positive cells) was infusion on the first day of the study; If the infusion dose level was 3 and 4, the total amount of TAEST16001 cells (calculated as TCR-T positive cells) was planned to be infusion at the ratio of 60% and 40% on the first and second days of the study. After the first infusion of TAEST16001 cells, patients will be subcutaneously injected with low-dose IL-2 (from the 1st day to the 14th day of the study), 500,000u/time. 12h). The first injection will be carried out within 30 minutes after the infusion of cells, twice a day (with an interval of 10-12h) for 14 days.

The safety and tolerance were observed before, during and after intravenous infusion (study day 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28) until 28 days after the first cell infusion. Among them, the DLT observation period is from the first cell infusion to 28 days after infusion for the patients enrolled in the dose escalation study. The first efficacy evaluation will be conducted on the 28th day after the first cell infusion (study day 28).

(3) **Follow-up period** (study day 29-270 [9 months]): safety and efficacy observation.

Safety: All AEs and SAEs will continue to be collected. Unless subsequent anti-tumor treatment is started due to ineffectiveness or disease progression from 90 days (3 months) to 270 days (9 months) after TAEST16001 cells infusion, from the beginning of replacement of anti-tumor treatment to the end of the study, all AEs will not be collected, only those related to treatment and all SAEs will be collected.

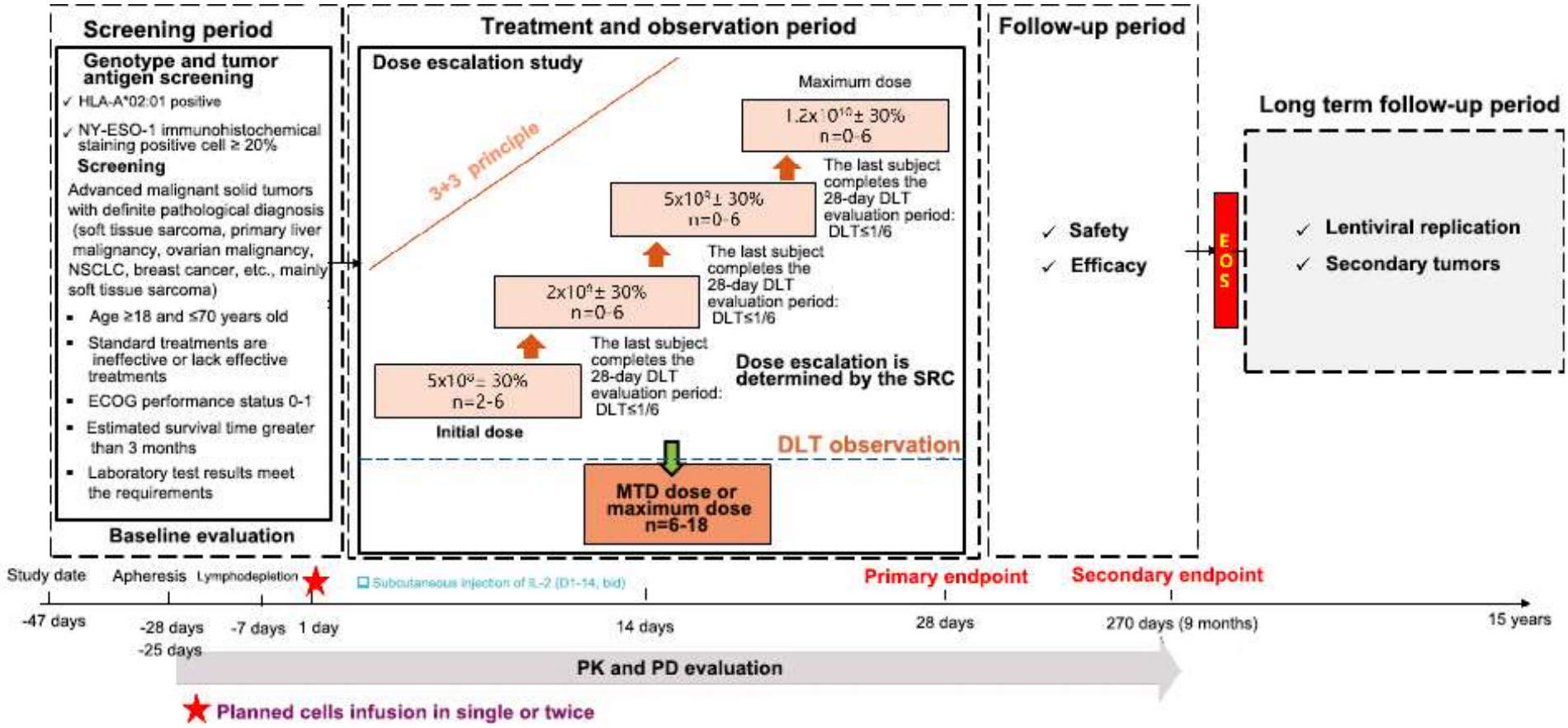
Efficacy: After entering the follow-up period, patients will be evaluated for tumor once at 2 months, 3 months, 6 months and 9 months after the first infusion of TAEST16001 cells, unless the patient started subsequent antitumor therapy is due to treatment failure/disease progression, withdrawal from the study, death or loss to follow-up, whichever occurred first.

The end of study (EOS) is defined as completion of protocol-specified follow-up (within 270 days [9 months] after infusion of TAEST16001 cells) for all subjects, early withdrawal from the study for any reason other than DLT, death or loss to follow-up, whichever occurs first.

Thereafter, patients will enter a long-term safety follow-up of up to 15 years for lentiviral replication and secondary tumor. The frequency is once a year from the 270 day of the study to 5 years after the cell infusion, and once every 3 years from 5 years to 15 years after the cell infusion.

See the overall study design diagram for details.

3.3 Study Design Diagram



3.4. Dose Escalation Study Design

3.4.1. Design basis for initial dose, maximum dose, and dose escalation group

According to an article published by Professor NCI Rosenberg in "Clinical Cancer Research" in 2014, the infusion dose of CD3+ T cells is between 8×10^9-13×10^{10}, and the ratio of NY-ESO-1 specific TCR expressed on the surface of transfected autologous T cells is between 18% and 90%, which varies from person to person. Therefore, the effective dose is between 1.44×10^9 and 11.7×10^{10}, and calculated as TCR-T positive cells. According to the results of previous human studies (see IB for details), the number of TCR-T-positive cells infusion was between 3.5×10^8 and 2.9×10^{10}. Based on safety considerations, in this clinical study, the dose of 5×10^8±30% TCR-T positive cells was used as the initial dose, and 1.2×10^{10}±30% was used as the maximum dose. The dose range was within the dose range used in previous studies in human. At the same time, the dose was increased by 4 times, 2.5 times and 2.4 times according to the principle of "fast firstly and then slow". If the maximum tolerated dose (MTD) was not explored in this study at 1.2×10^{10}±30%, no further dose escalation will be performed. See section 3.4.2 for specific dose groups.

3.4.2. Dose escalation study design

The dose increase is carried out according to the "3+3" increasing principle. A total of 4 dose levels are set (calculated as TCR-T positive cells):

dose level 1: $5\times10^8 \pm 30\%$;
dose level 2: $2\times10^9 \pm 30\%$;
dose level 3: $5\times10^9 \pm 30\%$;
dose level 4: $1.2\times10^{10} \pm 30\%$。

Three patients were enrolled first. If there is no DLT, they are enrolled in the next higher dose group; If one of the three patients in a dose group has DLT, the group will supplement three patients with the same dose. If DLT occurs in more than or equal to 1 of the 3 supplementary cases, the dose increase shall be stopped. The previous dose of this dose is defined as MTD; if DLT not occurs in 3 supplementary cases, the dose would be increased to the next group. Dose escalation is not allowed for the same patient.

In the dose level 1 and dose 2 groups, each patient needs to be observed for at least 14 days after receiving TAEST16001 cell infusion. If there is no DLT, the next patient in the same dose group is allowed to undergo cell infusion. In the dose level 3 and 4 groups, each patient needs to be observed for 14-28 days after receiving TAEST16001 cell infusion (the specific interval can be evaluated and adjusted by the researcher according to the cumulative safety results of patients in the dose level 1 and 2 groups). If there is no DLT, the next patient in the same dose group is allowed to undergo cell infusion. After receiving the first infusion of TAEST16001 cells, the last patient in the previous dose group was observed for at least 28 days, If there is no DLT, the first patient in the next dose group was allowed to undergo cell infusion after discussion and decision by the safety review committee (SRC).

MTD was defined as the previous lower dose of the dose group in which ≥2/6 patients experienced DLT.

If no MTD was detected at dose level 4 ($1.2 \times 10^{10} \pm 30\%$) in this study, no further dose

escalation will be performed.

In the course of dose increase, during the 28-day DLT observation period, if the patient withdraws from the DLT observation period due to reasons other than DLT and fails to complete the DLT observation period, the patient will be replaced and one additional case needs to be included.

3.5. Definition of Dose Limiting Toxicity

DLT is defined as the following adverse events that occur within 28 days after the completion of the first cell infusion in the dose escalation study and are were determinedto be related to cell therapy by the Safety Review Committee (SRC) (CRS and neurotoxicity are graded according to ASTCT 2018 standard, and the remaining AES will be evaluated according to CTCAE version 5.0 standard):

1. Grade 4 hematologic toxicity (except lymphopenia) not caused by underlying disease persists for more than 28 days from the date of cell infusion;
2. All Grade 3 CRS lasting >7 days and all Grade 4 CRS;
3. All grade 3 non-hematological toxicities lasting >7 days and all grade 4 non-hematological toxicities regardless of duration, except for the following:
   Grade 3 nausea and/or decreased appetite;
   - Aphasia/language impairment or blurred consciousness / cognitive impairment recovering to grade 1 or below within 2 weeks, or to baseline within 4 weeks;
   - Airway protection requires intubation ≤7 days;
   - Nephrotoxicity requiring dialysis ≤7 days;
   - Grade 3 alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphat-
     ase, elevated bilirubin, or other abnormal liver function, recovering to grade 2 or below within 14 days;
   - Grade 4 transient liver function abnormalities, recovering to Grade 3 or below within 72 hours;
   - Grade 3 or 4 fever;
   - Immediate hypersensitivity (related to cell infusion) occurred within 2 hours of cell infusion. After standard treatment, the hypersensitivity was reversed to grade 2 or below within 24 hours after taest16001 infusion.
4. Considering to be other AEs of DLT after SRC discussion

3.6. Definition of Maximum Tolerated Dose (MTD):

In the dose escalation study, the previous lower dose of the dose group in which ≥2/6 patients experienced DLT.

3.7. Expanded Enrollment Study Design

Under the premise of dose escalation study, with the consent of the investigator and the sponsor, and based on the cumulative safety, pk/pd, preliminary efficacy and other relevant data, after communication and approval with the regulatory authority (NMPA), about 6-18 patients with advanced soft tissue sarcoma with positive expression of tumor antigen NY-ESO-1 (genotype HLA-A*02:01) were enrolled at MTD dose level or dose level 4 (if MTD was not explored), to further evaluate the safety, tolerability, PK/PD characteristics and efficacy of TAEST16001 cell infusion.

3.8 Pharmacokinetic (PK) and Pharmacodynamic (PD) Study Design

Pharmacokinetic (PK) and pharmacodynamic (PD) studies will be conducted simultaneously during the dose escalation and expanded enrollment study. Blood samples of the enrolled patients will be collected at the designated time point (see the research flow chart for details) for PK and PD evaluation after single or fractional TAEST16001 cell infusion in total doses. PK/PD evaluation of TAEST16001 cells after infusion into human body was conducted according to the following criteria:

PK sample collection: detect taest16001 cells in peripheral blood. Collect them until 9 months after the first infusion of cells (study day 270). Tcr-t cells are not detected by flow cytometry for two consecutive times. The patient withdraws from the study, dies or loses follow-up for any reason, whichever occurs first; The copy number of tcr-t DNA detected by qPCR was collected until 9 months after the first infusion of cells (day 270 of the study), and the patient withdrew from the study, died or lost the follow-up for any reason, whichever occurred first. PD sample collection: until 9 months after the first infusion of cells (day 270 of the study), the patient withdraws from the study, dies or loses follow-up for any reason, whichever occurs first.

PK sample collection: Peripheral blood TAEST16001 cells are detected until 9 months after the first infusion of cells (study day 270), no TCR-T cells are detected by flow cytometry for two consecutive times, and patient withdraws from the study, dies or loses follow-up for any reason, whichever occurs first. The copy number of TCR-T DNA is detected by qPCR until 9 months after the first infusion of cells (day 270), patient withdraws from the study, dies or loses follow-up for any reason, whichever occurs first. PD sample collection: Until 9 months after the first infusion of cells (study day 270), or patient withdraws from the study, dies or loses follow-up for any reason, whichever occurs first.

The number of patients with complete PK and PD parameters collected in each dose group should be greater than or equal to 3. If the number of subjects whose complete PK parameters are collected is less than the number required by the protocol, after discussion between the sponsor and the investigator, it will be decided whether additional patients need to be enrolled.

4. Subject Selection

4.1 Inclusion Criteria

Patients must meet the inclusion criteria with * before genotype and tumor antigen detection, and all inclusion criteria must be met:

Patients must meet all of the following criteria to be enrolled in this study:

1. An informed consent form (ICF) should be signed before any research-related operations (genotype and tumor antigen screening and primary screening);
2. *Age ≥18 years old and ≤70 years old;
3. * Advanced malignant solid tumors with definite pathological diagnosis,

Note: The dose escalation research part intends to include patients with solid tumors such as soft tissue sarcoma, primary liver malignancy, ovarian malignancy, non-small cell lung cancer (NSCLC), breast cancer, etc., mainly patients with soft tissue sarcoma. Patients with soft tissue sarcoma will be included in the expanded study.

4. Unresectable advanced solid tumors that have failed to standard treatment (disease progression or recurrence or intolerance, such as chemotherapy, radiotherapy, targeted therapy, etc.) or lack effective treatment methods, including but not limited to the following tumor types:

1) Soft tissue sarcoma:

a) Soft tissue sarcomas failed to be treated with chemotherapy containing doxorubicin and ifosfamide;

2) Primary liver malignancies:

a) Child-Pugh liver function score grade A within 7 days before cell infusion;

3) Ovarian malignant tumor:

a) Failed to platinum based chemotherapy (such as paclitaxel combined with carboplatin).

4) Non-small cell lung cancer (NSCLC):

a) Failed (disease progression or toxicity intolerance) to previous standard therapy (platinum-containing chemotherapy regimen or driver gene-targeted therapy) or lack of effective treatment;

5) Breast cancer:

a) Patients who received standard treatment failed or did not suitable for standard treatment.

5. At least 1 measurable lesion (according to RECIST1.1 criteria [see Appendix 4 for details]);
6. Genotype and tumor antigen screening must meet the following two criteria:
   1) HLA-A*02:01 positive;
   2) Positive expression of NY-ESO-1: immunohistochemical staining positive cells ≥ 20%;
7. *ECOG score 0-1 and estimated survival time greater than 3 months;
8. Color-Doppler-Echocardiography indicates that the left ventricular ejection fraction is greater than or equal to 50%;
9. Laboratory inspection results should at least meet the following specified indicators:
   - White blood cell count ≥3.0×10^9/L;
   - Absolute neutrophil count (ANC)≥1.5×10^9/L (without G-CSF and GM-CSF support, at least 14 days before lymphodepleting chemotherapy);
   - Absolute lymphocyte count (ALC) ≥ 0.7×10^9/L;
   - Platelet (PLT) ≥ 75×10^9/L (no blood transfusion therapy 14 days before lymphodepleting chemotherapy);
   - Hemoglobin ≥ 9g/dL (no blood transfusion therapy 14 days before lymphodepleting chemotherapy);
   - International normalized ratio (INR) ≤1.5×ULN, unless receiving anticoagulant therapy;
   - Activated partial thromboplastin time (APTT)≤1.5×ULN, unless receiving anticoagulant therapy;
   - Serum creatinine≤1.5mg/dL (or 132.6μmol/L)
   - Creatinine clearance rate ≥60mL/min;
   - Aspartate aminotransferase (AST/SGOT)≤2.5×ULN;
   - Alanine aminotransferase (ALT/SGPT)≤2.5×ULN;
   - Total bilirubin (TBIL)≤1.5×ULN;

   Note: For patients with liver metastases or patients with primary liver tumor lesions, aspartate aminotransferase and alanine aminotransferase should be ≤5×ULN.
10. * Women of childbearing age who have not undergone sterilization before menopause must agree to take effective contraceptive measures from the beginning of study treatment (lymphodepleting chemotherapy) to one year after the last cell transfusion, and the serum pregnancy test is negative within 14 days before the first time of cell infusion.
11. * Men who have not undergone sterilization surgery must agree to use effective contraceptive measures from the beginning of the study treatment (lymphodepleting chemotherapy) until one year after the last cell infusion.

4.2 Exclusion Criteria

The exclusion criteria with * need to be verified before the patients are tested for genotype and tumor antigen, and all exclusion criteria need to be verified for enrollment

Patients who met any of the following criteria were not eligible for this study:

1. Received the last dose of anti-tumor therapy (chemotherapy, endocrine therapy, targeted therapy, immunotherapy, tumor embolization or Chinese medicine/Chinese herbal medicine treatment with anti-tumor indications, etc.) within 4 weeks before cell infusion;
2. Received live attenuated vaccine within 4 weeks before cell infusion;
3. * Any ingredient used in the treatment of this study is known to cause allergic reactions;
4. No recovered to <2 grade CTCAEv5.0 from previous surgery or treatment-related adverse reactions;
5. *Patients with a history of meningeal metastases or central nervous system metastases in the past, or patients with clear underlying diseases of the central nervous system and left significant symptoms within 6 months before cell infusion;
6. Hypertension with poor drug control (systolic blood pressure>160mmHg and/or diastolic blood pressure>90mmHg) or cardiovascular and cerebrovascular diseases with clinical significance (such as active disease), such as cerebrovascular accident (within 6 months before signing the master informed consent), myocardial infarction (within 6 months before signing the master informed consent), unstable angina, congestive heart failure with New York Heart Association (NYHA) class II or above, or severe arrhythmia that can not be controlled with drugs or have potential impact on study treatment; ECG results show clinically significant abnormality or average QTcF ≥ 450ms in 3 consecutive times (at least 5 minutes between each time) (see Appendix 2 for the formula);
7.* Complicated with other serious organic diseases or mental diseases;
8. Patients with systemic active infections requiring treatment, including but not limited to active tuberculosis, known HIV positive patients or patients with clinically active hepatitis A, B and C, including virus carriers, shall be excluded;
9.* Suffering from autoimmune diseases: Patients with a history of inflammatory bowel disease and autoimmune diseases determined by the researcher to be unsuitable for this study, such as systemic lupus erythematosus, vasculitis and invasive lung disease, should be excluded (except for vitiligo subjects);
10. Within 4 weeks before cell infusion and during the study period, it is planned to use (if there is long-term use) systemic pinesterols, hydroxyurea and immunomodulatory drugs (such as: α or γ interferon, GM-CSF, mTOR inhibitor, cyclosporin, thymosin, etc.);
11. *History of organ allotransplantation, allogeneic stem cell transplantation and renal replacement therapy;
12. * Known uncontrolled diabetes, pulmonary fibrosis, interstitial lung disease, acute lung disease or liver failure;
13.* Known alcohol and/or drug abuser;
14. * Pregnant or lactating women;
15. Subjects with any co-existing medical conditions or diseases that the investigator judges may affect the development of this trial;
16.*Subjects without legal capacity/restricted capacity;
17. Patients who have received similar gene therapy products before cell infusion, and the researchers believe that they are not suitable for enrollment;
18. Patients who are judged by the investigator to be difficult to complete all visits or operations required by the research protocol (including the follow-up period), or who have insufficient compliance to participate in this study; or patients who are deemed unsuitable for inclusion by the investigator.

4.3. Study termination and withdrawal

4.3.1. Termination of study

Termination of the study refers to the early termination of the treatment specified in the

protocol during the clinical study. The main purpose is to protect the rights and interests of the subjects, ensure the quality of the study and avoid unnecessary economic losses.

