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Supplementary information

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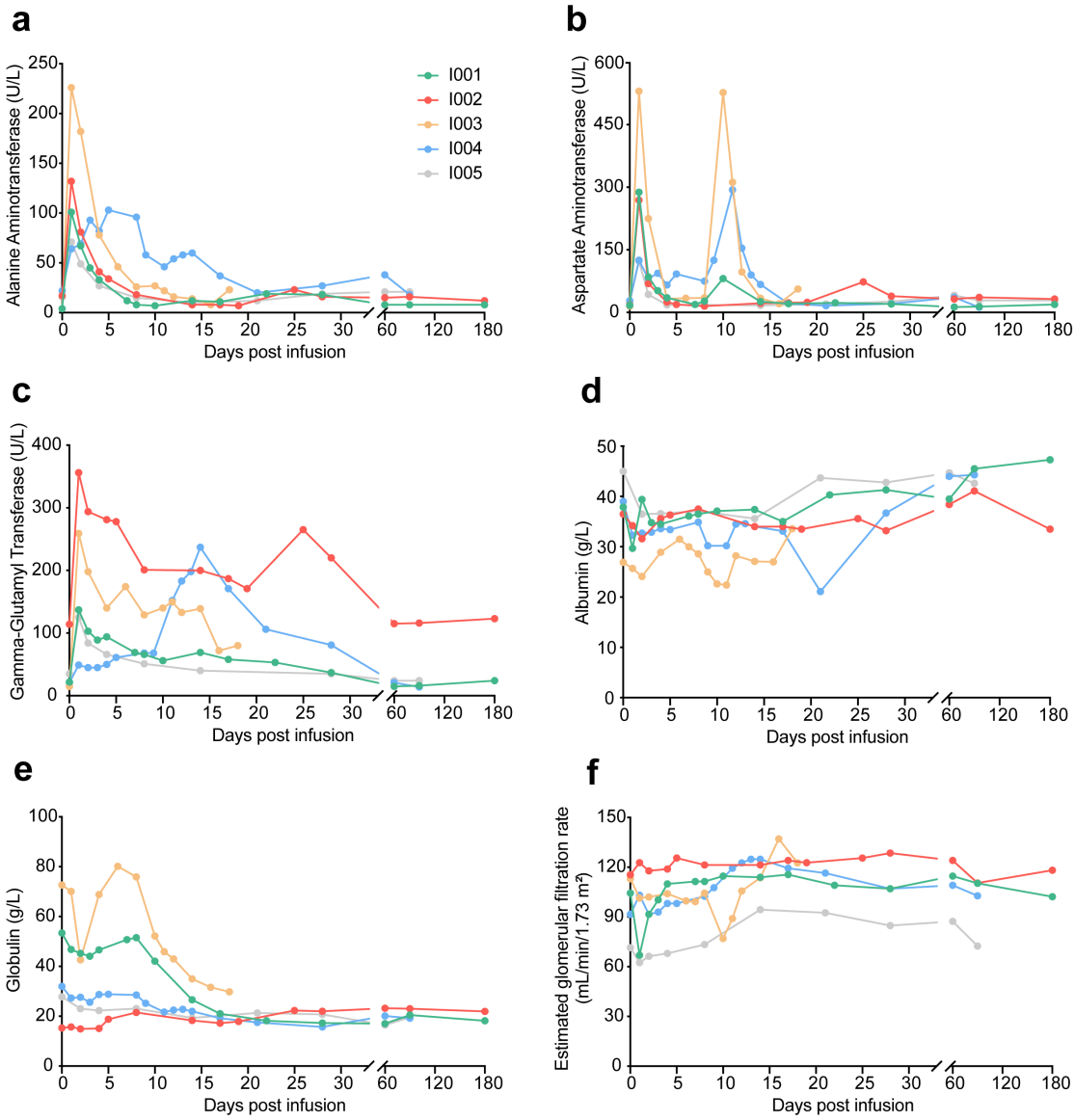
In vivo generation of anti-BCMA CAR-T cells in relapsed or refractory multiple myeloma: a phase 1 study

In the format provided by the authors and unedited



Supplementary Figure 1 | Serum biochemical indices after ESO-T01 infusion.

a, ALT showed transient elevations during the early post-infusion period, resolving by Day 14.

b, AST exhibited a similar pattern, with early increases followed by normalization.

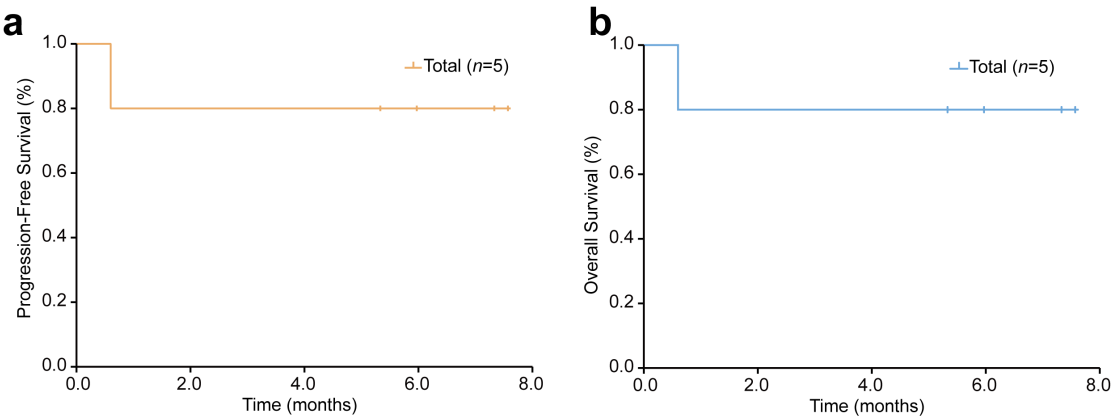
c, GGT rose transiently and declined by Day 14 in most patients.

d, Albumin levels showed moderate fluctuations but stabilized over time.

e, Globulin levels also fluctuated but recovered without persistent hypoproteinemia.

f, eGFR remained largely preserved, with no evidence of clinically significant renal impairment.

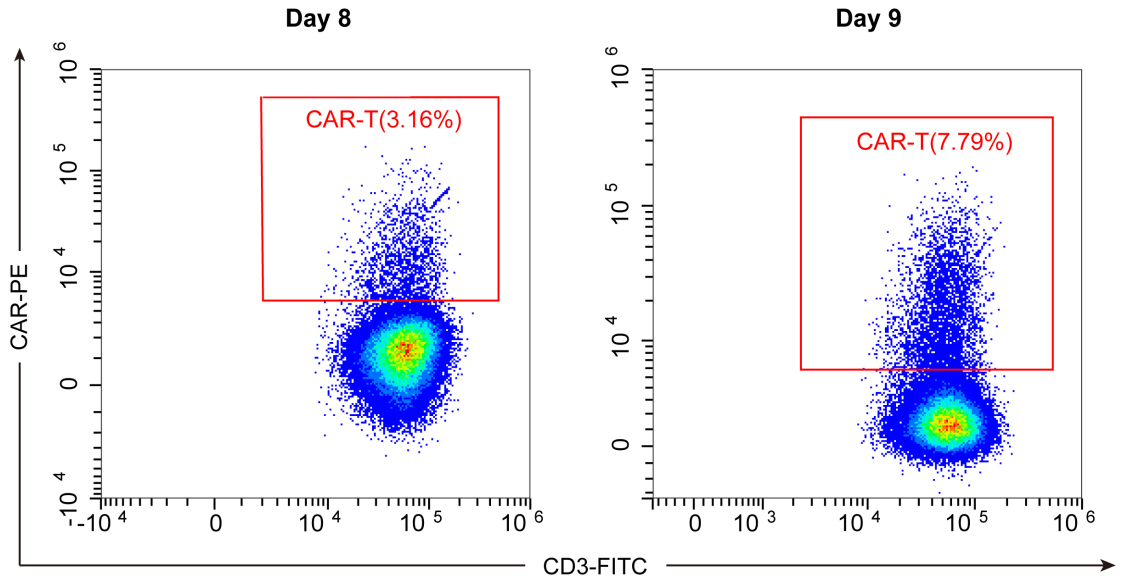
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; eGFR, estimated glomerular filtration rate.



Supplementary Figure 2 | Survival outcomes of patients treated with ESO-T01.

a, Kaplan–Meier curve of progression-free survival (PFS) among the five treated patients.

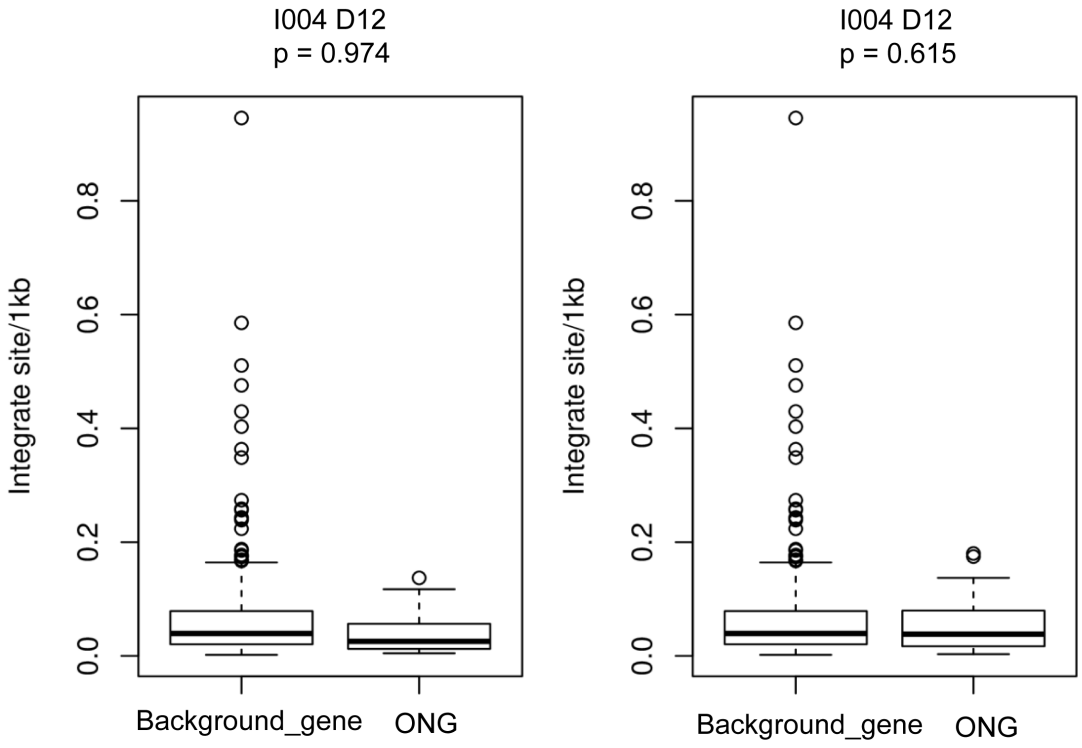
b, Kaplan–Meier curve of overall survival (OS) in the same cohort. Censoring is indicated by tick marks. Median follow-up was 6.0 months (range, 0.6–7.5 months).



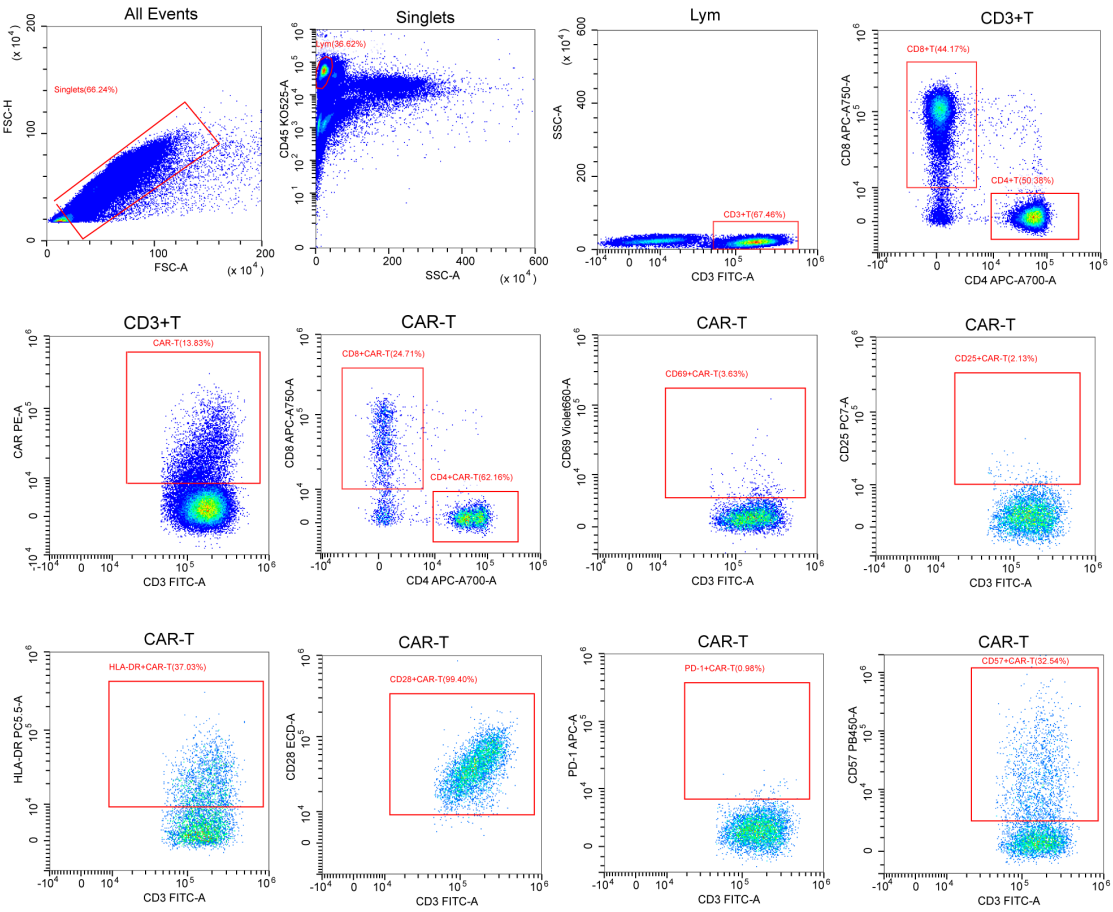
Supplementary Figure 3 | Flow cytometry analysis of CAR-T cells in pleural effusion of patient I002.

Representative dot plots show CAR-T cells in pleural effusion collected on Days 8 and 9 after ESO-T01 infusion. Gated $CAR^{+}CD3^{+}$ T cells (boxed region) increased from 3.16% to 7.79% of total $CD3^{+}$ T cells over 24 hours, consistent with infiltration and local expansion of CAR-T cells within the pleural cavity during the effector phase.

Abbreviations: CAR-T, chimeric antigen receptor T cell.

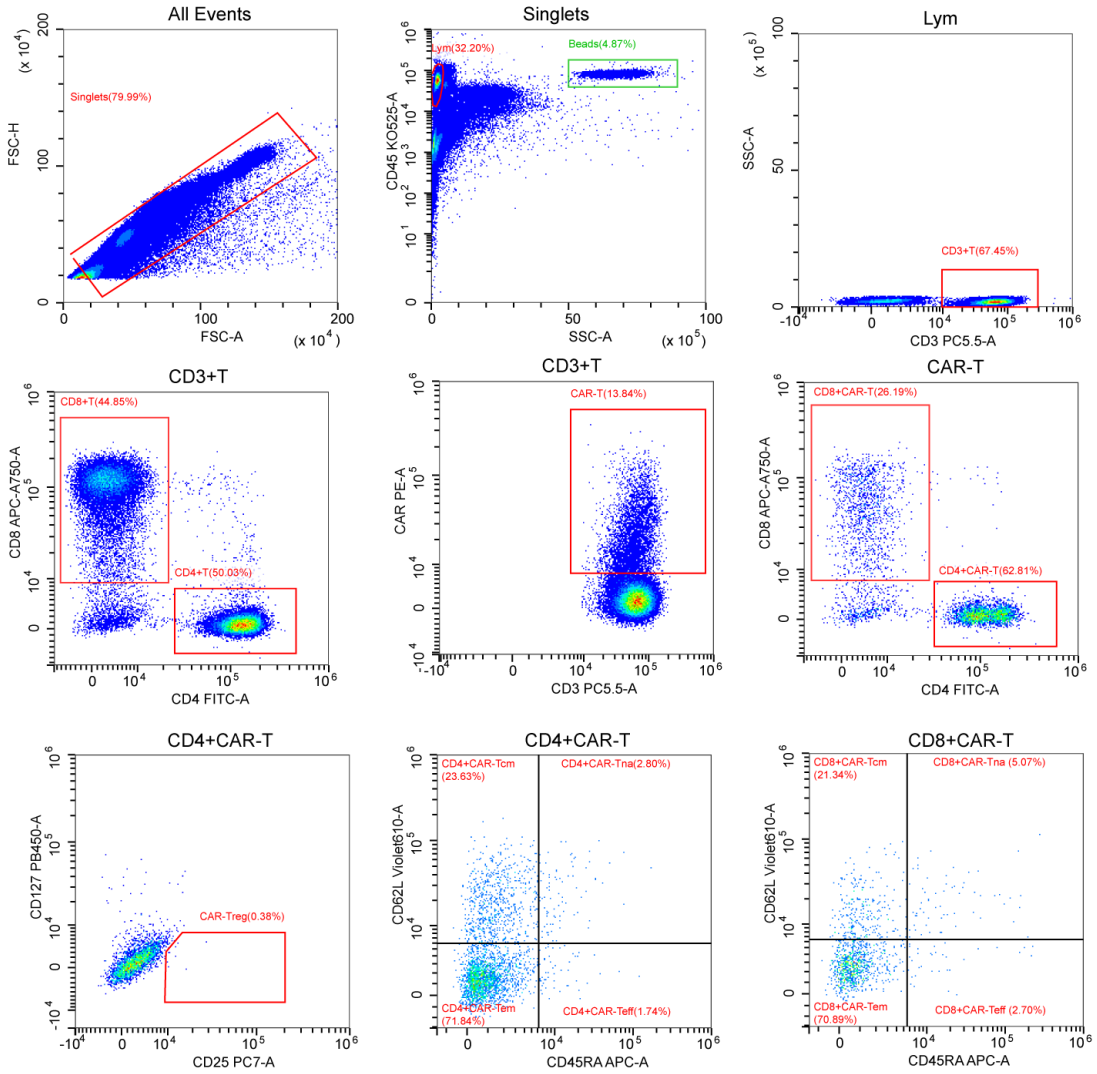


Supplementary Figure 4 | Integration-site analysis of ESO-T01 in peripheral blood.
Integration-site analysis was performed on peripheral blood from patient I004 at Day 12 post-infusion. Mapping of lentiviral vector integration events to a predefined set of tumor-associated genes revealed no enrichment relative to background, providing no evidence of genotoxicity. These findings are consistent with a favorable biosafety profile of ESO-T01 in vivo.



Supplementary Figure 5 | Gating strategy for CAR-T cell identification and functional marker assessment.

Time parameters were used to monitor cytometer stability. Doublets were excluded using FSC-H versus FSC-A, and lymphocytes were gated by CD45/SSC-A. $CD3^+$ T cells were then selected, followed by discrimination of CAR^+ and CAR^- subsets using PE-labeled Human BCMA in combination with CD3-FITC. Within gated CAR^+ T cells, the expression of activation markers (CD69, CD25, HLA-DR, CD28) and functional markers (PD-1, CD57) was evaluated.



Supplementary Figure 6 | Gating strategy for the detection of CAR and CAR-T subsets.
Time parameters were used to monitor instrument stability, doublets were excluded by FSC-H/FSC-A, lymphocytes were determined by CD45/SSC-A, and activated T cells were selected by $CD3^+$. The T cells were further divided into CAR^+ and CAR^- subsets. The $CD4^+$ CAR-T cell subset and $CD8^+$ CAR-T cell subset on CAR-T cells were analyzed.

Supplementary Table 1 | Prior therapeutic regimens of patients before ESO-T01 infusion.

Patient No.	Treatment history	Prior lines
I001	TAD, ARD, AIRD	3
I002	VD, IRD, DPD, KPD	4
I003	VRD, D-VRD, D-KRD	3
I004	VCD, VRD, ICD, Auto-SCT, IRD	5
I005	VD, DVD, DID	3

Treatment regimens are shown for each patient. Abbreviations: AIRD, doxorubicin, ixazomib, lenalidomide, dexamethasone; ARD, doxorubicin, lenalidomide, dexamethasone; Auto-HSCT, autologous hematopoietic stem cell transplantation; DID, daratumumab, ixazomib, dexamethasone; D-KRD, daratumumab, carfilzomib, lenalidomide, dexamethasone; DPD, daratumumab, pomalidomide, dexamethasone; DVD, daratumumab, bortezomib, dexamethasone; D-VRD, daratumumab, bortezomib, lenalidomide, dexamethasone; ICD, ixazomib, cyclophosphamide, dexamethasone; IRD, ixazomib, lenalidomide, dexamethasone; KPD, carfilzomib, pomalidomide, dexamethasone; TAD, thalidomide, doxorubicin, dexamethasone; VCD, bortezomib, cyclophosphamide, dexamethasone; VD, bortezomib, dexamethasone; VRD, bortezomib, lenalidomide, dexamethasone.

Supplementary Table 2 | Adverse events during the first four weeks after ESO-T01 infusion.

Patient No.	Early Phase (Day 0–2)	Late Phase (Day 3–28)	Preemptive Medications
I001	**Grade1-2:** Fever, Sinus Tachycardia, Hypotension, Hypoxia, Cardiac troponin I increased, Diarrhea, Thrombocytopenia, Lymphopenia, AST, LDH, GGT increased, Hypoalbuminemia, Creatinine increased, Hypocalcemia, CRS, Generalized Edema. **Grade 3-4:** Fever, Lymphopenia, Hypoxia, AST increased, CRS.	**Grade1-2:** Cardiac troponin I increased, Herpes simplex infection, Leukopenia, Neutropenia, Lymphopenia, Thrombocytopenia, GGT, LDH, ALP increased Hypocalcemia. **Grade 3-4:** Neutropenia.	Diphenhydramine, Acetaminophen.
I002	**Grade1-2:** Fever, Sinus tachycardia, Hypoxia, Leukopenia, Neutropenia, Lymphopenia, Thrombocytopenia, AST, LDH, GGT increased, Hypoalbuminemia, INR increased, Hypocalcemia, Fibrogenopenia, CRS. **Grade3-4:** Hypoxia, AST increased, Leukopenia, Lymphopenia, CRS.	**Grade1-2:** Peripheral edema, Localized edema**,** Hypotension, Pleural effusion, Leukopenia, Neutropenia, INR increased, Thrombocytopenia, APTT prolonged, GGT, LDH increased, Hypokalemia, Hypocalcemia, Fibrogenopenia. **Grade3-4:** Neutropenia, Thrombocytopenia.	Diphenhydramine, Acetaminophen, Dexamethasone.
I003	**Grade1-2:** Fever, Sinus tachycardia, Leukopenia, Neutropenia, Lymphopenia, Fibrogenopenia, Thrombocytopenia, ALT, AST, GGT increased, Hypoalbuminemia, INR increased, Hyperuricemia, CRS. **Grade3-4:** Leukopenia, Neutropenia, Lymphopenia, Thrombocytopenia, AST increased, Hypoalbuminemia.	**Grade1-2:** Fever, Sinus tachycardia, Hypertension, Hypoxia, Hypersomnia, Pain, Facial edema, Peripheral edema, Epistaxis, Movements involuntary, Lymphopenia, Thrombocytopenia, ALT, AST, GGT increased Hypoalbuminemia, INR increased, Hyperuricemia, APTT prolonged, Hypokalemia, Fibrogenopenia, Hyponatremia, Hypomagnesemia. CRS, ICANS.	Diphenhydramine, Acetaminophen, Dexamethasone.

		Grade3-4: Hypertension, Hypoxia, Lymphopenia, Thrombocytopenia, AST increased, Hypoalbuminemia, Hyponatremia, CRS. **Grade 5:** Spinal cord compression	
I004	**Grade1-2:** Fever, Sinus tachycardia, Hypotension, Anemia, Leukopenia, Neutropenia, Lymphopenia, Abdominal pain, Thrombocytopenia, AST, LDH increased, Hypoalbuminemia, APTT prolonged, Fibrogenopenia, Hypocalcemia, CRS. **Grade3-4:** Leukopenia, Neutropenia, Lymphopenia, Thrombocytopenia.	**Grade1-2:** Fever, Sinus tachycardia, Vomiting, Herpes simplex infection, Leukopenia, Neutropenia, Anemia, Lymphopenia, Abdominal pain, Thrombocytopenia, AST, GGT, LDH increased, Hypoglycemia, INR increased, APTT prolonged, Fibrogenopenia, Hyponatremia, Hypokalemia Hypocalcemia, CRS. **Grade3-4:** Anemia, Leukopenia, Neutropenia, Lymphopenia, Thrombocytopenia, AST increased.	Diphenhydramine, Acetaminophen, Dexamethasone.
I005	**Grade1-2:** Pain, Lymphopenia, AST, LDH, GGT increased, Thrombocytopenia, Hypoalbuminemia, Creatinine increased, Hypophosphatemia. **Grade 3-4:** Lymphopenia	**Grade1-2:** Pain, Lymphopenia, Thrombocytopenia, LDH increased, Hypoalbuminemia, Hypokalemia, Hypocalcemia, Hypophosphatemia, Hyponatremia	Diphenhydramine, Acetaminophen, Dexamethasone.

Adverse events are shown for each patient and stratified into early (Day 0–2) and late (Day 3–28) phases after infusion. Events were graded using CTCAE criteria, while CRS and ICANS were graded according to ASTCT consensus criteria. Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CRS, cytokine release syndrome; GGT, gamma-glutamyl transferase; ICANS, immune effector cell-associated neurotoxicity syndrome; INR, international normalized ratio; LDH, lactate dehydrogenase.

Supplementary Table 3 | Summary of all adverse events observed during follow-up in five patients treated with ESO-T01.

Adverse event	**Any grade** ***n*** **(%)**	**Grade 1-2** ***n*** **(%)**	**Grade 3** ***n*** **(%)**	**Grade 4-5** ***n*** **(%)**
DLT	0	0	0	0
General Disorders and Administration Site Conditions				
Pain	2 (40)	2 (40)	0	0
Fever	4 (80)	3 (60)	1 (20)	0
Peripheral Edema	2 (40)	2 (40)	0	0
Facial Edema	1 (20)	1 (20)	0	0
Generalized Edema	1 (20)	1 (20)	0	0
Respiratory, Thoracic and Mediastinal Disorders				
Hypoxia	3 (60)	0	3 (60)	0
Cough	1 (20)	0	0	0
Epistaxis	1 (20)	0	0	0
Pleural Effusion	1 (20)	1 (20)	0	0
Lung infection	1 (20)	1 (20)	0	0
Cardiac disorders				
Cardiac troponin I increased	1 (20)	1 (20)	0	0
Sinus Tachycardia	4 (80)	4 (80)	0	0
Hematologic Disorders				
Leukopenia	4 (80)	1 (20)	1 (20)	2 (40)
Neutropenia	4 (80)	0	1 (20)	3 (60)
Lymphopenia	5 (100)	0	0	5 (100)
Anemia	1 (20)	0	1 (20)	0
Thrombocytopenia	5 (100)	2 (40)	0	3 (60)
Vascular Disorders				
Hypotension	3 (60)	3 (60)	0	0
Deep vein thrombosis	1 (20)	1 (20)	0	0
Hypertension	1 (20)	0	1 (20)	0
Gastrointestinal disorders				
Vomiting	1 (20)	1 (20)	0	0
Diarrhea	1 (20)	1 (20)	0	0
Abdominal pain	1 (20)	1 (20)	0	0
Infections and Infestations				
Herpes simplex infection	2 (40)	2 (40)	0	0
Nervous system disorders				
Movements involuntary	1 (20)	1 (20)	0	0
Hypersomnia	1 (20)	1 (20)	0	0
Spinal cord compression	1 (20)	0	0	1 (20)

Skin disorders				
Eczema	1 (20)	1 (20)	0	0
Localized Edema	1 (20)	1 (20)	0	0
Investigations				
APTT prolonged	3 (60)	3 (60)	0	0
Fibrogenopenia	3 (60)	3 (60)	0	0
INR increased	3 (60)	3 (60)	0	0
ALT increased	1 (20)	1 (20)	0	0
AST increased	5 (100)	1 (20)	4 (80)	0
LDH increased	4 (80)	4 (80)	0	0
GGT increased	5 (100)	5 (100)	0	0
ALP increased	1 (20)	1 (20)	0	0
Creatinine increased	2 (40)	2 (40)	0	0
Metabolism and nutrition disoeders				
Hypoalbuminemia	5 (100)	4 (80)	1 (20)	0
Hypocalcemia	4 (80)	4 (80)	0	0
Hypokalemia	4 (80)	4 (80)	0	0
Hyponatremia	3 (60)	2 (40)	1 (20)	0
Hypomagnesemia	1 (20)	1 (20)	0	0
Hyperuricemia	1 (20)	1 (20)	0	0
Hypoglycemia	1 (20)	1 (20)	0	0
Hypophosphatemia	1 (20)	1 (20)	0	0
CRS	4 (80)	1 (20)	3 (60)	0
ICANS	1 (20)	1 (20)	0	0

The table consolidates all adverse events (AEs) by system organ class and grade, providing an overview of the safety profile in this first-in-human trial. Detailed listings of early and late AEs, as well as laboratory kinetics, are provided in Extended Data and Supplementary information. Values are shown as number of patients (%).

Abbreviations: ALT, alanine aminotransferase; ALP, Alkaline Phosphatase; APTT, activated partial thrombin time; AST, aspartate aminotransferase; CRS, Cytokine Release Syndrome; DLT, dose-limiting toxicity; GGT, Gamma-Glutamyl Transferase; ICANS, Immune effector cell-associated neurotoxicity syndrome; INR, international normalized ratio; LDH, lactate dehydrogenase.

Supplementary Table 4 | Longitudinal changes in peripheral blood counts after ESO-T01 infusion.

