Supplementary figures

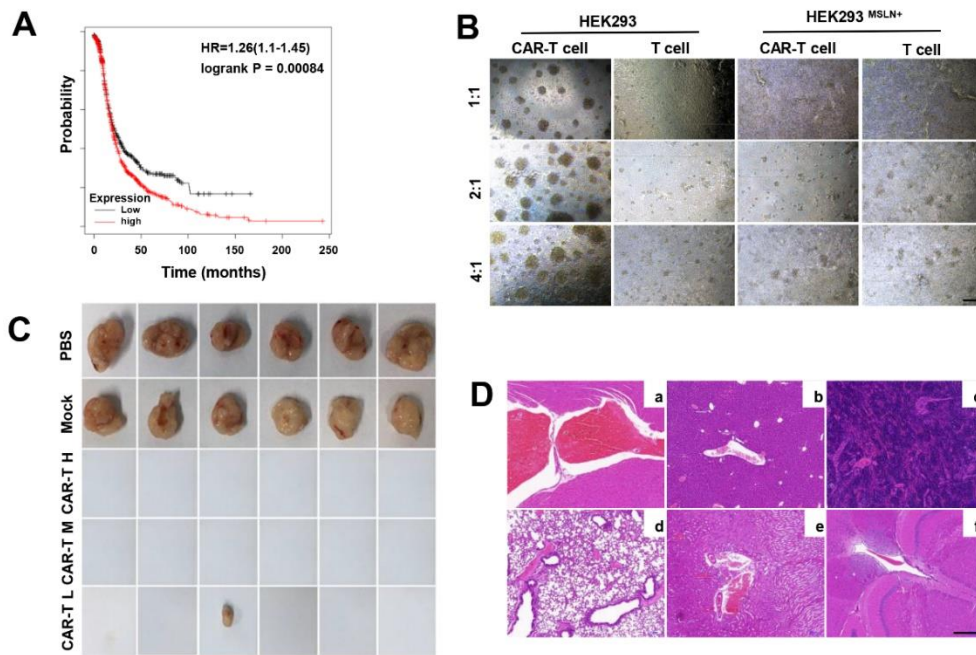**Figure S1. Anti-tumor activity of MSLN CAR-T *in vivo* and *in vitro*. A.** Kaplan-Meier plots summarize results from analysis of correlation between mRNA expression level and patient survival. Patients were divided based on level of expression into one of the two groups "low" (under cut off) or "high" (over cut off). X-axis shows time for survival (years) and y-axis shows the probability of survival, where 1.0 corresponds to 100 percent. **B.** Lysis of spheres of HEK293 cells overexpressing MSLN cultures and normal in the presence of MSLN CAR-T cells, or T cells (control) at a 1:1, 2:1, 4:1 effector: target ratio. (Scale bar: 250 μm). **C.