Reasons for termination of the study include but are not limited to the following:

1) During the dose escalation phase, the dose escalation between all dose groups will be based on the occurrence of DLT within 28 days after the first infusion of TAEST16001 cells. During the dose escalation process, if 2 or more subjects in any dose group have DLT, the dose escalation study will be terminated.
2) If serious safety problems occur during the research, the research should be terminated in time;
3) The quality of existing clinical data is poor, which is not conducive to the follow-up study;
4) Termination of research due to changes in regulations and policies of the National Drug Administration
5) The sponsor requests suspension (such as financial reasons, management reasons, etc.).

4.3.2. Subject withdrawal from treatment/study

Patients not complete the study protocol, including TAEST16001 cell infusion and follow-up assessments, are considered to have withdrawn from the study early. Reasons for withdrawal (eg, voluntary withdrawal, toxicity and death) must be recorded in the CRF and kept for a specified period of time as required by Good Clinical Practice (GCP). Possible reasons for early withdrawal include:

1) The investigator judges that the symptoms are aggravated;
2) Serious violations of the plan include but are not limited to:
   a) Fail to meet the inclusion / exclusion criteria and be entered by mistake;
   b) Combined use of prohibited drugs in this protocol.
3) The subjects do not cooperate with the treatment and follow-up;
4) Loss of follow-up;
5) Death of subjects;
6) pregnancy events;
7) The disease progressed rapidly before the infusion of TAEST16001 cells, and the investigators judge that other treatment regimens needed to be replaced based on the consideration of the maximum benefit of the subjects;
8) Lack of efficacy, the investigator determines that the subject has not benefited from the treatment, and continued participation in the study may put the patients at unacceptable risk;
9) Safety reasons considered by the researchers;
10) Subject voluntary discontinuation (subject is free to withdraw from study treatment or evaluation at any time);
11) The researcher judges the progress of the subject's disease, including PD and iCPD (PD: using RECIST1.1 evaluation standard; iCPD: using iRECIST evaluation standard)

Procedure for study termination/withdrawal

Subjects have the right to withdraw from the study (including TAEST16001 cell infusion and follow-up evaluation) at any time during the trial without any discrimination. Subjects withdrawing from the study should be asked about the reason for discontinuation and any adverse events. If possible, these patients should be evaluated by investigators and should be followed up for adverse events.

1) Those who withdraw from the study before TAEST16001 cell infusion will no longer conduct TAEST16001 cell infusion and follow-up examinations;
2) Subjects withdrawn from the study following TAEST16001 cell infusion, at the time of withdrawal, all AEs and SAEs pre-existing possibly or definitely related to TAEST16001 cell infusion must be followed until disappearance unless, in the opinion of the investigator,

the condition cannot disappear due to the presence of disease. In addition, unless the subjects explicitly require the researchers not to use the previously collected research data, these data should be included in the safety data set for analysis.

4.4 The definition of end of study

End-of-study (EOS) was defined as completion of protocol-specified follow-up (within 270 days [9 months] after infusion of TAEST16001 cells) for all subjects, early withdrawal from the study for any reason other than DLT, death or loss to follow-up, whichever occurs first.

5. Study Treatment

5.1. Lymphodepleting chemotherapy

The subjects received Lymphodepleting chemotherapy 7 days (D-7) before the infusion of TAEST16001 cells. The lymphodepleting chemotherapy regimen was FC regimen: cyclophosphamide (15 mg/kg/d) and fludarabine (20 mg/m^2/d) for 3 consecutive days.

If serum creatinine or creatinine clearance or glomerular filtration rate is abnormal on the day of lymphodepleting chemotherapy (before administration), if serum creatinine >1.5 times ULN, or creatinine clearance (Cockcroft-Gault formula) or radioisotope glomerular filtration rate (GFR)≤30mL/min/1.73m^2, chemotherapy should be suspended. If serum creatinine >2 mg/dL for more than 3 days, patients will be excluded from the study and regarded ed as dropout cases, and then screened and supplemented new cases.

During the period of lymphodepleting chemotherapy, if the subject's chemotherapy is interrupted due to fever, infection, cardiac insufficiency, liver and kidney dysfunction and other diseases, the investigator can decide whether to make up the subsequent chemotherapy course according to the degree of recovery of the subject's condition. If the subsequent chemotherapy course is made up within an acceptable time frame, it will not be considered a protocol violation. If lymphodepleting chemotherapy is only carried out for 1 or 2 days, and the subsequent lymphodepleting chemotherapy pretreatment is not performed: the investigator judges that the expected effect of lymphodepleting chemotherapy has been achieved, and subsequent chemotherapy does not need to be supplemented, which is not regarded as a protocol violation; It is up to the investigator to decide whether to enter the follow-up research process.

5.2 Standard for delayed cell infusion after lymphodepleting chemotherapy

If serious adverse reactions (such as heart, respiration, liver and kidney dysfunction) occur in patients receiving lymphodepleting chemotherapy, the infusion of TAEST16001 cells should be delayed, and the investigator should determine whether to delay the infusion time; if the delay exceeds 14 days, the investigator shall determine whether it is necessary to carry out lymphodepleting chemotherapy again. If the patient has symptoms such as uncontrollable infection and severe deterioration, the infusion of TAEST16001 cells should also be delayed.

In case of any of the above conditions, the investigator may decide to withdraw the patient from the study, and only collect and report all AEs within 28 days after the study related operation or treatment (such as leucocyte apheresis, lymphodepleting chemotherapy) or to the start of new anti-tumor treatment, whichever occurs first. Data related to efficacy evaluation will no longer be collected.

5.3. Other treatments before TAEST16001 cell infusion

In order to reduce the risk of infusion reaction as much as possible, it is recommended to give the patient 650mg of acetaminophen orally and 25-50mg of diphenhydramine hydrochloride (orally or intramuscularly) 30-60 minutes prior to the infusion of TAEST16001 cells. According to the investigator's assessment of symptoms, these drugs can be repeated every 6 hours as required. Prior intervention with steroid drugs should be avoided.

5.4 Dose and method of cell infusion

5.4.1 Dose of cell infusion

1) Dose escalation study:

Patients will receive 5×10^8 ±30%、2×10^9±30%、 5×10^9±30%, or 1.2×10^{10}±30% (calculated as TCR-T positive cells) TAEST16001 cells infusion, depending on the dose level (1-4).

2) Expanded enrollment study:

Patients will receive infusion of TAEST16001 cells (calculated as TCR-T positive cells) at the MTD dose level or at dose level 4: 1.2×10^{10}±30% (if no MTD is detected).

3) Infusion times:

- If the infusion dose levels are 1 and 2 (5×10^8±30% or 2×10^9±30%), the planned total amount of TAEST16001 cells (calculated as TCR-T positive cells) was infusion for a single time on the first day of the study;
- If the infusion dose levels are 3 and 4 (5×10^9±30% or 1.2×10^{10}±30%), the total amount of TAEST16001 cells (calculated as TCR-T positive cells) was planned to be infusion at the ratio of 60% and 40% on the first and second days of the study.

4) Application of IL-2:

After the first infusion of TAEST16001 cells, patients will be subcutaneously injected with low-dose IL-2 (from the 1st day to the 14th day of the study), 500,000u/time twice a day (with an interval of 10-12h), for 14 days. The first injection will be carried out within 30 minutes after the first cell infusion.

5.4.2. Infusion method

When re-infusion of TAEST16001 cells, use a disposable blood infusion set for administration, and the duration of infusion is about 30-60 minutes/100 mL of cell suspension.

Specific operation (see related SOP for details): One or two bags of antigen NY-ESO-1 specific TAEST16001 TCR-T cells will be stored in liquid nitrogen and delivered to the bedside of patients in the hospital, with a warm water bath at 36℃~38℃ for recovery. After complete thawing and resuscitation, the cells will be reinfused into the subject. Each infusion bag will be labeled with "self-use only". In addition, the label will identify at least two unique identifiers, such as the subject's initials, date of birth, and study number and subject number. Before infusion, two medical staff will independently verify the information on the infusion bag in front of the subjects to confirm that the information is correctly matched with the subjects.

In the process of infusion, emergency medical equipment (i.e. emergency cart) shall be prepared in the same observation room. The equipment will be used to deal with allergic reactions, or the crisis of severe hypotension and any other serious side effects. According to the study protocol (see the study flow chart for details), before and after infusion, observe the patient's vital signs (temperature, respiratory rate, pulse / heart rate and blood pressure) are observed until the vital signs stabilized. The patient is required not to leave the observation room until the investigator considers that the patient is in a safe state.

5.5. Concomitant medication

5.5.1 Permitted concomitant medications

Antiemetic and hemorrhagic cystitis prevention drugs, including granisetron or ondansetron, and mesna, are permitted before lymphodepleting chemotherapy.

The researcher is allowed to use (but not limited to) antibiotics, red blood cell infusion and platelet infusion, colony stimulating factors, bronchodilators, epinephrine or transfusions, colony-stimulating factors, bronchodilators, epinephrine, anti-inflammatory drugs, and other supportive treatments **after patient is enrolled**, according to the medical guidelines of the clinical research center.

The researcher is allowed to use, but not limited to, tocilizumab, glucocorticoid, in the treatment of drug-related AEs, such as CRS, after TAEST16001 cell infusion.

5.5.2 Prohibited/cautious medication

Pharmacological doses of corticosteroids (prednisone ≥ 20 mg/day or equivalent doses of other corticosteroids) and other immunosuppressive agents must be avoided within 7 days before leukocyte apheresis and within 5 days before the first infusion of TAEST16001 cells.

Corticosteroids and other immunosuppressive agents should be avoided within 3 months after the first infusion of TAEST16001 cells, unless they are used to control toxicity associated with TAEST16001 cell therapy. Other drugs that may affect the evaluation of the study, such as non-steroidal anti-inflammatory drugs, should also be avoided during this period unless necessary.

It is forbidden to receive other anti-tumor therapy (except lymphodepleting chemotherapy) from leukocyte apheresis until disease progression or study completion, including but not limited to chemotherapy, immunotherapy, targeted drugs, radiotherapy, autologous/allogeneic hematopoietic stem cell transplantation, and other clinical trial drugs. Traditional Chinese medicine / Chinese herbal medicine with anti-tumor indications cannot also be used.

5.6. Dosage adjustment

Patients should receive single or divided infusions of TAEST16001 cells in strict accordance with the cell dose assigned by the protocol.

5.7. Treatment discontinuation

5.7.1. Permanently stop infusion of TAEST16001 cells

In some cases, patients may need to permanently discontinue infusion of TAEST16001 cells after completion of leukocyte apheresis or lymphodepleting chemotherapy. All AEs reported up to 28 days after study-related procedures or treatments (eg, leukopheresis, lymphocytosis chemotherapy) or initiation of new antitumor therapy should be collected according to the study protocol, whichever occurs first. Data related to efficacy assessments are no longer collected.

5.7.2. If the production of TAEST16001 cells does not reach the specified dose

If the production dose fails to meet the set dose of the dose group, then:

- The patient should withdraw from the study and a new patient should be enrolled in this dose group to complete the study evaluation;
- Only collect all AES reported by the patient within 28 days after the last study related operation or treatment (such as leucocyte apheresis, lymphocyte clearance chemotherapy) or until the start of new anti-tumor treatment, whichever occurs first;
- Investigators need to provide patients with reasonable subsequent treatment recommendations.

5.8. Treatment after study

After the end of the study or withdrawal from the study, the investigators need to provide

reasonable subsequent treatment suggestions for the patients.

6. Research Process

This study is divided into 3 phases: screening period, treatment and observation period, and follow-up period. Patients need to be admitted to the ward during lymphodepleting chemotherapy and on the day of cell infusion, and the rest of the time is determined by the investigator.

6.1. Screening period: prescreening ~ study day -1

6.1.1. Genotype and tumor antigen screening: V1 (prescreening)

After obtaining the ICF signature of the subject for gene and tumor antigen detection, continue the follow-up clinical research procedures / steps:

1) Sign the pre-screening and tumor antigen detection informed consent;
2) Genotype and tumor antigen detection:
   HLA genotype detection: 2ml whole blood samples of the patient need to be collected and sent to a third-party central laboratory for detection;
   Tumor antigen marker detection: need to be performed after determining the HLA genotype as A*02:01. Patients are required to provide 3-5 unstained sections or consent to biopsy. The test is completed by the local laboratory of the clinical research center; if the test results are affected by problems such as the quality of the slices, the local laboratory of the clinical research center will decide whether it needs to be re-tested.
3) Demographic data: date of birth, gender, ethnicity, etc.;
4) Past medical history: Past medical history includes cardiovascular, pulmonary, gastrointestinal, liver and other related medical history and surgical history.
5) History of tumor disease: collect history of solid tumor, including but not limited to:
   Tumor diagnosis at initial diagnosis and screening;
   Previous antitumor drug treatment (including treatment scheme and drug name, treatment start and end date, reasons for discontinuation of drug treatment, best effect obtained by each treatment scheme), radiotherapy (including body site, total dose, unit, start and end time, reason for treatment, best response achieved and date of radiation site progression during or after treatment) and other treatments.
6) Review of inclusion and exclusion criteria: Only the items marked with * in the inclusion and exclusion criteria will be reviewed. See sections 4.1 and 4.2 for details.
7) SAE: Collect all SAEs related to operations of this study.

6.1.2. Primary Screening: V2 (Study Days -25 ~ -8)

For subjects whose genotypes are HLA-A*02:01 positive and tumor antigen positive (immunohistochemical results show that the positive cells ≥ 20%), after signing the main ICF, the subjects will receive leukocyte apheresis for the preparation of TCR -T cells.

The following information/assessments will be collected during this visit:

1) Review of inclusion and exclusion criteria: review all items in the inclusion and exclusion criteria, sections 4.1 and 4.2;
2) Height and weight;
3) Physical examination;
4) Vital signs;
5) ECOG performance status assessment, see Appendix 1 for details;
6) EORTC QLQ-C30 quality of life score, see Appendix 3 for details;
7) Imaging examination (CT/MRI);
8) Color-doppler-echocardiography (echocardiography);

9) 12-lead ECG;
10) Fingertip oxygen saturation;
11) Laboratory examination: the results of laboratory examination within 7 days before lymphodepleting chemotherapy can be accepted
    a) Blood routine: red blood cell count, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, platelet count, hemoglobin. If necessary, it can be repeated once to obtain acceptable measured values before the screening failure of subjects;
    b) Urine routine: urine red blood cells/urine occult blood, urine white blood cells, urine protein, urine glucose. If necessary, it can be repeated once to obtain acceptable measured values before the screening failure of subjects;
    c) Blood biochemistry: Including blood sodium, blood potassium, chloride, blood urea nitrogen/blood urea, creatinine, creatinine clearance (Cockcroft-Gault formula) (can also be replaced by radioisotope glomerular filtration rate [GFR]), blood sugar, ALT, AST, alkaline phosphatase, total bilirubin and lactate dehydrogenase. If necessary, it can be repeated once to obtain acceptable measured values before the screening failure of subjects;
    d) Coagulation function: prothrombin time, activated partial prothrombin time, thrombin time, plasma fibrinogen, and international normalized ratio. If necessary, it can be repeated once to obtain acceptable measured values before the screening failure of subjects;
    e) Cardiac enzymes: creatine kinase, lactate dehydrogenase, hydroxybutyrate dehydrogenase, and CK-MB. If necessary, it can be repeated once to obtain acceptable measured values before the screening failure of subjects;
    f) Virological test: including HCV antibody, five items of HBV, HIV antibody, and syphilis, and patients with positive infection should be excluded. Subjects who are positive for HCV antibody should be tested for HCV-RNA, those who are positive for HBV surface antigen and e-antigen should be excluded, and those who are positive for HBV core antibody should be tested for HBV-DNA. The results within 4 weeks before the signing of the master ICF are acceptable;
    g) Blood pregnancy test (for women of childbearing potential only)
12) Leukocyte apheresis: study day -25, time window -14 days (subject to the actual apheresis date);
13) Immune multi-factor detection: including IL-2, IL-6, IL-8, IL-10, IL-12, granzyme B, and TNF. 2ml whole blood samples of patients need to be collected for each teat and sent to the third-party central laboratory for detection;
14) Cytokine detection: IL-6, TNFα, and IFNγ: on the day of leukocyte apheresis;
15) Detection of inflammatory factors: CRP, and ferritin: on the day of leukocyte apheresis;
16) Tumor assessment: on the day of leucocyte apheresis;
17) Pharmacodynamic (PD) blood collection: on the day of leukocyte apheresis;
18) Concomitant medication;
19) AEs and SAEs: All AEs/SAEs are collected unless otherwise specified by the protocol (see section 10.1.6 for details).

6.1.3. Lymphodepleting chemotherapy: V3 (study days -7 to -5)

The following information/assessments will be collected on the day prior to the first dose of lymphodepleting chemotherapy:

1) vital signs;
2) ECOG performance status assessment;
3) Fingertip blood oxygen saturation;

4) Laboratory examination:
   a) Blood routine;
   b) Blood biochemistry: only rapid blood biochemistry, including blood urea nitrogen/blood urea, creatinine, creatinine clearance (Cockcroft-Gault formula) (can also be replaced by radioisotope glomerular filtration rate [GFR]), ALT, AST, and serum potassium;
5) Blood samples for PK testing: see Appendix 12 for details.
   Enrolled patients received lymphodepleting chemotherapy on study days -7 to -5: lymphodepleting chemotherapy regimen was FC regimen: cyclophosphamide (15 mg/kg/d) and fludarabine (20 mg/m2/d) for 3 consecutive days.
   The following information/assessment results will be collected on the day -5 after the completion of lymphadenectomy
6) Cytokine detection: IL-6, TNFα, and IFNγ;
7) Detection of inflammatory factors: CRP, and ferritin;
8) Replication of lentiviral cell (RCL) detection;
9) Concomitant medication;
10) AEs and SAEs: All AEs/SAEs are collected unless otherwise specified by the protocol (see section 10.1.6 for details).

6.1.4. Evaluation before cell infusion: V4 (study days -4 to -1)

During this visit, the subjects will be evaluated before infusion, and the following information/ evaluation results will be collected:

1) Vital signs;
2) ECOG performance status assessment;
3) Fingertip blood oxygen saturation;
4) Laboratory examination:
   a) Blood routine;
   b) Urine routine;
   c) Blood biochemistry: only rapid blood biochemistry, including blood urea nitrogen/blood urea, creatinine, creatinine clearance (Cockcroft-Gault formula) (can also be replaced by radioisotope glomerular filtration rate [GFR]), ALT, AST, and serum potassium;
   d) Blood pregnancy test (for women of childbearing potential only)
5) Immune multi-factor detection
6) Cytokine detection: IL-6, TNFα, and IFNγ;
7) Detection of inflammatory factors: CRP, and ferritin;
8) Concomitant medication;
10) AEs and SAEs: All AEs/SAEs are collected unless otherwise specified by the protocol (see section 10.1.6 for details).

6.2. Treatment and observation period (study days 1 to 28)

6.2.1. Cell infusion: V5 (study day 1)

On the 5th day after the completion of lymphoid chemotherapy, subjects will receive the cell infusion prescribed by the protocol: if the infusion dose level is 1 and 2, the total amount of TAEST16001 cells is infusion on the first day of the study; If the infusion dose level is 3 and 4, the total amount of TAEST16001 cells is planned to be infusion at the ratio of 60% and 40% on the first and second days of the study.

The visit will conduct the following research procedures and collect the following information/assessments:

Note: Unless otherwise specified, it should be completed before the cell infusion.

1) Physical examination;
2) Vital signs: Repeat measurement every 15 minutes (± 2 minutes) before and within 1h after cell infusion for at least one hour. After the first hour, the body temperature, respiration, blood pressure and pulse rate/heart rate are measured every hour (± 10 minutes) until the sixth hour;
3) 12-lead ECG: before the cell infusion, and 2h±15min after the infusion;
4) Fingertip blood oxygen saturation: before the cell infusion, and 2h±15min after the infusion;
5) TAEST16001 cells infusion;
6) Pharmacokinetics (PK) blood collection: within 2 hours before cell infusion and 1h ± 10min after cell infusion; see appendix 12 for details;
7) Pharmacodynamic (PD) blood collection: 1h ± 10min after cell infusion; see appendix 12 for details;
8) DLT/MTD evaluation: carried out after the start of cell infusion, only for patients enrolled in the dose escalation study;
9) Replication competent lentivirus (RCL) detection;
10) Combination medication；
11) AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.2.2. V6 (Study Day 2)

If cells were reinfused at dose levels of groups 3 and 4, the remaining 40% of cells were reinfused on study day 2.