Parameter (unit)	Patient	Baseline	2h	D1	D4	D8	D14	D28	2M	3M	6M
White blood count (×10⁹/L)	I001	3.73	–	8.97	6.70	3.97	5.19	5.11	6.12	6.61	8.47
	I002	7.36	1.35	6.09	6.27	2.90	5.53	4.14	5.81	5.05	9.29
	I003	1.79	0.07	1.38	2.37	4.41	1.69	–	–	–	–
	I004	2.68	0.52	1.51	2.45	1.26	0.97	2.76	4.05	2.34	–
	I005	6.69	8.50	12.98	6.76	7.47	7.00	6.74	6.05	5.46	–
Neutrophils (×10⁹/L)	I001	2.04	–	8.76	3.82	2.01	0.46	2.57	3.25	3.38	4.47
	I002	5.73	1.25	5.51	3.82	1.02	1.89	1.21	3.47	2.20	6.82
	I003	0.83	0	1.20	0.92	3.41	0.83	–	–	–	–
	I004	1.49	0.31	1.29	1.12	0.90	0.20	1.55	2.91	1.27	–
	I005	4.28	8.32	11.90	4.44	5.43	2.95	4.88	3.90	2.87	–
Lymphocytes (×10⁹/L)	I001	1.25	–	0.10	2.42	1.30	4.01	1.59	2.20	2.76	3.36
	I002	1.04	0.06	0.05	2.09	1.15	3.27	1.99	1.49	2.14	1.39
	I003	0.78	0	0.12	1.33	0.60	0.62	–	–	–	–
	I004	0.98	0.01	0.10	1.17	0.26	0.63	0.66	0.78	0.82	–
	I005	1.80	0.40	0.25	1.48	0.78	4.30	1.07	1.42	1.34	–
Monocytes (×10⁹/L)	I001	0.37	–	0.10	0.37	0.41	0.52	0.77	0.56	0.37	0.51
	I002	0.56	0.03	0.53	0.84	0.70	0.27	0.45	0.45	0.54	0.97
	I003	0.17	0	0.06	0.10	0.36	0.24	–	–	–	–
	I004	0.20	0.05	0.12	0.14	0.09	0.14	0.44	0.32	0.23	–
	I005	0.52	0.13	0.82	0.73	1.00	0.94	0.68	0.67	1.11	–
Hemoglobin (g/L)	I001	118	–	110	113	121	116	129	131	148	153
	I002	119	125	111	114	102	117	108	126	129	123
	I003	77	85	73	76	63	54	–	–	–	–
	I004	114	98	101	108	108	72	82	133	140	–
	I005	155	143	141	145	137	133	151	152	153	–
Platelets (×10⁹/L)	I001	241	–	137	96	119	95	241	200	222	202
	I002	111	86	57	70	63	61	33	170	182	235
	I003	82	62	45	34	23	44	–	–	–	–
	I004	83	40	42	33	19	33	53	156	85	–
	I005	191	104	120	124	135	138	222	185	177	–

Peripheral blood counts were assessed at baseline, 2 hours (2H), and on Days 1, 4, 8, 14 and 28, and at Months 2, 3 and 6 after infusion. Data are presented as absolute values, and missing values are denoted by "–". All values are absolute counts measured in peripheral blood. Values represent individual patient data. Abbreviations: WBC, white blood cell; D, day; M, month; 2h, 2 hours post-infusion.

Supplementary Table 5 | Longitudinal changes in biochemical indices after ESO-T01 infusion.

Parameter (unit)	Patient	Baseline	D1	D2	D4	D8	D14	D28	2M	3M	6M
ALT (U/L)	I001	4	101	67	33	8	12	18	8	8	8
	I002	17	132	81	41	18	8	16	15	16	12
	I003	4	226	182	78	26	14	–	–	–	–
	I004	22	64	69	82	96	60	27	38	18	–
	I005	16	71	49	27	15	10	19	21	21	–
AST (U/L)	I001	17	288	85	35	27	26	20	13	14	19
	I002	18	269	69	25	15	22	39	32	36	32
	I003	15	530	225	33	35	34	–	–	–	–
	I004	28	125	81	66	75	67	21	36	12	–
	I005	23	123	43	18	18	17	26	41	29	–
Creatinine (μmol/L)	I001	68	109	84	60	58	55	64	54	63	80
	I002	44	38	42	41	39	39	34	37	50	40
	I003	56	73	72	64	68	55	–	–	–	–
	I004	84	70	84	79	71	44	63	64	78	–
	I005	94	105	100	98	92	86	86	84	–	–
eGFR (mL/min/1.73 m²)	I001	104.4	66.9	91.6	109.9	111.4	113.9	107	114.7	110.3	102.2
	I002	115.5	122.7	117.8	118.9	121.4	121.4	128.5	124.1	110.5	118.2
	I003	113	101.4	102	104	104.4	113.7	–	–	–	–
	I004	91.6	103.1	91.6	98.1	102.5	124.8	107	109.1	102.8	–
	I005	71.6	62.5	66.3	68	73.4	94.4	84.8	87.3	72.5	–

Biochemical indices were assessed at baseline and on Days 1, 2, 4, 8, 14 and 28, and at Months 2, 3 and 6 after infusion. Data are presented as absolute values, and missing values are denoted by "–". All values are absolute measurements in serum. Values represent individual patient data. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate.

Supplementary Table 6 | Transient elevation of peripheral viral titers after ESO-T01 infusion.

Patient No.	Baseline (LPS·mL^{-1})	Day 2 (LPS·mL^{-1})	Day 4 (LPS·mL^{-1})
I001	0	2626.80	0
I002	0	1228.58	0
I003	0	2245.00	0
I004	0	38.51	0
I005	0	125.30	0

Peripheral blood samples were collected at baseline (pre-infusion), Day 2 and Day 4 after ESO-T01 administration. Viral load was measured by RT-qPCR and expressed as lipopolysaccharide equivalents per milliliter (LPS·ml^{-1}). All five patients exhibited a transient increase in viral titers at Day 2, which resolved completely by Day 4.

Supplementary Table 7 | Antibodies used for CAR-T functional and subset analyses.

Marker	Fluorophore	Manufacturer	Catalog #	Clone name
CD3	APC	BioLegend	317318	OKT3
TNFRSF17	PE	ACRO	BCA-HP2H2	/
CD4	FITC	BD	555346	RPA-T4
CD8	APC-Cy7	BD	557834	SK1
CD45RA	APC	BioLegend	304112	HI100
CD62L	Brilliant Violet 605™	BioLegend	304834	DREG-56
CD25	PE-Cy7	BD	557741	M-A251
CD45	BV510	BD	563204	HI30
CD3	FITC	BioLegend	317306	OKT3
CD4	R718	BD	566930	SK3
CD69	Brilliant Violet 650	BioLegend	310934	FN50
CD25	PE-Cy7	BD	557741	M-A251
HLA-DR	PerCP	BioLegend	307628	L243
CD28	PE/Dazzle 594	BioLegend	302942	CD28.2
PD-1	APC	BD	558694	MIH4
CD57	Brilliant Violet 421	BioLegend	393326	QA17A04

List of markers, fluorophores, manufacturers, and catalog numbers for antibodies applied in flow cytometry assays of CAR-T cell function and subset profiling. Some antibodies (e.g., CD3, CD4, CD25) were used in multiple fluorophore conjugates to accommodate different multicolor flow cytometry panels. All antibodies used for flow cytometry have been validated for flow cytometry applications, and the corresponding validation flow plots are available on the manufacturer's website.

Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology

Clinical Study Project Protocol

Project Name	Clinical Study on the Safety and Efficacy of ESO-T01 Injection in the Treatment of Relapsed/Refractory Multiple Myeloma
Protocol No.	PRG2402E2
Version No.	1.1
Version Date	January 13, 2025
Principal Investigator	Chunrui Li
Department	Department of Hematology
Estimated Start and End Dates	November 2024 to November 2027

PROTOCOL SIGNATURE PAGE

Project Name: Clinical Study on the Safety and Efficacy of ESO-T01 Injection in the Treatment of Relapsed/Refractory Multiple Myeloma

Version No./Version Date: 1.1/January 13, 2025

I have read and understood this protocol, and hereby confirm that the protocol includes all necessary information for the implementation of the study and clarifies the responsibilities of Investigators related to this study protocol. As the Principal Investigator, I will provide copies of this protocol and all related materials to all study personnel participating in this study. I will discuss these materials with them to ensure that they fully understand how to implement this study protocol. I agree to and will perform the relevant duties in strict accordance with all current applicable laws and regulations, the Declaration of Helsinki, Good Clinical Practice, and this study protocol.

Principal Investigator Signature (Printed):

Principal Investigator Signature/Date:

Clinical Research Unit:

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PROTOCOL SYNOPSIS

Study Name	Clinical Study on the Safety and Efficacy of ESO-T01 Injection in the Treatment of Relapsed/Refractory Multiple Myeloma
Study Objectives	**Primary Study Objective:** To evaluate the safety and tolerability of ESO-T01 Injection in the treatment of relapsed/refractory multiple myeloma (R/R MM). **Secondary Study Objectives:** 1. To preliminarily assess the efficacy of ESO-T01 Injection in the treatment of R/R MM; 2. To obtain the pharmacokinetic and pharmacodynamic data of ESO-T01 Injection in humans. **Exploratory Objective:** To assess the immunogenicity, viral shedding, and other characteristics of ESO-T01 Injection.
Study Endpoints	**Primary Study Endpoints:** Safety and tolerability of ESO-T01 Injection: Safety measures such as dose-limiting toxicities (DLTs), adverse events (AEs), laboratory tests, vital signs, physical examinations, and electrocardiograms (ECGs). **Secondary Study Endpoints:** 1. Efficacy parameters: Time to response (TTR), objective response rate (ORR), minimal residual disease (MRD) negativity rate (according to the 2016 International Myeloma Working Group (IMWG) response criteria [Appendix 1]), duration of response (DOR), progression-free survival (PFS), overall survival (OS), etc. Objective response rate includes stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR); 2. Pharmacokinetic parameters: Maximum concentration (C_{max}), time to maximum concentration (T_{max}), area under the curve from Time 0 to Day 28 ($AUC_{0\text{-}28d}$) etc. of the expansion of BCMA CAR-T in peripheral blood after ESO-T01 infusion. 3. Pharmacodynamic parameters: Testing of cytokine levels (IL-

	6, IL-10, IFN-γ, TNF-α, etc.), CRP, ferritin levels, immunoglobulin levels (IgG, IgA, IgM), and peripheral lymphocyte subpopulations (T, B, NK cell ratios and absolute counts) in peripheral blood after ESO-T01 infusion. **Exploratory Study Endpoint:** Immunogenicity of ESO-T01 and *in vivo* generated CAR-T by ESO-T01, and the presence of shedding of ESO-T01 in blood, saliva, urine, and feces.
Total Number of Patients Enrolled	4-24 subjects are planned to be enrolled in this study.
Study Phase	Investigator-initiated clinical trial
Study Duration	Main study evaluation and follow-up period of approximately 24 months
Inclusion Criteria	**Subjects must meet all of the following criteria to be eligible for participation in this study:** 1.Aged 18 years or older, any sex; 2.Diagnosed with multiple myeloma (MM) according to the IMWG response criteria (Appendix 2), with positive MM cells expressing BCMA target antigen confirmed by flow cytometry or bone marrow pathology and immunohistochemistry. 3.Received at least 2 lines of anti-MM therapy, with each line consisting of at least one complete treatment cycle, and experienced progressive disease as evidenced by test data within 12 months during or after the most recent anti-myeloma therapy; Or are determined by the Investigator to be refractory to both immunomodulators and proteasome inhibitors, and have experienced progressive disease (according to IMWG response criteria) during or within 2 months after the most recent anti-myeloma therapy. 4.Measurable disease at screening, i.e., meeting one or more of the following criteria: •Serum M-protein ≥ 0.5 g/dL; •Urine M protein level ≥ 200 mg/24 h

	•Or involved serum free light chain ≥ 10 mg/dL with abnormal serum free light chain κ/γ ratio. 5.ECOG score of 0-2 points (Appendix 3), with an expected survival of 3 months or more; 6.Bone marrow function test results (at screening or within 2 months before screening) meet the following criteria: •Hemoglobin ≥ 6 g/dL (no red blood cell transfusions within 1 week prior to screening), and the use of recombinant human erythropoietin is allowed. For patients who meet the inclusion criteria of hemoglobin ≥ 6 g/dL, red blood cell transfusions are permitted to maintain hemoglobin levels at ≥ 6 g/dL; •Absolute neutrophil count (ANC) ≥ 600/μL (no use of granulocyte-colony stimulating factor (G-CSF) within 1 week prior to screening or no use of pegylated G-CSF within 2 weeks prior to screening); •Platelet count (ANC) ≥ 50,000/μL; •Lymphocyte count (ANC) ≥ 500/μL; •Absolute CD3-positive T cell count ≥ 150/μL; 7.Normal renal function during the screening period or within 2 months prior to screening: Creatinine clearance (CrCl) (Cockcroft-Gault formula, Appendix 4) ≥ 45 mL/min; 8.Liver function must meet the following criteria during the screening period or within 2 months prior to screening: •Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 3.0 × upper limit of normal (ULN); •Total bilirubin (TBIL) and alkaline phosphatase (AKP or ALP) ≤ 2.0×ULN (except for congenital hyperbilirubinemia, such as Gilbert syndrome, where direct bilirubin can be ≤ 1.5×ULN); •Albumin ≥ 3 g/dL; 9.Cardiac function must meet the following criteria during the

	screening period or within 2 months prior to screening: •Left ventricular ejection fraction ≥ 40% (by echocardiography or MUGA scan); •No clinically significant pericardial effusion detected; •No clinically significant ECG abnormalities detected; 10.Lung function must meet the following criteria during the screening period or within 2 months prior to screening: •Oxygen saturation ≥ 90%; •No clinically significant pleural effusion detected; 11.A negative pregnancy test is required for women of childbearing potential at screening and before drug infusion, and they must also not be breastfeeding; 12.Men and women of childbearing potential must agree to use effective contraception (see Appendix 5) from the time they sign the informed consent form until one year after the use of study drug; 13.Men and women of childbearing potential must agree not to donate germ cells including sperm or ovum (oocytes) from the time they sign the informed consent form until one year after the use of study drug; 14.The subject or his/her legally authorized representative has provided consent by signing the informed consent form (ICF), indicating his/her understanding of the study purpose and procedures, and willingness to participate in the study.
Exclusion Criteria	**Subjects who meet any of the following criteria will be excluded from this study:** 1.Received other anticancer treatments during screening (primarily determined by the Investigator): •Received targeted therapy, epigenetic therapy, other treatments with clinical study drugs, or treatments involving invasive study medical devices within 5 half-lives; •Received immune/non-immune directed systemic therapy within 1 week;

	•Received cytotoxic therapy within 1 week; •Treated with a proteasome inhibitor and an immunomodulator within 2 weeks. •Received radiotherapy within 4 weeks (subjects are eligible regardless of the end date of radiotherapy if the radiation field covers ≤5% of the bone marrow reserve); 2.Received an allogeneic hematopoietic stem cell transplantation within 6 months before infusion or an autologous hematopoietic stem cell transplantation within 3 months before infusion. 3.A history of malignancies other than MM prior to screening, except for the following: Malignant tumors treated with curative intent, with no known active disease for ≥ 2 years prior to enrollment; Adequately treated non-melanoma skin cancer without current disease evidence; 4.Received treatment with any virus pseudotyped with vesicular stomatitis virus glycoprotein (VSVG); 5.Presence of serious, uncontrolled infections (bacterial, viral, fungal, etc.) during the screening period; 6.Positive for hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb), with the detected peripheral blood hepatitis B virus (HBV) DNA titer above the normal range within 6 months prior to infusion. Positive for hepatitis C virus (HCV) antibody, with the detected peripheral blood HCV RNA titer above the normal range. Positive for human immunodeficiency virus (HIV) antibody. Patients who test positive for syphilis; 7.Presence of symptomatic heart failure or other cardiac disorders such as serious arrhythmia; •New York Heart Association (NYHA) Class III or Class IV congestive heart failure; •Episodes of myocardial infarction, or prior coronary artery bypass grafting (CABG) or coronary arterial stent insertion within 6 months before signing the ICF; •Clinically significant ventricular arrhythmias or a history of

	unexplained syncope (excluding cases caused by vasovagal episodes or dehydration); •A history of severe non-ischemic cardiomyopathy; 8.Other clinically significant diseases, including: •Primary immunodeficiency; •Stroke or epileptic seizure within 6 months before screening; •Clear clinical evidence of dementia or mental status changes; •Having Parkinson's disease, parkinsonian dyskinesia, or a history of these conditions. 9.Underwent surgery within 2 weeks of administration or plans to receive surgery within 2 weeks after administration, excluding surgeries involving local anesthesia; 10.Immunization with a live attenuated vaccine within 1 month before administration; 11.Known severe allergic reactions to ESO-T01 or any of its preparation components; 12.Known severe allergic reactions to tocilizumab; 13.Patients unsuitable for the creation of venous access; 14.Other conditions deemed by the Investigator to be unsuitable for participation in the study.
Overall Design	This is a single-arm, open-label, single-center clinical study designed to assess the safety, tolerability, efficacy, and pharmacokinetic or pharmacodynamic profiles of the study drug ESO-T01. **1.Dose Escalation and Subject Enrollment Principles** This study uses a dose-escalation design with 4 dose groups: Dose A, Dose B, Dose C, and Dose D. A total of 4-24 subjects with relapsed/refractory multiple myeloma are enrolled. Subjects will be enrolled with rapid titration. In Dose A group, 7 days after infusion in the first subject, PK samples will be tested centrally and analyzed by the Principal Investigator, as well as the comparability with the

	Phase 1 clinical trial of BCMA autologous CAR-T, and a decision on whether to continue enrolling subsequent subjects or to increase the dose to Dose B will be made. Similarly, in Doses B, C, and D groups, a single subject will be enrolled first (titration) and administered infusion of ESO-T01. In Doses B, C, and D groups, 14 days after infusion in the first subject, PK samples will be tested centrally and analyzed by the Principal Investigator, as well as the comparability with the Phase 1 clinical trial of BCMA autologous CAR-T, and a decision on whether to continue enrolling subsequent subjects or to increase the dose to subsequent dose levels will be made. In Doses A, B, C, and D groups, if continued enrollment of subjects is required after the first subject has completed the titration, enrollment of 3-6 subjects of the dose group design may be completed. Within the same dose group, the enrollment interval for subsequent subjects is determined by the Principal Investigator, except that the enrollment interval between the first and second subjects is 7-14 days. MTD is the highest dose at which DLT is observed in ≤1/6 of subjects. Determination of MTD is made by the Investigator and the cooperating unit after discussion based on cumulative safety data from all subjects with dose-escalation. For 3 subjects at the assessed dose, ① if no DLT is observed, these subjects will be added to the next dose group; ② if DLT is observed in 1 subject, 3 additional subjects will be enrolled at this dose level. A. If DLT is observed in only 1 subject out of the 6 subjects in total, they will proceed to the next dose group; B. if DLT is observed in 2 (inclusive) or more subjects out of the 6 subjects in total, enrollment will be continued in the previous dose group up to 6 subjects to continue observing the DLT until the MTD is determined. **2.Dose Setting** 4 dose groups are established in this study, as shown in the table below.

ESO-T01 Dose Escalation Scheme

Dose Group	Dose A	Dose B	Dose C	Dose D
Dose (TU/subject)	0.2×10^9	0.6×10^9	1.2×10^9	2.4×10^9
Number of subjects	1-6	1-6	1-6	1-6

3.Follow-up Principles

After ESO-T01 infusion, subjects will undergo appropriate follow-up assessments (visit points as specified in the protocol or additional visit points as deemed necessary by the Investigator or the cooperating unit) for efficacy and safety evaluations until the end of the trial or withdrawal from the study. For subjects who have received ESO-T01 treatment, even if they withdraw from the trial prematurely, the Investigator should perform long-term safety follow-up for them according to the protocol to evaluate the long-term safety of this product.

Dose-limiting Toxicity (DLT)

Dose-limiting toxicity (DLT) is defined as ≥ Grade 3 toxicity occurring within the DLT observation period (28 days after ESO-T01 infusion) and considered by the Investigator or the cooperating unit to be reasonably related to ESO-T01 treatment. Toxicity will be graded according to the CTCAE v5.0, except for cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), which will be graded based on the 2019 ASBMT consensus criteria. Given the specificity of subjects with multiple myeloma (MM), DLT is defined as: A subject who, despite therapeutic measures, develops any of the following conditions related to the study drug:

(1) Grade 4 or higher CRS caused by ESO-T01 treatment;

(2) Grade 3 CRS caused by ESO-T01 treatment that does not resolve to Grade 2 or lower within 7 days of onset;

(3) Grade 3 or 4 neurotoxicity caused by ESO-T01 treatment

	(excluding cases where the neurotoxicity resolves to Grade 1 within 2 weeks of onset, or to baseline within 4 weeks of onset); (4) Grade 3-5 allergic reactions caused by ESO-T01 treatment (excluding cases of rapid allergic reactions related to ESO-T01 occurring with 2 hours of its infusion and resolving to Grade 2 or lower within 24 hours after systemic treatment); (5) Grade 3 or higher non-hematologic toxicity, except for the following: • Fever of any grade, including neutropenic fever; • Grade 3 or higher diarrhea lasting ≤ 72 hours; • Grade 3 or higher nausea and/or vomiting lasting ≤ 72 hours; • Grade 3 or higher fatigue lasting ≤ 7 days; • Elevated transaminases, bilirubin, or creatinine levels (Grade 3 or higher) lasting ≤ 14 days; • Any Grade 3 non-hematologic adverse events (AEs) that are rapidly reversible (resolve to baseline or Grade 2 and lower within 7 days). (6) Excluding hematocytopenia of any degree.
Study Drug Usage	Name: ESO-T01 Injection; Dosage and administration: Dosage of ESO-T01 is $0.2–2.4\times10^9$ TU/subject. The use of ESO-T01 is completed by intravenous infusion.
Drug Infusion	Drug infusion should not be started or should be delayed if the subject experiences any of the following: 1. Before drug infusion, the subject experiences any of the following, including but not limited to: New-onset arrhythmias that cannot be controlled with medications, hypotension requiring vasopressors, or uncontrolled active infection. The subject is not suitable for continued drug infusion as judged by the Investigator; 2. Body temperature > 38℃ within 48 hours before drug infusion (excluding tumor-related fever); 3. ECOG performance status score ≥ 3 before drug infusion;

	4. The subject is assessed as unable or unwilling to comply with the study protocol requirements by the Investigator. Subjects who pass the eligibility assessment for ESO-T01 administration will receive ESO-T01 via intravenous drip infusion. It is necessary to reconfirm that the study site has at least two doses of tocilizumab available. **Administration of prophylactic medications:** Administer acetaminophen (650 mg orally) and diphenhydramine (12.5 mg intramuscularly or 25 mg orally) within 1 hour before ESO-T01 infusion. Subjects may receive prophylactic medications such as hormones, as determined by the Investigator based on individual circumstances of subjects. **Study drug infusion:** ESO-T01 is administered by intravenous infusion at doses of 0.2-2.4×10^9 TU/subject. The use of a precision filtration infusion set is prohibited. Once the infusion process has begun, if there is any leakage of the drug solution, the administration should be halted, and the remaining uninfused drug solution should be retrieved and destroyed in accordance with requirements. First aid facilities should be available during the infusion to provide prompt treatment for severe allergic reactions, severe hypotension, or other reactions. **Criteria for Stopping Infusion** During the trial, the Investigator may decide whether the subject needs to stop the drug infusion or withdraw from the trial based on the following criteria: 1. Intolerable toxicity during infusion resulted in subjects being unable to continue ESO-T01 treatment; 2. Unwillingness of subjects to continue with the clinical study. Subjects can make requests to the Investigator and withdraw from the study at any time; 3. Exacerbation of disease in subjects before ESO-T01 infusion that is assessed as unsuitable for drug infusion by the Investigator;

	4. Other conditions that the Investigator considers unsuitable for drug infusion.
Criteria for Withdrawal from the Study	1)Intolerable adverse events occur, and the Investigator determines that the risks of continued participation in the study outweigh the benefits for the subject; 2)Poor subject compliance results in failure to complete follow-ups on time; 3)The subject or his/her legally authorized representative requests withdrawal from the trial or withdrawal of the ICF; 4)The subject experiences confirmed tumor progression during the trial and requires other medical interventions; 5)The Investigator considers the subject to have other conditions that make continued participation in the trial inappropriate. Note: For subjects who have received ESO-T01 treatment, even if they withdraw from the trial prematurely, the Investigator should perform long-term safety follow-up for them according to the protocol to evaluate the long-term safety of this product. All subjects who withdraw from the study with drug-related AEs should be followed up subsequently for AEs until AEs resolve to baseline, stabilize, the subject is lost to follow-up, or there is another reasonable explanation. If the AE is a chronic condition, it may be excluded from follow-up in consultation between the Investigator and the cooperating unit, but the consultation must be documented in the original medical record.
Criteria for Study Termination	Reasons for study termination include, but are not limited to: 1)The clinical trial terminated as required by the cooperating unit; 2)When the number and severity of adverse events occurring in this study indicate that continuing this study will pose greater risks than benefits to subjects; 3)Situations in which the National Medical Products Administration or the National Health Commission deems a clinical trial unsuitable for continuation.

Safety Evaluation	All adverse events will be monitored and recorded throughout this study. Abnormal changes in vital signs, physical examination, clinical laboratory tests, electrocardiogram (ECG), and other measures before and after ESO-T01 treatment are recorded. Each safety measure, including type, onset time, frequency, response time, severity, management measures, and correlation with ESO-T01 treatment, is described and statistically analyzed.
Pharmacokinetic Evaluation	Peripheral blood is collected from subjects at baseline and on D2, D4, D6, D8, D10, D12, D14, D17, D21, D24 and D28, and at M2, M3, M6, M9, M12, M18 and M24 after infusion to detect the CAR copy number in peripheral blood using validated qPCR method. Peripheral blood is collected from subjects at baseline and on D2, D4, D6, D8, D10, D12, D14, D17, D21, D24 and D28, and at M2 after infusion to detect changes in the number of CAR-T (CAR-T/mL blood) using flow cytometry methods. At the time point when CAR-T cells are detectable by flow cytometry, the CAR-T cell phenotype is further analyzed for CD4/CD8 ratio and naive, memory and effector cells, etc.
Pharmacodynamic Evaluation and Exploratory Study	Peripheral blood is collected at baseline, on D2, D4, D6, D8, D10, D12, D14, D17, D21, D24, D28, and at M2 to detect cytokine levels (IL-6, IL-10, IFN-γ, TNF-α, etc.), and CRP and ferritin levels. Peripheral lymphocyte subpopulations (T, B, and NK cell ratios and absolute counts) and immunoglobulin levels (IgG, IgA, and IgM) are detected at baseline, on D28, and at M2, M3 and M6. Exploratory immunogenicity and viral shedding study: Serum samples are collected and frozen at baseline, on D28, and at M3, M6, M9, M12, M18 and M24 for central detection of ESO-T01 and CAR-T cell immunogenicity. Blood, saliva, urine, and fecal samples are collected and frozen at baseline, and on D2, D4, D6, D8, D10, D14, D21 and D28 to detect viral shedding.