** Tumors harvested from control mice and CAR-T cell treated mice at necropsy following MSLN CAR-T treatment for 42 days. N=6 per group. **D.** HE staining of mouse parenchymal organs (heart, liver, spleen, lung and kidney). Immunohistochemical detection of MSLN expression of patients before enrollment. Scale bars, 200 μm.
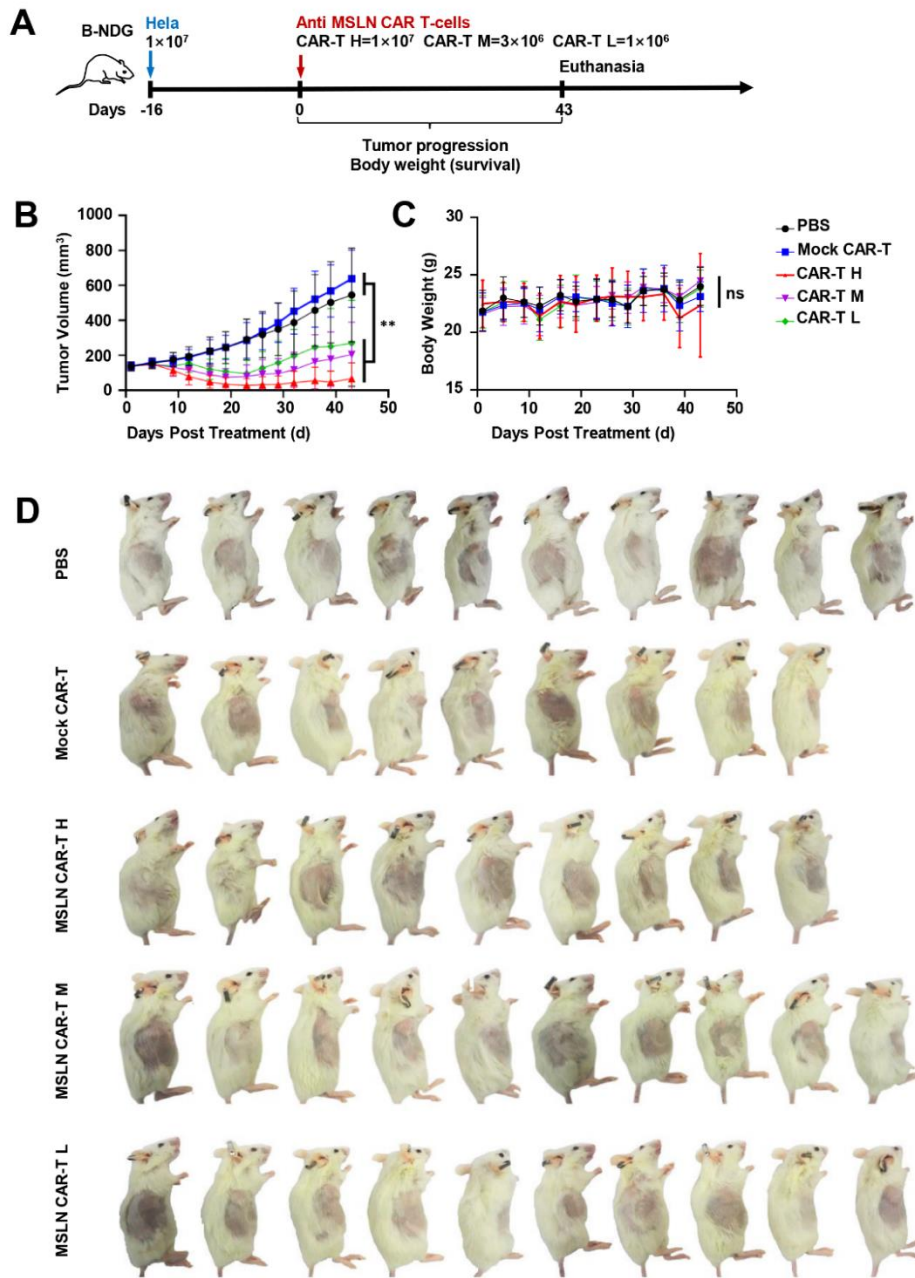
Figure S2. Specific tumor elimination capacity of anti-MSLN CAR-T cells in vivo. A. Schematic diagram of the experiment of subcutaneous inoculation HeLa cell. **B.** Tumor volumes were plotted versus time for each group. **C.** The body weight of NDG mice were measured before tumor formation and CAR-T cell injection, and continuously measured every 3-4 days after CAR-T cell injection. The line represents the mean ± SD.(n = 10). **D.** Pictures of mice