The visit will conduct the following research procedures and collect the following information/assessments:

1） Vital Signs: If the cells are reinfused on the same day, before the cell reinfusion, and within 1h after the reinfusion, repeat the measurement of the subject's vital signs every 15 minutes (± 2 minutes) for at least one hour; after the 1h, every 1h (±10 minutes) measure body temperature, respiration, blood pressure and pulse/heart rate for 1 time to 6h; otherwise, only need to measure once.
2） 12-lead ECG: If the cell reinfusion is performed on the same day, it should be performed before the cell reinfusion and 2h±15min after the reinfusion; otherwise, only one measurement is required.
3） Finger Oxygen Saturation: If the cell reinfusion is performed on the same day, it should be performed before the cell reinfusion and 2h±15min after the reinfusion; otherwise, only one measurement is required.
4） 2nd reinfusion of TAEST16001 cells (only for enrolled patients with cell reinfusion dose levels 3 and 4);
5） Pharmacokinetics (PK) blood collection: see Appendix 12 for details;
6） DLT/MTD evaluation: only partially enrolled patients in dose escalation studies;
7） Combination medication;
8） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.2.3. V7 (Study Day 3)

This visit collects the following information/assessments:

1） Vital Signs;
2） 12-lead ECG;
3） Finger Oxygen Saturation;
4） DLT/MTD evaluation: only partially enrolled patients in dose escalation studies;
5） Combination medication;
6） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.2.4. V8 (Study Day 4)

This visit collects the following information/assessments:

1） Vital Signs;
2） 12-lead ECG;
3） Finger Oxygen Saturation;
4） Laboratory examination:
   a) Blood routine;
   b) Urine routine;
   c) Blood biochemistry;
   d) Coagulation function;
   e) Myocardial enzymes
5） Cytokine detection: IL-6, TNFα, IFNγ;
6） Inflammatory factor detection: CRP, ferritin;
7） Pharmacokinetics (PK) blood collection: see Appendix 12 for details;
8） DLT/MTD evaluation: only partially enrolled patients in dose escalation studies;
9） Combination medication;
10） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.2.5. V9, V10 (Study Day 5, 6)

This visit collects the following information/assessments:

1） Vital Signs;
2） 12-lead ECG;
3） Finger Oxygen Saturation;
4） DLT/MTD evaluation: only partially enrolled patients in dose escalation studies;
5） Combination medication;
6） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.2.6. V11 (Study Day 7)

This visit collects the following information/assessments:

1） Physical examination;
2） Vital Signs;
3） 12-lead ECG;
4） Finger Oxygen Saturation;
5） Laboratory examination:
   a) Blood routine;

b) Urine routine;
c) Blood biochemistry;

6） Multi-factor immune detection;
7） Cytokine detection: IL-6, TNFα, IFNγ;
8） Inflammatory factor detection: CRP, ferritin;
9） Pharmacokinetics (PK) blood collection: see Appendix 12 for details;
10） Pharmacodynamic (PD) blood collection: see Appendix 12 for details;
11） DLT/MTD evaluation: only partially enrolled patients in dose escalation studies;
12） Combination medication;
13） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.2.7. V12 (Study Day 14)

This visit collects the following information/assessments:

1） Weight;
2） Physical examination;
3） Vital Signs;
4） ECOG performance status assessment, see Appendix 1 for details;
5） 12-lead ECG;
6） Finger Oxygen Saturation;
7） Laboratory examination:
a) Blood routine;
b) Urine routine;
c) Blood biochemistry;
d) Coagulation function;
e) Myocardial enzymes
8） Multi-factor immune detection;
9） Pharmacokinetics (PK) blood collection: see Appendix 12 for details;
10） DLT/MTD evaluation: only partially enrolled patients in dose escalation studies;
11） Combination medication;
12） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.2.8. V13 (Study Day 21)

This visit collects the following information/assessments:

1） Weight;
2） Vital Signs;
3） Laboratory examination:
a) Blood routine;
b) Urine routine;
c) Blood biochemistry;
4） Pharmacokinetics (PK) blood collection: see Appendix 12 for details;
5） DLT/MTD evaluation: only partially enrolled patients in dose escalation studies;
6） Combination medication;

7） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.2.9. V14 (Study Day 28±1)

This visit collects the following information/assessments:

1） Weight;
2） Physical examination;
3） Vital Signs;
4） ECOG performance status assessment, see Appendix 1 for details;
5） EORTC QLQ-C30 quality of life score, see Appendix 3 for details;
6） Imaging examination (CT/MRI);
7） 12-lead ECG;
8） Finger Oxygen Saturation;
9） Laboratory examination:
   f) Blood routine;
   g) Urine routine;
   h) Blood biochemistry;
   i) Coagulation function;
   j) Myocardial enzymes
10） Multi-factor immune detection;
11） Cytokine detection: IL-6, TNFα, IFNγ;
12） Inflammatory factor detection: CRP, ferritin;
13） Tumor assessment；
14） Pharmacokinetics (PK) blood collection: see Appendix 12 for details;
15） Pharmacodynamic (PD) blood collection: see Appendix 12 for details;
16） DLT/MTD evaluation: only partially enrolled patients in dose escalation studies;
17） Replication competent lentivirus (RCL) detection;
18） Combination medication;
19） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.3 Follow-up Period (Study Days 29-270)

V15, V16, V17 and End of Study (EOS) visits (Study Day 60±3 days, Study Day 90±3 days, Study Day 180±3 days and Study Day 270±3 days)

This visit collects the following information/assessments:

1） Weight;
2） Physical examination;
3） Vital Signs;
4） ECOG performance status assessment, see Appendix 1 for details;
5） EORTC QLQ-C30 quality of life score, see Appendix 3 for details;
6） Imaging examination (CT/MRI);
7） 12-lead ECG;
8） Finger Oxygen Saturation;
9） Laboratory examination:

a) Blood routine;
b) Urine routine;
c) Blood biochemistry;
d) Coagulation function;
e) Myocardial enzymes

10） Multi-factor immune detection;
11） Tumor assessment；
12） Serum Pregnancy: Females of Fertility Only, EOS Visits Only;
13） Pharmacokinetics (PK) blood collection: see Appendix 12 for details;
14） Pharmacodynamic (PD) blood collection: see Appendix 12 for details;
15） Combination medication;
16） RCL detection: V16 and EOS visits only;
17） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details).

6.4 Early Withdrawal/Unscheduled Visit

If the time interval between the early withdrawal visit and other visits specified in the plan is < 1 month, the relevant imaging examinations may not be repeated; if the time interval is < 1 week, the following visit items may not be repeated. (Note: If it is an unplanned visit, the visit item will be decided by the investigator according to the specific clinical situation of the subject):

1） Weight;
2） Physical examination;
3） Vital Signs;
4） ECOG performance status assessment, see Appendix 1 for details;
5） EORTC QLQ-C30 quality of life score, see Appendix 3 for details;
6） Imaging examination (CT/MRI);
7） 12-lead ECG;
8） Finger Oxygen Saturation;
9） Laboratory examination:
f) Blood routine;
g) Urine routine;
h) Blood biochemistry;
i) Coagulation function;
j) Myocardial enzymes
10） Multi-factor immune detection;
11） Tumor assessment；
12） Serum Pregnancy: Females of Fertility Only;
13） Pharmacokinetics (PK) blood collection: see Appendix 12 for details;
14） Pharmacodynamic (PD) blood collection: see Appendix 12 for details;
15） Combination medication;
16） RCL detection: V16 and EOS visits only;
17） AS/SAE: collect all AEs/SAEs unless otherwise specified by the scheme (see Section 10.1.6 for details);

18） Tumor Lesion Biopsy: Investigators can perform tumor biopsy biopsy according to the subject's condition. The inspection contents include: NY-ESO-1 detection, tumor immune microenvironment, gene mutation analysis.

6.5. Long-term Follow-up Period (Study Day 271/Early Withdrawal Visit to 15 Years after Cell Infusion)

From the end of the study to 15 years after cell reinfusion, only the following information/assessment results will be collected. Years, once every 3 years, with a time window of ±3 months; the start date of long-term follow-up is 1 year ±1 month since the end of study visit or early withdrawal visit:

1） RCL detection;
2） Secondary tumor.

7. Evaluation Indicators

7.1. Evaluation of Safety and Tolerability

7.1.1. Safety evaluation

Cytokine release syndrome (CRS) and neurotoxicity were graded using the ASTCT 2018 criteria (see Appendix 5 for details).

The remaining adverse events (AEs) will be rated using the CTCAE version 5.0 criteria. Unless otherwise specified by the protocol (see Section 10.1.6 for details), AEs will be assessed throughout the study period, DLT and MTD will also be observed in the dose escalation study portion, and the DLT observation period will be from the first cell infusion until the cell 28 days after reinfusion, DLT and MTD definitions are detailed in Sections 3.5 and 3.6.

To grade adverse events, indicators include vital signs, physical examination, 12-lead electrocardiogram, clinical laboratory examination indicators, ECOG score, EORTC-QLQ-C30 scale, AE and SAE, AESI, etc.

7.2. Pharmacokinetic (PK) and Pharmacodynamic (PD) evaluation

1) PK blood collection:

At each time point, 6 mL/test item of venous blood was collected and sent to the central laboratory for testing.

Collection time points: within 2 hours before lymphadenectomy and chemotherapy, within 2 hours before cell reinfusion, 1h ± 10min after the first reinfusion of cells (study day 1), study day 2, 4, 7, 14, 21, 28, 60, 90 , 180, 270 days.

Note: PK blood samples should be collected according to the research plan. For details, please refer to the laboratory manual. However, on the day when the subject develops AESI, SAE or withdraws from the study, unscheduled PK blood samples should be collected as soon as possible.

2) PK evaluation parameters:

Flow cytometry was used to detect peripheral blood TAEST16001 cells and qPCR to detect TCR-T DNA copy number at the same time, and the following parameters were calculated:

Peak (C_{max}), time to peak (T_{max}) and AUC_{0-28} of TAEST16001 cells in peripheral blood. If possible, AUC0-inf, terminal phase elimination rate constant (λz) and elimination half-life ($t_{1/2}$)

will be evaluated.

3) PD blood collection: send to the central laboratory for testing:

At each time point, 6mL/test item or 3mL/test item of venous blood was collected.

Collection time points: within 2h before leukocyte apheresis, 1h±10min after the first reinfusion of cells (study day 1), study days 7, 28,

60, 90, 180, 270 days.

4) PD evaluation parameters:

T cell subsets, peripheral blood antigen-specific CTL, effector cell activity assay.

Note: PD blood samples should be collected according to the research plan. For details, please refer to the laboratory manual. However, on the day when the subject develops AESI, SAE or withdraws from the study, unscheduled PD blood samples should be collected as soon as possible.

7.3. Efficacy evaluation

Efficacy evaluation criteria: Efficacy evaluation was conducted according to RECIST 1.1 (see Appendix 4 for details), and subjects who were evaluated as disease progression (PD) by RECIST 1.1 could be re-evaluated as iUPD according to iRECIST (see Appendix 10 for details). After 4-6 weeks, imaging reexamination was conducted to determine whether they were iCPD according to iRECIST; observe until disease progression (iCPD) or carry out other anti-tumor therapy, whichever occurs first.

Efficacy evaluation method: The tumor lesion size of the patients will be evaluated by imaging, and the tumor remission will be evaluated. Patients must have the same imaging modality at all efficacy assessment follow-up sites. The first efficacy evaluation was carried out 28 days after the first reinfusion of TAEST16001 cells, and then the efficacy evaluation was carried out at 2 months, every 3 months, 6 months and 9 months after the infusion.

1） Objective response rate (ORR): the best overall response (BOR) is the ratio of the number of PR or CR patients to the total number of cases;

2） Disease control rate (DCR): the best overall response (BOR) is the ratio of the number of PR or CR or SD patients to the total number of cases;

3） Progression-free survival (PFS): from patient enrollment (lymphatic chemotherapy) to disease progression (PD) or death due to any cause death interval.

In this study, an Independent Review Committee (IRC) was established. The IRC consisted of three independent reviewers.

When there is an inconsistency between the two IRC members, the tumor imaging evaluation must be arbitrated by a third IRC member, and the arbitration result will be the final tumor evaluation result of the IRC. IRC reading results will not be fed back to the research center.

7.4. Quality of life

Subjects' quality of life will be assessed using the EORTC QLQ-C30 scale (see Appendix 3 for details).

7.5. Safety Review Committee (SRC)

Composition of the Safety Review Committee (SRC):

- The principal investigator or his representative

- Sponsor representatives and CRO representatives
- Experts in clinical pharmacology, statisticians and other experts may be invited as necessary

All study-related safety issues should be consulted with the SRC. When necessary, clinical pharmacology experts, statisticians, and other experts can be invited to participate in the discussion of safety issues in this study.

Decisions made by the SRC include:

1） Dose escalation;
2） Increase the dose group by 3 evaluable patients;
3） Stop dose escalation;
4） Assess and/or supplement enrollment;
5） Explore other research programs.

If there are other patients on treatment at the time the SRC is being assessed, the SRC may decide to delay their decision until those patients can be assessed.

If a patient does not meet the inclusion or exclusion criteria at the start of the study but is still erroneously included in the study, and the patient meets the criteria for an evaluable patient, the SRC should assess whether the patient should be included in the dose escalation decision.

The SRC's decision on the next dose group should be recorded and provided to the investigator prior to enrolling new patients.

The timing and frequency of safety assessments can be adjusted as new data become available.

8. Statistical analysis

8.1. Determination of sample size

This study is divided into two parts: a dose escalation study and an expansion study.

The dose escalation study part was designed according to the 3+3 principle, with a total of 4 dose levels, and it is expected to enroll about 2 to 24 patients. Determination of dose cohorts and MTD. Expand the enrollment study part, plan to enroll 6-18 patients with target indications.

The total sample size of this study is expected to include 12-30 patients with the target indication.

8.2. Analysis Sets

8.2.1. Analysis Set Definitions

This study includes 4 data analysis sets: DLT analysis set, safety analysis set, pharmacokinetic (PK) analysis set and efficacy analysis set, which are defined as follows:

DLT Analysis Set: Part of the dose escalation study, patients who developed DLT after complete reinfusion of the target dose of TAEST16001 cells and patients who did not develop DLT and completed the 28-day follow-up after the first cell reinfusion.

Safety Analysis Set: Dose-escalation study portion and expansion-entry study portion, patients who received partial or full planned total dose of TAEST16001 reinfusion.

Pharmacokinetic (PK) Analysis Data Set: Dose escalation study portion and expansion-entry study portion, including patients who received partial or full planned total dose of TAEST16001

infusion therapy with no protocol violations or events that impacted the pharmacokinetic analysis.

Pharmacodynamic (PD) analysis set: dose-escalation study portion and expansion-entry study portion, including partial or complete planned total dose of TAEST16001 cell reinfusion therapy, with baseline and at least one post-baseline at least one evaluable drug effect patients with medical parameters.

Efficacy Analysis Data Set: Patients receiving partial or full planned total dose of TAEST16001 cell reinfusion therapy with baseline tumor assessment and at least one post-baseline tumor assessment.

Verdicts for all analysis sets will be determined at the data review meeting.

8.2.2. Determination of the analysis set

Patients in the safety analysis set will be analyzed according to the actual doses received at the start of the study.

At least the PK analysis lead, the investigator and the study statistician will determine the PK analysis set before the database is locked. Adverse events or protocol deviations that may result in patients being excluded from the PK analysis set will be discussed at the data review meeting.

The efficacy analysis data set will determine whether the patients in the dose escalation stage can be included in the analysis set according to the actual drug use of the patients, and it will be determined before locking the library.

The PD analysis set will be determined based on the patient's specific received dose, pharmacodynamic indicators, and adverse events or protocol deviations may lead to patients being excluded from the PD analysis set, which will be discussed at the data review meeting.

8.3. Statistical analysis methods

8.3.1. General analysis

After the research plan is determined, the statistical professional is responsible for formulating the Statistical Analysis Plan (SAP), and the detailed statistical analysis method will be

Detailed in SAP. For measurement data, use the number of cases, mean, standard deviation, median, maximum value, and minimum value for statistical description; for count data or grade data, use frequency and frequency.

The data from the most recent lymphodepleting chemotherapy will be used as the baseline data.

8.3.2. Demographic analysis

The safety analysis dataset will be used for demographic analysis.

Enrollment and Completion: According to the dose group and all subjects, describe and summarize the enrollment, dropout and exclusion, and completed cases, and list the dropout cases.

Patient characteristics included baseline medical history and disease characteristics, and the demographic characteristics and baseline characteristics of subjects in different dose groups were statistically described according to their data characteristics.

8.3.3. Safety and Tolerability Analysis

Security analysis will be done in the security analysis set.

Safety was assessed by a summary of adverse events/serious adverse events/adverse events of special interest (CRS, neurotoxicity), changes in laboratory findings, abnormal ECGs, vital signs, changes in ECOG scores.

Cytokine release syndrome (CRS) and neurotoxicity will be graded using the ASTCT 2018 criteria (see Appendix 5 for details), and the remaining adverse events (AEs) will be graded using the CTCAE version 5.0 criteria. Adverse events were coded using the International Dictionary of Medical Terms (MedDRA). The number and frequency of AEs that occurred in patients after lymphodepleting chemotherapy and after the first reinfusion of cells until the end of the study were summarized using the appropriate term according to the human organ system classification. All AESIs (CRS and neurotoxicity), SAEs (including death), and AEs leading to permanent discontinuation of cell reinfusion during cell reinfusion should be summarized in separate tabulations.

Changes in laboratory findings will be summarized according to the CTCAE version 5.0 grading. For laboratory indicators, the highest levels that occurred during the trial period were summarized in counts and percentages.

Descriptive statistics will be performed for ECG abnormalities, changes in vital signs and ECOG scores compared to baseline levels, and pre-cell transfusion levels. Some vital signs and laboratory test results will graph each subject's data over time.

In addition, a summary description (DLT set) of all DLTs will be made, and the details of the occurrence of DLTs will be listed in the list.

8.3.4. Pharmacokinetic (PK) Analysis

Will be done in PK set. Descriptive statistical analysis was performed on PK parameters at each visit time point, and the mean, standard deviation, median, minimum, maximum, geometric mean and coefficient of variation of the geometric mean were reported.

8.3.5. Pharmacodynamic (PD) Analysis

Will be done in PD set. Descriptive statistical analysis was performed on PD parameters at each visit time point, and the mean, standard deviation, median, minimum, maximum, geometric mean, and coefficient of variation of the geometric mean were reported.

8.3.6. Efficacy analysis

Efficacy analyses will be performed in the efficacy-evaluable sets.

Tumor assessment will be based on RECIST1.1 criteria (see Appendix 4 for details), and exploratory tumor assessment will also be based on iRECIST (see Appendix 10 for details). Tumor assessments were performed by investigators at the study center or designated investigators, and an IRC was established in this study to conduct independent imaging readings for all subjects. For statistical analysis, the tumor assessment results of the investigator and the IRC were analyzed separately.

Objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS) and their 95% confidence intervals were calculated after cell reinfusion. Median values and 95% confidence intervals for CR, PR, SD, and PFS were estimated using the Kaplan-Meier method.

Subjects who did not have disease progression or death on the analysis day, or who did not have disease progression while receiving any further anti-tumor therapy, will have their last full tumor before the cut-off date, or before the day of anti-tumor therapy. Censoring was performed

at the time of evaluation. If disease progression or death was recorded after a single missing tumor assessment, as a prespecified value, the actual event date of disease progression/death was used for the PFS event date. If disease progression or death was documented after ≥2 missing tumor assessments, the PFS time for these subjects was censored at the date of the last adequate tumor assessment without disease progression.