Efficacy Evaluation	This study will evaluate the treatment effect of the product on tumors within 24 months after ESO-T01 infusion to preliminarily observe the efficacy of ESO-T01 treatment in subjects with MM. 1.Objective response rate (ORR): Includes stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR). 2.MRD negativity rate; 3.Time to response (TTR); 4.Duration of Response (DOR); 5.Progression-free survival (PFS); 6.Overall survival (OS).
Statistical Analysis	Major statistical analysis sets for this study are as follows: (1) Full analysis set (FAS): All subjects enrolled who received ESO-T01 infusion. (2) Safety analysis set (SS): All subjects enrolled who received ESO-T01 infusion and had at least one safety measure recorded. (3) DLT analysis set (DLTS): All subjects who received ESO-T01 infusion and completed the DLT assessment, including subjects who developed DLT and those who did not develop DLT but completed all assessments during the DLT observation period. (4) Pharmacokinetic analysis set (PKS): Subjects with at least one quantifiable plasma concentration after administration and no major protocol deviations/violations or with no events that significantly affect pharmacokinetic results. (5) Efficacy analysis set (ES): All subjects who received ESO-T01 infusion, had a tumor assessment at baseline and had at least one available tumor assessment result after administration. **General Principles of Statistical Analysis** The analysis is performed using SAS 9.4 (or higher version) software. For continuous variables, descriptive statistics will include mean, median, standard deviation, maximum and minimum. For categorical variables, descriptive statistics will consist of the number and

	percentage of subjects. Detailed statistical analysis methods are described in the Statistical Analysis Plan. **Safety Analysis** **Adverse Events** The occurrence and frequency of DLT of ESO-T01 Injection is evaluated, and the MTD is determined. The number and percentage of subjects with adverse events, drug-related adverse events, adverse events of Grade 3 or higher in severity, and serious adverse events are summarized by dose group and overall. CRS and ICANS will be graded according to the 2019 ASTCT criteria, while other AEs will be graded per NCI CTCAE version 5.0. All AEs will be coded using the latest version of MedDRA. The incidence of adverse events will be summarized by system organ class (SOC) and preferred term (PT). **Laboratory Tests** Laboratory test data are graded in accordance with NCI CTCAE version 5.0. The data on laboratory test indicators such as blood routine examination, blood chemistry, coagulation function, and urine routine examination and their change values before and after baseline are summarized for each visit according to the different study phases and dose groups, and the positive and abnormal changes in blood routine examination, blood chemistry, coagulation function, and urine routine examination before and after baseline are summarized. All abnormal change values in laboratory tests are described using lists. **ECG, Physical Examination, and Vital Signs** Descriptive statistics are used to summarize the data for each visit and the change before and after baseline according to the different study phases and dose groups, and laboratory values are described in tabular statistics. See the Statistical Analysis Plan for detailed statistical analysis methods. **Efficacy Analysis** Objective response rate (ORR): Proportion of subjects achieving

	stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR), providing the efficacy to evaluate the subject number and percentage of each result. The overall response rate (ORR) and its two-sided 95% confidence interval (CI) will be calculated using the Clopper-Pearson method. MRD negativity rate: Calculations of subjects with negative MRD, MRD negativity rate and 95% confidence interval using the Clopper-Pearson method. Time to response (TTR), duration of response (DOR), progression-free survival (PFS), and overall survival (OS). Time to response (TTR) is defined as the time from the start of the drug infusion in subjects to the first occurrence of sCR, CR, VGPR, or PR. Duration of response (DOR) is defined as the time interval between the first occurrence of sCR, CR, VGPR, or PR after drug infusion in subjects and the first assessment of progressive disease or death, whichever occurs first. Progression-free survival (PFS) is defined as the time interval between drug infusion in subjects and the first assessment of progressive disease or death, whichever occurs first. Overall survival (OS) is defined as the time interval between drug infusion in subjects to death from any cause. The median survival is estimated using the Kaplan-Meier method, and the 95% confidence interval of the median survival is estimated using the Brookmeyer and Crowley method. Kaplan-Meier survival curve is plotted. DOR analysis is limited to subjects with documented responses. **Pharmacokinetic Analysis** PK analysis will be performed using the PK analysis set. Drug concentration data at each time point will be summarized using descriptive statistics. CAR copy numbers in peripheral blood will be detected, and individual plasma concentration-time curves and

	arithmetic mean concentration-time data plots will be created on both linear and semi-logarithmic scales. PK parameters are calculated using WinNonlin software and a non-compartmental model based on the actual blood sampling times. Evaluation indicators include: C_{max}, T_{max}, $AUC_{0\text{-}28d}$, etc. of expansion of ESO-T01 in peripheral blood after infusion. Statistics for PK parameters include, but are not limited to, the number of subjects, arithmetic mean, standard deviation, coefficient of variation, median, first quartile, third quartile, minimum and maximum.
Study Duration	November 2024 to November 2027
Subject Participation Duration	24 months
Study Facility/Location	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology

SCHEDULE OF ACTIVITIES

Visit Content	Screening Period[1]	Baseline Period*	Drug Infusion and Main Observation Period[2]															Follow-up Period[3]					Withdrawal from the Study[4]
	D-30 to D-3	D-2 to D-1	D0	D1	D2	D4	D6	D8	D10	D12	D14	D17	D21	D24	D28	M2	M3	M6	M9	M12	M18	M24	-
Time Window							±1 d	±1 d	±1 d	±1 d	±1 d	±1 d	±3 d	±3 d	±3 d	±7 d	±7 d	±7 d	±7 d	±7 d	±7 d	±7 d	
Informed consent	X																						
Inclusion/exclusion criteria	X																						
Infusion criteria			X																				
Demographic data[5]	X																						
Past medical history/medication history[6]	X																						
Height/weight/body surface area[7]	X																						
Physical examination[8]	X	X[a]		X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X
Vital signs[9]	X	X[a]		X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X
Oxygen saturation test[10]	X	X		X	X	X	X	X	X		X												
Infectious disease screening[11]	X																						
Blood pregnancy test[12]	X	X															X						X

Visit Content	Screening Period[1]	Baseline Period*	Drug Infusion and Main Observation Period[2]															Follow-up Period[3]					Withdrawal from the Study[4]
	D-30 to D-3	D-2 to D-1	D0	D1	D2	D4	D6	D8	D10	D12	D14	D17	D21	D24	D28	M2	M3	M6	M9	M12	M18	M24	-
Time Window							±1 d	±1 d	±1 d	±1 d	±1 d	±1 d	±3 d	±3 d	±3 d	±7 d	±7 d	±7 d	±7 d	±7 d	±7 d	±7 d	
FSH test[12]	X																						
Blood routine examination[13]	X	X[a]			X	X		X			X		X		X	X	X	X	X	X	X	X	X
Blood chemistry[14]	X	X[a]			X	X		X			X		X		X	X	X	X	X	X	X	X	X
Urine routine examination[15]	X	X[a]			X	X		X			X		X		X	X	X	X	X	X	X	X	X
Coagulation function[16]	X	X[a]			X	X		X			X		X		X	X	X	X	X	X	X	X	X
Serum protein electrophoresis	X	X[a]													X	X	X	X	X	X	X	X	X
Serum immunofixation electrophoresis	X	X[a]													X	X	X	X	X	X	X	X	X
Serum free light chain	X	X[a]													X	X	X	X	X	X	X	X	X
24-hour urine protein test	X	X[a]													X	X	X	X	X	X	X	X	X
24-hour urine protein electrophoresis	X	X[a]													X	X	X	X	X	X	X	X	X
24-hour urine immunofixation electrophoresis	X	X[a]													X	X	X	X	X	X	X	X	X
24-hour urine free light chain	X	X[a]													X	X	X	X	X	X	X	X	X

Visit Content	Screening Period[1]	Baseline Period*	Drug Infusion and Main Observation Period[2]															Follow-up Period[3]					Withdrawal from the Study[4]
	D-30 to D-3	D-2 to D-1	D0	D1	D2	D4	D6	D8	D10	D12	D14	D17	D21	D24	D28	M2	M3	M6	M9	M12	M18	M24	-
Time Window							±1 d	±1 d	±1 d	±1 d	±1 d	±1 d	±3 d	±3 d	±3 d	±7 d	±7 d	±7 d	±7 d	±7 d	±7 d	±7 d	
Electrocardiogram[17]	X	X[a]									X				X	X	X	X	X	X	X	X	X
Echocardiography[18]	X																						
Brain MRI[19]	X																						X
Imaging evaluation[20]	X		Follow up as clinically indicated.																				
ECOG score	X	X																X	X	X	X	X	X
Mini Mental State Examination (MMSE)	X																						
ICE score[21]		X		X	X	X	X	X	X		X		X		X								X
Cytokine[22]		X			X	X	X	X	X	X	X	X	X	X	X	X							X
CRP and ferritin		X			X	X	X	X	X	X	X	X	X	X	X	X							X
Immunoglobulins[23]		X													X	X	X	X					X
Bone marrow examination/biopsy[24]	X														X	X	X	X	X	X	X	X	X
Tumor assessment[25]	X														X	X	X	X	X	X	X	X	X

Visit Content	Screening Period[1]	Baseline Period*	Drug Infusion and Main Observation Period[2]															Follow-up Period[3]					Withdrawal from the Study[4]
	D-30 to D-3	D-2 to D-1	D0	D1	D2	D4	D6	D8	D10	D12	D14	D17	D21	D24	D28	M2	M3	M6	M9	M12	M18	M24	-
Time Window							±1 d	±1 d	±1 d	±1 d	±1 d	±1 d	±3 d	±3 d	±3 d	±7 d	±7 d	±7 d	±7 d	±7 d	±7 d	±7 d	
ESO-T01 Infusion[26]			X																				
ECG Monitoring			X																				
Peripheral blood PK testing[27]		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Peripheral blood lymphocyte subpopulations[28]	X	X[a]													X	X	X	X					
Immunogenicity and viral shedding[29]		X													X		X	X	X	X	X	X	X
Concomitant medication/treatment	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X
Adverse events[30]		X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X
Survival status[31]															X	X	X	X	X	X	X	X	X

Note:

*: Examinations during the baseline period can be completed just prior to drug infusion.

X[a]: Screening results are acceptable as baseline results unless the baseline needs to be retaken as assessed by the Investigator.

1.**Screening period:** Subjects who complete the appropriate visit content examination according to the visit schedule during the screening period and meet all

inclusion criteria and none of the exclusion criteria, as assessed by the Investigator, can be enrolled in the study. Results of subjects obtained within 7 days prior to screening may be accepted as screening results, as appropriate.

2.**Drug Infusion and Main Observation Period:** ESO-T01 infusion is performed on Day 0. At least 14 days of inpatient observation should be completed after infusion. The schedule of visits during the primary observation period is detailed below for the schedule of time points for each visit content, or until the withdrawal of informed consent, withdrawal from the study, occurrence of progressive disease, initiation of new anti-tumor therapy, and death, whichever occurs first.

3.**Follow-up period:** All subjects receiving treatment will be subsequently followed up once every 3 months until the end of the trial or withdrawal from the study.

4.**Withdrawal from the study:** A withdrawal visit should be conducted within 7 days after subjects withdraw from the study for any reason other than progressive disease, death or loss to follow-up: If the imaging evaluation for the withdrawal visit is within 4 weeks of the last examination, it does not need to be repeated. If more than 1 week has passed since other examinations and up to the withdrawal visit, those examinations need to be repeated. For subjects who have received ESO-T01 treatment, even if they withdraw from the trial prematurely, the Investigator should perform long-term safety follow-up for them according to the protocol to evaluate the long-term safety of this product. All subjects who withdraw from the study with drug-related AEs should be followed up subsequently for AEs until AEs resolve to baseline, stabilize, the subject is lost to follow-up, or there is another reasonable explanation. If the AE is a chronic condition, it may be excluded from follow-up in consultation between the Investigator and the cooperating unit, but the consultation must be documented in the original medical record. If a subject withdraws before drug infusion, the withdrawal visit can be omitted.

5.**Demographic data:** Includes date of birth, sex, ethnicity, etc.;

6.**Past medical history/medication history:** Includes, but is not limited to, disease diagnoses, history of tumor treatment, and history of other disease diagnoses and treatments;

7.**Height/weight/body surface area:** Height and weight should be measured at screening to calculate body surface area (BSA), and only weight needs to be recorded for other visits. Calculation formula of body surface area: BSA (m^2) = 0.00616 × Height (cm) + 0.01286 × Weight (kg) - 0.1529;

8.**Physical examinations:** Complete physical examinations include assessment of general condition, skin and mucosa, superficial lymph nodes, head and neck (including the thyroid gland), chest (breasts, thoracic cage, lungs, and heart), abdomen (liver, gallbladder, spleen, and kidneys), spine and extremities, and nervous system. Physical examinations are performed according to the assessment of the Investigator. If any abnormalities are found, real-time monitoring will be implemented until all indicators stabilize.

9.**Vital signs:** Vital signs, including body temperature (°C), respiratory rate (breaths/min), heart rate (beats/min), and blood pressure (mmHg), should be measured after the subject has rested in a seated position for 5 minutes and is in a calm state. Do not measure blood pressure on the arm receiving drug infusions or being used for blood sampling. Body temperature should be measured using the same method (e.g., axillary or ear temperature) for each subject throughout the study. On the day of drug infusion (Day 0), vital signs are measured at 15 minutes ± 5 minutes before the start of infusion and within 2 hours after the start of drug infusion (every 30 minutes ± 5 minutes) until the indicators stabilize. Vital signs are monitored twice a day (D1-D14) after drug infusion. If abnormalities occur, real-time monitoring will be adopted until the indicators stabilize.

10.**Oxygen saturation test:** Oxygen saturation is monitored once daily (D0-D14) after drug infusion. If abnormalities occur, real-time monitoring will be adopted until the indicators stabilize.

11.**Infectious disease screening:** Including five hepatitis B items (hepatitis B surface antigen [HBsAg], hepatitis B surface antibody [HBsAb], hepatitis B e antigen [HBeAg], hepatitis B e antibody [HBeAb], hepatitis B core antibody [HBcAb]), hepatitis C virus (HCV) antibody, human immunodeficiency virus (HIV) antibody, treponema pallidum antibody, and cytomegalovirus (CMV)-DNA. If HCV antibody-positive subjects are enrolled, they should be tested for HCV RNA and be RNA-negative before enrollment. Both HBV surface antigen and HBV core antibody should be negative. If any of these items are positive, a peripheral blood HBV DNA titer test is required, which should be less than the lower limit of the test value before enrollment. If a subject has completed some or all of the above virological/treponema

pallidum testing within 4 weeks prior to screening, it is up to the Investigator to determine if some or all of the testing needs to be repeated. HBV DNA test can be done concurrently in subjects with hepatitis B virus carriage directly based on the patient's medical history. Note: For subjects who are positive for HBV surface antigen or HBV core antibodies, viral activation needs to be monitored during treatment with CD19-BCMA TANCAR-T. The Investigator will decide whether or not to administer antiviral therapy based on the clinical situation. Regular viral quantification and liver function monitoring are recommended at the same time.

12.**Blood pregnancy test:** For women of childbearing potential only. Serum beta-human chorionic gonadotropin (β-HCG) test is performed. If pregnancy is suspected during the study, the Investigator may repeat the blood pregnancy test. A woman of childbearing potential is defined as a woman who is anatomically and physiologically capable of becoming pregnant after menarche and before menopause, without sterilization. Postmenopause is defined as cessation of menstruation for ≥12 months in the absence of alternative medical measures. Subjects with menolipsis or suspected menolipsis should undergo follicle-stimulating hormone (FSH) testing. An FSH level > 40 IU/L confirms menopause.

13.**Blood routine examination**: Includes red blood cell count, hemoglobin content, hematocrit, white blood cell count, platelet count, and white blood cell classification (including absolute counts of neutrophils/eosinophils/basophils and lymphocytes/monocytes).

14.**Blood chemistry:** Includes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γ-GT), total bilirubin (TBIL), alkaline phosphatase (ALP), albumin, total protein, lactate dehydrogenase (LDH), glucose (Glu), blood urea nitrogen (BUN), creatinine (Cr), blood uric acid, Na, K, Cl, Ca, Mg, and P.

15.**Urine routine examination:** Includes pH, urine white blood cells, urine protein, urine red blood cells, and urine glucose.

16.**Coagulation function:** Includes prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), international normalized ratio (INR), fibrinogen (FIB), and D-dimer.

17.**Electrocardiogram:** Rest for at least 5 minutes before each measurement, and measure in a calm state. The electrocardiographic examination should include heart rate, PR interval, QRS interval, QT interval, and QTc interval calculated using Fridericia's formula. Testing frequency can be increased if necessary.

18.**Echocardiogram:** Test results within 2 weeks prior to screening are accepted and additional assessments may be performed during the course of the trial depending on the clinical condition.

19.**Brain MRI:** This examination is performed in subjects with suspected central involvement during the screening period and is performed according to the assessment of the Investigator during the trial depending on the clinical condition.

20.**Imaging evaluation:** Whole-body X-ray plain film (including anteroposterior chest, cervical spine, thoracic spine, lumbar spine, humerus, femur, skull frontal and lateral position, and pelvis frontal position) or CT (local or whole-body low-dose) or MRI (whole-body or local, including cervical spine, thoracic spine, lumbosacral spine, and cranium) or PET/CT. Follow-up imaging should use the same modality. Test results with diagnostic quality achieved within 1 month prior to screening are acceptable.

21.**ICE score:** Assessments are made once a day (D0-D14) after drug infusion and at D21 and D28 follow-up.

22.**Cytokines:** At least IL-6, IL-10, TNF-α, and IFN-γ need to be tested. The Investigator may adjust the test frequency or test items according to clinical needs.

23.**Immunoglobulins:** Includes immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM);

24.**Bone marrow examination/biopsy:** Initial screening includes bone marrow cytology smear classification and flow cytometry. Subsequent follow-ups only require the test of bone marrow cytology smear classification and flow cytometry. It is recommended that flow cytometry include antibodies against the following molecules: CD19, CD38, CD45, CD56, CD20, CD138, κ light chain, λ light chain. Bone MRD test uses the next-generation flow cytometry or next-generation sequencing with a sensitivity of $\geq 10^{-5}$.

25.**Tumor assessment:** It will be assessed by the Investigator according to the IMWG response criteria (see Appendix 1 for details) based on the test results. Tumor assessments are performed at screening and baseline separately, and if the interval between the two assessments is within two weeks of each other, the tumor assessment

at baseline can be exempted, and the results of the tumor assessment closest to the pre-infusion period are used as the baseline value for efficacy assessment.

26.**ESO-T01 infusion:** Subjects should meet drug infusion criteria prior to drug infusion. If all infusion criteria are not met, ESO-T01 infusion must be delayed until recovery to meet all conditions.

27.**Peripheral blood PK testing:** CAR copy number in peripheral blood is detected by a validated qPCR method according to the visit points in the flow chart. Peripheral blood is collected at baseline, on D2, D4, D6, D8, D10, D12, D14, D17, D21, D24 and D28, and at M2 to detect the number of CAR-T (CAR-T/mL blood) by flow cytometry. At the time point when CAR-T cells are detectable by flow cytometry, the CAR-T cell phenotype is further analyzed for CD4/CD8 ratio and naive, memory and effector cells, etc.

28.**Peripheral blood lymphocyte subpopulations:** Peripheral lymphocyte subpopulations (T, B, and NK cell ratios and absolute counts) are detected at baseline, on D28, and at M2, M3 and M6.

29.Immunogenicity and viral shedding: Samples are collected and frozen at baseline, on D28, and at M3, M6, M9, M12, M18 and M24 for central detection of immunogenicity. Blood, saliva, urine, and feces are collected at baseline, and on D2, D4, D6, D8, D10, D14, D21 and D28 to detect viral shedding.

30.**Adverse events:** From drug infusion to the 24th month after infusion or 28 days after withdrawal from the trial. Results obtained prior to signing the ICF are not recorded as AEs unless they are caused by study procedures, and all abnormal results are recorded as medical history. All AEs and SAEs present at the time of withdrawal from the study will be continued in follow-up until the AE returns to baseline, stabilizes, the subject is lost to follow-up, or there are other reasonable explanations, unless, in the judgment of the Investigator, the adverse event is impossible to resolve due to the subject's medical condition.

31.**Survival status:** Survival status, including survival, relapse, and death are recorded.

In addition to the above examinations, if subjects experience any signs and symptoms deemed related to ESO-T01 treatment by the Investigator during the treatment period, the Investigator has the right to perform unscheduled examinations.

LIST OF ABBREVIATIONS

Abbreviation/Terminology	Definition/Explanation
AE	Adverse event
AESI	Adverse event of special interest
ASTCT	American Society for Transplantation and Cellular Therapy
AUC	Area under the serum drug concentration-time curve
$AUC_{0\text{-}28d}$	Area under the plasma concentration-time curve from time 0 to 28 days
$AUC_{0\text{-}inf}$	area under the plasma concentration-time curve from time 0 extrapolated to infinity
CAR	Chimeric antigen receptor
C_{max}	Maximum plasma concentration
CI	Confidence interval
CR	Complete response
CRO	Contract research organization
CRP	C-reactive protein
CRS	Cytokine release syndrome
CT	Computerised tomography
CTCAE	Common terminology criteria for adverse events
D/d	Day
DLT	Dose-limiting toxicity
DOR	Duration of response
IEC/IRB	Independent Ethics Committee/Institutional Review Board
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	(Electronic) case report form
EDC	Electronic data capture
ES	Efficacy analysis set
FAS	Full analysis set
G-CSF	Granulocyte colony-stimulating factor
GCP	Good Clinical Practice
GvHD	Graft versus host disease
h	Hour

Abbreviation/Terminology	Definition/Explanation
HSCT	Hematopoietic stem cell transplantation
ICANS	Immune effector cell-associated neurotoxicity syndrome
ICF	Informed consent form
IIT	Investigator-initiated trial
IFN-γ	Interferon γ
min	Minute
MM	Multiple myeloma
MMSE	Minimum Mental State Examination
MTD	Maximum tolerated dose
MR	Minimal response
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
NA	Not applicable
NCI	National Cancer Institute
ORR	Objective response rate
OS	Overall survival
PD	Pharmacodynamics
PD	Progressive disease
PET-CT	Positron emission tomography-computed tomography
PFS	Progression-free survival
PK	Pharmacokinetics
PKS	Pharmacokinetic analysis set
PT	Preferred term
RP2D	Recommended phase 2 dose
r/r MM	Relapsed/refractory multiple myeloma
PR	Partial response
SAE	Serious adverse event
sCR	Stringent complete response
SOC	System organ class
SOP	Standard operating procedure

Abbreviation/Terminology	Definition/Explanation
SRC	Safety Monitoring Committee
SS	Safety analysis set
SUSAR	Suspected unexpected serious adverse reactions
$t_{1/2}$	Elimination half-life
TLS	Tumor lysis syndrome
T_{max}	Time to maximum concentration
TNF-α	tumor necrosis factor α
ULN	Upper limit of normal
VGPR	Very good partial response

1 INTRODUCTION

1.1 Disease Background

Multiple myeloma (MM) is a malignant tumor characterized by the abnormal proliferation of clonal plasma cells, for which conventional clinical treatments are often ineffective.

MM is characterized by abnormal proliferation of clonal plasma cells and secretion of large amounts of monoclonal immunoglobulins, leading to end-organ damage. Due to the suppression of normal immunoglobulin production, various bacterial infections are prone to occur. MM accounts for about 1% of all tumors and about 10% of hematologic tumors, making it the second most common hematologic malignancy. Multiple myeloma is more common in middle-aged and elderly people, but in recent years, its incidence has increased and there is a trend towards earlier age of onset. In China, the peak incidence appears in people aged 50-65 years, with the incidence higher in males than females. Most cases are primary, and a small number of cases develop from monoclonal gammopathy of undetermined significance (MGUS).

Currently, significant advancements have been made in the treatment of MM with the application of new therapies, including proteasome inhibitors, immunomodulators, and CD38 monoclonal antibodies, as well as the recommendation of autologous hematopoietic stem cell transplantation as a consolidation therapy. However, MM remains largely incurable, and treatment primarily focuses on controlling disease progression, improving quality of life, and prolonging survival. The vast majority of patients will eventually experience disease recurrence or progression. Regarding the treatment for relapsed/refractory MM, the median progression-free survival (PFS) for R/R MM at the current level of treatment is 3.7 months to 26.3 months according to the statistical data from several studies. As patient survival increases and the disease progresses, patients will eventually face a situation where no effective treatment options remain. Another disadvantage of current new therapies is the serious systemic adverse reactions caused by off-target toxicity, which can lead to side effects such as vomiting, nausea, and alopecia. After the failure of autologous hematopoietic stem cell transplantation, allogeneic hematopoietic stem cell transplantation and donor lymphocyte infusion have been attempted in a small number of patients, but the benefit is unclear due to transplantation-related mortality and graft versus host disease (GVHD). All of these treatments may impose heavy financial and physical burdens on patients, therefore, there is an urgent need for new treatments with better safety and efficacy.

1.2 Background of *In Vivo* BCMA CAR-T Cell Therapy

In the search for new MM therapies, CAR-T cell products targeting MM cell surface antigens offer a new treatment approach for MM. Currently, only four CAR-T products are approved globally for the treatment of MM, all of which target BCMA, as shown in Table 1. Of them, Carvykti® and Abecma® have been marketed in the United States and Europe successively, addressing some of the current clinical needs of patients with MM. Two CAR-T products are approved in China for the treatment of MM: equecabtagene autoleucel from IASO Biotherapeutics, approved in June 2023, and zevorcabtagene autoleucel injection from CARsgen Therapeutics, approved in March 2024. According to the data shown in Table 1, BCMA-targeted CAR-T products have a low probability of Grade 3 or higher CRS and NT, demonstrating a favorable safety profile.

Table 1 Clinical Data of Marketed BCMA CAR-T

Manufacturer	Product Name	Safety Data	Efficacy Data
BMS/Bluebird Bio	ABECMA (idecabtagene vicleucel)	**KarMMa Phase 2 (median follow-up time = 10.7 months)**	
		N=127 • CRS (≥ Grade 3): 9% • NT (≥ Grade 3): 4%	N=100 ORR: 72% (72) • CR: 28% (28) • PR: 44% (44) • mDOR (PR or better): 11.0 months
Legend Biotech	*CARVYKTI* LCAR-B38M (ciltacabtagene autoleucel)	**CARTITUDE-1 (N=97) Phase 1b/2 (median follow-up time = 18 months)**	
		• CRS (≥ Grade 3): 5% • NT (≥ Grade 3): 11% Median time to onset of CRS (any grade): 7 days	ORR: 98% (95) • CR/sCR: 78.4% (76) • VGPR: 16.5% (16) mDOR: 22 months
IASO Biotherapeutics	Equecabtagene autoleucel	**FUMANBA-1 Phase 1b/2 (median follow-up time = 13.8 months)**	
		N=103: • CRS (≥ Grade 3): 1.0% (1)	N=101: • ORR: 96.0% (97) • ≥CR: 74.3% (75)

		• NT (≥ Grade 3): 0%	• ≥VGPR: 91.1% (92) Patients who have received CAR-T therapy N = 12: • ORR: 75% • ≥CR: 42%
CARsgen Therapeutics	Zevorcabtagene autoleucel injection	**LUMMICAR STUDY 1 Phase 2 (N = 102) (median follow-up time = 9 months)**	
		• CRS (≥ Grade 3): 6.9% • NT (≥ Grade 3): 0%	ORR: 92.2% • CR/sCR: 45.1%

Since the first clinical trial of BCMA CAR-T initiated by the US NCI in 2014, numerous clinical trials targeting BCMA for the treatment of multiple myeloma have been started continuously. The CAR-T cell doses used in these trials range from 0.07×10^6-10×10^8 CAR+ T cells. CAR-T cells are typically administered as a single intravenous infusion, and lymphodepleting preconditioning with cyclophosphamide/fludarabine is usually performed prior to infusion. The overall response rate of BCMA CAR-T against MM in clinical trials consistently exceeds 70%.

Despite the emergence of BCMA CAR-T targeted therapies as new and effective treatments for MM, access to CAR-T cell therapies is constrained by a number of factors, including limited production capacity and number of sites, production failures, complex logistics and supply chains, toxicity of preconditioning regimens, safety precautions required for treatment, and the cost of treatment, which is too high for many patients. For example, a recent survey of real-world experience of patients using commercialized idecabtagene vicleucel showed that fewer than 25% of patients were treated within 6 months after being determined to be able to receive the treatment, and the cumulative mortality rate within 12 months for those on the waiting list to receive CAR-T treatment was 25.6%. This trend is likely to intensify as the approved BCMA CAR-T moves into earlier lines to treat patients with relapsed/refractory multiple myeloma.

Allogeneic CAR-T therapy offers an alternative to autologous CAR-T and can increase patient accessibility. However, the clearance of allogeneic CAR-T cells by the host immune system following recovery from lymphocyte depletion limits the duration of clinical response

and negatively impacts overall clinical efficacy. In addition, lymphodepleting preconditioning of patients is required for *in vitro* autologous and allogeneic CAR-T therapies, which is designed to provide for improved CAR-T cell expansion and survival (especially critical for allogeneic CAR-T cells) and may reduce initial tumor load. While the inclusion of preconditioning improves the durability and depth of response to *in vitro* CAR-T cell therapy, it is also associated with an increased incidence of cytokine release syndrome (CRS), severe neurotoxicity, severe cytopenia, and increased infection rates.

The *in vivo* preparation of CAR-T technology allows CAR-T to be generated directly *in vivo*, perfectly addressing the challenges associated with *in vitro* autologous and allogeneic CAR-T cell therapies. ESO-T01 is a product that carries lentiviruses targeting the BCMA CAR molecule. It is an "off-the-shelf" product that can be administered to patients immediately, reducing the occurrence of progressive disease due to the long waiting time for patients. This product is simpler to produce than *in vitro* CAR-T, as it does not require the use of cells from patients or donors to prepare CAR-T *in vitro*, which greatly reduces costs and improves clinical accessibility.

The current investigator-initiated clinical trial has a single-arm, open-label study design and plans to enroll 4-24 patients with r/r MM who meet the requirements of the trial and are ≥18 years of age. The purpose is to evaluate the safety, tolerability and other characteristics of ESO-T01 Injection in the treatment of r/r MM.

1.3 ESO-T01 Injection

Generic name: ESO-T01 Injection

English name: ESO-T01 Injection

Chinese pinyin: ESO-T01 Zhusheye

ESO-T01 is a third-generation non-replicative, self-inactivating lentiviral vector expressing the PRG1801 BCMA-targeted CAR under the control of a T cell-specific synthetic promoter. Its envelope is engineered to be MHC I-free, expresses human CD47, and expresses alpaca-derived membrane-anchored single-domain antibodies targeting the constant region of the T-cell αβ receptor (TCR) chain on the surface of T cells. ESO-T01 is produced by transiently transfecting MHC IKO/CD47hi/TCR VHH 293T producing cells with three helper plasmids (Gag/Pol plasmid encoding the Gag-Pol polyprotein, Rev plasmid encoding the Rev protein, and ENV plasmid encoding a targeting-defective VSV-GMUT mutant envelope protein) as well as a shuttle plasmid encoding the PRG1801 BCMA CAR transgene (expressed under the control of a T cell-specific synthetic promoter).

A natural lentiviral (LV) outgrowth process is utilized to generate lentiviral vectors with MHC-deficient envelopes and high expression of hCD47 and membrane-anchored single-domain antibodies. This process integrates the plasma membrane proteins of the producing cell line into the LV envelope. The key features of the ESO-T01 are summarized in Figure 1 below.

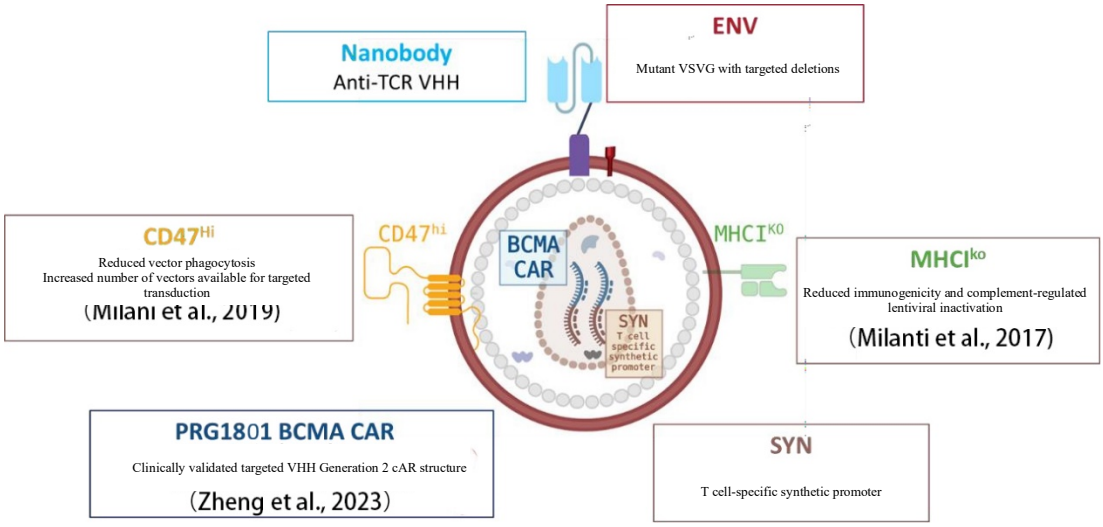

Figure 1 Schematic Diagram of Key Features of ESO-T01

1.4 Preclinical Studies

Preclinical studies of this product include pharmacokinetics and pharmacodynamics, etc. The cooperating unit conducted *in vitro* cell killing experiments of ESO-T01 and used tumor-bearing mouse model to study the preliminary toxicology, pharmacodynamics, and pharmacokinetics of ESO-T01 against MM. The following is a brief compilation and elaboration of the results of these preclinical studies, and more detailed experiment results can be found in the Investigator's Brochure or the preclinical study report.