sacrificed at the end of the experiment. The tumor volume grew to 2000 cm^3, and the mice of each group were sacrificed and photographed.

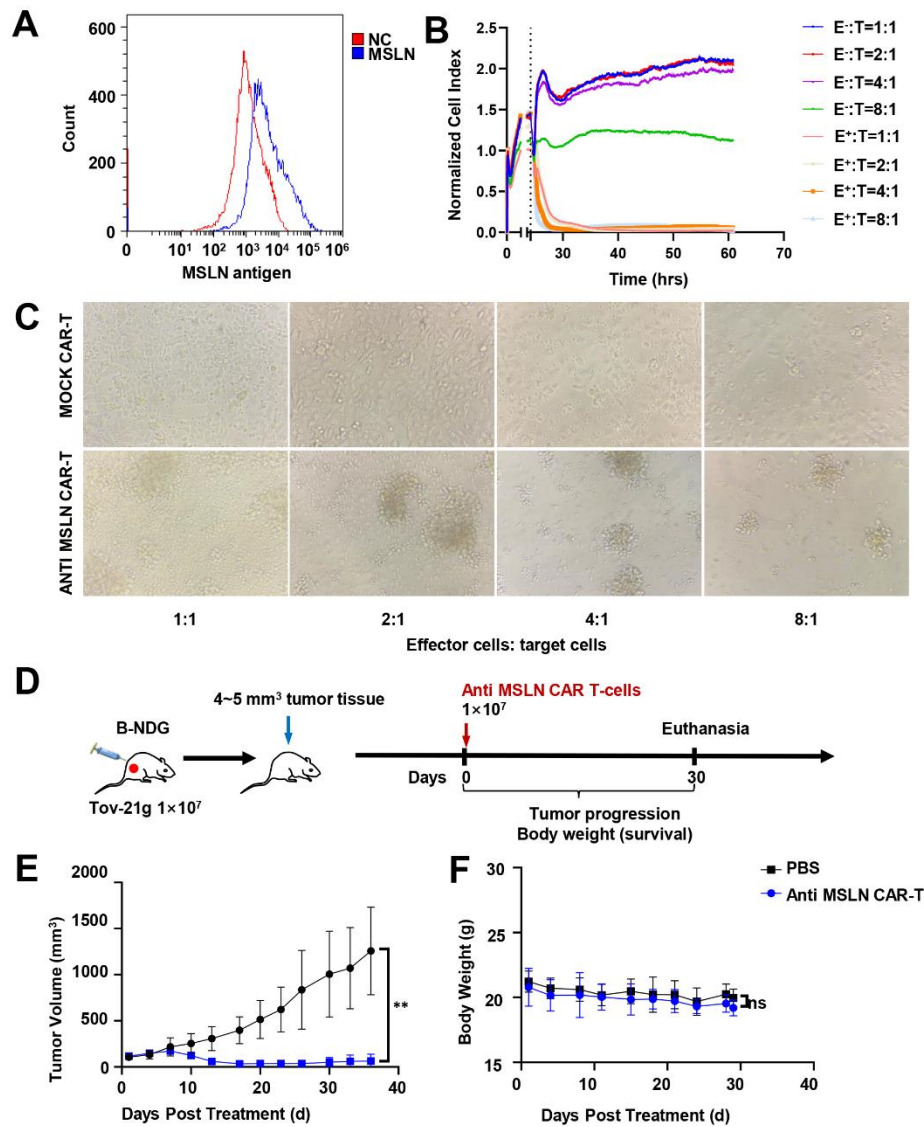**Figure S3. The efficacy test of Tov-21g cells *in vitro* and *in vivo*. A.** Detection of MSLN expression in Tov-21g cell by flow cytometry. **B.** A real-time cytotoxicity assay (xCELLigence RTCA SP) was used to evaluate the lysis of the indicated tumor cells treated with mock CAR-T (E$^-$) cells or MSLN CAR-T (E$^+$) cells at the indicated E/T ratios over a 40-h period. **C.** Lysis of spheres of

Tov-21g cell cultures in the presence of anti-MSLN CAR-T cells at different effector: target ratios (E: T) (Scale bar: 200 μm). **D**. Schematic diagram of the experiment of subcutaneous inoculation Tov-21g tumor tissues. **E.** Tumor volumes were plotted versus time for each group. **F.** The body weight of NDG mice were measured before tumor formation and MSLN CAR-T cell injection. Continuously measured every 3-4 days after CAR-T cell injection. The line represents the mean ± SD. (n = 6). Scale bars, 50 mm. Mean ±SD; two-way ANOVA, **P < 0. 01, ***P < 0.001.

Human IgG1(20 μg/mL)   G11 (20 μg/mL)   G11 (2 μg/mL)
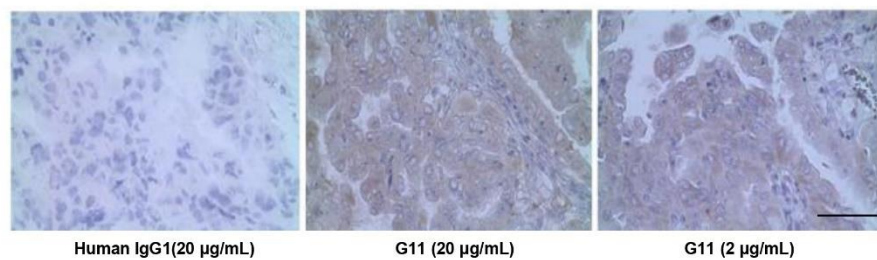
Figure S4.