Investigator-assessed PFS was described using the Kaplan-Meier method and appropriate summary statistics.

8.3.7. Quality of life analysis

The mean, standard deviation, median, minimum, maximum, geometric mean and coefficient of variation of the geometric mean of the EORTC-QLQ-C30 scale patient self-assessment results collected at different infusion dose groups at different measurement time points will be reported. If possible, appropriate methods will be used to plot the trend of statistical scores.

8.4. Description of plan analysis

The primary statistical analysis of this study will be the safety analysis, PK, PD analysis, and primary efficacy analysis after the last subject in the dose escalation study portion completes the DLT evaluation cycle (28 days after the first cell reinfusion or on the day of the DLT). This statistical analysis mainly provides data support for determining MTD. Final safety, efficacy analysis, PK, PD analysis will be performed as needed after the study.

9. Data Management

9.1. Data Management

9.1.1. Data traceability, filling and modification of electronic case report forms

Data management for this study was undertaken by the CRO's data department to ensure the integrity and accuracy of clinical study data.

Data Management Plan (DMP): It is a dynamic document written by the data management personnel according to the clinical research plan. It specifies and records the data management tasks of a specific clinical study in detail and comprehensively, including personnel roles, work content, and operating specifications, etc., as supporting documents and reference documents throughout the research process. The revisions and upgrades of the DMP accompany the entire research phase. The Clinical Research Database is a web-based EDC platform for collecting and reporting clinical data while complying with FDA 21 CRF part 11 requirements. Data managers will use the EDC system to perform a series of data management tasks such as eCRF construction, logic verification setup, data challenge management, database locking, data storage and export. If database migration is required due to eCRF modification during the study, approval from the sponsor must be obtained before the migration.

The data of the electronic case report form are the personnel authorized by the investigator enter all derived from the original medical records, and the data into the eCRF, and the completeness and accuracy of the information should be ensured. After data entry is complete, the EDC system is checked through programmed study-specific logic checks to ensure completeness and accuracy of the information. If there is any error that needs to be corrected,

the modification should be done in accordance with the eCRF filling instructions, and the EDC system will automatically record the name of the person who modified the data and the date of modification.

After the data in the EDC system has been confirmed by original data verification (SDV), data management personnel review and medical review, questioning and other processing and confirmation, before the data is locked, the researcher needs to carry out electronic signature confirmation.

9.1.2. Database design and establishment

The database of this study was established by the data department of the CRO, and should meet the requirements of FDA 21 CFR Part 11. The database should manage data traces such as system login, data entry, modification, and deletion, and the establishment of the database should adopt CDISC standards as much as possible.

9.1.3. Medical Coding

Adverse events and medical histories were coded according to system organ class (SOC) and preferred term (PT) using a fixed version of the MedDRA dictionary. Drug names are coded using a fixed version of the WHO DD.

9.1.4. Handling of doubts

After the data is entered and stored in the EDC system, system checks are activated and trigger questions that require review and response by the researcher. The data manager will review the researcher's answer and close the challenge if the answer is acceptable. Data administrators and medical staff also manually check the entered data to ensure the logic, consistency and accuracy of the data.

Programmatically generate patient data lists/reports to support manual data review throughout the study. Human challenges can be added to the EDC system when there is data that needs to be clarified/verified/confirmed by the investigator. Before locking the database, the data administrator should confirm that all questions have been resolved.

Data managers will compare the data points in the electronic data capture system (EDC) with the data points in the safety database or SAE report form according to the requirements of the data management plan for the SAE consistency check to ensure that the two The key data points of the subjects were consistent, such as subject number, adverse event name, start date, relationship to study drug, action taken, outcome, end date, etc.

9.1.5. Database locking

After the data review meeting confirms that the collected data is complete and correct, the principal investigator, sponsor, statistical analyst, data management

Personnel and others jointly confirm whether the database lock can be performed. After obtaining the lock library authorization, the data management personnel lock the database. In principle, no further changes are allowed to the locked database. Problems found after data locking shall be discussed jointly by the sponsor, principal investigator, statistician, and data manager, and finally decide whether it is necessary to open the database to trace the source of the data, modify and clarify the database, and then lock the database again.

After the database is locked, statistical analysts will conduct statistical analysis according to the pre-established statistical analysis plan, and the statistical department will provide

statistical analysis reports.

After the database is locked and transferred, the data manager will create a data management report according to CDE's requirements. The data management report is a work summary of the whole process of data management of the project, including the roles and responsibilities of participating in the data management project, main time nodes, CRF and database design, data cleaning, coding, SAE consistency check, external data management, data quality control, the final submitted dataset. The sponsor will review and sign off on the data management report.

9.2. Requirements for researchers to fill in data

1） The principal investigator or his authorized personnel must complete all eCRF pages that must be filled in, ensuring that the filling is in Chinese, accurate, complete and logically consistent, avoiding abbreviations and symbols as much as possible. All data filled in eCRF should be consistent with the source data.
2） All items in the research record need to be filled in, no blank items or missing items are allowed (the blanks without records should be filled in as required).
3） Laboratory inspection and auxiliary inspection items are complete.
4） When the database is questioned and the principal investigator or his authorized personnel handles the question, please confirm the data before answering the question and update the data at the corresponding eCRF data point if necessary. Personnel can update revision data.
5） This database must be entered by the principal investigator or his authorized and trained personnel. The authorized personnel should be familiar with the research protocol and master the principles of GCP.
6） The principal investigator or his authorized personnel fills in all the data on the eCRF page. After the monitor and data administrator complete the data verification and cleaning, the principal investigator needs to sign electronically with his user name and password. After signing, if the data is updated, the updated eCRF still needs to be re-signed by the principal investigator. Principal investigator electronic signatures are required for all eCRF pages for all screened and enrolled subjects.

9.3. Requirements for monitor data to be monitored

1） During the research process, the monitor should go to the research unit to check the informed consent of the subjects and the screening and inclusion status.
2） The monitor may discuss the review results of the eCRF and source files with the site staff. The Principal Investigator or his authorized personnel shall correct and answer all errors, omissions and questions in the database.
3） Confirm that all AEs have been recorded in the eCRF and SAEs have been reported and documented.
4） Verify whether research drugs are supplied, stored, distributed, and recovered in accordance with relevant regulations, and make corresponding records.
5） During the monitoring visit, the monitor can get in touch with the staff of the research-related center, obtain the source documents, and provide an appropriate environment

to complete the review of the research-related documents. Monitors will meet regularly with investigators during the study to provide feedback on the conduct of the study.

6） The monitor will compare the eCRF data with the hospital records (source files). The nature and location of all source documents will be clarified to ensure that all sources of the raw data required to complete the eCRF are understood, and the monitor can also contact the research center to review these data sources.

9.4. Recording and preservation of research materials

1） The researcher should keep all the detailed original documents of the subjects to ensure that the data in the database is accurate, complete and timely. Original documents, medical records, etc. should be clear, detailed and easily identifiable by personnel participating in this clinical study.

2） The data in the eCRF can only be modified by the principal investigator or their authorized personnel.

3） At the end of the study, the eCRF will be printed/engraved and archived as needed. Research data should be retained for 5 years after the end of the study. However, if required by existing regulations or an agreement with the sponsor, these data should be kept for a longer period of time. The sponsor will notify the investigator in writing when these data will no longer need to be kept.

10. Adverse Events And Serious Adverse Events

10.1. Adverse Events

10.1.1. Definition of Adverse Events

Adverse events (AEs) refer to adverse medical events that occur during clinical research, whether or not related to research methods. Therefore, AEs may be unfavorable and unexpected signs (e.g., including abnormal laboratory test results), symptoms, or diseases indirectly related to the study, whether related to the medicinal product or not.

A new condition or deterioration of an existing condition is considered an AE. Pre-existing stable chronic disease that did not worsen during the study period, such as arthritis, was not considered an AE. Abnormal laboratory test results, clinical symptoms or signs that were judged by the investigator to be clinically significant were considered AEs.

In this study, any events clearly caused by disease progression were not reported as adverse events. However, in the event of death due to disease progression within the study or safety reporting period, disease progression still needs to be reported as an SAE, that is, the event leading to death must be reported as an SAE with a grade of CTCAE level 5.

Unexpected adverse drug reaction (Unexpected Adverse Drug Reaction), the clinical manifestations and severity of adverse reactions in drug research, beyond the existing clinical research data and information, including the investigator's manual and drug instructions for unmarketed drugs.

10.1.2. Anticipation of Adverse Events

All adverse events that occurred after the patient signed the main ICF must be fully recorded

on the patient's case report form. Patients were asked each time questions about the occurrence of AEs by the investigator through open-ended questions (eg, "Have there been any changes since the last interview?" or "Are there any other questions?"). Investigators should avoid asking questions in a way that affects patients. The investigator will record and evaluate the date of onset, description of the AE, severity, measures taken, relationship to study drug, and outcome of the event.

For common adverse reactions related to treatment, please refer to Appendix 5, Appendix 6, Appendix 7, and Appendix 8.

During the study, once the above-mentioned adverse events occurred, the investigators immediately started relevant treatment or rescue, and recorded the corresponding adverse events, serious adverse events, concomitant medication, etc. in the original documents and eCRF.

10.1.3. Adverse event records and follow-up

The AE record form should be truthfully filled out during the study. The type, degree, appearance time, duration, treatment measures and treatment were recorded in detail, and the correlation with the study treatment method was evaluated on the basis of comprehensive consideration of comorbidities and concomitant medication. All changes determined to meet the AE definition should be recorded in the AE section of the eCRF when the investigator determines that the AE definition is met. All medical documentation regarding AEs should be recorded in the original documentation. According to regulatory requirements, after the signing of the ICF, unless otherwise specified in the protocol (see Section 10.1.6 for details), any adverse medical event that occurs up to the end of the study should be considered an AE. All adverse medical events that occur should be recorded as AEs.

10.1.4. Adverse Event Severity Criteria

All adverse events that occurred during the study should be described and recorded in the original medical records and eCRF. Severity was rated according to NCI-CTCAE version 5.0 on a scale of 1 to 5:

- Grade 1: mild; asymptomatic or mild; only clinical or diagnostic findings; no treatment required.
- Grade 2: Moderate; minor, topical, or non-invasive treatment required; age-appropriate instrumental ADL*.
- Grade 3: Severe or medically significant but not immediately life-threatening; leading to hospitalization or prolonged hospitalization; disabling; Individuals with limited activities of daily living**.
- Grade 4: Life-threatening consequences; urgent treatment required.
- Grade 5: AE-related death.

Activities of Daily Living (ADL)

** Instrumental activities of daily living refer to cooking, buying clothes, using the phone, managing money, etc.*

*** Personal activities of daily living refer to bathing, dressing and undressing, eating, washing, taking medicine, etc., without being bedridden.*

The distinction between severity and serious adverse events is important. Severity is used to describe intensity and is a measure of the degree of adverse events, not necessarily serious

adverse events. For example, nausea that lasts for several hours may be severe in magnitude but not a serious adverse event; headache may be grade 3 (severe) in intensity but cannot be included in the SAE unless it meets the criteria for an SAE. On the other hand, a cerebral infarction that only results in limited functional loss can be considered a mild cerebral infarction, but it should be considered a serious adverse event.

10.1.5. Judgment of causal relationship between adverse events and study drug

The investigator should evaluate the possible causal relationship between the adverse event and the study drug, referring to the following criteria:

Level 5 Classification	Judgment Standard
Definitely Related	It conforms to the known reaction type of the suspected drug, conforms to the reasonable time sequence after the drug is used, and the adverse reaction is alleviated or disappeared after the dose is reduced or stopped, and the reaction reappears when the drug is used again.
Probably Related	It conforms to the known reaction type of the suspected drug, conforms to the reasonable time sequence after the drug is used, and the adverse reaction is alleviated or disappeared after the dose reduction or drug withdrawal, but the clinical state of the subject or other reasons may also produce the reaction.
May be Related	It conforms to the known reaction type of the suspected drug, conforms to the reasonable time sequence after the drug is used, and the adverse reaction is alleviated or not obvious after the dose reduction or withdrawal, but the clinical status of the subject or other reasons can explain the reaction.
Probably Unrelated	It does not conform to the known type of reaction of the suspected drug, and it does not conform to the reasonable time sequence after the drug is administered. The clinical state of the subject or other reasons may also produce the reaction.
Unrelated	It does not conform to the known reaction type of the suspected drug, does not conform to a reasonable time sequence after the drug is administered, and the clinical state of the subject or other reasons can also explain the reaction. After excluding clinical symptoms or other reasons, the reaction is alleviated or disappeared.

If it is determined to be definitely related, likely related and possibly related, it should be regarded as an adverse reaction caused by the drug.

10.1.6. Treatment and follow-up of adverse events

When an AE occurs, regardless of whether the event has a causal relationship with the study drug, active treatment should be taken. Patients experiencing AEs should be treated with acceptable clinical measures. If it is absolutely necessary to apply the medical measures excluded by the research project, the patient should withdraw from the study after consultation with the sponsor.

In principle, the observation of AE starts from the signing of the ICF until the end of the study. But note the following:

- Only all SAEs related to study operations were collected from the time the patient signed the genotype and tumor antigen screening ICF to the main ICF.
- All AEs/SAEs will be collected for patients who received TAEST16001 cell reinfusion (either partial or complete) from the time the patient signed the primary ICF to the end of the study, unless 90 days after the patient's TAEST16001 cell reinfusion After (3 months) to 270 days (9 months) after reinfusion, follow-up anti-tumor therapy is started due to ineffectiveness or disease progression. From the start of the replacement of anti-tumor therapy to the end of the study, all AEs will no longer be collected. Treatment-related AEs and all SAEs.
- For patients who signed a primary ICF, but did not undergo any TAEST16001 cell reinfusion for any reason, collect only within 28 days of study-related procedures or treatments (eg, leukopheresis, lymphocytosis chemotherapy) or until initiation of new AEs of tumor treatment (whichever occurred first) and all SAEs during the study.

For some subjects, AEs related to the study drug that persist after the end of the study should continue to be followed at the end of treatment until any of the following conditions are achieved:

- Incident resolution;
- Events are stable;
- Events return to baseline levels;
- The event was attributable to a drug other than the study drug or was not related to the conduct of the study;
- When more information is not available (patient refuses to provide more information, or there is evidence that despite best efforts the patient is still lost to follow-up).

10.2. Serious Adverse Events

10.2.1. Definition of Serious Adverse Events

A serious adverse event (SAE) is an adverse event that occurs when the patient signs the ICF from the start of the study until the end of the study, with any dose of study drug, unless otherwise specified by the protocol (see Section 10.1.6 for details) and that meets one of the following or multiple criteria:

1） Cause death;

2） Life-threatening;

Note: Life-threatening means that the patient is immediately at risk of death in the event of a reaction, that is, it does not include adverse reactions that are assumed to occur in more severe forms that could result in death.

3） The patient requires hospitalization or prolonged hospitalization;

4） Cause permanent or severe disability or loss of function;

5） Congenital anomalies, birth defects.

Other circumstances require medical and scientific judgment to determine whether the event is reported under expedited reporting rules, such as a medically important event that may not be immediately life-threatening, or result in death, or a medically important event requiring hospitalization, but it may Endangers the patient, or may require intervention to prevent the occurrence of any of the definitions of serious adverse events. These medical events are generally considered serious adverse events. Examples of medically significant events include the need for intensive treatment in the emergency room or at home for allergic bronchospasm; cachexia or

seizures that did not result in hospitalization; and the development of drug dependence or drug abuse. Infections resulting from drug spoilage are considered medically significant events and are subject to expedited reporting requirements.

During the study or safety reporting period, the following events may not be reported as SAEs, but the investigators need to truthfully record them in the medical records:

- Emergency room visits;
- Hospital observation within 24 hours;
- Hospitalized for outpatient routine examination (less than 24 hours of hospital stay);
- Conditions described in the protocol: hospitalization for drug administration, hospitalization for completion of the tests required by the protocol, or hospitalization for the prescribed planned treatment for the target indication;
- Hospitalization for administrative or social reasons (e.g. convenience, homelessness, insufficient income, caregiver respite services, family issues, need for appropriate documentation);
- Previous hospitalizations that were planned prior to signing the ICF, requiring appropriate documentation (SAE if disease progression requires advance surgery);
- Hospitalization in rehabilitation institutions, nursing homes;
- Routine medical examination and hospitalization;
- Indication disease progression should not be considered an adverse event. However, if the symptoms and signs of disease progression/exacerbation meet the criteria for SAE, it can be reported as SAE. Death itself is a consequence and is not considered an SAE. The main cause of death (primary AE leading to death) should be recorded and reported as an SAE. Report "death" as a consequence of the corresponding AE. If the death has no definite cause to report, the death itself can be reported as an SAE.

10.2.2. Reporting and Follow-up of Serious Adverse Events

If a subject has SAE during the research process, regardless of whether it is related to the research treatment method, the investigator should immediately take appropriate treatment measures for the subject to ensure the safety of the subject. During the clinical research, the investigator must report to the sponsor immediately after learning of any SAE, and reports involving death events must be reported to the sponsor and the ethics committee of the research center at the same time. At the same time, the investigator must fill in the serious adverse event form, recording the occurrence time, severity, duration, measures taken and outcome of serious adverse events.

In this study, CRO Kunto Company will receive the SAE on behalf of the sponsor. Reporting contact: Kuntuo Clinical Safety Management Department, E-mail: Drugsafety@kuntuo.com.

If the investigator is unable to learn about the serious adverse event in time (for example, the patient first sees a doctor in another hospital), he should report and record the time when the serious adverse event was first learned immediately after being informed.

For all serious adverse events, the investigator is responsible for tracking and providing information to the sponsor in accordance with the reporting timeframe specified above. In addition, sponsors may request investigators to quickly collect specific supplemental

information. This information may be more detailed than the information recorded on the Serious Adverse Event Reporting Form. Typically, this information should include a detailed description of the serious adverse event to allow for a complete medical evaluation of the event and an initial independent judgment of probable cause. In addition to this, information on other possible causes, such as concomitant medications and concomitant diseases, must also be provided. In the event of a patient's death, the autopsy report must be submitted to the sponsor or its designated representative as soon as possible, if there is an autopsy result.

During the clinical research, if it is judged as an unexpected and serious adverse reaction related to the study drug, it is recommended to report quickly in the form of individual safety reports according to the "Standards and Procedures for Rapid Reporting of Safety Data During Drug Clinical Research".

In addition to individual safety reports of unexpected serious adverse reactions, other potential serious safety risk information identified by investigators and sponsors should also be reported to the national drug review agency as soon as possible, and a Make medical and scientific judgments. In general, this is the case for information that significantly affects the risk-benefit assessment of a drug or that may consider changes in drug usage, or that affect the overall drug development process, such as: (1) For known, serious adverse reactions, its incidence has increased and is judged to be of clinical importance; (2) significant harm to exposed populations, such as drug ineffectiveness in the treatment of life-threatening diseases; (3) significant safety findings in recently completed animal studies (such as carcinogenicity).

10.3. Adverse Events of Special Concern

10.3.1. Cytokine release syndrome

Cytokine Release Syndrome (CRS) refers to the fact that immune cells (including monocytes, macrophages, T cells and B cells) in the body are activated by drugs, antibodies or cell therapy and release a large number of cytokines, mainly A group of clinical syndromes including interleukin-6 (IL-6), tumor necrosis factor (TNF-α), interferon (IFN-γ), etc., with symptoms including flu-like symptoms, high fever, chills, and nausea to varying degrees , vomiting, muscle pain, hypotension, and difficulty breathing.

CRS was assessed according to the CRS grading scale of Lee DW et al. The original CRS grading criteria proposed by Lee et al. have been updated and published as the American Society for Blood and Marrow Transplantation (ASBMT) consensus guidelines.

See Appendix 5 and Appendix 7 for the toxicity, grading standards and intervention measures of various systems caused by CRS.

10.3.2. Neurotoxicity

The neurotoxicity grading and management of TCR-T cell therapy are detailed in Appendices 6 and 7.

10.4. Pregnancy events

Under the premise of taking adequate contraceptive measures, women of childbearing age can also participate in this study. At the time of screening, investigators must inform female patients of reproductive age about the importance of avoiding pregnancy during the study period and acceptable contraceptive methods, as well as the potential risk of unintended pregnancy.