1.4.1 *In Vitro* Pharmacodynamic Study

CAR-T Cells Prepared *In Vivo* by ESO-T01 Have Significant Killing Effect on MM Cell Lines

Two BCMA CAR constructs were formed using the BCMA-recognizing antibody sequence construct vector LCAR B38M, a marketed product of Nanjing Legend Biotech, and the PRG1801 antibody-recognizing sequence, a BCMA CAR-T product of Shenzhen Pregene. These two ENaBL-TT vectors (required for the production of the ESO-T01 lentiviral vector) were used to produce BCMA CAR-T cells *in vitro* from non-activated primary human PBMCs. Then, BCMA CAR-T cells were cocultured with NCI-H929 (BCMA-positive multiple myeloma cell line) or K562 (BCMA-negative chronic myelogenous leukemia cell line) at

different effector-to-target ratio. As shown in Figure 2 below, both BCMA CAR-T cells possessed potent specific anti-tumor activity, as demonstrated by the depletion of NCI-H929 cells over time, even at a low effector-to-target ratio (1:12) for ENaBL-TT PRG1801 CAR-T cells. Importantly, the toxicity of ENaBL-TT BCMA CAR-T cells was specific to targeted tumor cells and did not affect the survival of BCMA- K562 cells.

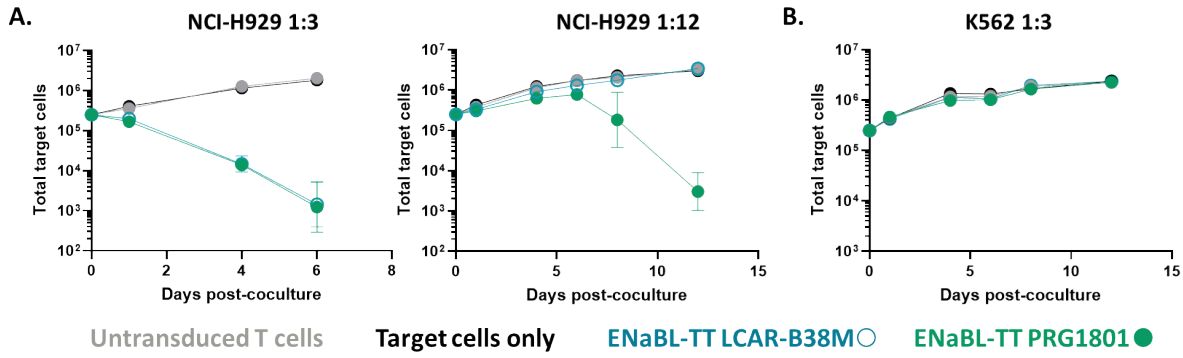

Figure 2 Killing of Target Cells by CAR-T Prepared with Different Lentiviruses

These results indicated that BCMA CAR-T cells reprogrammed *in vitro* using the ENaBL-TT vector had potent anti-tumor activity. It was noteworthy that the cytotoxic activity of PRG1801 BCMA CAR-T cells at low effector-to-target cell ratios suggested that the selected ESO-T01 CAR-T construct may have higher anti-tumor activity *in vivo*.

1.4.2 *In Vivo* Study (Experiment in Mice)

To assess the pharmacodynamics of ESO-T01 and its transduced cell population in a humanized mouse multiple myeloma tumor model. The transduction efficiency and specificity of ESO-T01, the biological distribution of the transduced cell subpopulation, and the anti-tumor activity and potential cytotoxicity associated with the generation and activation of CAR-T cells in a tumor-bearing mouse model were investigated *in vivo* in mice.

Immunodeficient NCG mice were humanized by chemoablation and transplantation of human hematopoietic stem cells ($CD34^+$ cells) and intravenously injected with 5×10^6 multiple myeloma cells (NCI-H929 cells) in order to establish a systemic multiple myeloma model in hCD34 NCG mice. 10 days after tumor establishment, tumor-transplanted animals were randomly grouped (D-3) and injected intravenously with a single ascending dose of ESO-T01 or vector. The experiment was performed with 4 groups, including the vector group and 3 dose groups (doses of 7.00×10^5, 1.75×10^6, and 3.50×10^6 TU/mouse, respectively), with 7-8 mice in each group. Lentiviral vector was injected on Day 0 and the study concluded on Day 49. Tumor

growth was assessed weekly by bioluminescence imaging, the transduction efficiency and specificity of ESO-T01 were measured weekly by flow cytometry. Meanwhile, plasma was collected to assess cytokine levels, and tissues were collected at the end of the study to assess the biological distribution of ESO-T01-transduced cells. At the same time, clinical scores and weight measurements were also monitored to identify any potential adverse reactions.

The results showed that:

1. ***In Vivo* Pharmacodynamics:**

As shown in Figures 3 and 4 below, the bioluminescent signal of NCI-H929 cells in the blank vector group increased exponentially, whereas tumor growth was controlled in all ESO-T01 groups on Day 25 after vector injection, and tumor control in ESO-T01-treated mice resulted in a superior overall survival in all groups compared to that of mice receiving vector at the end of the study.

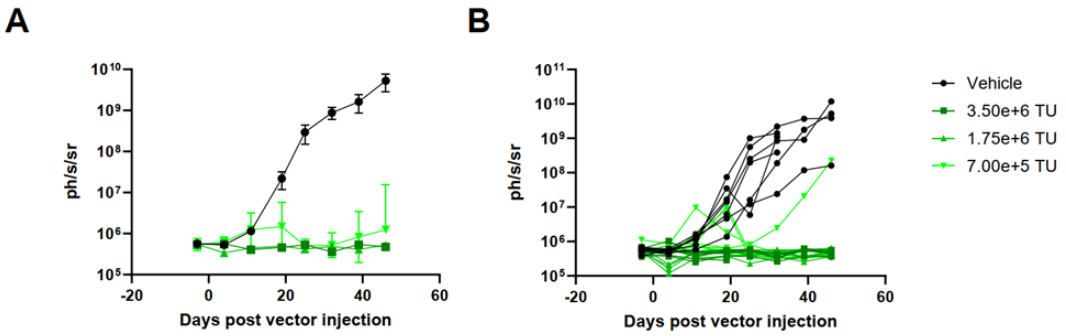

Figure 3 Bioluminescence Signal of Mice in Each Group After Administration

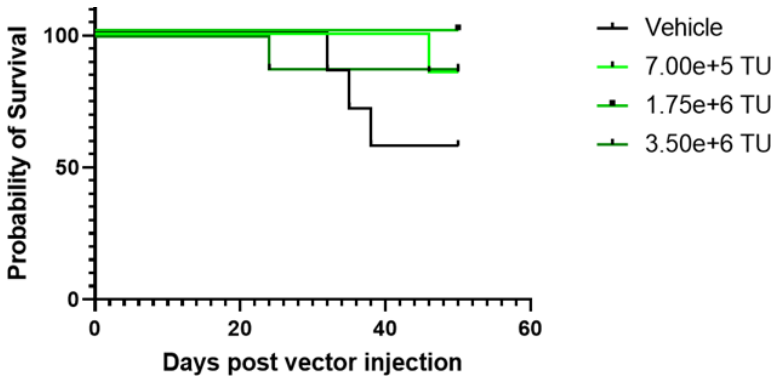

Figure 4 Survival Curves of Mice in Each Group

2. ***In Vivo* Transduction Efficiency:**

As shown in Figure 5 below, BCMA CAR-T cells were detected in peripheral blood by flow cytometry, and the percentage of BCMA CAR-T cells peaked at around Day 11 in the high-dose and medium-dose groups, while CAR-T cells peaked at around Day 20 in the low-dose group. The timing of peak CAR-T expansion correlated with maximal anti-tumor response,

as shown in the low-dose group, where transient and limited tumor growth was observed prior to peak CAR-T expansion (Day 20), followed by complete tumor clearance. After the CAR-T cell peak, CAR-T cell levels declined over time but remained detectable in all treatment groups at the end of the study (Day 49).

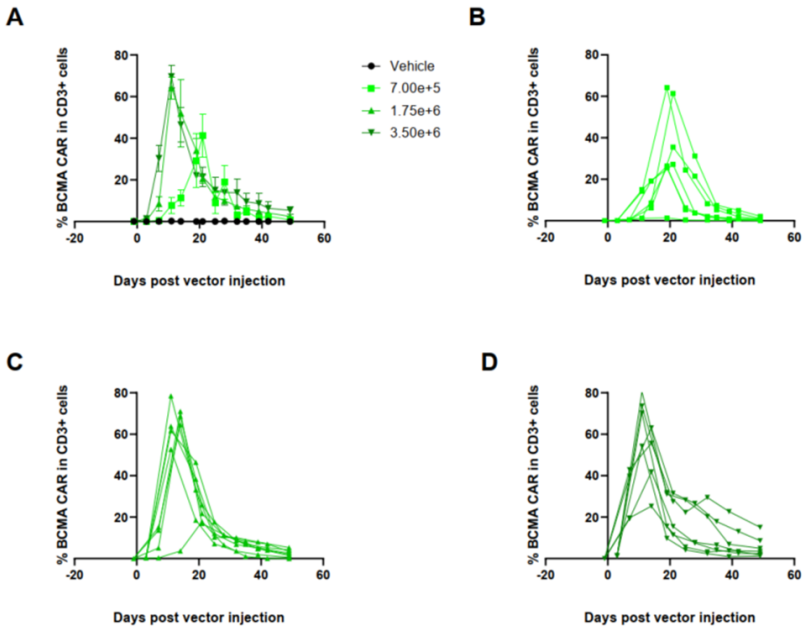

Figure 5. BCMA CAR Expression in Humanized Peripheral Blood T cells

Note: A) Mean BCMA CAR positivity rate in peripheral blood T cells (CD3+ cells) throughout the study. Since mice can only have blood collected once per week, each time point contains data from 3-4 animals per group. B) BCMA CAR positivity rate in T cells in the low-dose group (7.00E+5 TU per mouse). C) BCMA CAR positivity rate in T cells in the medium-dose group (1.75E+6 TU per mouse). D) BCMA CAR positivity rate in T cells in the high-dose group (3.50E+6 TU per mouse).

3. *In Vivo* Transduction Specificity:

As shown in Figure 6, the specificity of ESO-T01 was assessed by flow cytometry on Day 7 after vector injection. Results demonstrated dose-dependent transduction of $CD3^+$ cells by ESO-T01 in major immune cell populations (T cells, B cells, CD45-positive cells, and CD56-positive cells) in humanized mouse peripheral blood, i.e., transduction occurred only in T cells, and no BCMA CAR expression was detected in non-T cells populations.

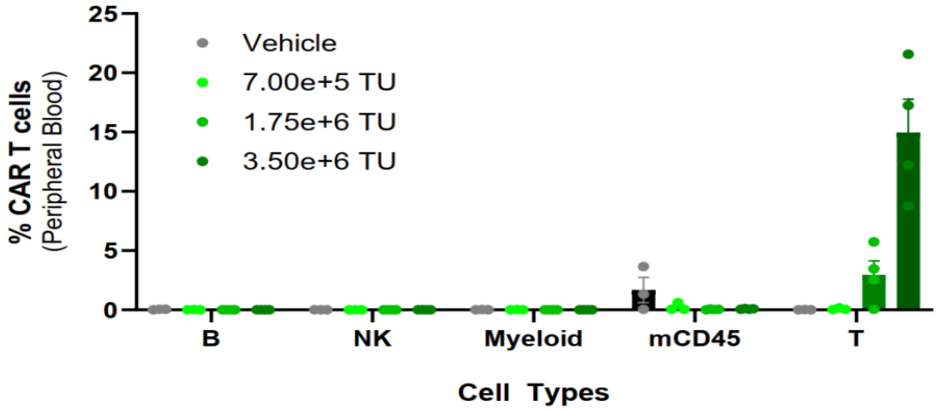

Figure 6. BCMA CAR Expression in Peripheral Blood Cells on Day 7 After Injection

4. *In Vivo* Safety Evaluation

a. Weight change and clinical score of mice: As shown in Figures 7, 8, and 9, ESO-T01 was well-tolerated in tumor-bearing hCD34+ NCG mice following intravenous administration: Weight of mice in the low- and medium-dose groups were stable and comparable to that of the vector group. Transient weight loss (approximately 15%) was observed in the high-dose group, which correlated with peak CAR-T cell expansion. No serious toxicity (clinical scores did not exceed 4) was observed with either medium or high doses.

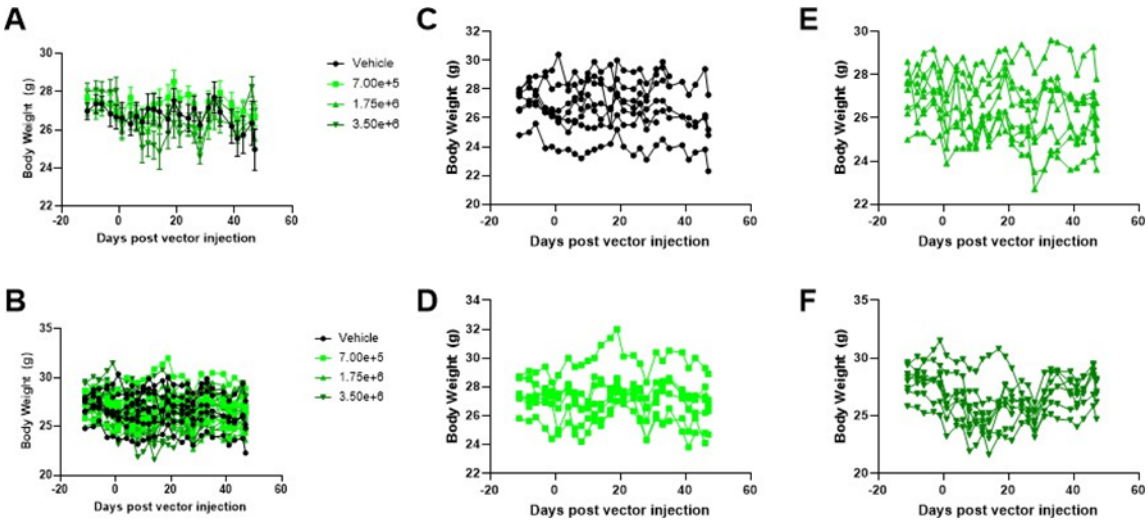

Figure 7 Body Weight Change of Mice

Note: A) Average body weight per group. n=7 - 8 mice per group. Error bars represent standard error. B) Individual body weight of all animals. C) Individual body weight of the vector group. D) Individual body weight of the 7.00E+5 TU/animal group. E) Individual body weight of the 1.75E+6 TU/animal group. F) Individual body weight of the 3.50E+6 TU/animal group.

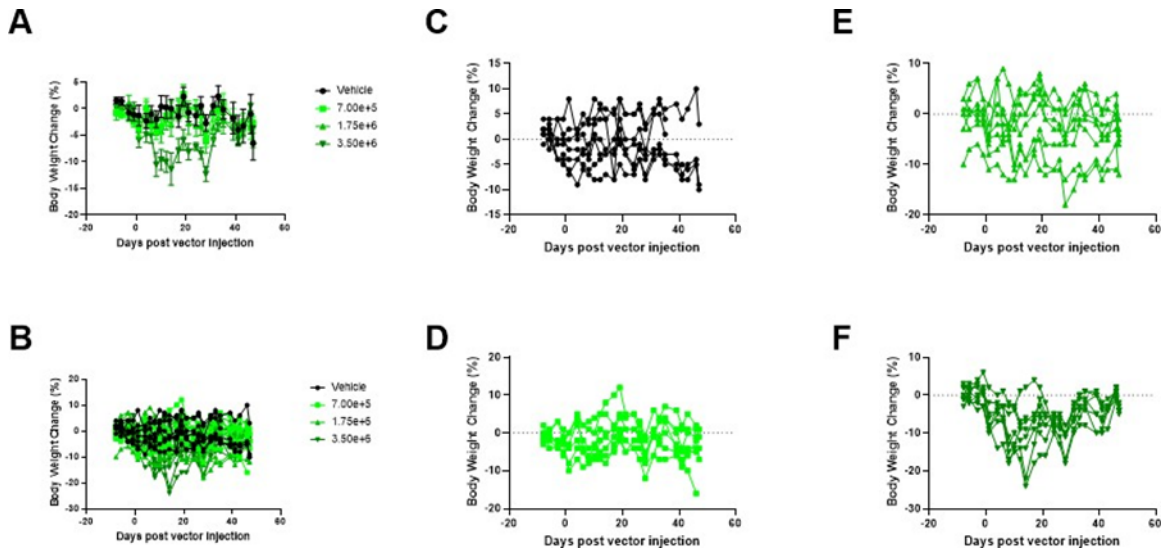

Figure 8. Relative weight change from baseline

Note: A) Average weight change per group. n=7 - 8 mice per group. Error bars represent SEM. B) Individual body weight changes in all animals. C) Individual body weight changes of the vector group. D) Individual body weight changes of the 7.00E+5 TU/animal group. D) Individual body weight changes of the 1.75E+6 TU/animal group. D) Individual body weight changes of the 3.50E+6 TU/animal group.

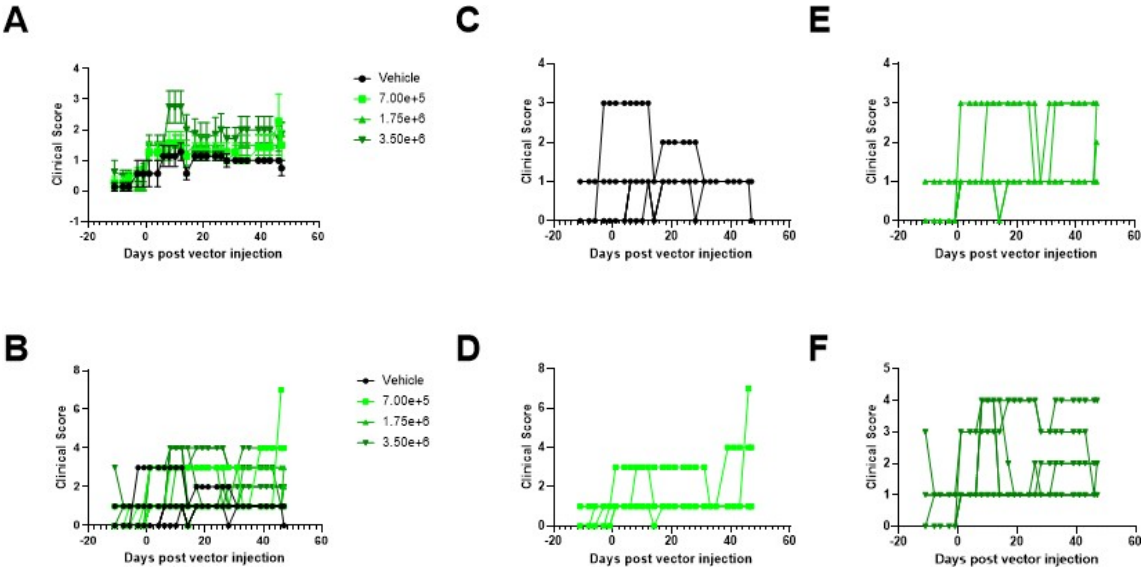

Figure 9. Overall Clinical Score

Note: The overall clinical score combines assessments of appearance, movement, activity, pallor, and body weight to evaluate an animal's general health. A maximum score of 2 is assigned for each category. A total clinical score greater than 7 is an ethical threshold requiring euthanasia. A) Mean overall clinical score of each group. n=7 - 8 mice per group, error bars represent SEM. B) Overall clinical scores of all animals. C) Overall clinical score of the vector group. D) Overall clinical score of the 7.00E+5 TU/animal group. E) Overall clinical score of the 1.75E+6 TU/animal group. F) Overall clinical score of the 3.50E+6 TU/animal group.

b. Cytokine level: As shown in Figure 10, low and medium doses did not cause an increase in CRS-related cytokine levels. Only at high doses were IFNγ, IL6, and IL10

slightly elevated, but they remained significantly lower than cytokine release toxicity levels previously reported for *in vivo* reprogrammed CD19 CAR T-cells.

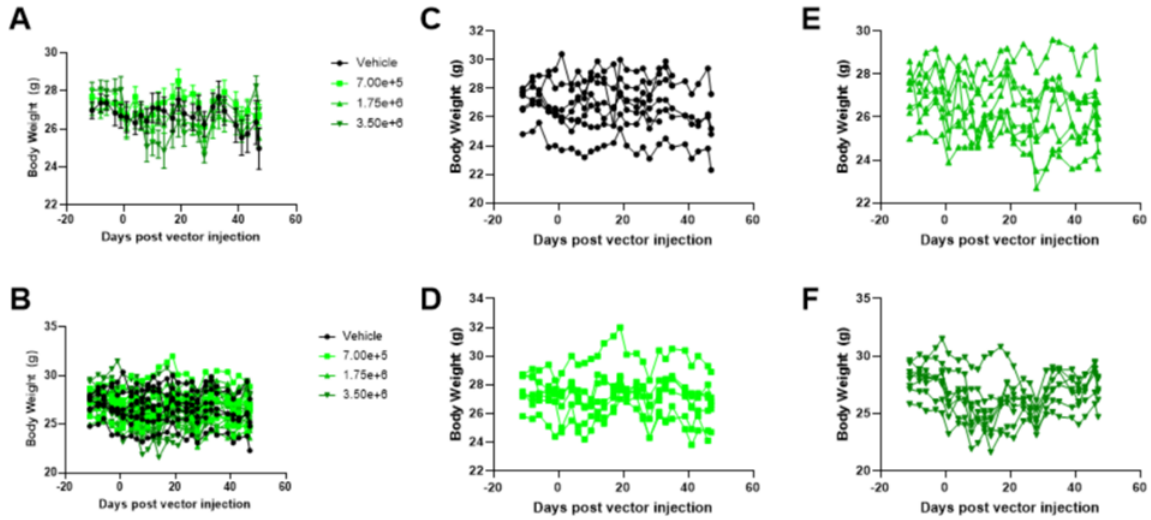

Figure 10. Human and Mouse Cytokine Levels in Plasma

Note: Multi-factor flow cytometry of cytokines in plasma based on magnetic beads. BD Pharmingen™ hTh1/Th2/Th17 Phenotyping Kit and a customized Biolegend Legendplex were used to detect mouse cytokines. Error bars represent SEM, n = 3 - 4 mice per group.

c. ***In vivo* distribution of BCMA CAR-T:** Vector copy number (VCN) analysis of bone marrow, liver, spleen, heart, lung, kidney, stomach, colon, ovary, brain, and spinal cord showed transduced cells present only in the bone marrow. The copy number of transduced cells was below the FDA-recommended threshold of ≤5 copies per cell in all dose groups (see Figure 11 below).

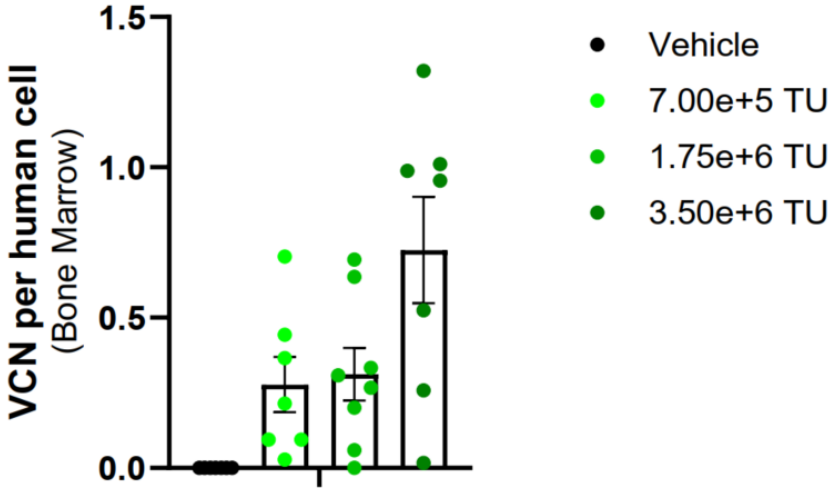

Figure 11 Vector Copy Number per Human Cell in Bone Marrow

Note: Vector copy number (VCN) in bone marrow. VCN is defined as the vector copy number per genome and is calculated by qPCR on genomic DNA. The vector copy number in each sample was determined using specific primers and compared to a standard curve. The total number of genome in each sample was determined using species-specific primers and compared

to a standard curve. The above VCN is represented as the vector copy number divided by the total number of human and mouse genomes in each sample. n = 7 - 8 mice per group, error bars represent SEM.

1.4.3 Study on the On-Target and Off-Target Toxicity of BCMA Sequences

The on-target and off-target toxicity studies included tissue cross-reactivity tests and human cell membrane protein cell array tests.

The tissue cross-reactivity test was conducted by immunohistochemical methods to detect the binding specificity of BCMA single-domain antibody-Fc fusion antibody (B77-linker-Fc protein 3.0-Biotin) with 34 normal human frozen tissue sections (3 sets of tissues from different individuals were selected for each normal human frozen tissue section) to evaluate the potential off-target risk of ESO-T01. The results showed that the Fc-tagged fusion recombinant protein of variable region fragments of the test article, anti-BCMA heavy chain antibody, could specifically bind to normal human splenic red pulp lymphocytes. This binding was due to the abundance of BCMA-expressing plasma cells in the spleen, and no off-target binding was observed during the study.

The human cell membrane protein cell array test was conducted by flow cytometry to detect the binding of BCMA single-domain antibody-Fc fusion antibody (B77-linker-Fc protein 3.0-Biotin) to HEK293T cells individually expressing 5344 human cell membrane proteins, thereby observing the potential off-target risk of ESO-T01. The results showed that BCMA protein was the only binding target of BCMA single-domain antibody-Fc fusion antibody (B77-linker-Fc protein 3.0-Biotin).

1.5 Clinical Studies of Similar Drugs

Genocury's *in vivo* CAR-T cell therapy targeting CD19 is currently undergoing an IIT at the 920th Hospital of the Joint Logistics Support Force of the People's Liberation Army of China. This clinical study enrolled a patient with relapsed/refractory B-ALL who had experienced relapse after 7 prior chemotherapy regimens. Complete response (CR) was achieved in the patient one month after CAR-T cell therapy, with no minimal residual disease (MRD negative) detected in the bone marrow. No cytokine storm above Grade 2 or neurotoxicity, which are common side effects of CAR T-cell therapy, were observed during treatment. This treatment was highly effective and safe. The patient showed stable vital signs and has been discharged. This suggests the feasibility, safety, and efficacy of CAR-T cell therapy *in vivo*.

1.5.1 Safety and Tolerability Evaluations

This product is a recombinant lentivirus product for the preparation of CAR-T *in vivo*. Lymphodepleting preconditioning is not required. However, because BCMA CAR-T cells are produced *in vivo*, side effects associated with BCMA CAR-T cell therapy may occur.

In current BCMA CAR-T clinical trials, patients with a higher MM disease burden or those receiving higher BCMA CAR-T cell doses experience more severe CRS, reaching Grade 3 or 4. This severe CRS is also associated with elevated serum cytokine levels, including IL-6, IFN-γ, and other cytokines. Symptoms of CRS include high fever, low blood pressure, difficulty in breathing, etc., which can be fatal in severe cases. In the trial conducted by Nanjing Legend Biotech, the median time to onset of CRS, regardless of grade, was 9 days (range: 1-19 days), and the median duration was 9 days (range: 3-57 days). In addition to supportive care such as fluid replacement and vasopressor support, treatment with the IL-6 receptor antagonist-tocilizumab and hormones can effectively alleviate toxicity without significantly impacting the efficacy of CAR-T cells. In some trials, CRS has been effectively prevented from the outset by modifying the composition of CAR, lowering disease burden in the inclusion criteria, or reducing the CAR-T cell dose.

Neurological adverse reactions with clinical manifestations of mild confusion, epilepsy, or coma requiring mechanical ventilation, may be observed during or after severe CRS. Pathophysiological abnormalities of severe neurotoxicity manifest as elevated cytokine levels in cerebrospinal fluid and increased blood-brain barrier permeability. Neurotoxicity can be effectively alleviated by using tocilizumab, high-dose hormones, and supportive therapy. Unlike CRS, tocilizumab’s effect on neurotoxicity outcomes is not yet well understood, and other treatment options are being explored and studied.

No unexpected damage to non-hematopoietic organs was observed in patients with MM after BCMA CAR-T cell infusion. Toxicities experienced by patients in the NCI trial, including increased creatine phosphokinase, were very similar to those observed with CD19 CAR-T cell therapy for ALL. The increased creatine phosphokinase occurred only in patients with severe CRS accompanied by high fever. Normal plasma cell reduction after BCMA CAR-T cell infusion is an expected toxicity response because normal plasma cells also express BCMA. Patients with MM who experience a decrease in polyclonal immunoglobulins after CAR-T cell therapy may be treated with immunoglobulin replacement therapy.

Based on clinical experience with anti-CD19 CAR-T cell therapy, patients with high disease burden of ALL may experience hematocytopenia. Hematologic toxicity is a common

adverse reaction after BCMA CAR-T cell infusion, including neutropenia, leukopenia, anemia, and thrombocytopenia. This is likely because the lymphodepleting preconditioning chemotherapy given before CAR-T infusion is expected to cause hematocytopenia. Additionally, the extensive anti-MM therapies that patients have received prior to enrollment can also contribute to chronic bone marrow damage. Overall, the toxicity/safety profile of CD19/BCMA CAR T-cell therapy is clinically manageable and transient.

In summary, adverse events that occur during ESO-T01 treatment should be monitored closely in clinical settings and a decision on whether to suspend or discontinue drug treatment should be made based on the patient's condition and the severity of adverse events. If a patient experiences an adverse event, active treatment should be administered. In the event of a serious adverse reaction that is life-threatening, rescue treatment should be immediately provided. For handling principles of adverse events related to the treatment of r/r MM patients with this product, please refer to Appendix 8.

A summary of pre-existing CAR-T clinical studies, potential adverse reactions during the treatment and risks are provided in Table 2 for reference in the ESO-T01 clinical trial.