Frozen slices of human ovarian cancer tissues were stained for IHC to detect the expression of MSLN at different concentrations. Human IgG1 was used as a negative control. The final concentration of 2 μg/mL and 20 μg/mL was used for human tissue crossover test. Scale bars, 20 μm.

Figure S5. Testing before the patient is enrolled. A. Immunohistochemical detection of MSLN expression of patients before enrollment. Three patients were assessed for MSLN positive grade. Patient 001, ++. Patient 002, +. Patient 003, +. **B.** Patient-derived MSLN CAR-T cells kill MSLN-positive tumor cell lines in vitro. Lysis of spheres of SKOV3 and Ovcar3 in the presence of MSLN CAR-T cells, or T cells (control) at a 1:1, 2:1, 4:1and 8:1 effector: target ratio. (Scale bar: 250 μm).

Figure S6. Characteristics of anti MSLN CAR-T cells successfully generated for three patients. A. Anti MSLN CAR expression of T cells in the 3 patients with Ovarian cancer. According to flow cytometry analysis, an average of 70% (range) of T cells express MSLN CAR. **B.** Representative histograms of infused MSLN CAR-T cell products in first time. The CAR-T cell data returned later is not shown.
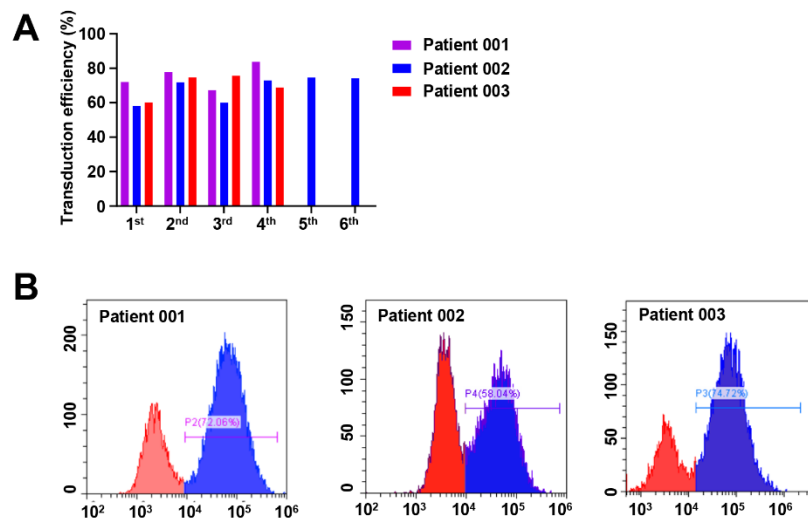
Supplementary tables

Table S1. Antibody preparation.

Antibody ID	Preparation amount	Expression amount (mg/L)	Antibody concentration （mg/ml）	Antibody purity （SDS-PAGE）%	Endotoxin content EU/mg
MB-2	5.7mg	190	6.3	>95	<1
MB-3	3.09mg	103	3.43	>95	<1
MB-7	2.12mg	70.7	3.26	>95	<1
MB 21	1.8mg	60	5.33	>95	<1
MB 37	1.6mg	53.4	5.5	>95	<1
MB 38	1.2mg	40	4.42	>95	<1
MB 50	4.27mg	142.3	4.27	>95	<1
MB 61	5.8mg	193.3	5.93	>95	<1
MB 92	1.5mg	50	3.41	>95	<1
B8	1.9mg	63.4	7.95	>95	<1
B12	1.825mg	60.8	0.75	>95	<1
E12	2.26mg	75.4	3.12	>95	<1
G11	3.71mg	123.7	3.23	>95	<1
H2	1.6mg	53.4	7.81	>95	<1

Table S2. Blood hematology test of B-NDG mice after MSLN CAR-T treatment.