During the study, if female patients suspect that they may be pregnant, they must contact the investigator immediately. The investigator must immediately allow the patient to perform a pregnancy test. If the result is positive, the investigator must contact the sponsor as soon as possible within 24 hours, and the sponsor will ultimately decide whether the pregnant woman should continue the study. Pregnancy itself is not reported as an adverse event, but each event should be assessed for reporting as an adverse event. For all subjects who are pregnant from the start of lymphatic depletion chemotherapy to 1 year after the last infusion of cells, the investigator must fill in the Pregnancy Report Form and report it to the sponsor within 24 hours after learning of the event.

Investigators need to continue follow-up until all relevant information on pregnancy outcomes is reached. If the subject is known to have spontaneous abortion or fetal congenital malformation, it will change from a simple pregnancy event to an SAE, and the investigator needs to fill in the SAE report form while filling in the pregnancy report form to report.

11. Research Management

11.1. Quality Control and Quality Assurance

Standard Operation Procedures (SOP) should be established for all research processes.

1） Qualification of research unit: The clinical research unit must be a drug clinical research base with clinical research qualification determined by the State Drug Administration.

2） Qualification of researchers: Researchers must be physicians, pharmacists or nurses trained in clinical research and work under the guidance of senior professionals.

3） Laboratory quality control measures: The laboratory shall establish standard operating procedures and quality control procedures for experimental observation indicators.

4） Quality control measures for clinical ward research:

5） The clinical ward of the pre-study inspection must meet the standardized requirements to ensure that the rescue equipment is fully equipped.

6） Train investigators (including nursing staff) on research protocols before the start of clinical research.

7） The operator checks that the instrument is functioning well and has no faults, and conducts a trial run of the instrument.

8） Subjects are cared for by professional nursing staff.

9） The research drugs are kept by professionally trained nursing staff, and the counters are locked and stored in accordance with drug storage requirements. The remaining research drugs are stored separately, and the remaining amount is registered on the "Clinical Research Drug Use Record Form", and at the end of the clinical study, they will be returned to the sponsor or destroyed.

10） All observed results and abnormal findings in clinical research should be carefully verified and recorded in time to ensure the reliability of the data. Various instruments, equipment, reagents, standards, etc. used in various inspection items in clinical research should have strict quality standards, and ensure that they work under normal

conditions. The recording and transfer of clinical data must be carried out by experienced physicians and supervised or checked by special personnel to ensure the scientificity and accuracy of the data. Various conclusions of clinical research must be derived from the original data.

11） The physician in charge of the research should fill in the eCRF completely, in detail and accurately. All research-related data should be centrally managed and analysed.

12） Establish procedures for data storage, data transmission, and data query. The data to be kept include: patients' medical records, imaging data, drug use registration form, patient code form, serious adverse event report form, GCP forms to be filled in by hospitals, and various related original medical documents, etc. The transmitted data includes: patient enrollment information, serious adverse event report form, and data and information to be used for summary data. The drug regulatory department, the sponsor and its entrusted supervisors, and the relevant leaders of the hospital have the right to consult the relevant research materials and original records with the consent of the principal investigator of the study.

13） When summarizing and analyzing clinical research results, standardized statistical analysis methods must be used, and the analysis shall be performed by professional biostatisticians.

14） In order to ensure the reliability and integrity of the research data, the principal investigator, the sponsor, and the inspectors entrusted by them shall conduct systematic inspections on the clinical hospital on a regular basis to determine whether the implementation of the research is in line with the research plan, and the reported results. Whether the data is consistent with the records of clinical participating units, a visit report should be written for each monitoring and visit.

11.2. Clinical Monitoring

The monitor will visit the research unit on a regular basis or according to the actual situation to conduct clinical monitoring work according to the "Quality Management Practice for Drug Clinical Research", and the investigator should actively cooperate with the monitor's work. The specific contents of the monitoring include:

1） Confirm that the research unit has appropriate conditions before the research, including staffing and training, various research-related inspections, the laboratory is well-equipped, working in good condition, and it is estimated that there are a sufficient number of subjects, and the participating researchers are familiar with requirements in the research proposal, etc.

2） Monitor the implementation of the research program by the investigator during the research process, confirm that the ICF of all subjects has been obtained before the research, understand the enrollment rate of the subjects and the progress of the research, and confirm the eligible subjects;

3） Confirm that all data records and reports are correct and complete, and that all eCRFs are filled in correctly and are consistent with the original data. All errors or omissions have been corrected or reasoned, signed and dated by the investigator. Verify that withdrawals and loss to follow-up of enrolled subjects are documented in the eCRF;

4） Confirm that all AEs are documented and SAEs report and document within the specified time.
5） Verify that the research drugs are supplied, stored, distributed, and recovered in accordance with relevant regulations, and make corresponding records.
6） Assist the researcher with necessary notification and application matters, and report the research data and results to the sponsor.
7） It should clearly and truthfully record the follow-up of the subjects that the researcher failed to do, the studies that were not conducted, the inspections that were not done, and whether errors or omissions were corrected;
8） Submit a written report to the sponsor after each visit. The report should state the date and time of the monitoring, the name of the monitor, the findings of the monitoring, etc., and is responsible for documenting the problems to be followed up discovered by the monitoring. Form feedback to the research center, and be responsible for following up the researcher's solution to the monitoring problem.

11.3. Data Management

The information provided by the sponsor to the investigator (including this clinical research protocol) is non-public information and must be kept confidential.

The collection and processing of personal data of subjects enrolled in this study is limited to that which is necessary for the purposes of this study.

Adequate precautions must be taken in the collection and processing of this data to ensure its confidentiality and compliance with applicable data privacy protection regulations. Appropriate technical and organizational measures must be available to prevent unauthorized disclosure or access to personal data, accidental or unlawful destruction of data, and accidental loss or alteration of data. According to their responsibilities, sponsor personnel who need to obtain personal data should agree to keep the subject's information confidential.

Informed consent of subjects (or their legal representatives) includes explicit consent to the processing of personal data, and explicit consent that investigators/research institutions may directly obtain subjects for research-related monitoring, auditing, ethics review board review, and regulatory agency review. Subject's original medical records (source data/documents).

The sponsor has the right to publish or publish information or data related to this study, or to report it to the Drug Administration. If other individuals or units related to this study wish to publish or publish the research results or related data, they must obtain the consent of the sponsor in advance. If the sponsor needs to appear the researcher's name in the content of publication, publication or advertisement, it should obtain the consent of the researcher.

12. Ethics related requirements

12.1. Ethical principles

This clinical research must comply with the ethical principles in the Declaration of Helsinki and China's ethical regulations and management practices and regulations related to clinical research. Prior to the start of the study, the investigator/research unit should obtain written approval from the ethics committee for investigator members, investigator handbook, study

protocol/protocol revision, written ICF, subject recruitment procedures, etc.

Investigational drugs may not be released to the study site prior to the start of the study. In the course of clinical research, any revisions to all research protocols should be reported to the ethics committee and implemented after approval. Any events that may affect patient safety or the continuation of clinical research, especially changes in safety, should be reported to the ethics committee.

If necessary, a progress report of the clinical study and a summary of the clinical results after the end of the clinical study should be submitted to the ethics committee every year.

12.2. Approval of Study Protocols and Protocol Amendments

The clinical research protocol and related materials must be submitted to the medical ethics committee of the hospital where the clinical research unit is located, and the ethics committee approval should be obtained before the start of the study. Any revisions to the research protocol during the research process also need to be submitted to the medical ethics committee of the hospital where the clinical research unit is responsible for implementation after obtaining the approval of the ethics committee; SAEs that may affect the safety of subjects and the process of research implementation or have not occurred during the research are implemented. Anticipated important adverse events should be reported to the ethics committee.

The sponsor will distribute revised and new versions of the protocol to the principal investigator.

12.3. Informed Consent

The ICF can only be used after it has been approved by the ethics committee.

Before participating in this study, each subject or his/her legal representative must read the ICF and give him/her sufficient time to understand the detailed content of this study after the detailed answer by the research doctor (subjects can choose to read and The order of explanation and notification by the researcher), so that the subjects or their legal representatives are fully informed, and the subjects have the right to voluntarily choose whether to participate in or withdraw from this study at any time.

Any revision of the ICF needs to be approved by the ethics committee of the research hospital, and patients who are not in the group must re-sign the new version of the ICF.

The ICF is voluntarily signed by the subject himself or his legal representative. The ICF signed by the investigator and the subject himself or his legal representative shall be in duplicate, and each party shall keep one copy.

12.4. Confidentiality of research data

The collection and processing of personal data of subjects enrolled in this study is limited to that which is necessary for the purposes of this study.

Adequate precautions must be taken in the collection and processing of this data to ensure its confidentiality and compliance with applicable data privacy protection regulations. Appropriate technical and organizational measures must be available to prevent unauthorized disclosure or access to personal data, accidental

or illegal destruction of data, and accidental loss or alteration of data. According to their

responsibilities, sponsor personnel who need to obtain personal data should agree to keep the subject's information confidential.

Informed consent of subjects (or their legal representatives) includes explicit consent to the processing of personal data, and explicit consent to direct access by investigators/research institutions for the purposes of research-related monitoring, audits, ethics review board reviews, and regulatory agency reviews. Subject's original medical records (source data/documents).

13. Responsibilities of the parties

13.1. Responsibilities of the sponsor

1） The sponsor is responsible for initiating this clinical study and providing research funding.
2） The sponsor provides the investigator's handbook, which includes the chemical, pharmaceutical, toxicological, pharmacological and clinical (including previous and ongoing studies) information and data of the investigational drug.
3） The sponsor, investigator, and contract research organization jointly design the clinical research plan and sign for confirmation.
4） The sponsor shall provide the research center with the investigational drug with easy identification, correct coding and special label, and ensure the quality is qualified. Research drugs should be properly packaged and stored according to the needs of the research protocol. The sponsor should establish a management system and record system for the investigational drug.
5） The sponsor should establish a quality control and quality assurance system for clinical research, and may organize audits of clinical research to ensure quality.
6） The sponsor and the researcher should promptly study the serious adverse events that occurred, take necessary measures to ensure the safety and rights of the subjects, and report to the drug supervision and administration department and the health administration department in a timely manner.
7） The sponsor is responsible for submitting the research summary report to the State Drug Administration.
8） The sponsor shall provide insurance for the subjects participating in the clinical research, and bear the cost of treatment and the corresponding economic compensation for the subjects who suffer damage or death related to the research. The sponsor shall provide the investigator with legal and financial guarantees, except those caused by medical malpractice.

13.2. Undertaking the responsibilities of clinical research medical institutions

1） You should be familiar with the research protocol provided by the sponsor, as well as the treatment methods for the relevant indications of the research, and the risk of discomfort that may be caused by the research drugs.
2） Participate in the formulation and revision of the clinical research protocol, and jointly sign/date the protocol.
3） Truthfully explain the details of the clinical research to the subjects. Before the

implementation of the clinical research, the subjects must be given sufficient time to consider whether to participate in the clinical research.

4） Truthfully record the side effects and AEs of the subjects and analyze the reasons; during the research process, after the investigator is informed of any SAE, the investigator must immediately report any SAE to the ethics committee and sponsor, and within 24 hours after the investigator learns of any SAE. Report to the relevant provinces, autonomous regions and municipalities directly under the Central Government for drug administration and the State Drug Administration. SAEs also need to be reported to the National Health Commission of the People's Republic of China.

5） In the event of AE, clinical researchers should make clinical judgments in a timely manner and take measures to protect the interests of subjects; if necessary, the ethics committee has the right to immediately suspend/terminate clinical research.

6） In case of suspension/termination of clinical research, subjects, sponsors, contract research organizations, research team leaders, ethics committees, NMPA, etc. shall be notified, and the reasons shall be explained.

7） Provide true, complete and reliable research data, and be responsible for the authenticity of the research data.

8） There is an obligation to keep the information provided by the sponsor confidential.

9） Investigators must follow relevant laws and regulations, comply with the protocol, and ensure that the research process is standardized.

13.3. Responsibilities of Contract Research Organizations (CROs)

1） Responsible for negotiating with the sponsor and investigator to determine the clinical research plan, signing and confirming it, and passing the ethical review.

2） According to the contract with the sponsor, a qualified monitor shall be appointed and accepted by the investigator.

3） Responsible for organizing and coordinating project implementation (screening of research institutions and principal investigators, organization of research conferences, application of research drugs, preparation of research materials, control of research schedules, data management and analysis statistics, research summary, etc.).

4） Responsible for the monitoring and quality control of the research implementation process (data integrity/authenticity verification, AE/SAE report verification, sample collection and transportation management, research document collection and management, research implementation process standardized management, monitoring reports, etc.), in strict accordance with GCP It is required to organize and supervise clinical research to ensure that clinical summary and clinical research data comply with the relevant regulatory requirements of GCP and the Declaration of Helsinki.

5） Report the project progress to the sponsor and sponsor regularly.

6） Responsible for formulating drug supply plans and assisting clinical research institutions in applying to sponsors for clinical research drugs required for research.

7） Responsible for assisting the sponsor to organize meetings related to the study, such

as investigator meetings, center kick-off meetings, etc.

14. Research Summary

A summary analysis was performed at the 1st statistical analysis (see Section 8.4 for details) and at the end of the study. Professional statisticians will assist the researcher to objectively summarize the research results and present the final statistical analysis report and update of the research analysis report. The researchers made an objective evaluation of the safety, tolerability, pharmacokinetics and efficacy of the drug based on the statistical results, and made a complete written summary report of the clinical study.

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Appendix 1：ECOG Performance Status

Grade	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 to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair

Appendix 2：Calculation formulas for QTcF and RR

QTcF Measured QTc corrected by Fridericia method (if not directly provided by ECG instrument):

$$QTcF(ms) = \frac{QT(ms)}{\sqrt[3]{RR(ms)/1000}}$$

RR interval formula (if not directly provided by ECG instrument):

RR (ms) = 60000/heart rate (bpm)

Appendix 3：EORTC QLQ-C30 Patient Quality of Life Assessment Scale

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling th number that best applies to you. There are no "right" or "wrong" answers. The information that you provide wi remain strictly confidential.

Please fill in your initials:
Your birthdate (Day, Month, Year):
Today's date (Day, Month, Year): 31

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4
16.	Have you been constipated?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Appendix 4：Response Evaluation Criteria in Solid Tumors 1.1 (RECIST1.1)

Response Evaluation Criteria for Solid Tumors Version 1.1 (RECIST v1.1): This version is an internal translation, please refer to the original English version for more details: http://ctep.cancer.gov/protocolDevelopment/docs/recist_guideline.pdf

Abstract

Background introduction

Assessing changes in tumor burden is an important feature of the clinical evaluation of cancer therapy. Both tumor shrinkage (objective response) and disease progression are meaningful endpoints in clinical trials. Since the publication of RECIST in 2000, many researchers, associations, industry, and government authorities have adopted this standard to evaluate treatment effects. However, a number of problems have arisen that led to the publication of this revised edition (version 1.1). Modifications (see topic in each chapter) result from evaluation of large databases (over 6500 patients), simulation studies, and literature reviews.

Important revisions to RECIST version 1.1

The main revisions are:

Determination of the number of lesions: In order to facilitate analysis, the evaluation of many trial data was combined into a database. According to this database, the total number of lesions required to determine the tumor burden of the response endpoint was reduced from the original maximum of 10 to the current maximum of 5. (reduced from 5 to 2 per organ).

The adjudication of pathological lymph nodes is now combined: nodules with a short-axis value of 15 mm are judged as measurable target lesions for evaluation. When determining tumor response, the short-axis value (of nodular lesions) must be included in the sum of lesions (of radii). Nodules are considered normal when they shrink to a short-axis value of <10 mm.

Efficacy confirmation is required in clinical trials with response rate as the primary endpoint, but is no longer required in randomized controlled clinical trials because control groups have become an effective way of interpreting trial data. Disease progression is addressed in the following ways: In addition to the original definition of a 20% increase in the sum of the target lesions (radius), if the total is small, an absolute (short axis of the lesion) must now be present to prevent overestimation of the degree of deterioration. The value is increased by 5 mm. In addition, guidance is provided on what constitutes "definite deterioration" in unmeasurable or non-target lesions—a point of confusion in the original RECIST guidelines. There is also a section at the end devoted to the detection of new lesions, including interpretation of FDG-PET scans. Imaging Guidelines: The revised RECIST includes a new imaging appendix with updated recommendations for optimal anatomical evaluation of lesions.

The next step of the job:

A key issue considered by the working group in revising RECIST v1.1 was the appropriateness of changing the assessment of tumor burden from a one-dimensional anatomical assessment to a three-dimensional anatomical assessment or functional assessment with PET and MRI. The current conclusion is that there are insufficient criteria or evidence to abandon anatomic assessment of tumor burden. The only explanation for this is the use of FDG-PET imaging as an adjunct to the diagnosis of disease progression. As discussed in detail in the

special topic of the last chapter, the use of these latest and promising technologies requires corresponding clinical validation studies.

Keywords: Efficacy Evaluation Criteria; Solid Tumors; Guidelines

1. Background

1.1. History of the RECIST Standard

Assessing changes in tumor burden is an important feature of the clinical evaluation of cancer therapy. Both tumor shrinkage (objective response) and time to disease progression are important adjudicative endpoints in cancer clinical trials. To screen for new antitumor drugs, years of research evidence support tumor shrinkage as an endpoint in phase II trials. These studies suggest that for a variety of solid tumors, drugs that shrink tumors in some patients may, albeit imperfectly, later be shown to improve patients' overall survival or have other opportunities to enter event evaluations in randomized phase III trials. . Objective response is currently more reliable than any other biomarker for evaluating treatment effect in phase II screening trials. Furthermore, in phase II and III clinical trials of drug development, clinical trials in critically ill conditions are increasingly using time to disease progression (or PFS) as an efficacy endpoint, which is also based on anatomic measurements of tumor size.

However, objective response and time to disease progression, two tumor adjudication endpoints, are only valuable if they are based on widely accepted and easy-to-use standard criteria based on tumor burden anatomy. The World Health Organization (WHO) first published tumor response criteria in 1981, mainly used in trials where tumor response is the primary endpoint. The WHO criteria introduce the concept of a global assessment of tumor burden by measuring the two-dimensional size of lesions and summarizing them, and assessing the response to treatment by assessing changes from baseline during treatment. However, in the more than a dozen years after the standard was published, the collaborating groups and pharmaceutical companies that used it often modified it to accommodate new technologies or raised ambiguities in the original literature, which led to trial results. Confusion of explanations. In fact, the application of various response criteria results in widely differing outcomes for the same treatment. In response to these problems with response, an international working group was formed in the mid-19th century to standardize and simplify the response criteria.

The new criteria, also known as RECIST (Response Evaluation Criteria in Solid Tumors), were published in 2000. The initial RECIST key features included determination of the smallest measurable lesion size, specification of the number of follow-up lesions (maximum 10; maximum 5 per organ), the use of one dimension instead of two, and an overall assessment of tumor burden. These criteria were later widely adopted by academic groups, collaborative groups, and the pharmaceutical industry, and the initial endpoint of the criteria was objective response or disease progression. In addition, the authorities accept RECIST as the appropriate standard for these evaluations.

2. Purpose of this guideline:

This guideline describes a standard method for measuring solid tumors and describes objective criteria for tumor size change used in clinical trials of adult and pediatric cancers. It is expected that these criteria will be used in all trials with objective response as the primary

endpoint and in trials using steady-state disease assessment, tumor progression, or time to progression by analysis as all measures of treatment effect are based on the anatomical tumor burden in the study and assessment of its changes. This paper makes no assumptions about the proportion of patients who meet the inclusion criteria with trial endpoints that predict the efficacy of a drug or treatment regimen: those definitions depend on the type of cancer in the ongoing trial and the particular drug being studied. The trial protocol must include an appropriate statistics section that defines the trial sample size and validity parameters on which the inclusion criteria are based. In addition to providing definitions and criteria for determining tumor response, this guideline also provides recommendations for standard reporting of clinical trial results using tumor response as a trial endpoint.