Table 2 Recommended Monitoring Items for Adverse Reactions Associated with CAR-T Cell Therapy

Local Adverse Reactions (Administration Site)	
Allergic reaction	
Systemic Adverse Reactions	
Non-hematological adverse events	**Hematology/laboratory test abnormalities**
Anomaly cardiac	Anemia
Tachycardia	Thrombocytopenia
Cardiac arrest	Decreased neutrophil count
Gastrointestinal discomfort	Decreased platelet count
Nausea	Decreased white blood cell count
Vomiting	Decreased hemoglobin
Abdominal pain	Lymphocytopenia
Diarrhea	Hypokalemia
Constipation	Elevated alanine aminotransferase (ALT)
Abnormal general conditions	Elevated aspartate aminotransferase (AST)
Fever	Elevated C-reactive protein (CRP)
Chills	Hyperphosphatemia
Fatigue	Prolonged partial thromboplastin time
Stomatitis	
Immune system disorders	
CRS	
Hypogammaglobulinemia	
Infectious and parasitosis	
The infectious pathogen is unknown	
Infection (bacterial, fungal, viral)	
Metabolic and nutritional disorders	
Decreased appetite	
Fluid retention	

Local Adverse Reactions (Administration Site)	
Allergic reaction	
Systemic Adverse Reactions	
Muscle and joint abnormalities Hand and foot pain Myalgia Joint pain Back pain Neurological abnormalities Headache Encephalopathy Tremors Mental disorder Confusion Delirium or excitation Anxiety Irritability Somnolence Cognitive disturbance Epileptic seizure Urinary system abnormalities Acute renal impairment (increase in serum creatinine) Respiratory system, thoracic cavity, and mediastinal abnormalities Tissue hypoxia Cough Pulmonary edema Tachypnoea Pleural effusion Nasal obstruction Respiratory failure Vascular abnormalities Hypotension Hypertension	

1.6 Risk/Benefit Assessment

Clinical trial data of CAR-T products suggest that an acceptable benefit/risk profile depends on several factors, including the specificity of the target antigen and the characteristics of the CAR itself, the latter encompassing the on-target, off-tumor activity.

From domestic and international clinical studies on BCMA CAR-T, it is evident that CAR-T cell therapy for patients with r/r MM has achieved an overall response rate of over 85%, with MRD consistently showing negative results. The risks of CAR-T cell therapy mainly focus on its mechanism of action, which involves the activation of T cells and the destruction of cells expressing BCMA, including tumor cells and normal plasma cells, and the resulting cytokine release effect can lead to a certain degree of CRS in human body, which in severe cases may be life-threatening or even cause death directly. A small number of patients may also experience

transient neurotoxicity reactions, or conditions such as hypogammaglobulinemia, thrombocytopenia, and pancytopenia lasting for months, necessitating monitoring and intervention. There are well-established clinical management principles and measures for these adverse events related to CAR-T therapy, available for effective control.

BCMA CAR-T therapy offers patients with r/r MM a high response rate without the need for further treatment, providing significant benefits for patients. Although some patients may require ICU monitoring due to severe CRS reactions, the safety of CAR-T products can be effectively ensured in qualified medical institutions. Compared to poor clinical outcomes of other existing therapies reported, CAR-T demonstrates superior clinical efficacy, with benefits outweighing the risks. Overall, BCMA CAR-T presents a positive benefit/risk assessment, meeting clinical needs and offering a new effective treatment modality for patients with r/r MM. Despite the limited clinical follow-up time, the persistence of CR/VGPR has been documented.

ESO-T01 utilizes the BCMA CAR molecule from PRG1801, a product independently developed by Shenzhen Pregene. PRG1801 anti-BCMA CAR is a second-generation CAR structure composed of an anti-BCMA single-domain antibody (VHH), CD8α hinge and transmembrane domain, 4-1BB ICD (intracellular co-stimulatory domain), and CD3ζ intracellular domain (CD3 zeta). In a phase 1 clinical trial conducted at the Bone Marrow Transplantation Center of the First Affiliated Hospital of Zhejiang University, PRG1801 CAR-T cells were tested in 15 patients with relapsed/refractory multiple myeloma. Treatment was well-tolerated at doses up to 5E+06 CAR-positive cells/kg, with no Grade 3 or higher CRS (cytokine release syndrome) or ICANS (immune effector cell-associated neurotoxicity syndrome). With a median follow-up of 14.42 months, the overall response rate was 100%, with 60% of patients achieving complete response and all patients achieving minimal residual disease (MRD) negative results.

ESO-T01 is a recombinant lentiviral product for *in vivo* CAR-T preparation. Recombinant lentiviruses are widely used in the preparation of CAR-T products and also have extensive application in some *in vivo* gene therapy products. ESO-T01 has undergone numerous *in vitro* and *in vivo* experiments on recognition specificity, immunogenicity, and drug toxicity, providing comprehensive safety data.

ESO-T01 can directly generate BCMA CAR-T *in vivo* after infusion, eliminating the need for lymphodepleting preconditioning required in traditional CAR-T preparation, thereby reducing the risks associated with lymphodepleting chemotherapy. ESO-01 is an off-the-shelf product that can be directly infused into patients without the need for blood collection and

preparation, allowing critically ill patients or those unsuitable for blood collection to undergo CAR-T therapy. Additionally, the drug cost of ESO-T01 is significantly lower than that of CAR-T products, improving drug accessibility.

These aspects indicate that ESO-T01, as an *in vivo* CAR-T preparation product, offers high safety and potential effective treatment for patients with r/r MM.

1.7 Strategies for Risk Mitigation

ESO-T01 is a recombinant lentiviral product, designed to be replication-incompetent, preventing viral proliferation in the body. However, precautions must be taken against potential toxic reactions such as viremia caused by viral expansion. Patients with viremia may experience symptoms such as fever, asthenia, fatigue, muscle pain, nausea, and vomiting, with severe cases potentially leading to organ dysfunction or life-threatening conditions. In such cases, antiviral medications can be administered based on the subject's clinical manifestations.

CAR-T products have a high incidence of adverse events, primarily CRS and neurotoxicity reactions, usually occurring within 6 weeks post-treatment, with CRS reactions potentially reaching Grades 3/4. CRS can cause a range of inflammatory symptoms, from flu-like symptoms to severe multi-organ dysfunction, and even death. Neurotoxicity symptoms mainly include: Aphasia, tremors, epileptic seizure, confusion, and encephalopathy, generally milder than CRS. Grades 3/4 CRS require ICU observation. CRS treatment follows a graded management principle, involving supportive therapy and the use of tocilizumab. Neurotoxicity response monitoring requires frequent neurological evaluations and treatment with glucocorticoids.

In this clinical trial, strategies will be developed and implemented to minimize and manage the risks associated with the use of ESO-T01. Risk assessment and mitigation strategies will ensure that the benefits to patients significantly outweigh the risks. These strategies include:

- According to the requirements for risk assessment and risk mitigation strategies, this investigational product is only available for patients to use at medical institutions with medical experience in applying CAR-T cell products;
- Provide on-site training for all involved parties from pharmacies and Investigators to clinical sites, followed by knowledge assessments to certify their qualifications for distributing and using ESO-T01; provide necessary training to subjects and their families on the principles and processes of treatment, as well as the observation and management of adverse reactions, and ensure the training are effectively implemented;

- Formulation of contingency plan and training on Investigators: Before the commencement of the trial, all Investigators must receive GCP and trial project training, familiarize themselves with the trial protocol, understand the properties of the drug, expected adverse reactions and treatment strategies, and proficiently master medical emergency measures. This aims to ensure that all staff participating in the trial are clear about their respective roles and responsibilities, prioritize the safety and health of subjects over scientific interests, and firmly establish the concept of "subject's safety first". Develop contingency plans for various emergencies, particularly the coordination with other wards, and conduct drills in advance, including the time required for emergency transport to the ICU and the optimal delivery route, so as to enhance the emergency response capabilities of medical staff;
- Select subjects suitable for ESO-T01 treatment in strict accordance with the inclusion/exclusion criteria established in the protocol;
- Before the trial, conduct a thorough assessment of the potential risks faced by subjects, and grade adverse events that may occur according to their severity; meanwhile, clearly define the different handling procedures for AEs of different grades and make the necessary preparations in advance. If needed, consult a specialist or timely transfer subjects to the ICU for treatment with the companion of medical staff;
- Monitor the vital signs of subjects (body temperature, respiratory rate, sitting blood pressure, pulse rate) and the functions of important organs, closely observe any adverse events, and conduct follow-up for subjects (including those who withdraw midway) after the end of the trial;
- Clinical sites using ESO-T01 must report all viremia, CRS and neurotoxicity reactions;
- Retain all documents processed and discussed to mitigate risks;
- Seek a CRO with relevant experience to monitor and audit this trial. Simultaneously, provide on-site training for Investigators to ensure that clinical adverse events are handled promptly and appropriately, and to ensure that the trial process is conducted in accordance with the protocol and GCP;
- Ensure that at least two doses of tocilizumab are available at the clinical site using ESO-T01 before the commencement of the trial. Adequate emergency medications for managing adverse reactions to CAR-T products must be available throughout the trial;
- Create corresponding patient/healthcare provider pocket cards.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Study Objectives

To evaluate the safety and tolerability of ESO-T01 Injection in the treatment of relapsed/refractory multiple myeloma.

2.1.2 Secondary Study Objectives

1. To preliminarily assess the efficacy of ESO-T01 Injection in the treatment of R/R MM;

2. To obtain the pharmacokinetic and pharmacodynamic data of ESO-T01 Injection in humans.

2.1.3 Exploratory Study Objectives

To assess the immunogenicity and viral shedding of ESO-T01 Injection;

2.2 Study Endpoints

2.2.1 Primary Study Endpoints

Safety and tolerability of ESO-T01: Safety measures such as dose-limiting toxicities (DLTs), adverse events (AEs), laboratory tests, vital signs, physical examinations, and electrocardiograms (ECGs).

2.2.2 Secondary Study Endpoints

1. Efficacy parameters: Time to response (TTR), objective response rate (ORR), minimal residual disease (MRD) negativity rate (according to the 2016 International Myeloma Working Group (IMWG) response criteria [Appendix 1]), duration of response (DOR), progression-free survival (PFS), overall survival (OS), etc. Objective response rate includes stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR);

2. Pharmacokinetic parameters: Maximum concentration (C_{max}), time to maximum concentration (T_{max}), area under the curve from Time 0 to Day 28 ($AUC_{0\text{-}28d}$) etc. of the expansion of BCMA CAR-T in peripheral blood after ESO-T01 infusion.

3. Pharmacodynamic parameters: Testing of cytokine levels (IL-6, IL-10, IFN-γ, TNF-α, etc.), CRP, ferritin levels, immunoglobulin levels (IgG, IgA, IgM), and peripheral lymphocyte subpopulations (T, B, NK cell ratios and absolute counts) in peripheral blood after ESO-T01 infusion.

2.2.3 Exploratory Study Endpoints

Immunogenicity of ESO-T01 and *in vivo* generated CAR-T by ESO-T01, and the presence shedding of ESO-T01 in blood, saliva, urine, and feces;

3 STUDY DESIGN

3.1 Study Overall Design

This is a single-arm, open-label, single-center clinical study designed to evaluate the safety, tolerability, efficacy, and pharmacokinetic or pharmacodynamic profiles of the study drug ESO-T01 Injection.

3.1.1 Dose Escalation and Subject Enrollment Principles

This study uses a dose-escalation design with 4 dose groups: Dose A, Dose B, Dose C, and Dose D. A total of 4-24 subjects with relapsed/refractory multiple myeloma are enrolled. Subjects will be enrolled with rapid titration. In Dose A group, 7 days after infusion in the first subject, PK samples will be tested centrally and analyzed by the Principal Investigator, as well as the comparability with the Phase 1 clinical trial of BCMA autologous CAR-T, and a decision on whether to continue enrolling subsequent subjects or to increase the dose to Dose B will be made. Similarly, in Doses B, C, and D groups, a single subject will be enrolled first (titration) and administered infusion of ESO-T01. In Doses B, C, and D groups, 14 days after infusion in the first subject, PK samples will be tested centrally and analyzed by the Principal Investigator, as well as the comparability with the Phase 1 clinical trial of BCMA autologous CAR-T, and a decision on whether to continue enrolling subsequent subjects or to increase the dose to subsequent dose levels will be made. In Doses A, B, C, and D groups, if continued enrollment of subjects is required after the first subject has completed the titration, enrollment of 3-6 subjects of the dose group design may be completed. Within the same dose group, the enrollment interval for subsequent subjects is determined by the Principal Investigator, except that the enrollment interval between the first and second subjects is 7-14 days.

MTD is the highest dose at which DLT is observed in ≤1/6 of subjects. Determination of MTD is made by the Investigator and the cooperating unit after discussion based on cumulative safety data from all dose-escalation subjects.

For 3 subjects at the assessed dose, ① if no DLT is observed, these subjects will be added to the next dose group; ② If DLT is observed in 1 subject, 3 additional subjects will be enrolled at this dose level. A. If DLT is observed in only 1 subject out of the 6 subjects in total, they will proceed to the next dose group; B. If DLT is observed in 2 (inclusive) or more subjects out of the 6 subjects in total, enrollment will be continued in the previous dose group up to 6 subjects to continue observing the DLT until the MTD is determined.

3.1.2 Dose Setting

4 dose groups are established in this study, as shown in Table 3.

Table 3 ESO-T01 Intravenous Infusion Dose Escalation Scheme

Dose Group	Dose A	Dose B	Dose C	Dose D
Dose (TU/subject)	0.2×10^9	0.6×10^9	1.2×10^9	2.4×10^9
Number of subjects	1-6	1-6	1-6	1-6

3.1.3 Follow-up Principles

After the infusion of ESO-T01, subjects will undergo corresponding follow-up assessments to evaluate efficacy and safety. Follow-up will continue until the trial concludes or the subject withdraws from the study. For subjects who have received ESO-T01 treatment, even if they withdraw from the trial prematurely, the Investigator should perform long-term safety follow-up for them according to the protocol to evaluate the long-term safety of this product.

3.2 Justification of Dose Selection

Dose selection is based on the safe and effective dose in humanized mice, as well as doses from similar products in non-human primate studies, extrapolated by body weight, with the following considerations:

1) The minimum effective dose in this humanized mice model is 0.7×10^6 TU. Based on an average mouse weight of 20 g, the minimum effective dose is 35×10^6 TU/kg (0.35×10^8 TU/kg); The effective dose of similar products in non-human primates is $1\text{-}7\times10^8$ TU/kg;

2) Based on an average subject weight of 60 kg, the effective dose should be 21×10^8 TU/subject (0.35×10^8 TU/kg×60 kg = 21×10^8 TU); For safety considerations, the clinical starting dose is typically 1/10 of the effective dose in animal experiments, resulting in a calculated safe starting dose of approximately 2×10^8 TU/subject, or 0.2×10^9 TU/subject; Four dose levels were designed, with the maximum dose (Dose D) set at 2.4×10^9 TU/subject. This dose was determined to be effective based on observations in humanized mice model.

In summary, this study plans to use 0.2×10^9 TU/subject of ESO-T01 injection as the starting dose level, with four dose groups planned (0.2×10^9, 0.6×10^9, 1.2×10^9, 2.4×10^9 TU/subject).

3.3 Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity (DLT) is defined as ≥ Grade 3 toxicity occurring within the DLT observation period (28 days after ESO-T01 infusion) and considered by the Investigator or the cooperating unit to be reasonably related to ESO-T01 treatment. Toxicity will be graded according to the CTCAE v5.0, except for cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), which will be graded based on the 2019 ASBMT consensus criteria. Given the specificity of subjects with multiple myeloma (MM), DLT is defined as: A subject who, despite therapeutic measures, develops any of the following conditions related to the study drug:

(1) Grade 4 or higher CRS caused by ESO-T01 treatment;

(2) Grade 3 CRS caused by ESO-T01 treatment that does not resolve to Grade 2 or lower within 7 days of onset;

(3) Grade 3 or 4 neurotoxicity caused by ESO-T01 treatment (excluding cases where the neurotoxicity resolves to Grade 1 within 2 weeks of onset, or to baseline within 4 weeks of onset);

(4) Grade 3-5 allergic reactions caused by ESO-T01 treatment (excluding cases of rapid allergic reactions related to ESO-T01 occurring within 2 hours of its infusion and resolving to Grade 2 or lower within 24 hours after systemic treatment);

(5) Grade 3 or higher non-hematologic toxicity, except for the following:

- Fever of any grade, including neutropenic fever;
- Grade 3 or higher diarrhea lasting ≤ 72 hours;
- Grade 3 or higher nausea and/or vomiting lasting ≤ 72 hours.
- Grade 3 or higher fatigue lasting ≤ 7 days;
- Elevated transaminases, bilirubin, or creatinine levels (Grade 3 or higher) lasting ≤ 14 days;
- Any Grade 3 non-hematologic adverse events (AEs) that are rapidly reversible (resolve to baseline or Grade 2 and lower within 7 days).

(6) Excluding hematocytopenia of any degree.

3.4 Sample Size

4-24 subjects are planned to be enrolled in this study.

3.5 Study Duration

3.5.1 Study Duration

Main study evaluation and follow-up period of approximately 24 months. However, the study duration may vary among individuals due to differences in screening requirements, treatment efficacy, or survival duration.

3.5.2 Study Completion

Study completion is defined as the last subject completing 24 months of follow-up after ESO-T01 infusion, loss to follow-up, or death, whichever occurs first.

4 SUBJECT SCREENING AND ENROLLMENT

No study-related procedures or activities may be conducted until a signed and dated informed consent form, approved by the Ethics Committee, has been obtained.

Prior to initiating any study procedures or activities, each subject entering the screening period will be assigned a screening number. Subjects are permitted one repeat screening under the same screening number for the specific item that initially led to their screening failure. Subjects who fail rescreening are considered screening failures and are out group. Subjects who are screening failures and out group are allowed one opportunity to be rescreened. They must re-sign the informed consent form and will be assigned a new screening number.

5 STUDY POPULATION

5.1 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for participation in this study:

1. Aged 18 years or older, any sex;
2. Diagnosed with multiple myeloma (MM) according to the IMWG response criteria (Appendix 2), with positive MM cells expressing BCMA target antigen confirmed by flow cytometry or bone marrow pathology and immunohistochemistry.
3. Received at least two lines of anti-MM therapy, with each line consisting of at least one complete treatment cycle, and experienced progressive disease as evidenced by test data within 12 months during or after the most recent anti-myeloma therapy; Or are determined by the Investigator to be refractory to both immunomodulators and proteasome inhibitors, and have experienced progressive disease (according to IMWG response criteria) during or within 2 months after the most recent anti-myeloma therapy.
4. Measurable disease at screening, meaning meeting one or more of the following criteria:

- Serum M-protein ≥ 0.5 g/dL;
- Urine M protein level ≥ 200 mg/24 h
- Or involved serum free light chain ≥ 10 mg/dL with abnormal serum free light chain κ/γ ratio.

5. ECOG score of 0-2 points (Appendix 3), with an expected survival of 3 months or more;
6. Bone marrow function test results (at screening or within 2 months before screening) meet the following criteria:

- Hemoglobin ≥ 6 g/dL (no red blood cell transfusions within 1 week prior to screening), and the use of recombinant human erythropoietin is allowed; For patients who meet the inclusion criteria of hemoglobin ≥6 g/dL, red blood cell transfusions are permitted to maintain hemoglobin levels at ≥ 6 g/dL;
- Absolute neutrophil count (ANC) ≥ 600/μL (no use of granulocyte-colony stimulating factor (G-CSF) within 1 week prior to screening or no use of pegylated G-CSF within 2 weeks prior to screening);
- Platelet count (ANC) ≥ 50,000/μL;
- Lymphocyte count (ANC) ≥ 500/μL;
- Absolute CD3-positive T cell count ≥ 150/μL;

7. Normal renal function during the screening period or within 2 months prior to screening:

Creatinine clearance (CrCl) (Cockcroft-Gault formula, Appendix 4) ≥ 45 mL/min;

8. Liver function must meet the following criteria during the screening period or within 2 months prior to screening:

- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 3.0 × upper limit of normal (ULN);
- Total bilirubin (TBIL) and alkaline phosphatase (AKP or ALP) ≤ 2.0×ULN (except for congenital hyperbilirubinemia, such as Gilbert syndrome, where direct bilirubin can be ≤ 1.5×ULN);
- Albumin ≥ 3 g/dL;

9. Cardiac function must meet the following criteria during the screening period or within 2 months prior to screening:

- Left ventricular ejection fraction ≥ 40% (by echocardiography or MUGA scan);
- No clinically significant pericardial effusion detected;
- No clinically significant ECG abnormalities detected;

10. Lung function must meet the following criteria during the screening period or within 2 months prior to screening:

- Oxygen saturation ≥ 90%;
- No clinically significant pleural effusion detected;

11. A negative pregnancy test is required for women of childbearing potential at screening and before drug infusion, and they must also not be breastfeeding;
12. Men and women of childbearing potential must agree to use effective contraception (see Appendix 5) from the time they sign the informed consent form until one year after the use of study drug;
13. Men and women of childbearing potential must agree not to donate germ cells including sperm or ovum (oocytes) from the time they sign the informed consent form until one year after the use of study drug;
14. The subject or his/her legally authorized representative has provided consent by signing the informed consent form (ICF), indicating his/her understanding of the study purpose and procedures, and willingness to participate in the study.

5.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from this study:

1. Received other anticancer treatments during screening (primarily determined by the Investigator):

- Received targeted therapy, epigenetic therapy, other treatments with clinical study drugs, or treatments involving invasive study medical devices within 5 half-lives;
- Received immune/non-immune directed systemic therapy within 1 week;
- Received cytotoxic therapy within 1 week;
- Treated with a proteasome inhibitor and an immunomodulator within 2 weeks.
- Received radiotherapy within 4 weeks (subjects are eligible regardless of the end date of radiotherapy if the radiation field covers ≤5% of the bone marrow reserve);

2. Received an allogeneic hematopoietic stem cell transplantation within 6 months before infusion or an autologous hematopoietic stem cell transplantation within 3 months before infusion.
3. A history of malignancies other than MM prior to screening, except for the following: Malignant tumors treated with curative intent, with no known active disease for ≥ 2 years prior to enrollment; Adequately treated non-melanoma skin cancer without current disease evidence;
4. Received treatment with any virus pseudotyped with vesicular stomatitis virus glycoprotein (VSVG);
5. Presence of serious, uncontrolled infections (bacterial, viral, fungal, etc.) during the screening period;
6. Positive for hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb), with the detected peripheral blood hepatitis B virus (HBV) DNA titer above the normal range within 6 months prior to infusion. Positive for hepatitis C virus (HCV) antibody, with the detected peripheral blood HCV RNA titer above the normal range. Positive for human immunodeficiency virus (HIV) antibody. Patients who test positive for syphilis;
7. Presence of symptomatic heart failure or other cardiac disorders such as serious arrhythmia;

- New York Heart Association (NYHA) Class III or Class IV congestive heart failure;
- Episodes of myocardial infarction, or prior coronary artery bypass grafting (CABG) or coronary arterial stent insertion within 6 months before signing the ICF;
- Clinically significant ventricular arrhythmias or a history of unexplained syncope (excluding cases caused by vasovagal episodes or dehydration);
- A history of severe non-ischemic cardiomyopathy;

8. Other clinically significant diseases, including:

- Primary immunodeficiency;
- Stroke or epileptic seizure within 6 months before screening;

- Clear clinical evidence of dementia or mental status changes;
- Having Parkinson's disease, parkinsonian dyskinesia, or a history of these conditions.

9. Underwent surgery within 2 weeks of administration or plans to receive surgery within 2 weeks after administration, excluding surgeries involving local anesthesia;
10. Immunization with a live attenuated vaccine within 1 month before administration;
11. Known severe allergic reactions to ESO-T01 or any of its preparation components;
12. Known severe allergic reactions to tocilizumab;
13. Patients unsuitable for creation of venous access;
14. Other conditions deemed by the Investigator to be unsuitable for participation in the study.

5.3 Bridging Therapy

The Investigator, after assessing the subject's condition, may administer bridging therapy, including but not limited to hormones (such as dexamethasone, prednisone), chemotherapy (such as vincristine, etoposide), or radiation therapy (such as total body irradiation), if deemed necessary to reduce tumor burden and if the potential benefits outweigh the risks for the patient.

Bridging therapy must be short-term. Efficacy must be assessed before or at baseline after bridging therapy, and subjects who achieve CR after bridging therapy are not allowed to receive ESO-T01 treatment.

Recommended washout periods for bridging therapy medications prior to drug administration:

Monoclonal antibody	MM patients with IgG type are not recommended to receive CD38 monoclonal antibody bridging therapy for 21 days.
Chemotherapy, targeted therapy, epigenetic therapy	Within 14 days or 5 half-lives, whichever is shorter.
Proteasome inhibitors	14 days
Radiotherapy (radiation field covering ≤ 5% of bone marrow reserve)	7 days, lung radiotherapy for 14 days
Immunomodulators	7 days

5.4 Drug Infusion Criteria

Drug infusion should not be started or should be delayed if the subject experiences any of the following:

1. Before drug infusion, the subject experiences any of the following, including but not

limited to: New-onset arrhythmias that cannot be controlled with medications, hypotension requiring vasopressors, or uncontrolled active infection. The subject is not suitable for continued drug infusion as judged by the Investigator;

2. Body temperature > 38℃ within 48 hours before drug infusion (excluding tumor-related fever);
3. ECOG performance status score ≥ 3 before drug infusion;
4. The subject is assessed as unable or unwilling to comply with the study protocol requirements by the Investigator.

5.5 Criteria for Stopping Infusion

During the trial, the Investigator may decide whether the subject needs to stop the drug infusion or withdraw from the trial based on the following criteria:

1. Intolerable toxicity during infusion resulted in subjects being unable to continue ESO-T01 Injection treatment;
2. Unwillingness of subjects to continue with the clinical study. Subjects can make request to the Investigator and withdraw from the study at any time;
3. Exacerbation of disease in subjects before ESO-T01 Injection infusion that is assessed as unsuitable for drug infusion by the Investigator;
4. Other conditions that the Investigator considers unsuitable for drug infusion.

5.6 Criteria for Withdrawal from the Study

1) Intolerable adverse events occur, and the Investigator determines that the risks of continued participation in the study outweigh the benefits for the subject;
2) Poor subject compliance results in failure to complete follow-ups on time.
3) The subject or his/her legally authorized representative requests withdrawal from the trial or withdrawal of the ICF;
4) The subject experiences confirmed tumor progression during the trial and requires other medical interventions;
5) The Investigator considers the subject to have other conditions that make continued participation in the trial inappropriate.

Note: For subjects who have received ESO-T01 treatment, even if they withdraw from the trial prematurely, the Investigator should perform long-term safety follow-up for them according to the protocol to evaluate the long-term safety of this product. All subjects who withdraw from the study with drug-related AEs should be followed up subsequently for AEs until AEs resolve to baseline, stabilize, the subject is lost to follow-up, or there is another

reasonable explanation. If the AE is a chronic condition, it may be excluded from follow-up in consultation between the Investigator and the cooperating unit, but the consultation must be documented in the original medical record.

5.7 Criteria for Study Termination

Reasons for study termination include, but are not limited to:

1) The clinical trial terminated as required by the cooperating unit;
2) When the number and severity of adverse events occurring in this study indicate that continuing this study will pose greater risks than benefits to subjects;
3) Situations in which the National Medical Products Administration or the National Health Commission deem a clinical trial unsuitable for continuation.

6 TREATMENT REGIMENT

6.1 Treatment Terminology

The following terms will be used to describe and define the treatment in this study:

- In this study, study drug refers to ESO-T01 Injection;
- All treatments required by the treatment protocol in this study reference study.

6.2 Study Drug

6.2.1 Physical and Chemical Characteristics of Study drug

he product is generally a turbid liquid, available in four strengths:

0.2×10^9 TU/subject per dose;

0.6×10^9 TU/subject per dose;

1.2×10^9 TU/subject per dose

2.4×10^9 TU/subject per dose

6.2.2 Dosage and Administration of Study Drug

The ESO-T01 dose is 0.2-2.4×10⁹ TU/subject, administered by intravenous infusion at a rate of 2-5 mL/min.

6.2.3 Packaging and Labeling of Study drug

The cooperating unit will package and label study drug.

Product label content includes: Clinical study number, subject number, product name, strengths, usage instructions, expiration date and time, and the statement “For Clinical study Use Only”. It is recommended to include storage conditions and the cooperating party’s name, address, and contact information.

6.2.4 Storage and Transportation of Study drug

ESO-T01 injection must be stored and transported at -80°C. Transportation to the clinical site is handled by the cooperating unit or a qualified professional biopharmaceutical cold chain company commissioned by the cooperating unit.

6.2.5 Management and Use of Study Drug

The site should establish complete drug receipt procedures, with designated personnel responsible for receiving the study drugs and signing the drug receipt form. Only authorized clinical study drug management personnel may access the study drug. Upon receiving ESO-T01 injection, the Investigator, study director, or pharmacist at the clinical site must confirm

and sign for it. After the infusion, the ESO-T01 Injection bag should be disposed of by the hospital as biohazardous medical waste according to relevant regulations.

The site must ensure that the storage conditions for study drug meet specified requirements, maintain records of these conditions, and contact the cooperating unit immediately if any deviations from these requirements are detected.

6.3 Study Treatment

6.3.1 ESO-T01 Injection Infusion Procedure

6.3.1.1 Infusion Procedure

Subjects who pass the eligibility assessment for ESO-T01 administration will receive ESO-T01 via intravenous drip infusion. Before the infusion, medical professionals should verify the subject's information is in line with that on labels attached to the packaging bags, and confirm once again that the clinical site has at least two doses of tocilizumab available for use.

Before the official infusion of ESO-T01, it must be confirmed: Authorized Investigators have signed the cell usage record page to confirm that the subject meets the criteria for infusion and is free from infection.

Study drug infusion: ESO-T01 is administered by intravenous infusion at doses of 0.2-2.4×10^9 TU/subject. The use of precision filtration infusion set is prohibited. Once the infusion process has begun, if there is any leakage of the drug solution, the administration should be halted, and the remaining uninfused drug solution should be retrieved and destroyed in accordance with requirements. First aid facilities should be available during the infusion to provide prompt treatment for severe allergic reactions, severe hypotension, or other reactions.

6.3.1.2 Observation and Management of Infusion Reactions

Infusion reactions mostly occur during or within 1-2 h after infusion. Preventive and therapeutic measures can be taken at the start and during the infusion.

NCI-CTCAE Grading	Treatment Modifications
Grade 1-Mild Mild, transient reactions;	No need to interrupt infusion; no intervention required. Reduce ESO-T01 Injection infusion rate by 50% and closely monitor for any worsening until the reaction resolves.
Grade 2-Moderate Requires treatment or interruption of infusion; symptoms resolve promptly with symptomatic treatment (e.g., antihistamines, NSAIDs, anesthetics, IV fluid replacement, etc.); Preventive treatment	Pause ESO-T01 Injection infusion. Once the infusion-related reaction resolves or severity reduces to Grade 1 or below, resume infusion at 50% of the previous rate and closely monitor for any worsening.