PARAMETER (MEAN±SD)	B-NDG	PBS	CAR-T Cell Therapy		
			MSLN-H	MSLN-M	MSLN-L
WBC (10^3/μL)	1.7±.063	6.02±2.41 **	1.67±0.05	1.52±0.34	3.9±3.06 *
RBC (10^6/μL)	8.49±0.23	6.91±3.75	6.85±3.72	8.8±0.39	7.28±3.87
HGB (g/Dl)	14.13±0.29	14.18±0.43	14.8±0.72	15±0.33	14.8±1.24
HCT (%)	43.55±1.05	41.98±1.03	44.33±1.79	44.88±0.9	44.33±3.22
MCV (fL)	51.33±0.21	49.07±0.59 *	50.4±0.68 *	49.65±0.71	49.32±1.22
MCH (pg)	16.65±0.14	16.58±0.28	16.82±0.33	16.58±0.3	16.45±0.63
MCHC（g/dL）	32.45±0.16	33.77±0.42	33.38±0.39	33.43±0.25	33.37±0.51
PLT (10^3/μL)	1060.33±49.83	1234.50±105.36***	1043.83±119.97	1043.5±53.14	918.73±437.81
RDW (%)	14.82±0.08	14.12±0.1	14.5±0.6	14.3±0.33	14.35±0.26
MPV (fL)	3.38±0.08	3.4±0.09	3.28±0.42	3.12±0.15	3.02±0.15
PDW (%)	16.25±0.45	16.32±0.41	15.92±0.37	16.3±0.15	15.98±0.69

Mean ±SD; two-way ANOVA, ***P < 0.001, ****P < 0.0001. n=6.

Table S3. Human cross reaction.

Biotin-G111	Brain	Cerebellum	Pituitary gland	Eyeball	Thyroid	Thymus	Heart
2 (µg/mL)	-	-	-	-	-	2/3	1/3
20 (µg/mL)	-	-	-	-	-	2/3	2/3
Binding site	-	-	-	-	-	Lymphocytes	Cardiomyocytes

Biotin-G111	Lung	Galactophore	Spinal cord	Vascular endothelium	Liver	Pancreas	Spleen
2 (µg/mL)	1/3	-	-	-	-	-	-
20 (µg/mL)	3/3	-	-	-	-	-	-
Binding site	Alveolar cells	-	-	-	-	-	-

Biotin-G111	Small intestine	Colon	Lymph nodes	Adrenal glands	Kidney	Bladder	Ureter
2 (µg/mL)	-	-	-	-	-	-	-
20 (µg/mL)	-	-	-	-	1/3	-	-
Binding site	-	-	-	-	Renal tubules	-	-

Biotin-G111	Ovaries	Oviduct	Uterus	Testis	Prostate	Placenta	Skin
2 (µg/mL)	-	2/3	-	-	--	-	1/3
20 (µg/mL)	-	2/3	3/3	-	-	3/3	1/3
Binding site	-	Mucosal layer	Muscle layer	-	-	Fluff	Epithelial Cells

Biotin-G111	Skeletal muscle	blood cells	Bone marrow	Salivary glands	Stomach		
2 (µg/mL)	-	-	-	-	-		
20 (µg/mL)	-	-	-	-	-		
Binding site	-	-	-	-	-		

Table S4. The amount of CART cells infused per patient.

Patient ID	The 1st infusion cell/kg	The 2nd infusion cell/kg	The 3rd infusion cell/kg	The 4th infusion cell/kg	The 5th infusion cell/kg	The 6th infusion cell/kg
1	1.0E+6	5.0E+6	1.5E+7	1.5E+7	-	-
2	1.0E+6	5.0E+6	1.5E+7	2.3E+7	2.3E+7	3.0E+7
3	1.0E+6	5.0E+6	1.5E+7	-	-	-

Table S5. Hematological indexes after CAR-T cell transfusion in Patients.