Although these guidelines can be used in the study of malignant brain tumors, separate criteria have been published for the assessment of response in this area. Since the international guidelines for the assessment of lymphoma response have also been published separately, this guideline was not used in the study of malignant lymphomas.

Finally, many oncologists rely on multiple imaging studies in their daily clinical practice to track patients' malignancy and to decide on further treatment options on the basis of a double standard of objective and symptomatic. These RECIST guidelines will play an important role in decision-making only when the treating oncologist judges them soundly.

3. Baseline Tumor Measurements

3.1. Definitions

Tumor lesions/lymph nodes were classified as measurable and non-measurable at baseline, as follows

3.1.1. Measurable **neoplastic lesions:** must accurately measure at least one dimension not less than the (instrument detection) lower limit (the longest diameter on the measuring instrument will be recorded):

- 10mm with CT scan (CT scan slice thickness not greater than 5mm).
- Clinical examination 10mm measured with calipers (lesions that cannot be accurately measured with calipers should be recorded as non-measurable).
- 20mm with chest X-ray.

Malignant lymph nodes: When assessed by CT scan (CT scan slice thickness is recommended to be no greater than 5 mm), the short axis of the lymph node must reach 15 mm to be considered pathologically enlarged and measurable. Preoperatively and during follow-up, only the short-axis length was measured and tracked. Information on lymph node measurements can also be obtained from the notes under "Preoperative Documentation for Target and Non-Target Lesions".

3.1.2. Unmeasurable (tumor)

All other lesions, including small lesions (less than 10 mm in longest diameter or 10 mm to less than 15 mm in the short axis of pathological lymph nodes) and true non-measurable lesions. Lesions considered truly unmeasurable include: meningeal disease as determined by pharmacological examination, ascites, pleural or pericardial effusion, inflammatory breast disease, skin or lung involvement of lymphatic vessels, abdominal mass/abdominal organ macrosomia, which are Reproduces what imaging techniques cannot measure.

3.1.3. Special considerations for measurable lesions

Special attention should be paid to bone lesions, cystic lesions, and lesions previously treated locally:

Bone lesions:

- Bone scans, PET scans, or plain radiographs are considered inadequate imaging techniques for measuring bone lesions. However, pharmacologically these techniques can be used to confirm the presence or disappearance of bone lesions.
- Osteolytic lesions or mixed acute osteolytic lesions with identifiable soft tissue can be considered measurable when they can be assessed by cross-imaging techniques such as CT or MRI if the soft tissue component meets the above definition of measurability. Sexual disease.
- Osteogenic lesions were not measurable.

Cystic lesions:

- X-ray-defined simple cysts that meet the inclusion criteria should not be considered malignant (neither measurable nor non-measurable) because by their definition they are simple cysts.
- "Cystic lesions" that present with cystic metastases can be considered measurable lesions if they meet the above definition of measurable lesions. However, if there are non-cystic lesions in the same patient, the target lesions are preferentially selected.

Lesions previously treated with topical therapy:

- Tumor lesions located in previously irradiated areas or sites that have received other local treatment are generally not considered measurable unless it has been demonstrated that the lesions are continuing. The study protocol should detail the conditions under which such lesions would be considered measurable.

3.2. Specification of measurement methods

3.2.1. Measurement of lesions

Clinical assessments were measured with calipers (calipers) and all measurements were recorded in metric units. All baseline assessments must be performed as close as possible to the start of treatment, but no earlier than four weeks.

3.2.2. Measurement method

At baseline and follow-up, the same adjudication methods and techniques should be used to describe each reported lesion. Unless follow-up reveals that the lesions are not suitable for imaging examination, imaging examination should usually be used for evaluation rather than clinical examination.

Clinical lesions: Only superficial lesions (eg, subcutaneous nodules) greater than 10 mm in diameter using calipers were considered measurable. For cases of skin lesions, it is recommended to use color photographs for recording, with the photographs attached to the scales for measuring the size of the lesions. As mentioned earlier, when lesions are available both clinically and by imaging, imaging should be used because imaging assessments are more objective and can be used for final review of clinical studies.

Chest X-ray: Chest CT is preferred over chest X-ray, especially when disease progression is used as an important endpoint because CT scan is more sensitive than X-ray in identifying new

lesions. However, lesions were considered measurable if they were clearly demarcated on x-ray and surrounded by air-filled lungs.

CT, MRI: CT is currently the most effective and reproducible detection method for evaluating the efficacy of lesions. Guidelines define measurable lesions on CT scans based on slice thickness not exceeding 5 mm. As shown in Appendix II, when the CT slice thickness exceeds 5 mm, the minimum measurable lesion should be twice the slice thickness. MRI may also be used in certain situations (eg, a full-body scan). Additional comments on the use of CT and MRI for detection of solid tumors to assess response are provided in Appendix II.

Ultrasonography: Ultrasonography is not suitable for assessing lesion size and should not be used as a measurement method. Ultrasound examinations are not completely reproducible between two adjacent observations, and the results are examiner-dependent, with no guarantee of the same technique and measurement results from one examination to the next. If new lesions are identified by ultrasound during the study, verification by CT or MRI is recommended. If the radiation exposure of CT is a concern, MRI can be used instead to detect the lesion to be examined.

Endoscopy, Laparoscopy: These techniques are not recommended for objective tumor evaluation. However, they are beneficial when biopsy confirms complete pathological remission or confirms complete remission or recurrence after surgical resection.

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. However, when tumor markers begin to rise above the upper limit of normal, markers must be standardized if they are to be used to judge patients in complete remission. Because tumor markers are disease-specific, measurement specifications should label records of baseline testing for a particular disease. Specific guidelines for CA-125 changes (in ovarian cancer recurrence) and PSA changes (in prostate cancer recurrence) have been published. In addition, the International Group of Gynecologic Oncology (InterCohort) developed the CA125 progression criteria, which apply the overall first-line application to the objective evaluation of tumors in ovarian cancer trials.

Cytology, Histology: These techniques can often be used to differentiate PR from CR in individual cases (eg, residual benign neoplastic lesions with germ cell tumors) if required by the clinical research protocol. When exudate is known to be a potentially serious adverse outcome of treatment (eg, certain paclitaxel-based chemotherapeutics or angiogenesis inhibitors), in order to distinguish between response to treatment (eg, stable disease) and disease progression, even if measurable tumors are consistent with response or Stable criteria require attention to any neoplastic exudate that appears or worsens during therapy as demonstrated by cytology.

4. Evaluation of tumor efficacy

4.1. Assessment of total tumor and measurable lesions

In order to evaluate objective response or possible future progression, it is necessary to perform a baseline assessment of the total tumor burden of all tumor lesions as a reference for subsequent measurements. In clinical programs with objective response as the primary treatment endpoint, only patients with measurable disease at baseline were enrolled. Measurable lesions were defined as the presence of at least one measurable lesion. For those trials with disease progression (time to disease progression or degree of progression on a fixed date) as the primary treatment endpoint, the inclusion criteria of the protocol must clarify

whether it is limited to patients with measurable lesions, or patients without measurable lesions can also be enrolled.

4.2. Baseline recording of target and non-target lesions

When there is more than one measurable lesion at baseline assessment, all lesions should be recorded and measured, with a total of no more than 5 (no more than 2 per organ), as target lesions representing all involved organs (that is, only one or two). Patients with cumulative organs selected up to two or four target lesions as baseline measurement lesions).

Target lesions must be selected based on size (longest diameter), be representative of all involved organs, and measurements must be reproducible. Sometimes a reproducible largest lesion can be re-selected when the largest lesion cannot be re-measured.

Lymph nodes require special attention because they are normal tissues and are still detectable on imaging even in the absence of tumor metastasis. Pathological lymph nodes defined as measurable nodules or even target lesions must meet the following criteria: CT-measured short diameter ≥ 15 mm. Baseline only needs to detect short diameters. Radiologists usually use the short diameter of a nodule to determine whether a nodule has metastasized. The nodule size is generally represented by the two-dimensional data of image detection (CT uses the axial plane, and MRI selects a plane from the axial, sagittal or coronal plane). Take the smallest value as the short diameter. For example, a 20 mm × 30 mm abdominal nodule with a short diameter of 20 mm can be considered a malignant, measurable nodule. In this example, 20 mm is the nodule measurement. Nodules ≥ 10 mm but < 15 mm in diameter should not be considered target lesions. Nodules <10 mm are not classified as pathological nodules and need not be recorded and further observed.

The calculated sum of the diameters of all target lesions (including the longest diameter of non-nodular lesions and the short diameter of nodular lesions) will be reported as the sum of the baseline diameters. If the diameter of the lymph node is included, as mentioned above, only the short diameter is counted. The sum of the baseline diameters will serve as the reference value for the baseline level of disease.

All remaining lesions, including pathological lymph nodes, can be considered non-target lesions and do not need to be measured, but should be recorded at the baseline assessment. Such as recorded as "presence", "absence" or in rare cases "clear progression". Extensive target lesions can be documented with target organs (eg, massively enlarged pelvic lymph nodes or massive liver metastases).

4.3. Efficacy evaluation criteria

This section defines the criteria used to determine ORR in tumor target lesions.

4.3.1. Efficacy evaluation of target lesions

Complete remission (CR): All target lesions disappeared, and the short-axis value of any pathological lymph nodes (whether target lesions or not) must be <10 mm.

Partial remission (PR): At least a 30% reduction in the sum of the diameters of all target lesions compared to the total diameter at baseline.

Disease progression: The sum of the diameters of all target lesions increased by at least 20% with reference to the sum of the smallest lesion diameters (including the sum of the diameters of the lesions at baseline, if it was the smallest). In addition, in addition to a 20% relative increase

in the sum of diameters, the absolute value of the sum must also increase by at least 5 mm (Note: the appearance of one or more new lesions can also be considered as disease progression).

Stable disease (SD): Taking the sum of the smallest lesion diameters during the study as a reference, the reduction of the lesions is neither consistent with PR, nor the increase of the lesions is consistent with the progression of the disease.

4.3.2. Precautions for efficacy evaluation of target lesions

When the target lesion is a lymph node:

The actual short-axis measurement (on the same anatomical plane as the baseline measurement) should usually be recorded, even if all lymph nodes in the study regressed to less than 10 mm. This means that when the target lesion is a lymph node, even if the criteria for complete remission are achieved, the sum of the target lesion diameters will not be 0, because lymph nodes with a short-axis value <10 mm are defined as normal lymph nodes. Case report forms or other data collection methods may be designed to record nodular target lesions separately, and each nodule must have a short-axis value <10 mm in order to determine complete remission. For PR, SD, and disease progression, the sum of target lesions (diameters) will include the actual short-axis value of the nodule.

Target lesions too small to measure:

All lesions (nodular and non-nodular) recorded at baseline in the study had to have their actual measurements recorded at subsequent assessments, albeit small (eg, 2 mm).

However, sometimes a lesion or lymph node is recorded at a critical value because the signal is too weak on a CT scan, and the radiologist may be reluctant to give an accurate measurement, but instead report it as "too small to measure". It is important to note a measurement on the case report form when this occurs. If the radiologist believes that the lesion may disappear, the measurement can be recorded as 0mm. If the lesion does exist and the signal is too weak, the default value of 5 mm can be recorded (this rule is not suitable for lymph nodes, because normal lymph nodes have a well-defined size and are often surrounded by fatty tissue, such as those in the retroperitoneal space; but , if the lymph node does exist but the signal is too weak to measure, it can also be recorded as the default value of 5mm).

The default value of 5mm is derived from the thickness of the CT scan slice (if the thickness is changed, the default value of 5mm should not be changed). Measurements for such (too small to measure) lesions may lack reproducibility, and giving a default value prevents false cures or false deteriorations in the case of measurement errors. Again, if the radiologist can give an actual measurement, even if it is less than 5mm, it should be recorded.

Lesions that ruptured or fused during treatment：

As noted in Appendix II, when a non-nodular lesion is "fragmented", the longest diameters of all fragments must be added together to calculate the sum of the target lesion diameters. Also, when the lesions are merged, the long diameter between them can be preserved, which helps to obtain the maximum diameter value of each lesion before the merger. If the lesions are fully fused and no longer separated from each other, the vector of the longest diameter in this case is the largest longest diameter of the fusion lesions.

4.3.3 Assessment of non-target lesions

This section defines the response criteria for non-target tumors. Although some non-target

lesions are actually measurable, measurement is not required, and only qualitative assessments are required at protocol-specified time points. Complete remission (CR): All non-target lesions disappeared, and tumor markers returned to normal levels. All lymph nodes were of non-pathological size (<10 mm short diameter).

Incomplete response (non-CR)/non-disease progression: presence of one or more non-target lesions and/or persistence of tumor marker levels above normal. Disease progression: Definite progression of existing non-target lesions. NOTE: The appearance of one or more new lesions is also considered disease progression.

4.3.4 Special Considerations Regarding the Assessment of Non-Target Lesion Progression

The supplementary explanation for the definition of non-target progression is as follows: When a patient has measurable non-target lesions, even if the target lesion is assessed as stable or partial response, to make a clear definition of progression on the basis of non-target lesions, non-target lesions must be met. The overall deterioration has reached a point where treatment must be discontinued. However, a general increase in the size of one or more non-target lesions is often insufficient to meet the criteria for progression. Therefore, when the target lesion is stable or in partial response, it is almost impossible to define overall tumor progression solely on the basis of changes in non-target lesions. very rare.

When none of the patient's non-target lesions is measurable: In some phase III trials, this occurs when the inclusion criteria do not specify that measurable lesions must be present. The overall assessment is still based on the above criteria, but because there are no measurable data on the lesions in this case. Deterioration of non-target lesions is not easy to assess (by definition: all non-target lesions must be truly unmeasurable), so when changes in non-target lesions result in an increase in the overall disease burden equivalent to disease progression in target lesions, decisions are made on the basis of non-target lesions. A clear definition of progression requires the establishment of an effective detection method for evaluation. An increase in tumor burden as described corresponds to an additional 73% increase in volume (equivalent to a 20% increase in measurable lesion diameter). Another example is peritoneal effusion from "minor" to "massive"; lymphangiopathies from "localized" to "widespread"; or described in the protocol as "sufficient to change treatment". Examples include pleural effusions ranging from trace to massive, lymphatic involvement spreading from the primary site to distant sites, or what may be described in the protocol as "the need for therapeutic changes". If definite progression is found, the patient should generally be considered progressive at that point. It is desirable to have objective criteria applicable to the assessment of non-measurable lesions, note that the added criteria must be reliable.

4.3.5 New lesions

The appearance of new malignant lesions heralds disease progression; therefore some evaluation of new lesions is very important. There are currently no specific criteria for imaging lesions, however the discovery of a new lesion should be definitive. For example, progression cannot be attributed to differences in imaging techniques, changes in imaging morphology, or pathology other than the tumor (eg, some so-called new bone lesions are only cures of the original lesions, or recurrence of the original lesions). This is important when a patient has a partial or complete response to a baseline lesion, for example: a case of necrosis of a liver lesion

may be classified as a new cystic lesion on CT report, when it is not.

Lesions detected at follow-up but not at baseline are considered new and suggestive of disease progression. For example, a patient with visceral lesions found at baseline, and metastases are found on CT or MRI head examination, the patient's intracranial metastases will be regarded as the basis for disease progression, even if he is at baseline. No head examination was done.

If a new lesion is ambiguous, such as due to its small size, further treatment and follow-up evaluation are required to confirm whether it is a new lesion. If repeated examinations confirm that it is a new lesion, the time of disease progression should be counted from the time of its initial discovery.

FDG-PET evaluation of lesions generally requires additional testing for supplementary confirmation, and it is reasonable to combine FDG-PET and supplemental CT findings to evaluate progress (especially for new suspicious diseases). New lesions can be identified by FDG-PET, according to the following procedures:

Baseline FDG-PET findings were negative and subsequent follow-up FDG-PET findings were positive, indicating disease progression.

No baseline FDG-PET was performed and subsequent FDG-PET results were positive:

If the new lesions found by the positive FDG-PET results at follow-up are consistent with the CT results, it is proved that the disease is progressive.

If the new lesions found by the positive results of the follow-up FDG-PET cannot be confirmed by the CT examination results, a CT examination is required for confirmation (if confirmed, the disease progression time is counted from the abnormality found in the previous FDG-PET examination.).

If the positive results of follow-up FDG-PET are consistent with pre-existing lesions on CT, and the lesions do not progress on imaging, the disease is non-progressive.

4.4. Best Global Response Evaluation

The best overall assessment of efficacy is the best-documented response from the start of the trial to the end of the trial, taking into account any necessary conditions for confirmation. Efficacy responses sometimes occur after the end of treatment, so the protocol should clarify whether the evaluation of the efficacy after the end of treatment is considered within the best overall efficacy evaluation. Protocols must address how any new treatment prior to progression affects optimal efficacy response. Optimal patient response is largely dependent on the outcome of target and non-target lesions and the presentation of new lesions. In addition, it depends on the nature of the trial, protocol requirements, and outcome measures. Specifically, in non-randomized trials, where response profile is the primary objective, efficacy confirmation of PR or CR is required to determine which is the best overall response.

4.4.1. Time-point responses

Efficacy responses were assumed to occur at specific time points for each regimen. Supplementary Table 1 will provide a summary of the overall efficacy response at each time point in the patient population with measurable disease at baseline. If the patient has no measurable lesions (no target lesions), the assessment can be found in Supplementary Table 2.

4.4.2. Missing and non-evaluable statements

If imaging or measurement of the lesion cannot be performed at a particular time point, the patient is not evaluable at that time point. If only some lesions can be evaluated in a single evaluation, this situation is generally considered to be unevaluable at that time point, unless there is evidence that the missing lesions will not affect the evaluation of response at the specified time point. This is likely to occur as the disease progresses. For example: a patient who has 3 lesions with a sum of 50 mm at baseline, but then only has 2 lesions that are evaluable with a sum of 80 mm, will be assessed for disease progression, regardless of the impact of the missing lesions.

4.4.3. Best overall response: all time points

Once all patient data are available, the best overall response can be determined.

Assessment of best overall response when the study does not require confirmation of complete or partial response: The best response in the trial is the best response at all time points (eg: a patient with SD in cycle 1, SD in cycle 1) The second cycle was evaluated as PR, and the last cycle was evaluated as disease progression, but the best overall response was evaluated as PR. When the best overall response was evaluated as SD, it must meet the shortest time from the baseline level specified by the protocol. If the criteria for the shortest time are not met, even the best overall response evaluation of SD is not acceptable, and the patient's best overall response will depend on subsequent evaluations. For example: a patient was evaluated as SD in the first cycle, and the second cycle For disease progression, but it did not meet the minimum time requirement of SD, its best overall response was evaluated as disease progression. The same patients who were lost to follow-up after evaluation of SD in the first cycle will be considered as non-evaluable.

Evaluation of best overall response when the study requires confirmation of complete or partial response: only when each subject meets trial-specified criteria for partial or complete response and is specifically mentioned in the protocol at subsequent time points (generally After four weeks), the efficacy can be confirmed again before it can be declared as complete or partial remission. In this case, the best total response is described in Supplementary Table 3.

4.4.4. Special hints for efficacy assessment

When nodular lesions are included in the total target lesion assessment and the nodule size is reduced to a "normal" size (<10 mm), they will still have a lesion size scan report. In order to avoid overestimating what is reflected by an increase in nodule size, measurements will be recorded even if the nodule is normal. As already mentioned, this means that subjects in complete remission will not be recorded as 0 on the CRF.

Repeated "unmeasurable" time points will complicate optimal efficacy assessment if efficacy confirmation is required during the trial. The analysis plan for the trial must state that these missing data/assessments can be accounted for when determining efficacy. For example, in most trials, a subject's response to PR-NE-PR can be used as confirmation of efficacy.

Symptomatic progression should be reported when there is no objective evidence to support an overall deterioration in the subject's health that requires discontinuation of the drug. Even after treatment discontinuation, objective progress should be assessed whenever possible. Symptomatic worsening is not an assessment description of an objective response: it is a reason for discontinuation of treatment. The objective response of such subjects will be assessed by the

target and non-target lesions shown in Schedules 1 to 3.