NCI-CTCAE Grading	Treatment Modifications
duration ≤24 h.	
Grade 3-Severe Delayed symptom relief (e.g., not promptly resolved after symptomatic treatment and/or brief infusion interruption); Initial improvement followed by symptom relapse; Hospitalization or clinical consequences (e.g., renal injury, pulmonary infiltration, etc.).	Based on the Investigator's judgment, decide whether to continue treatment. It is recommended to immediately stop ESO-T01 Injection infusion and disconnect the infusion line. If treatment continues, reduce the infusion rate to below 50% of the previous rate and closely monitor for any worsening.
Grade 4-Life-threatening Requires urgent intervention (e.g., ventilator support, etc.).	Immediately stop ESO-T01 Injection treatment, and the patient must not receive ESO-T01 Injection again.

NCI-CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events; NSAID=Non-Steroidal Anti-Inflammatory Drug.

If systemic anaphylaxis/anaphylactoid reactions occur (typical manifestations within minutes of administration, characterized by Distress respiratory, edema of larynx, and/or severe bronchospasm; Vascular collapse or shock typically occurs afterward, but without preceding difficulty in breathing. Skin manifestations, such as pruritus and hives, with or without swelling. If gastrointestinal symptoms, such as nausea, vomiting, abdominal colic, and diarrhea, occur, the infusion must be stopped and the patient closely monitored. If hypersensitivity occurs, it must be treated with the best available medical care patient. Subjects are advised to report any delayed reactions to the Investigator immediately. If hypersensitivity is observed, administer epinephrine injection and dexamethasone infusion, followed by immediate monitoring.

6.3.2 Concomitant Medication/Treatment

Concomitant medications/treatments refer to the simultaneous use of two or more drugs/treatment methods. In this study, it refers to other drugs (including chemical drugs, biologics, traditional Chinese medicines, etc.) or treatments received in addition to the routine study treatment drugs.

Concomitant treatments are collected from the signing of the ICF. Except for physiological saline or glucose injections used as drug solvents without therapeutic effects, all medications or non-drug therapies must be recorded until the subject withdraws from the study or 24 months after infusion, whichever occurs first. If the reason for concomitant medications/treatments meets the definition of an AE, the relevant information must also be recorded in the subject's original documents and eCRF.

6.3.2.1 Permitted Concomitant Medications/Treatments

During the study, except for prohibited concomitant treatments listed in the study, the

Investigator must provide adequate supportive treatment to subjects.

1. Standard of care and palliative care (excluding anti-tumor therapy) for existing diseases, medical and/or surgical complications, with all medications recorded.
2. Bridging therapy is permitted for subjects with high tumor burden or rapid disease progression after screening.
3. Medications to prevent infusion-related reactions; concomitant medications for adverse events are permitted.
4. If significant neutropenia or febrile neutropenia/infection is observed, the Investigator may refer to the American Society of Clinical Oncology (ASCO) guidelines for prophylactic use of growth factors (e.g., granulocyte colony-stimulating factor [G-CSF]). G-CSF should be used with caution within 14 days after infusion or before CRS resolution; GM-CSF and pegylated G-CSF are prohibited.
5. Others: Medications such as prophylactic antiemetics, gastric mucosal protectants, and antihypertensive medications deemed appropriate by the Investigator.

6.3.2.2 Prohibited Concomitant Medication/Therapy

From the start of ESO-T01 Injection infusion until disease progression or treatment change, subjects must not use the following treatments unless otherwise specified in the protocol:

1. Anti-tumor treatments (including traditional Chinese medicines, proprietary Chinese medicines, and Chinese medicine preparations approved by the National Medical Products Administration for anti-tumor treatment);
2. Hematopoietic stem cell transplantation;
3. Any other drugs that may affect the Investigator’s assessment of efficacy or subject safety.

7 STUDY EVALUATION

7.1 Safety Evaluation

All adverse events will be monitored and recorded throughout this study. Abnormal changes in vital signs, physical examination, clinical laboratory tests, electrocardiogram (ECG) and other measures before and after ESO-T01 treatment are recorded. Each safety measure, including type, onset time, frequency, response time, severity, management measures, and correlation with ESO-T01 treatment, are described and statistically analyzed.

7.2 Efficacy Evaluation

This study will evaluate the treatment effect on tumors within 24 months after ESO-T01 infusion to preliminarily observe the efficacy of ESO-T01 treatment in subjects with MM.

1. Objective response rate (ORR): Includes stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR).
2. MRD negativity rate;
3. Time to response (TTR);
4. Duration of Response (DOR);
5. Progression-free survival (PFS);
6. Overall survival (OS).

7.3 Pharmacokinetic Evaluation

Peripheral blood is collected from subjects at baseline and on D2, D4, D6, D8, D10, D12, D14, D17, D21, D24 and D28, and at M2, M3, M6, M9, M12, M18 and M24 after infusion to detect the CAR copy number in peripheral blood using validated qPCR method.

Peripheral blood is collected from subjects at baseline and on D2, D4, D6, D8, D10, D12, D14, D17, D21, D24 and D28, and at M2 after infusion to detect changes in the number of CAR-T (CAR-T/mL blood) using flow cytometry methods. At the time point when CAR-T cells are detectable by flow cytometry, the CAR-T cell phenotype is further analyzed for CD4/CD8 ratio and naive, memory and effector cells, etc.

Pharmacokinetic parameters: Maximum concentration (C_{max}) of ESO-T01 in peripheral blood after reinfusion, time to reach maximum concentration (T_{max}), and area under the curve over 28 days ($AUC_{0\text{-}28d}$).

The collection tubes and other conditions for pharmacokinetic blood sampling must comply with the requirements of the bioanalytical laboratory. It is recommended to collect PK blood samples according to the time points listed in the table below.

Table 4 PK Blood Sample Collection Schedule

PK blood Sampling Time Point	Sampling Points	Schedule	Time Window
Baseline	1	Baseline	NA
2 days after the end of infusion	2	D2	±4 h
4 days after the end of infusion	3	D4	±6 h
6 days after the end of infusion	4	D6	±6 h

8 days after the end of infusion	5	D8	±8 h
10 days after the end of infusion	6	D10	±10 h
12 days after the end of infusion	7	D12	±10 h
14 days after the end of infusion	8	D14	±10 h
17 days after the end of infusion	9	D17	±10 h
21 days after the end of infusion	10	D21	±24 h
24 days after the end of infusion	11	D24	±24 h
28 days after the end of infusion	12	D28	±24 h
2 months after the end of infusion[1]	13	M2	±7 d
3 months after the end of infusion	14	M3	±7 d
Follow-up period	15	M6	±7 d
	16	M9	±7 d
	17	M12	±7 d
	18	M18	±7 d
	19	M24	±7 d
Withdrawal visit	NA	Early withdrawal	7 d

Note: 1. Peripheral blood CART cell levels will only be collected until M2;

7.4 Pharmacodynamics Evaluation

Peripheral blood is collected at baseline, on D2, D4, D6, D8, D10, D12, D14, D17, D21, D24, D28, and at M2 to detect cytokine levels (IL-6, IL-10, IFN-γ, TNF-α, etc.), and CRP and ferritin levels.

Peripheral lymphocyte subpopulations (T, B, and NK cell ratios and absolute counts) and immunoglobulin levels (IgG, IgA, and IgM) are detected at baseline, on D28, and at M2, M3 and M6.

Exploratory immunogenicity and viral shedding study: Serum samples are collected and frozen at baseline, on D28, and at M3, M6, M9, M12, M18 and M24 for central detection of ESO-T01 and CAR-T cell immunogenicity. Blood, saliva, urine, and fecal samples are collected and frozen at baseline, and on D2, D4, D6, D8, D10, D14, D21 and D28 to detect

viral shedding.

8 ADVERSE EVENTS

8.1 Definition

8.1.1 Adverse Events

Adverse Event (AE) refers to any unfavorable medical occurrence in a subject after receiving the infusion up to 24 months or within 28 days after withdrawal from the trial. This may manifest as a symptom, sign, disease, or laboratory abnormalities, but not necessarily be causally related to the study drug. This includes any newly occurring events or events that worsen in severity or frequency compared to baseline conditions, including abnormal laboratory results.

AE does not include:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, infusion), although the conditions leading to these procedures should be reported as AEs;
- Pre-existing conditions or abnormalities detected during screening that do not worsen, including abnormal laboratory results;
- Expected progression of the study disease and/or expected progression of symptoms or signs of the study disease, unless the severity or frequency exceeds expectations.

If an adverse event or serious adverse event is caused by the study disease, specific symptoms and signs should be reported. Worsening of symptoms and signs due to the study disease should also be reported as adverse events and recorded in the eCRF.

The Investigator will determine whether a subject needs to withdraw from treatment due to an adverse event based on clinical judgment. If a subject requests to withdraw from treatment or the study due to an adverse event, the subject should complete the required examinations and procedures for the withdrawal visit.

8.1.2 Serious Adverse Events

A Serious Adverse Event (SAE) refers to any unfavorable medical occurrence in a subject after receiving the drug up to 24 months or within 28 days after withdrawal from the trial (at any dose) that meets one or more of the following criteria:

1) Death;
2) Life-threatening: The event places the subject at immediate risk of death at the time of occurrence. It does not include adverse events that hypothetically might have caused death if more severe.

3) Hospitalization or prolongation of hospitalization: Generally, hospitalization refers to a subject being admitted to a hospital or emergency ward for observation (usually at least overnight) and/or receiving treatment not suitable for a doctor's office or outpatient clinic. Complications occurring during hospitalization were AEs. If a complication prolonged hospitalization or met any other criteria for seriousness, the event was an SAE. When it is uncertain whether an AE requires "hospitalization", the AE should also be considered an SAE.

AEs do not include pre-existing conditions managed with elective treatment or that have not worsened since screening hospitalization.

Note: The following hospitalizations are not considered SAEs:

- *Temporary care hospitalization, e.g., hospitalization for conditions that do not meet the criteria for hospitalization but require temporary care;*
- *Hospitalization due to social reasons, e.g., hospitalization for convenience of care;*
- *Planned hospitalization required by the study protocol, e.g., hospitalization for the administration of the investigational product or protocol-required laboratory tests;*
- *Planned hospitalization, elective surgery, or examination for pre-existing conditions before informed consent (provided the condition requiring hospitalization does not worsen or develop into a new disease after the investigational product is administered, with documented evidence);*
- *Routine maintenance hospitalization for devices implanted before study participation (e.g., battery replacement).*

4) Permanent or severe disability or loss of function: Disability refers to a substantial disruption of a person's ability to carry out normal life functions. This definition excludes clinically insignificant events such as simple headaches, nausea, vomiting, diarrhea, flu, or accidental injuries (e.g., ankle sprains) that may affect daily functions but do not cause significant functional loss.
5) Congenital anomalies or birth defects.
6) Other important medical events: In some cases, medical and scientific judgment is required to determine whether expedited reporting is necessary. Medically important events may not be immediately life-threatening, result in death, or require hospitalization, but if medical intervention is required to prevent one of the above

outcomes, they are generally considered serious.

8.1.3 Suspected Unexpected Serious Adverse Reactions

Suspected Unexpected Serious Adverse Reactions (SUSAR): SUSAR refers to any suspected and unexpected serious adverse reaction whose nature and severity of clinical manifestations are beyond the information already available in the Investigator's Brochure of the study drug, instructions of the marketed drug or summary of product characteristics.

Suspected refers to a potential causal relationship with the study drug, as detailed in Section 8.4 Causality Assessment of Study Drug.

8.1.4 Adverse Events of Special Interest

The Adverse Events of Special Interest (AESI) in this study include: CRS, ICANS, prolonged hematocytopenia (defined as ≥ Grade 3 platelet count decreased, neutrophil count decreased, or anemia (hemoglobin <80 g/L, not related to MM) persisting on day 28 after reinfusion), tumor lysis syndrome, and secondary malignancies.

8.2 Collection and Reporting

8.2.1 Collection and Recording of Adverse Events

AEs and SAEs are collected and recorded from the time of drug infusion to the 24th month after infusion or 28 days after withdrawal from the trial.

Results from the signing of the ICF to the time of infusion are not recorded as AEs unless caused by study procedures.

8.2.2 Reporting of Adverse Events

During the study, subjects are encouraged to report any AEs. Investigators should report all AEs, whether observed directly or reported by the subject, in concise language.

Investigators are responsible for reviewing all laboratory results for all subjects and determining whether they constitute AEs. Medical and scientific judgment should be used to decide whether isolated laboratory abnormalities should be classified as AEs.

If an Investigator determines that an abnormal laboratory finding (e.g., clinical chemistry or hematology) or other abnormal assessment (e.g., ECG or vital signs) that is not a known feature of the study disease is clinically significant, it should be recorded as an AE if it meets the AE definition or as an SAE if it meets the serious adverse event definition.

All AEs occurring during study must be documented in the source documents. This documentation should include the name of the AE, start and end dates, severity, the study's assessment of the relationship between the lymphodepleting preconditioning medication/study

drug and the event, actions taken, and the outcome of the AE. The relationship of the AE to study drug should be evaluated, taking into account comorbidities and concomitant medications. CRS and ICANS should be recorded separately as AEs. Abnormal inflammatory markers related to CRS are not recorded as AEs.

For SAEs, the following data elements should be collected: date the AE became an SAE, date the Investigator became aware of the SAE, reason for the AE becoming an SAE, dates of hospitalization and discharge, possible cause of death, date of death, autopsy results, assessment of the relationship to study procedures, and event description.

8.2.3 Reporting a Serious Adverse Event

Any SAE occurring during the trial, regardless of its relationship to the investigational product, must be actively managed by the Investigator to ensure subject safety. The Investigator must report the SAE to the cooperating unit or designated representative (email: wangml@pregene.com) within 24 hours of becoming aware of it.

The Investigator must follow up on SAEs as required by the protocol (see Section 8.5) and provide a detailed, written follow-up report within 24 hours of obtaining follow-up information, using the same reporting method.

8.2.4 Suspected Unexpected Serious Adverse Reaction Report

After receiving an SAE report from the Investigator, the cooperating unit must analyze each SAE according to the latest applicable drug clinical trial regulations and company standard operating procedures, including expectedness assessment, causality evaluation, and whether it is unexpected. If an SAE is determined to be a SUSAR, the cooperating unit must promptly report it to all participating Investigators, trial institutions, Ethics Committees, drug regulatory authorities, and health authorities.

Reporting timelines for the cooperating unit:

1)For fatal or life-threatening SUSARs, the cooperating unit must report within 7 days of initial awareness and provide follow-up information within the next 8 days.

2)For non-fatal or non-life-threatening SUSARs, the cooperating unit must report within 15 days of initial awareness.

3)After the initial report, the cooperating unit must continue tracking and submit follow-up reports within 15 days of obtaining new information or updates to previous reports.

If the Investigator and cooperating unit cannot agree on the causality assessment of an adverse event, and either party cannot exclude a relationship with the study drug, the event should be reported within the timelines specified in 2) and 3).

Upon receiving safety information related to the investigational product from the cooperating unit, the Investigator must promptly acknowledge, review, and consider adjustments to the subject's treatment. If necessary, the Investigator should communicate with the subject as soon as possible. And report the SUSAR to the clinical trial institution and Ethics Committee.

The Investigator must follow up on SAEs/SUSARs as required by the protocol and promptly report new information or updates to the cooperating unit within 24 h of becoming aware of the information.

8.2.5 Report of Adverse Events of Special Interest

If an adverse event of special interest meets the criteria for an SAE, it must be handled and reported according to the SAE process.

8.3 Judgment Criteria for Severity of Adverse Events

The Investigator should assess the severity of adverse events using clinical judgment. The severity of AEs is graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 5.0). Use the above definition of severity to describe the highest severity of adverse events. If an adverse event is determined to be an SAE, the CTCAE grade recorded in the eCRF must match the description in the narrative section of the SAE report. CRS and ICANS are graded using the guidelines in Sections 16.8.1 and Table 16.8.3, not CTCAE.

According to NCI CTCAE version 5.0, all AEs are graded from Grade 1 to Grade 5 as follows:

Grade	Clinical Description of Severity
1	Mild; Asymptomatic or mild symptoms; For clinical or diagnostic findings only; No treatment is required.
2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) *.
3	Serious or medically significant but not immediately life-threatening; May lead to hospitalization or prolong the duration of hospitalization; Disabling; Self-care activities of daily living are limited**
4	Life threatening; requiring urgent treatment.
5	Death related to AE.

Activities of daily living(ADL)

*Instrumental activities of daily living refer to cooking, shopping of clothes, using the telephone, managing finances, etc.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not being bedridden.

Note the difference between the severity and seriousness of adverse events. A severe adverse event is not necessarily a serious adverse event. For example, a headache can be severe (significantly affecting the subject's daily function) but may not be a serious adverse event unless it meets one of the criteria for serious adverse events listed above.

8.4 Causal Relationship to Study Drug

The causality of an adverse event (AE) in relation to the investigational product is categorized as related, probably related, possibly related, possibly unrelated, or unrelated. Among these, related, probably related, and possibly related are considered related to the study drug. For causality assessments judged as possibly unrelated by the Investigator, the cooperating unit should carefully evaluate; if there is reasonable evidence supporting a possible causal relationship, it should also be considered related to the study drug.

Criteria for determining the causality of adverse events in relation to the investigational product:

1. Related:
   - There is a reasonable temporal relationship
   - It is consistent with the known mechanism of action, properties, or known adverse reactions of the drug
   - Positive dechallenge
   - No other reasonable explanation exists
   - Positive rechallenge
2. Probably related
   - There is a reasonable temporal relationship
   - It is consistent with the known mechanism of action, properties, or known adverse reactions of the drug
   - Positive dechallenge
   - No other reasonable explanation exists
   - Lack of positive rechallenge
3. Possibly related:
   - There is a reasonable temporal relationship
   - Lack of positive rechallenge evidence
   - Exhibits any of the following:

① Consistent with the known mechanism of action, properties, or known adverse reactions of the drug, with positive dechallenge, but other reasonable explanations exist;

② Consistent with the known mechanism of action, properties, or known adverse reactions of the drug, with no positive dechallenge evidence, and no other reasonable explanation exists;

③ Inconsistent with the known mechanism of action, properties, or known adverse reactions of the drug, with positive dechallenge, and no other reasonable explanation exists;

④ Inconsistent with the known mechanism of action, properties, or known adverse reactions of the drug, with positive dechallenge, but other reasonable explanations exist;

⑤ Inconsistent with the known mechanism of action, properties, or known adverse reactions of the drug, with no positive dechallenge evidence, and no other reasonable explanation exists;

4. Possibly unrelated
   - There is a reasonable temporal relationship
   - Lack of positive dechallenge evidence
   - Lack of positive rechallenge evidence
   - Exhibits any of the following:

   ① Inconsistent with the known mechanism of action, properties, or known adverse reactions of the drug, and other reasonable explanations exist;

   ② Consistent with the known mechanism of action, properties, or known adverse reactions of the drug, but other more reasonable explanations exist;

5. Unrelated
   - There is no reasonable temporal relationship (medically determined)
   - Inconsistent with the known mechanism of action, properties, or known adverse reactions of the drug
   - Lack of positive dechallenge evidence
   - Lack of positive rechallenge evidence
   - Other reasonable explanations exist

Table 5: Causality Assessment of Adverse Events in Relation to the Investigational Product

	Related	Probably Related	Possibly Related				Possibly Unrelated		Unrelated
Whether have a reasonable temporal relationship	+	+	+				+		—
Whether consistent with the known mechanism of action, properties, or known adverse reactions of the drug	+	+	+		—		+	—	—
Dechallenge result	+	+	+	—	+	—	—		—
Rechallenge result	+	—	—				—		—
Whether have other reasonable explanations	—	—	+	—	—/+	—	++	+	+

Note: +: Positive or affirmative, -: Negative or no result yet, ++: Other "more" reasonable explanations exist

When the Investigator judges an event as possibly unrelated, the report must provide a detailed explanation of the reasoning, confirm the basis for each Grade 3 or higher SAE with serious clinical consequences judged as possibly unrelated, and provide detailed records to the technical review agency.

8.5 Follow-Up of Adverse Events/Serious Adverse Events

The Investigator should follow up on every adverse event. All SAEs that persist until the end of AE/SAE collection and recording, as well as AEs related to the investigational product, should be followed up until:

- The event resolves or returns to baseline or stabilizes;
- The Investigator determines no further improvement is expected;
- The subject starts a new treatment protocol for the study disease;
- When no further information is available (e.g., subject death, subject refuses to provide more information, or there is evidence indicating that the subject is still lost to follow-up despite best efforts).

During the study, the recovering time of adverse events (with dates) should be recorded in the source documents to verify raw data.

For serious adverse events, adverse events of special interest, and pregnancy events, the cooperating unit or other designated personnel may obtain additional subject information through phone, fax, email, and/or monitoring to conduct a more comprehensive medical evaluation of these reported cases.

8.6 Outcomes of Adverse Events

The Investigator should determine the outcome of adverse events based on the subject's condition. The outcomes of adverse events include the following scenarios:

Table 6 Outcomes of Adverse Events

Resolved/recovered	The event has returned to normal or baseline levels, or the Investigator considers it stable. Record the event stop date.
Resolving	The event has improved but not resolved. Follow-up is required, and the event stop date is not recorded.
Resolved with sequelae	The subject has recovered but still has residual effects or damage. These residual effects may be temporary but are still present at the time of reporting. If the sequelae are thought to be temporary, updated information should be provided at follow-up visits if the situation changes.
Ongoing	The subject's event-related signs and symptoms remain unchanged, and their condition is stable; If the subject dies due to another AE rather than this AE, this AE is considered ongoing.
Death	Only SAEs that lead to death can select "death" as the outcome. All other AEs/SAEs present at death should be reported.
Unknown	When the subject is lost to follow-up, and the Investigator cannot determine the outcome.

8.7 Pregnancy and Lactation

There is currently no clinical experience with the use of ESO-T01 injection in pregnant or lactating women, nor have animal reproductive studies been conducted. Based on the potential risks of the preconditioning chemotherapy in the study to the fetus, all women of childbearing potential must have a negative serum pregnancy test before enrollment. Pregnant or lactating women should not receive this investigational treatment.

If a female subject or the female partner of a male subject becomes pregnant within 1 year after ESO-T01 injection treatment, the pregnancy event must be reported to the cooperating unit contact. The cooperating unit may require the Investigator to provide additional information about the pregnancy and/or clinical outcomes. In addition to reporting pregnancy events during the study, the Investigator should also monitor pregnancy events occurring within 1 year after ESO-T01 injection treatment. The Investigator must report the pregnancy event to the cooperating unit contact (email address is the same as that used in the above-mentioned serious adverse events report) within 24 hours of becoming aware of it. All pregnancy events must be followed up until the pregnancy outcome, until the newborn reaches full term (generally 6-8 weeks after the due date) or until the pregnancy is terminated early (including spontaneous or elective abortion).

If a lactating event occurs in a female subject undergoing treatment, it must be reported to the cooperating unit contact. In addition to reporting lactating events during the study, the Investigator should also monitor lactating events occurring within 1 year after ESO-T01 injection treatment. The Investigator must report the lactating event to the cooperating unit contact (using the same method as above) within 24 hours of becoming aware of it.

9 STATISTICAL METHODS AND ANALYSIS

9.1 Determination of Sample Size

As this study is an IIT to evaluate the tolerability, safety, and efficacy of ESO-T01 Injection in treating r/r MM, the sample size is not determined based on statistical hypotheses.

This study plans to enroll 4 to 24 subjects diagnosed with r/r MM.

9.2 General Principles of Statistical Analysis

Analysis is performed using SAS 9.4 (or higher version) software.

For continuous variables, descriptive statistics will include mean, median, standard deviation, maximum and minimum. For categorical variables, descriptive statistics will consist of the number and percentage of subjects. Detailed statistical analysis methods are described in the Statistical Analysis Plan.

9.3 Analysis Sets

Major statistical analysis sets for this study are as follows:

(1) Full analysis set (FAS): All subjects enrolled who received ESO-T01 reinfusion.

(2) Safety analysis set (SS): All subjects enrolled who received ESO-T01 reinfusion and had at least one safety measure recorded.

(3) DLT analysis set (DLTS): All subjects who received ESO-T01 Injection infusion and completed the DLT assessment, including subjects who developed DLT and those who did not develop DLT but completed all assessments during the DLT observation period.

(4) Pharmacokinetic analysis set (PKS): Subjects with at least one quantifiable plasma concentration after administration and no major protocol deviations/violations or with no events that significantly affect pharmacokinetic results.

(5) Efficacy analysis set (ES): All subjects who received ESO-T01 reinfusion, had a tumor assessment at baseline and had at least one available tumor assessment result after administration.

9.4 Analytical Procedures

9.4.1 Subject Disposition

Subject enrollment and completion status will be described using the number (percentage) of subjects. List the reasons for withdrawal from the study for both dropout and rejected subjects by the subject disposition of each data set.

9.4.2 Demographic Data and Baseline Characteristics Analysis

Descriptive statistics of demographic data and other baseline characteristic values are

provided, and the number of subjects, mean, standard deviation, median, minimum and maximum are calculated for non-missing observation results by continuous variables. The number and percentage of subjects are calculated for count and ordinal data.

9.4.3 Safety Analysis

Adverse Events

Evaluate the occurrence and frequency of dose-limiting toxicity (DLT) of ESO-T01 Injection and determine the MTD.

Number and percentage of subjects with adverse events, drug-related adverse events, adverse events of Grade 3 or higher in severity, and serious adverse events are summarized by dose group and overall. CRS and ICANS will be graded according to the 2019 ASTCT criteria, while other AEs will be graded per NCI CTCAE version 5.0. All AEs will be coded using the latest version of MedDRA. The incidence of adverse events will be summarized by system organ class (SOC) and preferred term (PT).

Laboratory Tests

Laboratory test data are graded in accordance with NCI CTCAE version 5.0. The data on laboratory test indicators such as blood routine examination, blood chemistry, coagulation function, and urine routine examination and their change values before and after baseline are summarized for each visit according to the different study phases and dose groups, and the positive and abnormal changes in blood routine examination, blood chemistry, coagulation function, and urine routine examination before and after baseline are summarized.

All abnormal change values in laboratory tests are described using lists.

ECG, Physical Examination, and Vital Signs

Descriptive statistics are used to summarize the data for each visit and the change before and after baseline according to the different study phases and dose groups, and laboratory values are described in tabular statistics.

9.4.4 Efficacy Analysis

Objective response rate (ORR): Proportion of subjects achieving stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR), providing the efficacy to evaluate the subject number and percentage of each result. The overall response rate (ORR) and its two-sided 95% confidence interval (CI) will be calculated using the Clopper-Pearson method.

MRD negativity rate: Calculations of subjects with negative MRD, MRD negativity rate and 95% confidence interval using the Clopper-Pearson method.

Time to response (TTR), duration of response (DOR), progression-free survival (PFS), and overall survival (OS).

Time to response (TTR) is defined as the time from the start of the drug infusion in subjects to the first occurrence of sCR, CR, VGPR, or PR.

Duration of response (DOR) is defined as the time interval between the first occurrence of sCR, CR, VGPR, or PR after drug infusion in subjects and the first assessment of progressive disease or death, whichever occurs first.

Progression-free survival (PFS) is defined as the time interval between drug infusion in subjects and the first assessment of progressive disease or death, whichever occurs first.

Overall survival (OS) is defined as the time interval between drug infusion in subjects to death from any cause.

The median survival is estimated using the Kaplan-Meier method, and the 95% confidence interval of the median survival is estimated using the Brookmeyer and Crowley method. Kaplan-Meier survival curve is plotted. DOR analysis is limited to subjects with documented response.

9.4.5 Pharmacokinetic Analysis

PK analysis will be performed using the PK analysis set. Drug concentration data at each time point will be summarized using descriptive statistics. CAR copy numbers in peripheral blood will be detected, and individual plasma concentration-time curves and arithmetic mean concentration-time data plots will be created on both linear and semi-logarithmic scales.

PK parameters are calculated using WinNonlin software and a non-compartmental model based on the actual blood sampling times. Evaluation indicators include: C_{max} of expansion, T_{max}, and $AUC_{0\text{-}28d}$ of ESO-T01 after reinfusion in peripheral blood. Statistics for PK parameters include, but are not limited to, the number of subjects, arithmetic mean, standard deviation, coefficient of variation, median, first quartile, third quartile, minimum and maximum.

9.5 Interim Analysis

No interim analyses were planned for this study.

10 ETHICS

10.1 National Regulations and the Declaration of Helsinki

The study must be conducted in accordance with *Good Clinical Practice* (GCP) in the Declaration of Helsinki and with all applicable laws and regulations of the country where the clinical trial was conducted, so as to protect subjects to the greatest extent.

10.2 Informed Consent

The ICF (along with the study protocol) must be reviewed and approved by the Ethics Committee.

Subjects must be informed of the study information both verbally and in writing by the Investigator. If subjects or their guardians are unable to read, an impartial witness must read the Informed Consent Form (ICF) and any other written information (if any) to them and witness the informed consent process. The Investigator or their designee is responsible for explaining the Informed Consent Form (ICF) to subjects (or their guardians/witness) in a language and manner they can understand, making it readily understood by subjects or their guardians/witness. When new information that may affect subjects' willingness to continue in the study becomes available to the Investigator, subjects (or their guardians) should be promptly informed, and this communication should be documented.

The final ICF and other materials provided to subjects should include the following: study overview, study objectives, study procedures and duration, study procedures that should be followed by subjects, obligations of subjects, potential benefits to subjects (including the possibility of no benefit) and risks; treatment and compensation available to subjects in the event of study-related injury; compensation that may be received by subjects for participating in the clinical trial; Subjects may voluntarily participate in this study and have the right to withdraw from the study at any stage of the study at any time; principles of confidentiality of subject's personal data.

Before signing the Informed Consent Form (ICF), the Investigator or the designated study personnel should give subjects (or their guardians) adequate time and opportunity to understand the details of the clinical trial. Subjects (or their guardians) and the Investigator performing the informed consent should each sign and date the Informed Consent Form (ICF) (if it's not signed in person, the relationship to the subject should be documented). Both the Investigator and the subject should retain a copy of the ICF. If significant new information related to the study drug is discovered, the ICF must be revised in writing, submitted to the Ethics Committee for approval, and then re-consented by subjects (or guardians).

10.3 Independent Ethics Committee/Institutional Review Board (IEC/IRB)

Clinical trial protocols and their amendments, investigator brochure, informed consent forms, relevant recruitment materials (such as advertisements for enrollment), and other essential documents must be reviewed and approved by the IEC/IRB.

IEC/IRB approval must be obtained before initiating the study. The approval letter to the Investigator should state the dates of the committee meeting and approval. Any amendment to the protocol must be formally approved or filed by the IEC/IRB. During the study, if any protocol deviation occurs that may increase the risk to subjects, the Investigator should promptly report it to the IEC/IRB.

All serious adverse events will be reported to the relevant ethics committees and regulatory authorities as required by applicable regulations.