Patient ID	Days after first infusion	HCT (0.35-0.5) %	HB (115-175) g/L	WBC (3.5-9.5) 10⁹/L	ANC (1.8-6.3） 10⁹/L	LY （1.1-3.2） 10⁹/L	MONO （0.1-0.6） 10⁹/L	EOS （0.02-0.52） 10⁹/L	BASO （0-0.06）	CRP mg/L
001	39	0.281	89	2.8	1.25	0.89	0.24	0.26	0.07	17.7
	46	0.308	100	5.2	3.47	1.04	0.34	0.10	0.09	1.4
	123	0.276	90	3.9	2.52	0.96	0.21	0.05	0.01	2.8
	139	0.233	77	2.7	2.22	0.15	0.21	0.11	0.00	40.9
	144	0.221	72	-	2.41	0.14	0.35	0.02	0.00	174.9
	149	0.300	92	4.9	3.85	0.26	0.31	0.12	0.09	34.8
	249	0.228	74	4.3	3.23	0.70	0.19	0.06	0.02	5.6
	253	0.212	71	2.5	1.93	0.27	0.20	0.09	0.01	-
	258	0.207	69	5.3	4.26	0.45	0.26	0.19	0.02	-
	262	0.225	73	5.9	5.15	0.41	0.22	0.06	0.02	-
002	-1	0.322	105	4.5	2.86	1.05	0.33	0.13	0.01	-
	3	0.330	108	4.1	2.46	0.98	0.40	0.16	0.02	7.5
	12	0.459	160	7.8	4.91	1.96	0.60	0.13	0.03	3.3
	21	0.331	109	4.4	2.85	1.09	0.21	0.10	0.01	10.3
	27	0.353	113	4.6	2.87	1.10	0.34	0.14	0.03	-
	31	0.320	105	4.9	3.14	1.07	0.37	0.21	0.02	81.4
	48	0.345	107	4.4	2.79	1.10	0.26	0.11	0.03	5.0
	57	0.320	103	6.1	4.27	1.14	0.58	0.12	0.02	18.2
	62	0.353	113	5.1	3.02	1.40	0.39	0.20	0.02	-
	66	0.354	111	6.0	4.19	0.95	0.39	0.29	0.08	28.6
	79	0.324	102	6.3	4.40	1.2	0.54	0.11	0.04	30.3
	90	0.347	110	4.9	2.83	1.22	0.30	0.13	0.02	1.2
	96	0.335	109	4.8	2.19	1.27	0.39	0.16	0.02	0.7
	101	0.359	117	19.7	16.49	1.49	1.06	0.43	0.05	192.6
003	-2	0.341	117	3.8	2.5	0.87	0.23	0.11	0.02	-
	3	0.332	112	4.3	2.99	0.74	0.25	0.20	0.02	4.6
	9	0.335	116	5.3	4.01	0.79	0.27	0.1	0.02	4.5
	27	0.340	117	3.7	2.37	0.91	0.23	0.11	0.02	-
	31	'0.330	114	4.1	2.68	0.72	0.35	0.24	0.01	6.5
	57	0.330	109	3.9	2.82	0.63	0.20	0.14	0.02	-
	63	0.341	114	8.5	7.73	0.29	0.26	0.12	0	52.4
	77	0.299	97	3.5	2.57	0.59	0.25	0.10	0.02	-
	87	0.291	97	4.3	3.31	0.66	0.14	0.13	0.03	8.0
	93	0.284	93	2.7	1.74	0.60	0.18	0.11	0.01	-
	100	0.321	104	3.4	2.2	0.66	0.25	0.22	0.02	6.3

Hematocrit, HCT. Hemoglobin, HB. Leukocyte, white blood cell, WBC. Absolute Neutrophil Count, ANC. Lymphocyte count, LY. Monocyte count, MONO. Eosinophil count, EOS. Basophil count, BASO. C-reactive protein, CRP. Cholesterol, CHOL. Total cholesterol.

Supplementary patients' information

Patient 001 was first diagnosed with ovarian tumor by B-ultrasound and then with ovarian serous papillary cystadenocarcinoma *via* pathological detection after surgery. No significant improvement in pathology was observed after 6 weeks of chemotherapy and 2 weeks of DC/CIK cell immunotherapy. The patient then received a conditioning chemotherapy regimen of cyclophosphamide and fludarabine, followed by three rounds of anti-MSLN CAR-T cells infusion. The first and second rounds were separated by 1 month, but the third round was postponed due to the COVID-19 pandemic.

Patient 002 was diagnosed with bilateral ovarian adenocarcinoma, which recurred several times following surgical resection and did not resolve after treatment with various chemotherapy regimens. On August 25, 2020, the patient joined the anti-MSLN CAR-T therapy clinical study for her first apheresis. Due to her physical condition, the patient could not continue chemotherapy; thus, lymphatic clearance was not performed.

Patient 003 had irregular tumors on both sides of the ovary. After surgical resection, the pathological examination revealed high-grade serous carcinoma of the ovary on both sides, and cancer foci were observed in the greater omentum. Before CAR-T cell treatment, she received multiple cycles of chemotherapy and DC/CIK cell immunotherapy. The cancer of this patient was still progressing, and CT examination revealed liver tissue metastases.