Defined as early progression, early death and non-evaluable conditions are study exceptions and should be clearly described in each protocol (depending on treatment interval and treatment cycle).

In some cases, it is difficult to distinguish local lesions from normal tissue. When the assessment of complete response is based on this definition, we recommend biopsy prior to the assessment of response to locoregional complete response. FDG-PET was used as an evaluation criterion similar to biopsy to confirm response to complete remission when in some subjects abnormal imaging findings of local lesions were thought to represent fibrosis or scarring of the lesions. In such cases, the use of FDG-PET should be described prospectively in the protocol, supported by reports from the specialist medical literature for the situation. However, it must be realized that due to the limitations of FDG-PET and biopsy itself (including the resolution and sensitivity of both), it will lead to false positive results in the assessment of complete remission.

Schedule 1 Time-point responses: subjects with target lesions (with or without non-target lesions)

Target Lesions	Non-Target Lesions	New Lesions	Total Relief
CR	CR	No	CR
CR	non-CR/non-disease progression	No	PR
CR	Cannot Be Assessed	No	PR
PR	Non-progress or incomplete assessment	No	PR
SD	Non-progress or incomplete assessment	No	SD
Cannot Be Fully Assessed	Non-progress	No	NE
Disease progression	Any Situation	Yes or No	Disease progression
Any Situation	Disease progression	Yes or No	Disease progression
Any Situation	Any Situation	Yes	Disease progression
CR=Complete Remission	PR=Partial Remission	SD=Stable Disease	NE=Not Evaluable

Schedule 2 Time Point Responses - Subjects with Non-Target Lesions Only

Non-Target Lesions	New Lesions	Total Relief
CR	No	CR
non-CR/non-disease progression	No	non-CR/non-disease progression
Cannot Be Fully Assessed	No	Cannot Be Assessed
Indeterminate Disease Progression	Yes or No	Disease progression
Any Situation	Yes	Disease progression

Note: For non-target lesions, "non-CR/non-progression" refers to efficacy over SD. As SD is increasingly used as an endpoint to evaluate efficacy, non-CR/non-progressive efficacy was developed to address the absence of measurable lesions that were not specified.

For indeterminate progressive findings (eg, very small indeterminate new lesions; cystic or necrotic lesions in pre-existing lesions) treatment can be continued until the next evaluation. If, at the next assessment, disease progression is confirmed, the date of progression should be the date of the previous suspected progression.

Schedule 3 Best overall responses for CR and PR efficacy needs to be confirmed

First Time Total Remission	Later Time Total Remission	Best Total Remission
CR	CR	CR
CR	PR	SD, disease progression or PRa
CR	SD	SD if SD persists for sufficient time, otherwise disease progression
CR	Disease progression	SD if SD persists for sufficient time, otherwise disease progression
CR	NE	SD if SD lasts long enough, otherwise should be NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	Disease progression	SD if SD persists for sufficient time, otherwise disease progression
PR	NE	SD if SD lasts long enough, otherwise should be NE
NE	NE	NE

Notes: CR=complete remission, PR=partial remission, SD=stable disease; NE=not evaluable.

Superscript "a": If CR does occur at the first time point, and any disease that occurs at subsequent time points, then even if the subject's efficacy relative to the baseline meets the PR criteria, the efficacy evaluation at subsequent time points remains for PD (because the disease will reappear after CR). Optimal response depends on whether SD occurs within the shortest treatment interval. However, sometimes the first assessment is CR, but subsequent time point scans show that small lesions still appear, so in fact the subject response should be PR rather than CR at the first time point. In this case, the first CR judgment should be revised to PR, while the best response is PR.

4.5. Frequency of tumor re-evaluation

The frequency of tumor re-evaluation during treatment depends on the treatment regimen and should be consistent with the type and schedule of treatment. However, in phase II trials where the benefit of treatment is unclear, follow-up every 6 to 8 weeks (time designed at the end of a cycle) is reasonable, and the length of the interval can be adjusted in special protocols or circumstances. Protocols should specify which tissue sites need to be assessed at baseline (usually those most likely to be closely associated with metastases in the tumor type under study) and how often the assessment is repeated. Under normal circumstances, target lesions and non-target lesions should be evaluated at each assessment. In some optional situations, some non-

target lesions may be evaluated less frequently, for example, the response evaluation of target disease is confirmed as CR or CR. Repeat bone scans should only be performed when skeletal disease progression is suspected.

After treatment, re-evaluation of the tumor depends on whether the response rate or the time to an event (progression/death) are used as clinical trial endpoints. If a certain event time (eg: TTP/DFS/PFS) occurs, routine repeated evaluations as specified in the protocol are required. Especially in RCTs, scheduled evaluations should be included in the schedule (eg, 6-8 weeks during treatment, or 3-4 months after treatment) and should not be influenced by other factors, such as treatment delays , dosing interval, and any other events that may lead to an imbalance in the treatment arm in the timing of disease assessment.

4.6. Efficacy assessment/confirmation of remission period

4.6.1. Confirmation

For non-randomized clinical studies with efficacy as the primary research endpoint, the efficacy of PR and CR must be confirmed to ensure that the efficacy is not the result of evaluation errors. This also allows for a reasonable interpretation of the results where historical data are available, but the efficacy in the historical data from these trials should also be confirmed. But in all other cases, such as randomized trials (phase II or III) or studies with stable disease or disease progression as the primary endpoint, efficacy confirmation is no longer required, as it has no value in the interpretation of trial results. However, removing the requirement for efficacy confirmation would make central review to prevent bias even more important, especially in unblinded experimental studies.

In the case of SD, within the shortest time interval after the start of the test (generally not less than 6-8 weeks), at least one measurement meets the SD criteria specified in the protocol.

4.6.2. Total remission period

The overall response period was the time from the measurement of the first meeting of the criteria for CR or PR (whichever was measured first) to the first real recording of disease recurrence or progression (using the smallest measurement recorded in the trial as a reference for disease progression). Total complete response time was the time from the measurement of the first meeting of the CR criteria to the first real recording of disease recurrence or progression.

4.6.3. Stable disease

is the time from the start of treatment to disease progression (in randomized trials, from the time of randomization), with the smallest sum in the trial as a reference (if the baseline sum is the smallest, it is used as a reference for the calculation of disease progression). The clinical relevance of stable disease varies from study to study and disease to disease. If, in a particular trial, the proportion of patients who maintain the shortest period of stability is the end point, the protocol should specifically state the shortest time interval between the two measurements in the definition of SD.

Note: Periods of remission, stabilization, and PFS are affected by the frequency of follow-up after the baseline evaluation. Defining standard follow-up frequencies is outside the scope of this guideline. The frequency of follow-up should take into account many factors, such as disease type and stage, treatment cycle and standard norms. However, if inter-trial comparisons are required, the limitations on the accuracy of these measurement endpoints should be considered.

4.7. PFS/TTP

4.7.1. Phase II clinical trials

This guideline focuses on the use of objective response as an end point in phase II clinical trials. In some cases, the response rate may not be the optimal choice for evaluating the potential anticancer activity of a new drug/regimen. In these cases, PFS/PPF at the cut-off time point can be considered a suitable surrogate to provide a raw signal of the biological activity of the new drug. But clearly, in an uncontrolled trial, these assessments would be questioned, since plausible observations may be related to biological factors such as patient selection, rather than the effect of drug interventions. Therefore, phase II clinical trials with these as research endpoints are best designed to be randomized controlled. However, some tumors are clinically consistent (often consistently poor), and non-randomized trials are justified. However, in these cases, due to the lack of an active control, care should be taken to document evidence of efficacy when assessing the expected PFS or PPF.

The follow-up content is the end point of phase III evaluation, independent evaluation, and result report, etc., see the English version for details.

Appendix 5: Cytokine release syndrome (CRS) systemic toxicity and grading criteria

CRS was assessed according to the CRS grading scale of Lee DW et al. At the end of 2018, the original CRS grading criteria proposed by Lee et al. were updated and published as the American Society for Blood and Marrow Transplantation (ASBMT) consensus guidelines (ASTCT consensus).

Toxic and side effects of various systems caused by CRS

System Distribution	Toxic Reaction
Systemic Symptoms	Fever ± chills, general malaise, fatigue, myalgia, arthralgia, headache
Digestive Tract	Nausea, vomiting, diarrhea, anorexia
Respiratory Tract	Shortness of breath, hypoxia
Cardiovascular	Tachycardia, increased pulse pressure, hypotension, increased cardiac output (early stage), possibly decreased cardiac output (late stage)
Coagulation	Elevated D-dimer, hypofibrinemia ± bleeding
Kidney	Azotemia
Liver	Elevated transaminases, hyperbilirubinemia
Nervous System	Headache, poor mental status, confusion, delirium, gibberish or aphasia, hallucinations, tremors, dystopia, gait changes, seizures

Grading criteria for cytokine release syndrome (CRS)

CRS Grading Evaluation Criteria				
CRS Performance	**Level 1**	**Level 2**	**Level 3**	**Level 4**
Fever†	Body temperature ≥38°C	Body temperature ≥38°C	Body temperature ≥38°C	Body temperature ≥38°C
Hypotension	No	No need for vasopressors	Requires a vasopressor (without vasopressin)	Need for multiple vasopressors (without vasopressin)
And/Or Hypoxia	No	Requires low-flow nasal cannula^ or purge	Requires a high-flow nasal cannula^, a disposable respirator or ventilator	Positive pressure required (eg, continuous positive airway pressure/CPAP, bilevel positive airway pressure/BiPAP, intubation and mechanical ventilation)

Note:

†: CRS-related fever is defined as a temperature above 38°C that cannot be explained by other causes. For patients with CRS on antipyretics or anti-cytokine drugs (eg, tocilizumab, steroids), body temperature is no longer used to assess the severity of CRS, and hypotension/hypoxia is

used to grade CSR.

‡: Hypotension and hypoxia could not be explained by other causes, and the more severe of hypotension or hypoxia was used to determine the grading of CRS. ^: Low-flow nasal cannula means: Oxygen delivery rate ≤6 L/min, low-flow conditions also include purging oxygen delivery (sometimes used in children). High-flow nasal cannula means: Oxygen delivery rate >6 L/min.

Reference: ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biol Blood Marrow Transplant. 2019. 4: 625-638.

Appendix 6: Neurotoxicity grading and management recommendations

According to ASTCT consensus

1. Immune effector cell-associated neurotoxicity syndrome (ICANS) grading scale

Neurotoxicity	Grade 1	Grade 2	Grade 3	Grade 4
Immune Effector Cell-Associated Encephalopathy (ICE) Score^	7-9	3-6	0-2	0 (cannot wake the patient)
Degree of inhibition of consciousness[v]	Awakened naturally	Wake up by sound	Can only be awakened by tactile stimuli	Inability to be aroused or only aroused by forceful or repetitive tactile stimuli, coma
Epilepsy (Any Age)	N/A	N/A	Clinically manifested epilepsy (localized or generalized) that can be rapidly controlled; or nonconvulsive EEG epilepsy that can be controlled	Life-threatening seizures (>5min); or repetitive clinical or EEG seizures with no return to baseline during seizures
Dyskinesia (Any Age)[§]	N/A	N/A	N/A	Deep central movement disorders, such as hemiparesis or paraparesis
Elevated Intracranial Pressure/ Cerebral Edema (Any Age)	N/A	N/A	Neuroimaging central/local edema[#]	Diffuse intracranial edema on neuroimaging; decerebral or cortical state; or cranial nerve VI palsy; or papilledema; or Cushing's sign

Note:

‡ ICANS was graded by the most severe of all items that could not be explained by other causes (ICE score, degree of depression of consciousness, epilepsy, dyskinesia, increased intracranial pressure/cerebral edema).

^ For patients with an ICE score of 0: if the patient is awake and with complete aphasia, the CANS is grade 3; if the patient cannot be awakened, the ICANS is grade 4.

The degree of suppression of V consciousness cannot be explained by other reasons (eg, sedatives).

§ Immune effector cell-associated tremor and myoclonus can be graded according to CTCAE v5.0, but they do not affect the ICANN classification.

Intracranial hemorrhage (with/without edema) can be graded according to CTCAE v5.0, but intracranial hemorrhage is not a manifestation of neurotoxicity and its grade does not affect the ICANS classification.

2. The ICE scoring criteria are as follows

ICE Scoring Criteria		
Project	Content	Total Score
Environmental Judgment	Can correctly locate the environment: year, month, city, hospital	4
Naming	Able to correctly name 3 objects (eg clock, pen, button)	3
Execute Instruction	Ability to execute simple commands (eg, show 2 fingers, close eyes and stick out tongue)	1
Writing	Able to write a standard sentence (eg, China's national flag is a five-star red flag)	1
Attention	Ability to start at 100 and count down to 10	1

Note: One point for each correct completion of any of the above tasks, 10 points, normal nervous system; 7-9 points, mild nervous system damage (level I); 3-6 points, moderate nervous system damage (level II) ; 0-2 points, severe nervous system damage (grade III)

3. Recommendations for the management of status epilepticus after cell therapy

1) Nonconvulsive status epilepticus

- Assess airway, breathing and circulation, check blood sugar
- Lorazepam* 0.5mg IV to control epilepsy, if necessary, increase by 0.5mg every 5 minutes until a total of 2mg is used
- Levetiracetam 500mg IV bolus for maintenance dose
- If seizures persist, transfer to ICU, phenobarbital 60mg IV bolus
- After remission of nonconvulsive epilepsy maintenance status, maintain the following doses: lorazepam 0.5mg q8h IV, 3 times a day; levetiracetam 1000mg q12h IV; phenobarbital 30mg q12h IV

2) Convulsive status epilepticus

- Assess airway, breathing and circulation, check blood sugar
- Transfer to ICU monitoring
- Lorazepam* 2mg IV, if not controlled, additional 2mg IV to control epilepsy, total dose 4mg
- Levetiracetam 500mg IV bolus for maintenance dose
- If the seizures persist and cannot be controlled, add phenobarbital 15mg/kg
- Maintenance of the following doses after the maintenance of convulsive epilepsy: lorazepam 0.5mg q8h IV, 3 times a day; levetiracetam 1000 mg q12h IV; phenobarbital 30mg q12h IV
- If seizures are difficult to control, continuous EEG monitoring should be performed.

All drug doses are for adults.

*Lorazepam is the recommended benzodiazepines. Compared with tranquilizers, benzodiazepines are short-acting drugs and are widely used in the treatment of epilepsy.

4. Recommendations for the management of increased intracranial pressure after cell therapy

1) Papilledema* grade 1 or 2 with cerebrospinal fluid pressure (CSF) <20mmHg, no

cerebral edema

- Acetazolamide 1000 mg IV, followed by 250-1000 mg IV q12h (dose adjusted according to renal function and acid-base balance, monitor 1-2 times a day)

2) Ppilledema* grade 3, 4 or 5 with any imaging evidence of cerebral edema, or cerebrospinal fluid pressure (CSF) ≥ 20 mmHg

- CRES level 4 recommends the use of high-dose glucocorticoids such as methylprednisolone 1g/day (Table 2)
- Raise the head of the bed 30 degrees
- Hyperventilation to achieve arterial carbon dioxide (PaCO2) of 28-30mmHg and maintain this state for no more than 24h
- Hypertonicity treatment: use mannitol (20g/dl solution) or hypertonic saline (3% or 23.4%, as shown below)
  - Mannitol: initial dose 0.5-1g/kg; maintenance dose 0.25-1g/kg q6h, while monitoring metabolism and serum osmolality every 6h, if serum osmolality ≥ 320 mOsm/kg, or osmotic pressure difference ≥ 40, stop with mannitol
  - Hypertonic saline: initial dose of 250ml of 3% hypertonic saline; maintain a rate of 50-75ml/h while monitoring electrolytes every 4h and discontinue infusion if serum sodium level ≥155mEq/l
  - For those with impending herniation: Initially administer 30ml of 23.4% hypertonic saline; if needed, repeat after 15 minutes
- If the patient has an ommaya reservoir, the CSF drainage pressure is less than 20mmHg
- Invite neurology and anesthesiology consultations during EEG bursts of inhibitory electrical activity
- Metabolic analysis every 6 hours, daily head CT scan, and dose adjustment of the above drugs to prevent recurrent cerebral edema, renal failure, electrolyte abnormalities, hypovolemia, and hypotension

All drug doses are for adult subjects;

*Papilloedema was graded according to the modified Frisén criteria.

References:

1. *ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biol Blood Marrow Transplant. 2019. 4: 625-638.*
2. *Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. Nat Rev Clin Oncol. 2018 15:47-62.*

Appendix 7: Reference points for pharmacological interventions for CRS and neurotoxicity

Medicine	Indication	Dose
Tocilizumab	• Echocardiogram showing left ventricular ejection fraction <40%; • Creatinine level is 2.5 times higher than before CAR-T cell infusion; • 48-hour required dose of norepinephrine >2 μg/min; • Norepinephrine cannot relieve hypotension; • Oxygen FiO2> 50% for more than 2 consecutive hours; • Difficulty breathing and need a ventilator; • Activated partial thromboplastin time (APTT) higher than 2 times the upper limit of normal; • Clinically significant bleeding; • Creatine kinase level 5 times higher than the upper limit of normal for more than 2 consecutive days.	4-8 mg/kg, the total amount should not exceed 800 mg, each infusion time is more than 1h
Methyl Prednisolone	Tocilizumab-Refractory CRS Toxicity	1-2 mg/kg IV q 12 h
Other Glucocorticoids	• Grade 3 neurotoxicity lasting more than 24 hours, except headache; • Grade 4 neurotoxicity occurred; • Symptoms of epilepsy appear.	10 mg/kg IV q 6 h, administered at least 8 times, or toxicity reduced to grade 1 or less

References: Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. Nat Rev Clin Oncol. 2018 15:47-62.

Appendix 8: Additional Security Risks and Controls

1. **Chills:** In order to prevent the occurrence of chills, it is recommended to routinely inject anti-allergic drugs (such as promethazine hydrochloride 25mg or similar drugs) before reinfusion, and keep warm during the whole process of reinfusion; if chills still occur after treatment, you can Give the same dose of anti-allergic drugs again.
2. **Fever:** drug ± physical cooling measures. Drugs can be considered intravenous infusion of propatamol hydrochloride 1g or oral ibuprofen sustained-release capsules for antipyretic treatment. For those with poor antipyretic effect and grade 4 fever after drug treatment, it is recommended to take physical cooling measures such as drugs + ice blankets and ice caps.
3. **Critical illness:**

   The reinfusion of TAEST16001 cells may also cause some unexpected emergencies, including but not limited to:

   1） Anaphylactic shock;
   2） Acute respiratory distress syndrome;
   3） Acute pulmonary edema;
   4） Respiratory failure;
   5） Acute heart failure;
   6） Others.

   The above-mentioned critical and critical treatment can be carried out in accordance with the critical treatment procedures of the research center. The drugs listed in the list of emergency drugs in Appendix 9 are recommended as the necessary drugs for the clinical trial of TAEST16001 cell therapy, and are always available in the rescue cart. In addition to the above-mentioned first aid medicines, tracheal intubation and items needed for tracheotomy should be prepared, including laryngoscope, laryngoscope guide wire, mouth gag, tooth pad, tongue forceps, tongue depressor, tracheal tube, etc.
4. **Tumor Lysis Syndrome (TLS):**

   It refers to a group of symptoms that cause significant metabolic disorders and electrolyte disorders due to the massive disintegration of tumor cells and the release of cell contents and metabolites during the treatment of hematological or other malignant tumors, including hyperuricemia. , hyperphosphatemia, hypocalcemia, hyperkalemia, acute uric acid nephropathy and renal insufficiency. The main clinical manifestations are persistent high fever, nausea, vomiting, convulsions, and oliguria. If oncolytic syndrome occurs, the study treatment should be stopped first, and adequate fluid replacement, alkalization of urine, oral allopurinol, and correction of water and electrolyte imbalance should be the principles to protect the heart, liver, and kidney functions of important organs. Hemodialysis should be performed for hyperkalemia.
5. **Adverse reactions caused by the use of IL-2:**

   Chapuis, AG, Yee, C. et al. (PNAS 2012) published "Transferred melanoma-specific CD8+ T cells persist, mediate tumor regression, and acquire central memory phenotype". The clinical trial involved in the article is an FDA-approved clinical trial. A dose-finding study of IL-2 was conducted in this trial, and the results showed that exogenous IL-2 was beneficial

to establish an environment that promotes the proliferation of infused CTLs, and subjects tolerated low doses of IL-2 well. The use of IL-2 (interleukin-2) in this protocol is to promote the proliferation of TCR-T cells that are reinfused into the subject.