11 DATA MANAGEMENT

11.1 Establishment of Database

This study uses eCRFs remote data entry to collect clinical study data. The data manager (DM) designs the electronic data capture (EDC) system database and tests it with simulated or real eCRF data to ensure its accuracy.

11.2 Data Acquisition

Information will be entered into the EDC database by the Investigator or an authorized designee as soon as possible after each visit, adhering strictly to the principle of "what you see is what you record". After entry of raw data, any changes made to the eCRF will be automatically logged by the system.

11.3 Database Review

Data verification methods during the data management include: data logical checks, manual verification, medical verification, and statistical pre-analysis verification. Data queries will be electronically documented in the EDC system for the site to address. Close queries if the answer is satisfactory. If, after the previous query resolution or database update based on the previous query response, further questions arise, a new response should be provided by the Investigator or clinical coordinator after consulting with the Investigator. This process will be repeated until all data in the database have been verified without error.

11.4 Database Locking

The DM prepares a data review report based on the protocol, data review criteria and database. The project manager then organizes a data review meeting, attended by the cooperating unit, Principal Investigator, statisticians and the data manager, to review the data. All party representatives should sign the data review resolution. After all parties approve and lock data, the DM Group will lock the data. The locked data will be submitted to the statisticians for statistical analysis. Data may be locked when all of the following criteria are met:

- All data have been collected and entered into the database;
- Raw data verification has been completed;
- Verification of consistency of serious adverse events has been completed;
- External data has been received and consistency has been validated;
- Medical monitoring has been completed;
- All queries have been resolved (including queries raised during the data review);
- All codes have been verified and confirmed;

•Final review data has been complete;

•Electronic signatures have been obtained from all Investigators;

•All analysis sets have been divided;

•The Statistical Analysis Plan has been signed.

12 QUALITY CONTROL

12.1 Training

According to GCP guidelines, the clinical research associate (CRA) must be qualified and acceptable to the cooperating unit. Before the clinical study begins, the head of study site must train the Investigators on the study protocol to thoroughly read and understand this clinical study protocol, be familiar with GCP principles, and use standardized recording methods and assessment criteria. The protocol must be followed rigorously.

12.2 Standard Operations

The cooperating unit will adhere to regulations in the GCP and the *Provisions for Drug Registration*, and the study drug should meet the relevant requirements of Good Manufacturing Practice for Drugs, and undergo rigorous quality control.

Laboratory test is performed by the site following standard operating procedures.

Methods for site laboratory test and quality control should be standardized. The site clinical laboratory must conduct internal quality control according to regulations and obtain a Certificate of Quality Assessment from the Ministry of Health's Center for Clinical Laboratories.

12.3 Auditing and Inspecting

The Investigator should understand that the cooperating unit or the delegated contract research organization (CRO) with clinical experience, or other units in the field of cell clinical translation, will assign clinical monitors and auditors to conduct regular monitoring visits to ensure adherence to the clinical trial protocol. They will also inspect the raw data to guarantee its accuracy, reliability, timeliness and completeness.

12.4 Study Monitoring

The clinical research associate (CRA) will regularly contact and visit the Investigator. If the subject privacy is protected as required by regulations, the CRA will review various trial records (eCRF and other data of subjects) as needed.

The CRA will verify whether the completion of the eCRF meets the protocol requirements during routine visits, ensuring its completeness, consistency and accuracy. The CRA will also access laboratory test and the subject's other records to verify the accuracy of the data entered in the eCRF. The Investigator (or his/her assistant) should assist the CRA in resolving the issues detected in the course of monitoring.

13 MANAGEMENT OF THE STUDY

13.1 Protocol Amendment

The revised protocol must be submitted to the Ethics Committee for approval. Before obtaining approval from the Ethics Committee, the Investigator will continue to follow the original study protocol unless the revisions can immediately eliminate potential harm to subjects, or if the revisions only involve administrative changes to the study (such as changes to phone number).

13.2 Protocol Violations or Deviations

All requirements stipulated in the protocol must be strictly followed. Any intentional or unintentional deviation or violation of the study protocol and GCP principles is classified as a protocol deviation or protocol violation. Deviations from the protocol must be documented in a deviation record, which should detail the time, process, and cause of the deviation, along with any corrective actions taken. The Ethics Committee and the cooperating unit must be notified of any deviations.

When a major protocol deviation occurs, an assessment should be performed. If necessary, the cooperating unit may terminate this study early.

13.3 Study Documents and Storage

The Investigator must fully and accurately document the study process so that the study data can be verified. These documents are categorized into two types: Investigator files and subjects' raw data.

The Investigator files include the protocol and its amendments, case report forms (CRFs) and data query forms, approval documentation and correspondence with the Ethics Committee and regulatory authorities, sample of Informed Consent Forms (ICFs), drug accountability records, CVs of study personnel and authorization forms, along with other essential documents and correspondence.

The subject's original documents (which predefine the key efficacy/safety data to be recorded) include inpatient/outpatient records, physician and nurse orders, appointment visit dates, raw laboratory test results, ECG, UCG, PET-CT, MRI, pathological reports, special assessment reports, signed informed consent forms, consultation records, and subject screening logs.

The Investigator is required to retain these documents for five years after the completion or termination of study. After five years, these documents may be destroyed in accordance with applicable regulations. If the Investigator cannot secure the storage of these documents at the

study site, they may arrange for storage at an alternative location. These documents must be sealed and readily retrievable for inspection by the regulatory authorities. If these documents are still needed, copies can be kept elsewhere.

13.4 Original Document and Data

When the CRF is illegible or there's an error during data transfer, the Investigator needs to provide the raw data from study documents or medical records. Complete study records should also be accessible in the event of specific queries, regulatory authority questions, and/or inspection requests, provided subject confidentiality is maintained.

14 PUBLICATION OF STUDY RESULTS

The results of this study may be presented at scientific conferences and published in journals. Authorship will be granted to those principal Investigators who have made significant contributions to the implementation and management of this study and to staff (e.g., staff or consultants of the cooperating unit) who have made substantial contributions to the design study, interpretation or analysis of this study. Authorship will be determined according to the International Committee of Medical Journal Editors (ICMJE) authorship requirements.

Any publication resulting from this study in any form must be submitted to the cooperating unit for review and approval. The Investigator has the right to publish the results of this study, but must comply with requirements for protecting confidential information.

The intellectual property rights of this confidential information belong exclusively to Shenzhen Pregene Biopharma Co., Ltd. It must not be disclosed to any third party without the written consent of Shenzhen Pregene Biopharma Co., Ltd., and may not be used for any purpose other than this study protocol.

15 APPENDIXES

15.1 Appendix 1 International Myeloma Working Group (IMWG) Criteria for Evaluating Efficacy in Multiple Myeloma (2016)

Response Category	Response Criteria
Stringent complete response (sCR)	In addition to meeting CR criteria, the serum free light chain (FLC) ratio must be normal, and immunohistochemical analysis of the bone marrow must show no clonal cells. Definition of ***clonal plasma cells in bone marrow***: Use immunohistochemical methods for testing, with two consecutive readings of κ/λ>4:1 or <1:2 (for patients with κ and λ types, respectively, plasma cells count ≥ 100). If bone marrow pathology is unavailable, polychromatic flow cytometry with a sensitivity of 10^{-4} can be used to monitor bone marrow specimens for the absence of clonal plasma cells.
Complete response (CR)	Serum and urine immunofixation electrophoresis negative, soft tissue plasmacytoma resolved, plasma cells in bone marrow < 5%; For patients whose measurable disease is solely based on serum FLC levels, in addition to meeting the above CR criteria, the involved serum FLC ratio must also normalize on two consecutive assessments. Daratumumab administration may interfere with the assessment of CR (complete response) in patients with IgG κ-type multiple myeloma.
Very good partial response (VGPR)	M-protein is not detected by serum protein electrophoresis, but serum and urine immunofixation electrophoresis remain positive; or serum M-protein decreased by ≥ 90% and urine M-protein < 100 mg/24h; In addition to meeting the VGPR criteria mentioned above, patients whose measurable disease is assessed solely by serum FLC also require a greater than 90% reduction in the difference between involved and uninvolved serum FLC levels on two consecutive measurements.
Partial response (PR)	- Serum M-protein decreased by ≥ 50%, and 24h urine M-protein decreased by ≥ 90%, or decreased to < 200mg/24h; - If M-protein is unmeasurable in serum and urine, the difference between involved and uninvolved free light chain (FLC) must be reduced by ≥ 50%. - If serum and urine M-protein and serum FLC are all unmeasurable, and the proportion of bone marrow plasma cells at baseline is ≥ 30%, a ≥ 50% reduction in bone marrow plasma cell count is required; - In addition to the above criteria, if soft tissue plasmacytoma is present at baseline, a ≥50% reduction in the sum of the products of the diameters (SPD) of measurable lesions is required. The above serum and urine M-protein levels must be assessed twice successively, with no new bone lesions or evidence of existing bone lesion progression.
Minor response (MR)	- Serum M-protein decreased by 25% to 49% and 24h urine M-protein decreased by 50% to 89%; - If a soft tissue plasmacytoma is present, a ≥50% reduction in the SPD of measurable lesions is required. There is no increase in the number or size of lytic lesions (although compression fractures may occur).

Response Category	Response Criteria
Stable disease (SD)	Does not meet the criteria for CR, VGPR, PR, MR, and PD. No evidence of new bone lesions or progression of existing bone lesions.
Disease progression (PD)	Meeting one of the following criteria (all of the following data values are compared to the lowest obtained value): a. Serum M-protein increased by ≥25% (an absolute increase ≥ 5 g/L), or an increase in M-protein of ≥ 10 g/L (when baseline serum M-protein is ≥ 50 g/L); b. Urine M-protein increased by ≥ 25% (an absolute increase ≥ 200 mg/24h). c. If M-protein is unmeasurable in serum and urine, the difference between involved and uninvolved FLC levels must be increased ≥ 25% (an absolute increase > 100 mg/L). d. If serum and urine M-protein and serum FLC are all unmeasurable, a ≥ 25% increase in bone marrow plasma cell proportion is required (an absolute increase ≥ 10%); e. New soft tissue plasmacytoma present: More than one original measurable lesion increased by ≥ 50% from the lowest point or the long diameter of the original lesion sized ≥ 1 cm increased by ≥ 50%. f. Circulating plasma cells increased by ≥ 50% (applicable when circulating plasma cells are the only measurable lesion, with an absolute count of at least 200 cells/μL).
Clinical relapse	Meeting one or more of the following criteria: a. a. New bone lesions or soft tissue plasmacytomas (excluding osteoporotic fractures); b. Confirmed increase (≥ 50% increase in SPD of measurable lesions with an absolute value of ≥ 1 cm) in pre-existing plasmacytomas or bone lesions; c. Hypercalcemia (> 2.75 mmol/L); d. Hemoglobin concentration decreased by ≥ 20 g/L (unrelated to treatment or non-MM factors). e. From the start of MM treatment, a rise in serum creatinine of ≥ 176.8 μmol/L (2 mg/dL) that is related to the MM; f. Serum M-protein-related hyperviscosity syndrome
Recurrence after CR	Meeting one of the following criteria: a. Confirmation of M-protein reappearance in blood or urine by immunofixation electrophoresis. b. Proportion of bone marrow plasma cells ≥ 5%; c. Meeting one of the above criteria for PD.

IMWG MRD Criteria

(1) Persistent MRD negative: Two negative results, at least one year apart, on both bone marrow MRD testing using next-generation flow cytometry (NGF) or next-generation sequencing (NGS) and imaging testing. Further assessments are described by the duration of MRD-negative, for example, “5-year MRD-negative”;

(2) MRD-negative by NGF: NGF flow cytometry should be performed to test the phenotypically normal plasma cells in bone marrow, using EuroFlow standard operating procedures (or an equivalent validated method) with a minimum sensitivity of 1 clonal plasma cell per 10^5 nucleated cells; 8-color flow antigen panel includes: cyκ,

cyλ, CD19, CD27, CD138, CD45, CD56 and CD38, with the minimum sensitivity of 10^{-5}.

(3) MRD-negative by NGS: Negative clonal rearrangements of tumor plasma cell IgH (VDJH), IgH (DJH) or Ig-Kappa (IGK) in whole bone marrow cells from patients were detected using nested PCR amplification combined with deep NGS on the LymphoSIGHT platform or an equivalent validated method, with a minimum sensitivity of 1 clonal plasma cell per 10^5 nucleated cells;

(4) MRD-negative with pre-existing positive imaging findings: MRD-negative by NGF or NGS testing is required, and complete resolution of all hypermetabolic lesions on the original PET-CT scan, or the standardized uptake value (SUV) of the lesions must be lower than that of the mediastinal blood pool or the surrounding normal tissue;

(5) Relapse after achieving MRD-negative: MRD-negative converting to positive (presence of clonal plasma cells confirmed by NGF or NGS, or imaging evidence of MM relapse); confirmation of M-protein reproduction in serum or urine by immunofixation or protein electrophoresis; bone marrow clonal plasma cells ≥ 5%; Any other signs of disease progression (e.g., new plasmacytomas, osteolytic lesions, or hypercalcemia).

References:

Rajkumar, S.V., et al., International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol, 2014. 15(12): p. e538-48.

15.2 Appendix 2 International Myeloma Working Group (IMWG) Criteria for the Diagnosis of Multiple Myeloma (2014)

Must meet both Criteria 1 and 2, in addition to any one of the following in Criterion 3:

1.Proportion of bone marrow monoclonal plasma cells ≥ 10% and/or biopsy-proven plasmacytoma;

2.Presence of monoclonal M-protein in serum and/or urine*;

3.Myeloma-related manifestations:

(1) Evidence of target organ damage due to plasma cell malignant proliferation:

•Hypercalcemia: Serum calcium > 0.25mmol/L (≤ 1 mg/dL) above the upper limit of normal, or serum calcium > 2.75 mmol/L (≤ 11 mg/dL);

•Renal impairment: Creatinine clearance < 30 mL/min, or creatinine > 177μmol/L (2mg/dL);

•Anemia: Hemoglobin below the lower limit of normal 20 g/L or < 100 g/L;

•Osteolytic lesion: Imaging examinations (X-ray, CT or PET/CT) show one or more osteolytic lesions.

(2) No target organ damage, but one or more of the following abnormalities:

•Proportion of bone marrow monoclonal plasma cells ≥ 60%;

•Involved/uninvolved serum free light chain ratio ≥ 100;

•Focal bone destruction > 5 mm in more than one location seen on MRI.

Note*:No restrictions on blood or urine M-protein levels. If M-protein is not detected (diagnosed as non-secretory MM), bone marrow monoclonal plasma cells must be ≥ 30%, or a biopsy must confirm plasmacytoma.

References:

Lokhorst, H.M., et al., Targeting CD38 with Daratumumab Monotherapy in Multiple Myeloma. N Engl J Med, 2015. 373(13): p. 1207-19.

15.3 Appendix 3 ECOG Performance Status Scale

ECOG Performance Status Scale

Grade	Description
0	Fully active, no significant difference from pre-onset
1	Ambulatory and capable of light activity, but unable to perform strenuous physical activities
2	Ambulatory, independent in daily activities, but unable to work, and out of bed for more than half of the daytime
3	Partially dependent in daily activities, requiring bed rest or wheelchair use for more than half of the daytime
4	Bedridden and totally dependent in daily activities
5	Death

References:

ken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982; 5:649-655

15.4 Appendix 4 Cockcroft-Gault Equation

Calculate the creatinine clearance (CrCl) as follows:

Serum creatinine concentration in mg/dL:

Male CrCl (mL/min) = [(140 - age) × (weight)a]/ [(72) × (serum creatinine)]

Female CrCl (mL/min) = [(0.85) × (140 - age) × (weight)a]/ [(72) × (serum creatinine)]

Serum creatinine concentration in μmol/L:

Male CrCl (mL/min) = [(140 - age) × (weight)a]/ [(0.818) × (serum creatinine)]

Female creatinine clearance (mL/min) = [(0.85) × (140 - age) × weight (a)]/ [(0.818) × (serum creatinine)]

a Age is in years and body weight is in kilograms.

15.5 Appendix 5 Definition of Women of Childbearing Potential, Contraceptive Measures, and Contraceptive Requirements

Definition of woman of childbearing potential:

A woman of childbearing potential is defined as a woman who is anatomically and physiologically capable of becoming pregnant after menarche and before menopause, without sterilization. Sterilization procedures include bilateral tubal ligation or bilateral oophorectomy or hysterectomy.

Women of non-childbearing potential are defined as postmenopausal women and premenopausal women who have undergone sterilization procedures. Postmenopause is defined as cessation of menstruation for ≥12 months in the absence of alternative medical measures. Subjects with amenorrhea or suspected amenorrhea should undergo follicle-stimulating hormone (FSH) testing. An FSH level > 40 IU/L confirms menopause.

Contraceptive Measures and Contraceptive Requirements

All female subjects of childbearing potential must have a negative pregnancy test during the screening period.

Female subjects of childbearing potential must agree to use one of the following contraceptive measures during the study and for at least 1 year after the drug infusion:

- Complete abstinence: Periodic abstinence (e.g., calendar, ovulation, symptothermal and post-ovulation methods) is not acceptable.
- Or the male partner correctly uses condoms, along with one of the following contraceptive methods:
  - Intrauterine device (IUD)
  - Hormonal contraceptives: Oral contraceptive pills, contraceptive injections, sustained-release contraceptives (implants, vaginal rings, patches, and hormone-releasing intrauterine devices)
  - Female barrier method: cervical cap or Dutch cap
  - Topical spermicide: Vaginal contraceptives such as suppositories, tablets, jellies, gels, and films
- Tubal sterilization.
- The male partner undergoes vasoligation.

Male subjects must agree not to donate sperm to female partners during the study until 1 year after the drug infusion. If their female partners have childbearing potential, their female

partners must use one of the contraceptive methods listed above during the study until 1 year after the drug infusion.

Procedures to be Followed During Pregnancy

If a subject (or their partner) becomes pregnant at any time during the study (from signing the Informed Consent Form until 1 year after the drug infusion), they must immediately report this information to the Investigator. Pregnant or suspected pregnant subjects must report this information to the Investigator immediately.

15.6 Appendix 6 ICE Assessment Scale

ICE score

Each Point Represents the Ability to Correctly Perform One of the Following Tasks	**Score**
Orientation: The patient accurately states the current year, month, city, and hospital	4
Naming skill: Name three objects (e.g., pen, watch, button)	3
Ability to follow instructions: Can follow simple instructions (such as showing two fingers, closing eyes, sticking out tongue)	1
Writing ability: Write a standard sentence (e.g. “The Chinese national flag is the Five-starred Red Flag”)	1
Count ability: Count backward from 100 in units of 10	1

Note: A total score of 10 on the ICE indicates normal cognitive function, with no impairment.

15.7 Appendix 7 Minimum Mental State Examination (MMSE)

Note: Ask the questions in the order listed. Each correct answer gets 1 point. Incorrect answers or "I don't know" responses get 0 points. Refusal to answer or failure to understand the question gets 8 points. When calculating the total score, scores of 8 and 9 are counted as 0. The maximum score is 30.

Highest Score	Patient Score	Questions
5		What is the current date, including day of the week, day of the month, month, season, and year?
5		Where are we now: (Province/Municipality) (District/County) (Street/Township) (Specific Location) (Floor No.)
3		I'm going to name three items. Please repeat them back to me after I'm finished. Remember these three items, as I'll ask you about them again in a few minutes. (Please clearly explain each item, one second per item). (3 unrelated objects, such as "ball", "flag", "tree" Please repeat these three items. (Your first answer will be scored)
5		Please subtract 7 from 100 and then subtract 7 from the resulting number, keep calculating in this way, and tell me the resulting number each time, until I say "stop". (If the current answer is incorrect, but the next answer is correct, only one error is counted) 93…, 86…, 79…, 72…, 65…
3		Could you please tell me the three items I asked you to remember? (Such as "ball", "flag", "tree")
1		(Showing a watch) What is this?
1		(Showing a pencil) What is this?
1		I'm going to speak a sentence. Repeat this sentence clearly after me. "Forty-four stone lions"
3		I'll give you a sheet of paper. Please follow my instructions. Let's begin. "Hold this paper in your right hand, fold it in half with both hands, and then place it on your lap". (Do not repeat instructions or give examples)
1		Please read this sentence aloud and follow the instructions. "Close your eyes"
1		Write and show me a complete sentence. (Sentences must have a subject and a verb, and express a complete thought)
1		It's a drawing below. Copy this drawing on the same piece of paper. (Yes: Two pentagonal patterns, with a small quadrilateral shape at crossover) 

Clinical impression: _____Signature of evaluation physician: _____

Evaluation time: _____

This scale assesses the following seven domains: Orientation to time and place, immediate memory, attention and calculation, delayed recall, language, visuospatial skills. A total of 30 items, with a total score ranging from 0 to 30. Testing scores are closely related to educational level and the standard for dividing cutoff values is as follows: illiteracy: score > 17; elementary school: score > 20; junior high school and above: score > 24.

Interpretation of scores:

Method	Score	
Education	≤17 ≤20 ≤24	Cognitive disturbance as illiteracy Cognitive disturbance with elementary school education Cognitive disturbance with junior high school education and above.
Severity	24-30 18-23 0-17	No cognitive disturbance Mild cognitive disturbance Severe cognitive disturbance
Scope	<21 >25	Increased likelihood of senile dementia Reduced likelihood of senile dementia

15.8 Appendix 8 Guidelines on Management of Adverse Reactions

This appendix is for the Investigator's reference only during the clinical trial and is not a mandatory executive document.

Note: The clinical site must ensure adequate reserve of medications for treating various AEs associated with CAR T-cell therapy, as this clinical trial involves *in vivo* CAR T-cell generation. The Investigators should closely monitor any adverse events occurring during this study and determine, based on the patient's condition and the severity of the adverse event, whether it is necessary to suspend or terminate the ESO-T01 infusion treatment for the patient; If a patient experiences an adverse event, active treatment should be administered. In the event of a serious adverse reaction that is life-threatening, rescue treatment should be promptly provided.

15.8.1 Cytokine Release Syndrome (CRS)

The adoptive cell transfer therapy (ACT) with CAR T-cell therapy is associated with tumor lysis and CRS, which can cause significant toxicity. This may indicate a syndrome associated with exogenous drug infusions, which can begin at any time point during the drug infusion therapy and up to several days after it ends, causing severe illness in some cases. IL-6 is a key mediator of CRS and can trigger a pro-inflammatory cascade response. This treatment, a form of ACT therapy, can kill tumor cells in the patient who received infusion. However, it also carries the risk of cytokine release syndrome (CRS) and neurotoxicity due to cytokine secretion.

These symptoms range from mild to severe and can lead to high fever (up to 40°C), life-threatening circulatory failure, adult respiratory distress syndrome (ARDS), kidney failure, liver failure or catastrophic neurological events. Fever and low blood pressure are typical symptoms of CRS.

Related Clinical Symptoms of Cytokine Release Syndrome (CRS)

System	Symptom
Systemic symptoms	Fever ± chills, general discomfort, tiredness, loss of appetite, muscle pain, joint pain, vomiting, nausea, headache
Skin	Rash
Digestive tract	Nausea, vomiting, diarrhea
Respiratory tract	Shortness of breath, hypoxia
Cardiovascular	Tachycardia, widened pulse pressure, hypotension, increased cardiac output (early), possibly decreased cardiac output (late)
Coagulation	Elevated D-dimer, hypofibrinogenemia ± hemorrhage
Kidney	Azotemia
Liver	Increased transaminases, hyperbilirubinemia
Nervous system	Headache, poor mental state, confusion, delirium, incoherent speech or aphasia, hallucinations, tremors, biocular dysmetria, gait disturbance, epileptic seizure

CRS Grading Criteria

CRS Grading Criteria				
CRS manifestations	Grade 1	Grade 2	Grade 3	Grade 4
Fever[1]	Body temperature ≥ 38°C	Body temperature ≥ 38°C	Body temperature ≥ 38°C	Body temperature ≥ 38°C
Hypotension	No	No vasopressor is required	Require a vasopressor (excluding vasopressin)	Require multiple vasopressors (excluding vasopressin)
And/or[2] hypoxia	No	Require low-flow nasal cannula[3] or air purging	Require high-flow nasal cannula[3], disposable respiratory mask, or Venturi mask.	Require positive pressure ventilation (e.g., continuous positive airway pressure/CPAP, bilevel positive air pressure/BiPAP, intubation and mechanical ventilation)

Note: 1. Fever related to CRS is defined as a temperature exceeding 38°C that cannot be explained by other causes. For CRS patients managed with antipyretics or anticytokine agents (e.g., tocilizumab, steroid hormone), temperature is no longer used to assess CRS severity, but hypotension/hypoxia will be used for CRS grading.

2. If hypotension and hypoxia cannot be attributed to other causes, the CRS grade is determined by the more severe of the two (hypotension and hypoxia).

3. Low-flow nasal cannula refers to: Oxygen delivery rate of ≤ 6 liters/min, including oxygen delivery by air purging (sometimes used in children). High-flow nasal cannula refers to: Oxygen delivery rate > 6 liters/min.

CRS Management Recommendations

CRS Grade	Symptom or Manifestations	Treatment Method
Grade 1	Fever	Acetaminophen, ibuprofen, and physical cooling measures can be used to treat fever Assess for infection If the patient has reduced neutrophils, broad-spectrum antibiotics and filgrastim may be used Maintain intravenous fluids for hydration IV injection of tocilizumab at 8 mg/kg can be considered
Grade 2	Hypotension Hypoxemia	**Hypotension** Administer 500-1000 mL of normal saline intravenously For systolic blood pressure (SBP) less than 90 mmHg, a second IV fluid may be considered Tocilizumab 8 mg/kg * IV is indicated for hypotension refractory to IV infusion in patients, and tocilizumab may be repeated after 6 hours if needed If hypotension persists after two fluid challenges and tocilizumab administration, initiate vasopressor support and consider transfer to the Intensive Care Unit (ICU) for echocardiography and initiate other hemodynamic testing methods. For high-risk # patients or those with persistent hypotension after 1-2 doses of tocilizumab, dexamethasone at 10 mg every 6 hours can be administered by intravenous injection. If ineffective, the dose can be increased to 20 mg every 6 hours. Management of fever and constitutional symptoms for Grade 1 CRS

		Hypoxemia **Supplemental oxygen** **Tocilizumab ± corticosteroids and supportive treatment** are recommended for the treatment of hypotension
Grade 3	Hypotension Hypoxemia	**Hypotension** If CRS develops without prior administration, treatment with tocilizumab is recommended. Dosage should follow the treatment for Grade 2 CRS Transfer to ICU, obtain an echocardiogram, and perform hemodynamic testing, as the management of Grade 2 CRS Administer **dexamethasone** 10 mg intravenously every 6 hours; If the effect is poor, the dose can be increased to 20 mg every 6 hours
		Hypoxemia Supplemental oxygen includes **high-flow** oxygen therapy and noninvasive **positive pressure** ventilation Tocilizumab ± corticosteroids and supportive treatment, as described above
Grade 4	Hypotension Hypoxemia	**Hypotension** Intravenous fluids, tocilizumab 8 mg/kg for treatment, vasopressors, and hemodynamic testing, as the management of Grade 3 CRS Intravenous infusion of **methylprednisolone** 1 g/day
		Hypoxemia Mechanical ventilation Tocilizumab ± corticosteroids and supportive treatment, as described above

All drug dosages shown are for adults

*Maximum single dose of tocilizumab: 800 mg

#High-risk patients include those with higher body weight, comorbidities, and those who experience early-onset CRS within 3 days of drug infusion

Organ toxicities that may be associated with CRS include: 1. Heart: Tachycardia, arrhythmia, heart block, low ejection fraction. 2. Respiratory system: Tachypnoea, pleural effusion, pulmonary edema. 3. GI tract: Nausea, vomiting, diarrhea. 4. Liver: Serum ALT, AST, or bilirubin levels elevation. 5. Kidneys: Acute kidney injury (serum creatinine level elevation) with reduced urine output. 6. Skin diseases: Skin rash (uncommon). 7. Coagulation disorders: Disseminated intravascular coagulation (uncommon). When organ toxicity occurs, conduct appropriate examinations and provide symptomatic support. Consult with specialists if necessary.

Organ toxicity related to CRS can be graded according to NCI-CTCAE v5.0, but it does not affect the grading of CRS.

References:

Lee D W, Santomasso B D, Locke F L, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells[J]. Biology of Blood and Marrow Transplantation, 2019, 25(4):625-638.

15.8.2 Fever and Chills

Mechanism of its occurrence: It may be caused by CRS or infusion reactions. Infusion reactions usually manifest as chills within 10-20 minutes after infusion, with varying intensity

and lasting for 10-50 minutes, followed by fever.

Recommended treatment: Before infusion, simple preventative measures can be taken. Patients are advised to drink plenty of warm water and stay warm. If sweating occurs, avoid going outside to prevent catching cold, and intramuscular injection of 25mg of promethazine hydrochloride is given. Fever ≤ 38.5°C can be managed with physical cooling methods and increased fluid intake. Fever > 38.5°C can be managed with symptomatic treatments of physical cooling methods, antipyretic medications (such as ibuprofen sustained-release capsules or propacetamol hydrochloride for injection), and fluid replacement therapy. Blood bacterial cultures, urine cultures, and a complete physical examination should be performed in the presence of fever to rule out bacterial infection. If infection cannot be ruled out and the patient has neutropenia, broad-spectrum antibiotics should be administered.

If the patient has a persistent temperature > 38.0°C for consecutive 3 days without evidence of infection, tocilizumab and/or dexamethasone should be administered in combination with cytokine changes and CRS diagnostic criteria.

15.8.3 Neurotoxicity

Reported neurotoxicity symptoms presented as: Obnubilation, delirium, aphasia, obtundation, muscle spasms and epilepsy. For severe neurotoxicity response, cerebrospinal fluid analysis should be performed. Due to its excellent penetration, dexamethasone is generally the preferred treatment. When significant central nervous system toxicity (≥ Grade 3) or epilepsy occurs, administer dexamethasone 10 mg every 6 hours for a total of 8 doses or until toxicity improves significantly (≤ Grade 1). Consult a neurologist for treatment promptly. Patients experiencing seizures require immediate antiepileptic therapy.