The adverse reactions of IL-2 are usually related to the route of use, dose, dosing interval, infusion rate and duration of treatment. The most common dose-limiting adverse reactions of high-dose IL-2 are: hypotension, edema, and renal dysfunction; common adverse reactions are: fever, chills, nausea, vomiting, diarrhea, hypotension, increased neutrophils, lymphatic and Decreased monocytes, decreased blood calcium or phosphorus, vitamin C deficiency, etc.

The pioneer of cell therapy, Professor Rosenberg (NIH, USA), used a large dose of IL-2 (720,000 IU/kg, once a day, up to 15 days) intravenously after TCR-T reinfusion. The subjects were calculated on an average of 60kg, and the dose of IL-2 reached 43.2 million IU, while this clinical program adopted a small dose of IL-2 (500,000 IU), subcutaneously injected, and the dose was about 1% of the dose of Professor Rosenberg's research group. , so the above systemic side effects will not occur.

Based on Professor Cassian Yee (director of the Center for Cellular Immunotherapy at MD Anderson Cancer Hospital, and an internationally renowned tumor immunotherapy expert)'s report on the side effects caused by subcutaneous injection and the applicant's TCR-T clinical trial patient experience, the side effects of subcutaneous injection of this dose of IL-2 are: Mild, mainly local irritation, redness, rash, etc., can be recovered within a few days after discontinuation.

6. Proliferating lentivirus:

Proliferating lentivirus refers to a lentivirus that can replicate and multiply after the lentiviral vector is transferred into human cells. In the lentiviral vector quality control standard, the applicant conducts strict safety and quality control testing (RCR/RCL) on the vector used in TAEST16001 injection according to international standards. For the production of TAEST16001 injection. The applicant adopts a four-plasmid co-transformation system and a viral vector containing a self-inactivating (Self Inactivating, SIN) structure at the end, and the risk of generating RCL during the production process has been significantly reduced. In the virus product quality research, the applicant used the sensitive cell infection test method, that is, the sample to be tested was inoculated on the sensitive cell C8166, and passed multiple passages. At the end of the culture, the expression of p24 antigen was detected by ELISA and the specific gene psi- gag expression is sufficient to detect viral replication. For cell products, Applicants have PCR assays for specific gene psi-gag expression. At the same time, the applicant has strict QC quality standards, uses different methods for RCL detection on each batch of viral vector and cell products prepared, and has sufficient data to verify the safety of the products. In addition, more than 20 years of experience at home and abroad have generated a large amount of data on the safety of retroviral or lentiviral vectors in the clinical application of gene therapy, including the use of different vector designs, vector generation cells, vector batches, and RCR/RCL. Laboratory testing of ex vivo transduced cells and monitoring of patient samples collected during clinical studies lacked positive results for RCR/RCL testing. There are no relevant reports in clinical

trials at home and abroad for many years to show that subjects are infected with proliferating lentivirus after reinfusion of TCR-T cells.

Applicants will refer to the FDA's "Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up (Draft Guidance for Industry)" published in August 2018 to follow up on the subjects. Enter. Lentiviral DNA was detected before and 180 days after subject cell reinfusion. In the event of the proliferation and infection of the proliferating lentivirus, the applicant will take corresponding measures to deal with it in a timely manner in accordance with the clinical diagnosis and treatment guidelines for related viral infections.

7. Clonal and insertional carcinogenesis

Nature Medicine reported a case of a patient with B-cell leukemia treated with CAR-T targeting CD19 who relapsed and developed CAR19-B-cell tumor after 9 months of reinfusion (Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell), no RCL was detected in the patient, so the CAR19-B cell tumor was not caused by lentiviral replication, but was contaminated by the patient's tumor cells (B-cell leukemia cells) during the preparation of CAR-T cells , CAR is inserted into a tumor B cell and forms a clone, which leads to a large number of proliferation of the tumor cell clone in the patient after reinfusion and recurrence occurs.

Most of the indications for CAR-T therapy are hematological tumors. There are a large number of leukemia cells (including B cells) in the blood circulation, so it is very possible to collect such tumor cells. The indications for the TAEST16001 cell product developed by the applicant are: In soft tissue sarcomas (solid tumors), the peripheral blood of patients with solid tumors contains much less tumor cells than patients with hematological tumors. So far, no such related reports have been seen in all TCR-T clinical trials, and the applicant has not found such clonal and insertional carcinogenesis in previous clinical studies.

In TCR-T treatment of solid tumors such as synovial sarcoma, liver cancer, ovarian cancer, it is theoretically impossible to have clonal and insertional carcinogenesis like CAR-T CD19 (Ruella et al. Nature Medicine October 2018). Even if the lentiviral vector transduces tumor cells during production, it is theoretically impossible for the subject's tumor cells to express TCR (because tumor cells have not yet been found to have the key molecules necessary to express antigen-binding TCRs. reported), but tumor cells may express CAR (because tumor cells naturally express antibody molecules); therefore, based on the data reported so far, lentivirus-transduced cancer cells targeted by TAEST16001 will also be eliminated. However, in order to ensure the safety of subjects to the greatest extent, the applicant will prepare TCR-T in strict accordance with the repeatedly verified safe operation procedures to prevent any contamination, so that clonal and insertional cancers will not be detected in the applicant's experiments. appear in. Further, the applicant will pay close attention to the related research on clonal and insertional carcinogenesis, and study the scientific issues of related detection, and will follow up the recurrence or disease progression of the subjects according to the actual needs. The latest detection methods are used to detect subjects and give corresponding clinical treatment.

8. Lymphatic chemotherapy

Cyclophosphamide, a widely used anticancer drug, is effective against malignant lymphoma, acute or chronic lymphocytic leukemia, and other tumors (such as breast cancer, testicular tumors, ovarian cancer, non-small cell lung cancer (NSCLC), head and neck Squamous cell carcinoma, nasopharyngeal carcinoma, neuroblastoma, rhabdomyosarcoma and osteosarcoma) have certain curative effect. Its usage is as follows: single-drug intravenous injection of 500-1000 mg/m2, once a week, 2 times in a row, repeated after 1-2 weeks of rest; 500-600 mg/m2 in combination.

Common adverse reactions: 1) Myelosuppression: leukopenia, thrombocytopenia, the lowest value is 1-2 weeks after treatment, and mostly recovers after 2-3 weeks; 2) It affects liver function and causes gastrointestinal reactions: including appetite 3) Urinary tract reactions: when large doses of cyclophosphamide are administered intravenously without effective preventive measures, it can cause hemorrhagic cystitis, manifested as bladder Irritation symptoms, oliguria, hematuria and proteinuria are caused by the stimulation of the bladder by its metabolite acrolein, but the incidence is low when cyclophosphamide is used in conventional doses; 4) Other adverse reactions include alopecia, stomatitis, Toxic hepatitis, skin pigmentation, menstrual disorders, azoospermia or oligospermia, and pulmonary fibrosis.

The main indication of fludarabine is B-cell chronic lymphocytic leukemia, its clinical usage: intravenous injection or intravenous drip: 25mg/m2 per day, continuous intravenous drug for 5 days every 28 days. The adverse reactions of fludarabine are mainly dose-dependent bone marrow suppression, such as neutropenia and anemia. Other side effects include nausea, vomiting, diarrhea, anorexia, drug eruption, cough, opportunistic infections, and pneumonia. Severe cases can also cause blindness.

Subjects will receive low-dose cyclophosphamide (15mg/kg/d) and low-dose fludarabine (20mg/m2/d) for 3 consecutive days starting 7 days before TCR-T cell reinfusion (d-7) Clear lymphocytes were treated for 3 consecutive days, and after 4 days, TCR-T cells were reinfused.

The combination of cyclophosphamide and fludarabine is mainly used for lymphocyte depletion before T cell reinfusion to reduce or eliminate suppressive lymphocyte Treg, while allowing the infused T cells to obtain enough living space and nutrition to maintain long-term survival. Amplify and survive. The dose of this combination regimen is significantly lower than that of conventional chemotherapy. This regimen has been adopted by many CAR-T and TCR-T clinical trials at home and abroad, and the foreseeable side effects are mainly: lymphopenia (grade 4), granulocytopenia, gastrointestinal reactions, edema, mild transaminases Increased, bladder irritation symptoms, occasional urine red blood cells, etc. Among them, the reduction of lymphocytes is the precondition for the efficacy of T cell infusion, and it is the purpose of its application. For these symptoms such as lymphopenia due to clearing, the applicant will observe the patients in a laminar flow environment according to the plan to avoid infection, thus avoiding the risk of the test.

This program is a standard program for pretreatment of CAR-T clinical trials at home and abroad. So far, thousands of subjects have used it, and no serious adverse reactions have been found. The aforementioned adverse reactions generally recover within 1-2 weeks after

drug withdrawal.

Appendix 9: List of Emergency Medicines

No.	Drug Name	Dose	Quantity	Indication	Effects	Side Effects
1	Nicotine (Koramine)	0.375g/1.5ml	10 sticks	**Respiratory stimulants:** for respiratory depression of various causes	Selectively excite the medulla oblongata respiratory center to deepen and speed up breathing	Increased blood pressure, palpitations, cardiac arrhythmias, convulsions in overdose
2	Lobeline Hydrochloride (Shamrock Hydrochloride)	3mg/1ml	10 sticks	**Respiratory stimulants:** for central respiratory depression, carbon monoxide and opioid poisoning, neonatal asphyxia	Stimulate the carotid sinus and aortic body chemoreceptors, make breathing deepen and accelerate, and have no direct stimulating effect on the respiratory center	May have malignant, vomiting, choking, headache, palpitations, etc.
3	Epinephrine Hydrochloride	1mg/1ml	10 sticks	**Anti-shock drugs:** used to rescue cardiac arrest, anaphylactic shock, dyspnea, prolong the action time of infiltration anesthesia	Excite the myocardium, dilate coronary blood vessels, improve myocardial blood supply, increase blood pressure, relax bronchial and gastrointestinal smooth muscles	Palpitations, irritability, increased blood pressure, etc.
3	Norepinephrine Bitartrate	2mg/1ml	10 sticks	**Anti-shock drugs:** for shock, hypotension, acute myocardial infarction	vasoconstriction, increase blood pressure	Drug solution extravasation can cause local necrosis; slow heart rate, arrhythmia
4	Isoproterenol Hydrochloride	1mg/2ml	10 sticks	**Anti-shock drugs:** for shock, cardiac arrest, atrioventricular block	Enhance myocardial contractility, increase heart rate; relax bronchial and gastrointestinal smooth muscles	Palpitations, dizziness; angina pectoris, myocardial infarction, hyperthyroidism disabled
5	Metahydroxylamine Bitartrate (Alamin)	10mg/1ml	10 sticks	**Anti-shock drugs:** for acute hypotension, cardiogenic shock	Increase myocardial contractility, increase blood pressure	Excessive hypertensive response can cause acute pulmonary edema and arrhythmia; drug solution extravasation can cause local necrosis or induration
6	Atropine Sulfate	0.5mg/1ml	10 sticks	**Anti-shock drugs:** used for anti-shock, pre-administration of general anesthesia, various visceral	Relieve gastrointestinal smooth muscle spasm, inhibit glandular secretion, increase intraocular pressure, increase	Dry mouth, less sweating, dilated pupils, blurred vision, fast pulse, coma in severe poisoning, etc.

				colic, rescue of organophosphorus poisoning	heart rate; bronchiectasis, dilate blood vessels in large doses, improve microcirculation	
7	Dopamine Hydrochloride	20mg/2ml	10 sticks	**Vasopressors:** for shock, cardiac insufficiency, myocardial infarction, renal failure, heart failure	Increases urine output at low doses; increases myocardial contractility at moderate doses; decreases urine output at high doses	Drug solution extravasation can cause local necrosis; large doses can cause arrhythmias; chest pain, dyspnea, palpitations
8	Deacetylanthoside (Cidylland)	0.4mg/2ml	10 sticks	**Cardiac drugs:** for heart failure, acute cardiac insufficiency, atrial fibrillation	Positive inotropic effect can increase myocardial contractility; negative frequency effect can slow down heart rate and delay atrioventricular conduction	Arrhythmia, nausea, vomiting, and rarely blurred vision or "yellow vision" poisoning
9	Lidocaine Hydrochloride	40mg/2ml	10 sticks	**Antiarrhythmic drugs:** for ventricular arrhythmias, also for local anesthesia	At low doses, it can reduce myocardial automaticity and resist ventricular arrhythmias; high concentrations can slow down cardiac conduction velocity and inhibit myocardial contractility; it can also be used for infiltration and topical anesthesia	Adverse reactions such as drowsiness, paresthesia, muscle tremor, and respiratory depression may occur, as well as hypotension and bradycardia.
10	Sulfamethoxamine Injection	1g/5ml	10 sticks	**Hemostatic drugs:** used for bleeding before and after various operations, bleeding caused by poor platelet function and increased vascular fragility	Constrict blood vessels, shorten bleeding time; promote platelet release of coagulation active substances, shorten coagulation time	Nausea, headache, rash; occasional reports of anaphylactic shock following IV administration
11	Vitamin K1	10mg/1ml	10 sticks	**Hemostatic agents:** for bleeding due to vitamin K deficiency, low prothrombin	Vitamin K deficiency can lead to impaired or abnormal coagulation factor synthesis	Large or overdose may aggravate liver damage; intramuscular injection may cause local redness and pain
12	Racemic Anisodamine Hydrochloride	10mg/1ml	10 sticks	**Antispasmodic and microcirculation-improving drugs:** used for gastrointestinal colic, biliary spasm, acute	It can significantly relax smooth muscles, relieve vasospasm, and also have analgesic effect.	Dry mouth, flushed face, mild pupil dilation, blurred vision near objects

	(654-2)			microcirculation disturbance, organophosphorus poisoning		
13	Dexamethasone	5mg/1ml	10 sticks	**Hormone drugs (long-acting):** used for allergic and autoimmune inflammatory diseases, comprehensive treatment of severe infection and poisoning, malignant lymphoma, severe bronchial asthma, rescue of critically ill patients	Adrenal corticosteroids. Anti-inflammatory, anti-allergic, anti-rheumatic, immunosuppressive	Cushing-like syndrome; psychiatric symptoms, such as euphoria, delirium, disorientation, etc., complicated by infection; glucocorticoid withdrawal syndrome
14	Methylprednisolone	40mg/1ml	20 sticks	**Hormonal drugs (moderate effect):** mainly used for allergic and inflammatory diseases. Due to the weak sodium retention effect of this product, it is generally not used as a replacement therapy for adrenal insufficiency.	Adrenal corticosteroids. Anti-inflammatory, anti-allergic, anti-rheumatic, immunosuppressive	Rapid intravenous administration of large doses may cause systemic allergic reactions, including facial, nasal mucosa, eyelid swelling, urticaria, shortness of breath, chest tightness, and stridor.

Appendix 10: Immunotherapy Response Evaluation Criteria in Solid Tumors (iRECIST)

For details, please refer to the original English version:
Lesley S, Jan B, Andrea P, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. The lancet oncology, RPe143-e152.

	Timepoint response with no previous iUPD in any category	Timepoint response with previous iUPD in any category*
Target lesions: iCR; non-target lesions: iCR; new lesions: no	iCR	iCR
Target lesions: iCR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iPR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iSD; non-target lesions: non-iCR/non-iUPD; new lesions: no	iSD	iSD
Target lesions: iUPD with no change, or with a decrease from last timepoint; non-target lesions: iUPD with no change, or decrease from last timepoint; new lesions: yes	Not applicable	New lesions confirm iCPD if new lesions were previously identified and they have increased in size (≥5 mm in sum of measures for new lesion target or any increase for new lesion non-target) or number; if no change is seen in new lesions (size or number) from last timepoint, assignment remains iUPD
Target lesions: iSD, iPR, iCR; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in the size of non-target disease (does not need to meet RECIST 1.1 criteria for unequivocal progression)
Target lesions: iUPD; non-target lesions: non-iCR/non-iUPD, or iCR; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in sum of measures ≥5 mm; otherwise, assignment remains iUPD
Target lesions: iUPD; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed based on a further increase in previously identified target lesion iUPD in sum of measures ≥5 mm or non-target lesion iUPD (previous assessment need not have shown unequivocal progression)
Target lesions: iUPD; non-target lesions: iUPD; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in previously identified target lesion iUPD sum of measures ≥5 mm, previously identified non-target lesion iUPD (does not need to be unequivocal), or an increase in the size or number of new lesions previously identified
Target lesions: non-iUPD or progression; non-target lesions: non-iUPD or progression; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of an increase in the size or number of new lesions previously identified

Target lesions, non-target lesions, and new lesions defined according to RECIST 1.1 principles; if no pseudoprogression occurs, RECIST 1.1 and iRECIST categories for complete response, partial response, and stable disease would be the same. *Previously identified in assessment immediately before this timepoint. "i" indicates immune responses assigned using iRECIST. iCR=complete response. iPR=partial response. iSD=stable disease. iUPD=unconfirmed progression. non-iCR/non-iUPD=criteria for neither CR nor PD have been met. iCPD=confirmed progression. RECIST=Response Evaluation Criteria in Solid Tumours.

Table 2: **Assignment of timepoint response using iRECIST**

Appendix 11: Child-Pugh Liver Function Score

		1 Point	2 Point	3 Point
Serum Total Bilirubin	mg/dl	<2	2-3	>3
	μmol/L	<34.2	34-51	>51
Albumin		>35	28-35	<28
Ascites		None	Mild, Controllable	Refractory Ascites
Hepatic Encephalopathy		None	Mild	Moderate to Severe
Prothrombin Time Prolongation		< 4s	4-6s	> 6s
		(<1.7)*	(1.7-2.2)	(>2.2)

Note: *International Normalized Ratio (INR)

Grading: Grade A: 5-6 points, Grade B: 7-9 points, Grade C: >10 points (including 10 points)

Appendix 12: PK, PD Sample Collection Schedule

Study Operations/Visits, Study Days	Time Window (H or D)	PK Blood Collection	PD Blood Collection
Leukopheresis/ V2 Visit, Day 25	Within 2 hours before apheresis		×
Lymphatic Chemotherapy/ Visit V3, Day 7	Within 2 hours before the first dose of chemotherapy	×	
Day of cell infusion/ V5, Day 1	Within 2 hours before cell reinfusion	×	
Day of cell infusion/ V5, Day 1	1h ±10min after cell reinfusion	×	×
Day 2	±2h	×	
Day 4	±2h	×	
Day 7	±6h	×	×
Day 14	±6h	×	
Day 21	±6h	×	
Day 28	±6h	×	×
Day 60	±6h	×	×
Day 90	±6h	×	×
Day 180	±6h	×	×
Day 270	±6h	×	×

1. PK sample collection:

Blood collection items: Peripheral blood TAEST16001 cells were collected until 9 months after the first reinfusion of cells (study day 270), no TCR-T cells were detected by two consecutive flow cytometry, the patient was withdrawn from the study early for any reason, died or Lost to follow-up, whichever occurs first.

Blood collection items: qPCR detection of TCR-T DNA copy number collection until 9 months after the first reinfusion of cells (study day 270), the patient withdraws from the study early for any reason, died or was lost to follow-up, whichever occurred first.

PD samples were collected until 9 months after the first reinfusion of cells (study day 270), early withdrawal of the patient from the study for any reason, death, or loss to follow-up, whichever occurred first.

√ The time window for PK and PD sample collection after the first cell reinfusion on the first day of the study should be calculated based on the time after the first cell reinfusion on the first day of the study. See the table above for the time window.

√ × represents collection. Collection times should be recorded on a 24-hour clock, accurate to the minute, such as 09:35.

√ Detection: PK will be detected by flow cytometry and qPCR method simultaneously; PD will be detected by flow cytometry and ELISPOT method.