ASTCT Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) Grading Criteria

Neurotoxicity	Grade 1	Grade 2	Grade 3	Grade 4
Immune Effector Cell-Associated Encephalopathy (ICE) score[1]	7-9	3-6	0-2	0 (Patient unresponsive)
Depressed level of consciousness[2]	Spontaneous awakening	Awakened by sound	Awakened only by tactile stimulation	Cannot be awakened or awakened only by forceful or repeated tactile stimulation
Epilepsy (at any age)	N/A	N/A	Clinically evident epilepsy (focal or generalized) that can be rapidly controlled; or controllable epilepsy with non-spasmodic	Life-threatening epilepsy (> 5 min); or recurrent clinical or electrographic epilepsy, during which the electrical waves do not

			EEG	return to baseline
Dyskinesia (at any age)[3]	N/A	N/A	N/A	Deep central dyskinaesia, such as mild hemiparesis or mild paraparesis
Elevated intracranial pressure/cerebral edema (at any age)	N/A	N/A	Central/focal edema on neuroimaging[4]	Diffuse intracranial edema on neuroimaging; decorticate or decerebrate status; or cranial nerve VI palsy; or papilledema; or Cushingoid signs

Note: ICANS grade is determined by the most severe manifestation among all items that cannot be explained by other causes (ICE score, level of depressed consciousness, epilepsy, dyskinesia, and elevated intracranial pressure/cerebral edema).
1. For patients with an ICE score of 0: If the patient is conscious but has global aphasia, the ICANS grade is 3; if the patient cannot be awakened, the ICANS grade is 4.
2. The level of depressed consciousness cannot be explained by other causes (such as sedative use).
3. Tremor and myoclonus related to immune effector cells can be graded according to NCI-CTCAE v5.0, but they do not affect the grading of ICANS.
4. Intracranial hemorrhage (with or without edema) can be graded according to NCI CTCAE v5.0. However, intracranial hemorrhage is not a manifestation of neurotoxicity, and its grading does not affect ICANS grading.

ICANS Management Recommendations

ICANS Grade	Management Recommendations
Grade 1	Avoid oral ingestion of foods, medications and liquids. Your ability to swallow will be assessed If swallowing is impaired, switch all oral medications or nutritional supplements to intravenous administration Avoid using medications that suppress the central nervous system For patients experiencing emotional instability, low-dose lorazepam (0.25-0.5 mg IV every 8 hours) or haloperidol (0.5 mg IV every 6 hours) may be administered Perform ophthalmoscopy to assess for papilledema Brain MRI examination; Lumbar puncture to measure opening pressure; If the patient has focal peripheral neurological impairment, an MRI of the spine should be performed; if an MRI of the brain cannot be performed, a CT scan of the brain should be obtained 30-minute EEG daily until resolution of toxic symptoms If no related seizures are detected on the EEG, continue to use levetiracetam 750 mg every 12 hours. If the EEG shows non-convulsive status epilepticus, manage it accordingly If ICANS is associated with concurrent cytokine release syndrome (CRS), consider treatment with tocilizumab 8 mg/kg * IV
Grade 2	Provide supportive care and neurological examinations as described for Grade 1 ICANS If concurrent CRS develops, administer tocilizumab 8 mg/kg * IV for treatment If tocilizumab treatment is unavailable or impractical, or for ICANS without concurrent CRS, administer dexamethasone 10 mg intravenously every 6 hours or methylprednisolone 1 mg/kg IV If ICANS is associated with ≥ Grade 2 CRS, consider transferring the patient to the Intensive Care Unit (ICU)
Grade 3	It's recommended to transfer the patient to the ICU If tocilizumab is suspected to be related to the development of CRS in a patient who was not previously treated, manage it as described in the Grade 2 ICANS management recommendations. If papilledema of stage 1 or 2 is present and cerebrospinal fluid (CSF) pressure is < 20 mmHg,

	provide management based on related method If ≥ Grade 3 ICANS persists in patients, repeat neuroimaging (CT or MRI) examination every 2-3 days
Grade 4	ICU monitoring; Consider using mechanical ventilation for airway protection Tocilizumab treatment and repeated neuroimaging as described for Grade 3 CRES High-dose glucocorticoid should be maintained until Grade 1 ICANS is achieved, then tapered gradually. For example, methylprednisolone IV 1 g/day for 3 days, followed by a reduction of 250 mg every 12 hours for 2 days; reduction of 125 mg every 12 hours for 2 days; reduction of 60 mg every 12 hours for 2 days. For convulsive status epilepticus, manage it accordingly For papilledema ≥ stage 3, CSF pressure ≥ 20 mmHg or cerebral edema, manage it accordingly

Note: All drug dosages shown are for adults
*The maximum single dose of tocilizumab is 800 mg

Management Recommendations for Status Epilepticus Following CAR-T Cell Therapy

Non-convulsive status epilepticus
• Assess airway, breathing, and circulation, and check blood glucose
• Administer lorazepam* 0.5 mg IV to control epilepsy. If necessary, increase the dose by 0.5 mg every 5 minutes, up to a maximum total dose of 2 mg
• Administer levetiracetam 500 mg IV push as a maintenance dose
• If the epilepsy continues, transfer the patient to the ICU for monitoring and administer phenobarbital 60 mg IV push
• After the non-convulsive status epilepsy resolves, maintain the following dose: Lorazepam 0.5 mg q8h IV, TID; Levetiracetam 1000 mg q12h IV; Phenobarbital 30mg q12h IV
Convulsive status epilepticus
• Assess airway, breathing, and circulation, and check blood glucose
• Transfer to ICU for monitoring
• Administer lorazepam 2 mg IV. If seizures are not controlled, give an additional 2 mg IV for epilepsy control, with a total dose of 4 mg
• Administer levetiracetam 500 mg IV push as a maintenance dose
• If the epilepsy continues and cannot be controlled, administer an additional 15 mg/kg of phenobarbital.
• After the convulsive status epilepsy resolves, maintain the following dose: Lorazepam 0.5 mg q8h IV, TID; Levetiracetam 1000 mg q12h IV; Phenobarbital 30 mg q12h IV
• If epileptic seizure is difficult to control, continuous EEG monitoring should be performed.
All medication doses are for adults.
*Lorazepam is the recommended benzodiazepines. Compared to tranquilizers, benzodiazepines are short-acting and widely used in the treatment of epilepsy.

Management Recommendations for Intracranial Hypertension Following CAR T-Cell Therapy

Grade 1 or 2 papilledema* with cerebrospinal fluid (CSF) pressure < 20 mmHg, without cerebral edema
• Acetazolamide 1000 mg IV, followed by 250-1000 mg IV q12h (adjust dose based on renal function and acid-base balance, monitor 1-2 times daily)
Grade 3, 4 or 5 papilledema* with any imaging evidence of brain edema, or CSF pressure ≥ 20 mmHg
• For Grade 4 CRES, high-dose of glucocorticoids is recommended, for example: Methylprednisolone 1 g/day
• Elevate the head of the bed to 30 degrees
• Make the patient hyperventilate to achieve a partial pressure of arterial carbon dioxide ($PaCO_2$) of 28-30 mmHg and maintain this level for no longer than 24 hours
• Hypertonic therapy: Use mannitol (20 g/dl solution) or hypertonic saline (3% or 23.4%, as indicated below)
-Mannitol: initial dose: 0.5-1 g/kg; maintenance dose: 0.25-1 g/kg q6h. Monitor metabolic status and serum osmolality every 6 hours. Discontinue mannitol if serum osmolality is ≥ 320 mOsm/kg or if the

osmolality difference is ≥ 40
- Hypertonic saline: initial dose of 3% hypertonic saline: 250 mL; maintain an infusion rate of 50-75 mL/h, and monitor electrolyte every 4 hours. Discontinue the infusion if the serum sodium level reaches ≥ 155 mEq/L
- For patients with impending brain herniation: Initial administration of 30 mL of 23.4% hypertonic saline; repeat after 15 minutes if necessary
• If the patient has an Ommaya reservoir, the cerebrospinal fluid drainage pressure should be less than 20 mmHg
• Consult neurology and anesthesiology when burst-suppressive electrical activity is observed on EEG
• Metabolic analysis should be performed every 6 hours and a daily head CT scan should be done, with dose adjustments to the above medications to prevent recurrent cerebral edema, renal failure, electrolyte abnormalities, hypovolemia and hypotension.
All drug doses are for adult patients;
*Papilledema grading will follow the modified Frisén scale.

References:

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

15.8.4 Viremia

Symptoms and severity of viremia depend on the type of virus, the individual's immune status and the extent of the infection. In general, patients with viremia may experience symptoms such as fever, fatigue, muscle pain, nausea, and vomiting, with severe cases potentially leading to organ dysfunction, even life-threatening conditions.

If the patient experiences viremia, general treatment and drug therapy can be delivered under the doctor's guidance. General treatment typically includes rest, dietary adjustments, and adequate hydration. The drug therapy may include antiviral medications such as remdesivir, azvudine and immunoglobulins. If the patient develops fever, ibuprofen sustained-release capsules or acetaminophen tablets can be used to manage the fever.

15.8.5 Capillary Leak Syndrome

Mechanism of its occurrence: Caused by CRS, characterized by edema in the limbs and dependent areas with normal serum protein levels, which is unresponsive to simple albumin supplementation and diuretics.

Recommended treatment: Use macromolecular substances (such as plasma, hydroxyethyl starch, etc.) for volume expansion in combination with appropriate diuretics, and monitor cardiac function and electrolyte balance.

15.8.6 Tumor Lysis Syndrome (TLS)

Mechanism of its occurrence: The intracellular substances will be released rapidly after the massive lysis and destruction of tumor cells, exceeding the liver's metabolic and kidney's

excretion capacity, causing the accumulation of metabolic products and leading to hyperuricemia, hyperkalemia, hyperphosphatemia, hypocalcemia, metabolic acidosis and a series of metabolic disorders, which in turn lead to severe cardiac arrhythmias or acute renal failure, thus posting life-threatening risks.

Recommended treatment: After infusion, if fever occurs, active hydration and other symptomatic treatments should be administered, and the Investigator should decide whether to administer allopurinol to prevent complications of tumor lysis syndrome. If the patient is allergic to allopurinol, the Investigator may decide to use other drugs such as febuxostat. For TLS leading to renal insufficiency, rapid increase in uric acid levels, or organ dysfunction symptoms, treatments such as dialysis will be performed as needed at the discretion of the Investigator.

1) Hyperuricemia: Active treatment such as hydration, diuretics, medication with allopurinol and rasburicase.

Allopurinol: 100 mg/m^2/8 h (10 mg/kg/d, q8h), oral: maximum dose 800 mg/d, IV injection: maximum dose: 600 mg/d;

In case of renal failure, reduce the dose by half or more as appropriate.

Rasburicase: Routine monitoring of uric acid levels, medication based on uric acid levels.

2) Hyperphosphatemia: Aluminum hydroxide 50-150 mg/kg/d, oral or nasogastric, q6h for 1-2 days.

Hemodialysis is more effective in removing phosphate.

3) Hyperkalemia: For mild or asymptomatic cases, avoid potassium supplements, closely monitor ECG or arrhythmias, and administer polystyrene sulfonate sodium 1 g/kg + 50% sorbitol, orally or rectally (contraindicated in patients with leukopenia);

In severe cases, close monitoring, insulin 0.1U/kg, intravenous infusion; 2 mL/kg 25% dextran infusion; sodium bicarbonate 1-2 mEq/kg, intravenous bolus; dialysis;

4) Hypocalcemia: Calcium gluconate 50-100 mg/kg, slow intravenous infusion, cardiac monitoring.

5) Hypercalcemia: Recommended treatment includes enhanced hydration and alkalinization; if urine output is normal, 2000-3000 mL of fluid replacement per day is appropriate; at the time of fluid infusion, use diuretics reasonably to maintain urine output >1500 mL/d; drug therapy includes glucocorticoids, calcitonin, and bisphosphonates, etc.; hemodialysis or peritoneal dialysis can be performed when consolidated with renal insufficiency.

15.8.7 Macrophage Activation Syndrome

Mechanism of its occurrence: Elevated cytokines activate a large number of macrophages, leading to uncontrolled phagocytic activity of macrophages, which can result in persistent fever, hepatosplenomegaly, generalized lymphadenopathy, pancytopenia, high triglycerides, low fibrinogen, elevated serum ferritin, severe hepatic impairment, coagulation disorders, and hemophagocytosis in bone marrow macrophages.

Recommended treatment: Treatment in the same way as clinical hematology hemophagocytic syndrome, by administering chemotherapy drugs to eliminate the excessively activated macrophage count, with appropriate supportive care.

15.8.8 Renal Insufficiency

Hydration, alkalization, and diuresis to prevent renal insufficiency; for individuals with renal failure, dialysis should be performed; Avoid using nephrotoxic drugs; avoid using intravenous contrast agents; for patients receiving long-term bisphosphonate therapy, their renal function should be monitored.

15.8.9 Hypoimmunoglobulinemia

Clinical manifestations: After infusion, patients who develop an immunization response experience a reduction in plasma cells, leading to decreased levels of immunoglobulin. This side effect can serve as one of the indicators of the sustained activity of CART cells in the patients. If necessary, test of the levels of IgG, IgA or IgM may be performed.

15.8.10 Off-target Effects

Off-target effects refer to the phenomenon of failing to reach and deviating from the pre-set goal. In ACT CAR T-cell therapy, off-target effect occurs because the target antigens recognized by CAR T-cells are often tumor-associated, not tumor-specific. These target antigens can be expressed at varying levels in normal tissues. Consequently, with high affinity for target antigens and strong killing potency, CAR T-cells that effectively eliminate tumors can also attack healthy tissues, leading to organ damage. Morgan et al. reported a case of colorectal cancer with liver and lung metastases patients who experienced adverse events of death following CAR-T cell therapy with first-generation human epidermal growth factor receptor 2 (HER2). This was mainly attributed to the low expression of HER2 in some normal tissues, such as pulmonary vasculature. While the CAR-T cells were effective in killing the tumor, they also damaged the lung tissues, leading to respiratory failure and death.

Lamers et al., in their first-generation carbonic anhydrase IX (CAIX) CAR-T cell therapy

study for renal cell carcinoma, found that off-target effects could cause autoimmune cholangitis and severe liver damage due to the low expression of CAIX in normal pancreaticobiliary duct epithelium, gastric mucosa, and small intestinal crypt cells. In clinical trials for malignant pleural mesothelioma and metastatic pancreatic cancer, Beatty et al. found that mesothelin is expressed on normal peritoneal, pleural and pericardial mesothelial cells. Off-target effects can cause toxic and side effects of cardiac arrest, respiratory failure, bowel obstruction and abdominal pain. CD19 is expressed in most B-cell malignancies. It is also found on mature B cells, precursor B cells, and plasma cells in normal tissues. B-cell hypoplasia is a result of off-target effects from anti-CD19 CAR T-cells and can serve as an indicator of duration of CAR T-cell efficacy. Fortunately, B-cell hypoplasia can be treated with immunoglobulin infusion as replacement therapy.

References:

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15.8.11 Allergic Reactions

Allergic reaction is a type I hypersensitivity, which refers to hypersensitivity occurring within minutes after a sensitized body is re-exposed to the same antigen. Its major characteristics include: ①Rapid onset and rapid resolution; ① Mainly mediated by specific IgE; ① Usually causes physiological dysfunction but generally does not result in lasting tissue damage; ④Shows significant individual differences and genetic predisposition. Based on the pathogenesis of type I hypersensitivity, its development can be divided into three phases: sensitization phase, activation phase and effector phase.

Sensitization phase refers to the initial entry of an allergen into a body with allergic constitution, stimulating the production of allergen-specific IgE antibodies. Activation phase

refers to the phase where the same allergen re-enters the body and binds specifically to IgE on sensitized cells to achieve degranulation, leading to release and synthesis of active mediators. Effector phase refers to the phase where the active mediator binds to the corresponding receptors on the effector organ, causing local or systemic pathological changes.

This product's antigen-recognition region is derived from a humanized monoclonal antibody. Therefore, infusion should not trigger host cellular or humoral immune responses due to the immunogenicity of foreign proteins. However, the possibility of allergic reactions caused by residual excipients from the *in vitro* expansion of ESO-T01 injection cannot be ruled out. Mild allergic reactions typically require no treatment. In the event of a severe allergic reaction, the Investigator should provide immediate emergency treatment according to relevant diagnosis and treatment routines.

15.8.12 Infections

Because the primary disease causes a weakened immune system, or because chemotherapeutics, immunosuppressants and steroids used during treatment further compromise the immune system, the subject is at increased risk of infection. If an infection occurs during treatment, considering that hospital-acquired infections are predominantly gram-negative, antibiotics effective against gram-negative bacteria, with coverage for gram-positive bacteria as well, should be administered. Simultaneously, symptomatic treatment should be provided based on the subject's symptoms. Antibiotic treatment should follow the principles of combination therapy, regular administration, quantity sufficient and completion of the full course. To ensure antibiotic effectiveness, appropriate dosage and treatment duration should be observed to prevent the development of drug-resistant bacteria. Antifungal prophylaxis should be routinely administered after 5-7 days of continuous antibiotic use.

15.8.13 Secondary Tumor

During CAR-T cell production, retroviral or lentiviral vectors are commonly used at present. These vectors randomly insert the CAR gene into the T-cell genome. This random insertion may activate oncogenes, disrupt tumor suppressor genes, or increase the expression of growth factors, potentially leading to tumor formation. Therefore, long-term follow-up is necessary. Currently, there are no known effective preventive measures to reduce the risk of secondary tumors and it is also impossible to predict which patient will develop them. Regular check-ups are the best way to detect and treat secondary tumors as early as possible.

Study protocol amendment summary

The following revisions have been made to the clinical study protocol (Version number/Version date: Version 1.0, October 6, 2024) based on specific clinical circumstances:

Page number/line number	**Original clinical study protocol (Version 1.0, October 6, 2024)**	**Revised clinical study protocol (Version 1.1, January 13, 2025)**	**Reason for revision**
Cover page, protocol signature page, header	Version number: 1.0 Version date: October 6, 2024	Version: 1.1 Version date: January 13, 2025	Update version and date
Page 6, Page 50 / Primary study objectives.	To evaluate the safety and tolerability of a single intravenous ESO-T01 Injection in the treatment of relapsed/refractory multiple myeloma (R/R MM).	To evaluate the safety and tolerability of ESO-T01 Injection in the treatment of relapsed/refractory multiple myeloma (R/R MM).	Remove the term "single." Based on the infusion reactions (elevated cytokines and CRP) observed in the first enrolled subject, and the similar reactions and mechanisms of action when infusing bispecific antibodies, we plan to follow the strategies for managing infusion reactions associated with bispecific antibodies (TECVAYLI/teclistamab, a BCMA-CD3 bispecific antibody). For subsequent enrollments, we intend to administer either one dose or multiple doses.
Pages 11-12, 52 / Dose Escalation and Subject Enrollment Principles	Subjects will be enrolled with rapid titration. In Dose A group, 14 days after infusion in the first subject, PK samples will be tested centrally and analyzed	Subjects will be enrolled with rapid titration. In Dose A group, 7 days after infusion in the first subject, PK samples will be tested centrally and analyzed by	The subject enrollment interval has been adjusted. This clinical study is being conducted simultaneously at Union Hospital and Tongji Hospital, both

	Cmax and Tmax by the Principal Investigator, as well as the comparability with the Phase 1 clinical trial of BCMA autologous CAR-T, and a decision on whether to continue enrolling subsequent subjects or to increase the dose to Dose B will be made. Similarly, in Doses B, C, and D groups, a single subject will be enrolled first (titration) and administered a single intravenous infusion of ESO-T01. In Doses B, C, and D groups, 14 days after infusion in the first subject, PK samples will be tested centrally and analyzed Cmax and Tmax by the Principal Investigator, as well as the comparability with the Phase 1 clinical trial of BCMA autologous CAR-T, and a decision on whether to continue enrolling subsequent subjects or to increase the dose to subsequent dose levels will be made. In Doses A, B, C, and D groups, if continued enrollment of subjects is required after the first subject has completed the titration, enrollment of 3-6 subjects of the dose group design may be completed. Within the same dose	the Principal Investigator, as well as the comparability with the Phase 1 clinical trial of BCMA autologous CAR-T, and a decision on whether to continue enrolling subsequent subjects or to increase the dose to Dose B will be made. Similarly, in Doses B, C, and D groups, a single subject will be enrolled first (titration) and administered a single intravenous infusion of ESO-T01. In Doses B, C, and D groups, 14 days after infusion in the first subject, PK samples will be tested centrally and analyzed by the Principal Investigator, as well as the comparability with the Phase 1 clinical trial of BCMA autologous CAR-T, and a decision on whether to continue enrolling subsequent subjects or to increase the dose to subsequent dose levels will be made. In Doses A, B, C, and D groups, if continued enrollment of subjects is required after the first subject has completed the titration, enrollment of 3-6 subjects of the dose group design may be completed. Within the same dose group, the enrollment interval for	affiliated to Tongji Medical College of Huazhong University of Science and Technology. Currently: Union Hospital enrolled the first subject on November 27, 2024, and the second subject on January 3, 2025; Tongji Hospital enrolled its first subject on January 10, 2025. The first subject at Union Hospital has completed the safety and efficacy follow-up at the 1-month post-administration time point. Data indicate that safety is manageable, and initial efficacy has been achieved—suggesting that Dose A may be a safe and effective dose, which requires enrolling more subjects for further observation. The second subject at Union Hospital has completed 9 days of post-administration follow-up, and the first subject at Tongji Hospital has completed 2 days of post-administration follow-up; both sets of data confirm that safety remains manageable after infusion, and the post-infusion reactions of the three subjects are similar.

	group, the enrollment interval for subsequent subjects is determined by the Principal Investigator, except that the enrollment interval between the first and second subjects is 14 days.	subsequent subjects is determined by the Principal Investigator, except that the enrollment interval between the first and second subjects is 7-14 days.	Therefore, based on the safety and initial efficacy data (45 days post-infusion for the first subject, 9 days post-infusion for the second subject, and 2 days post-infusion for the third subject), it is planned to enroll more subjects for further observation in the Dose A group, with the infusion interval for this dose group adjusted to 7 days.
Page 15 / Drug Infusion	Administration of prophylactic medications: Administer acetaminophen (650 mg orally) and diphenhydramine (12.5 mg intramuscularly or 25 mg orally) within 1 hour before ESO-T01 infusion. Considering that corticosteroids may affect T-cell persistence, the use of systemic corticosteroids such as hydrocortisone, prednisone, methylprednisolone, or dexamethasone is not recommended, except in life-threatening emergencies.	Administration of prophylactic medications: Administer acetaminophen (650 mg orally) and diphenhydramine (12.5 mg intramuscularly or 25 mg orally) within 1 hour before ESO-T01 infusion. Subjects may receive prophylactic medications such as hormones, as determined by the Investigator based on individual circumstances of subjects.	Adjustment of Prophylactic Medication RegimenEnrolled subjects developed infusion reactions (characterized by elevated cytokines and elevated CRP) post-administration; symptomatic treatment (including corticosteroids, etc.) was administered, which achieved effective control. Currently, with reference to the measures for managing infusion reactions during bispecific antibody (BsAbs) administration, we are exploring prophylactic medication regimens—with no restriction on the use of corticosteroids and other agents as prophylactic medications.

Page 66 / Prohibited Concomitant Medication/Therapy	From the start of ESO-T01 Injection infusion until disease progression or treatment change, subjects must not use the following treatments unless otherwise specified in the protocol: 1.Anti-tumor treatments (including traditional Chinese medicines, proprietary Chinese medicines, and Chinese medicine preparations approved by the National Medical Products Administration for anti-tumor treatment); 2.Hematopoietic stem cell transplantation; 3.Corticosteroids (avoid use within 72 hours before infusion; avoid prophylactic use after infusion, and only use when necessary, such as in the case of CRS) and other immunosuppressive drugs. 4.Any other drugs that may affect the Investigator's assessment of efficacy or subject safety.	From the start of ESO-T01 Injection infusion until disease progression or treatment change, subjects must not use the following treatments unless otherwise specified in the protocol: 1.Anti-tumor treatments (including traditional Chinese medicines, proprietary Chinese medicines, and Chinese medicine preparations approved by the National Medical Products Administration for anti-tumor treatment); 2.Hematopoietic stem cell transplantation; 3.Any other drugs that may affect the Investigator's assessment of efficacy or subject safety.	The reason for the modification is the same as above; this clause stating that "hormones are prohibited concomitant medications" is deleted.
Pages 23/ SCHEDULE OF ACTIVITIES	Urine protein electrophoresis，Urine immunofixation electrophoresis，Urine free light chain	24-hours urine protein electrophoresis，24-hours urine immunofixation	Add the notation "24h". Clarify that urine samples refer to 24-hour

		electrophoresis， 24-hours urine free light chain	collected urine, followed by sampling and analysis.
Pages 25/ SCHEDULE OF ACTIVITIES	1. Screening period: Subjects who complete the appropriate visit content examination according to the visit schedule during the screening period and meet all inclusion criteria and none of the exclusion criteria, as assessed by the Investigator, can be enrolled in the study.	2. Screening period: Subjects who complete the appropriate visit content examination according to the visit schedule during the screening period and meet all inclusion criteria and none of the exclusion criteria, as assessed by the Investigator, can be enrolled in the study. Results of subjects obtained within 7 days prior to screening may be accepted as screening results, as appropriate.	Add the provision that " Results of subjects obtained within 7 days prior to screening may be accepted as screening results, as appropriate". Considering that some subjects have undergone invasive procedures (such as bone marrow aspiration) prior to screening, the protocol is revised to allow the discretionary acceptance of results from 7 days prior to screening, so as to avoid repeated invasive procedures.

9 STATISTICAL METHODS AND ANALYSIS

9.1 Determination of Sample Size

As this study is an IIT to evaluate the tolerability, safety, and efficacy of ESO-T01 Injection in treating r/r MM, the sample size is not determined based on statistical hypotheses.

This study plans to enroll 4 to 24 subjects diagnosed with r/r MM.

9.2 General Principles of Statistical Analysis

Analysis is performed using SAS 9.4 (or higher version) software.

For continuous variables, descriptive statistics will include mean, median, standard deviation, maximum and minimum. For categorical variables, descriptive statistics will consist of the number and percentage of subjects. Detailed statistical analysis methods are described in the Statistical Analysis Plan.

9.3 Analysis Sets

Major statistical analysis sets for this study are as follows:

(1) Full analysis set (FAS): All subjects enrolled who received ESO-T01 reinfusion.

(2) Safety analysis set (SS): All subjects enrolled who received ESO-T01 reinfusion and had at least one safety measure recorded.

(3) DLT analysis set (DLTS): All subjects who received ESO-T01 Injection infusion and completed the DLT assessment, including subjects who developed DLT and those who did not develop DLT but completed all assessments during the DLT observation period.

(4) Pharmacokinetic analysis set (PKS): Subjects with at least one quantifiable plasma concentration after administration and no major protocol deviations/violations or with no events that significantly affect pharmacokinetic results.

(5) Efficacy analysis set (ES): All subjects who received ESO-T01 reinfusion, had a tumor assessment at baseline and had at least one available tumor assessment result after administration.

9.4 Analytical Procedures

9.4.1 Subject Disposition

Subject enrollment and completion status will be described using the number (percentage) of subjects. List the reasons for withdrawal from the study for both dropout and rejected subjects by the subject disposition of each data set.

9.4.2 Demographic Data and Baseline Characteristics Analysis

Descriptive statistics of demographic data and other baseline characteristic values are

provided, and the number of subjects, mean, standard deviation, median, minimum and maximum are calculated for non-missing observation results by continuous variables. The number and percentage of subjects are calculated for count and ordinal data.

9.4.3 Safety Analysis

Adverse Events

Evaluate the occurrence and frequency of dose-limiting toxicity (DLT) of ESO-T01 Injection and determine the MTD.

Number and percentage of subjects with adverse events, drug-related adverse events, adverse events of Grade 3 or higher in severity, and serious adverse events are summarized by dose group and overall. CRS and ICANS will be graded according to the 2019 ASTCT criteria, while other AEs will be graded per NCI CTCAE version 5.0. All AEs will be coded using the latest version of MedDRA. The incidence of adverse events will be summarized by system organ class (SOC) and preferred term (PT).

Laboratory Tests

Laboratory test data are graded in accordance with NCI CTCAE version 5.0. The data on laboratory test indicators such as blood routine examination, blood chemistry, coagulation function, and urine routine examination and their change values before and after baseline are summarized for each visit according to the different study phases and dose groups, and the positive and abnormal changes in blood routine examination, blood chemistry, coagulation function, and urine routine examination before and after baseline are summarized.

All abnormal change values in laboratory tests are described using lists.

ECG, Physical Examination, and Vital Signs

Descriptive statistics are used to summarize the data for each visit and the change before and after baseline according to the different study phases and dose groups, and laboratory values are described in tabular statistics.

9.4.4 Efficacy Analysis

Objective response rate (ORR): Proportion of subjects achieving stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR), providing the efficacy to evaluate the subject number and percentage of each result. The overall response rate (ORR) and its two-sided 95% confidence interval (CI) will be calculated using the Clopper-Pearson method.

MRD negativity rate: Calculations of subjects with negative MRD, MRD negativity rate and 95% confidence interval using the Clopper-Pearson method.

Time to response (TTR), duration of response (DOR), progression-free survival (PFS), and overall survival (OS).

Time to response (TTR) is defined as the time from the start of the drug infusion in subjects to the first occurrence of sCR, CR, VGPR, or PR.

Duration of response (DOR) is defined as the time interval between the first occurrence of sCR, CR, VGPR, or PR after drug infusion in subjects and the first assessment of progressive disease or death, whichever occurs first.

Progression-free survival (PFS) is defined as the time interval between drug infusion in subjects and the first assessment of progressive disease or death, whichever occurs first.

Overall survival (OS) is defined as the time interval between drug infusion in subjects to death from any cause.

The median survival is estimated using the Kaplan-Meier method, and the 95% confidence interval of the median survival is estimated using the Brookmeyer and Crowley method. Kaplan-Meier survival curve is plotted. DOR analysis is limited to subjects with documented response.

9.4.5 Pharmacokinetic Analysis

PK analysis will be performed using the PK analysis set. Drug concentration data at each time point will be summarized using descriptive statistics. CAR copy numbers in peripheral blood will be detected, and individual plasma concentration-time curves and arithmetic mean concentration-time data plots will be created on both linear and semi-logarithmic scales.

PK parameters are calculated using WinNonlin software and a non-compartmental model based on the actual blood sampling times. Evaluation indicators include: C_{max} of expansion, T_{max}, and $AUC_{0\text{-}28d}$ of ESO-T01 after reinfusion in peripheral blood. Statistics for PK parameters include, but are not limited to, the number of subjects, arithmetic mean, standard deviation, coefficient of variation, median, first quartile, third quartile, minimum and maximum.

9.5 Interim Analysis

No interim analyses were planned for this study.