Supplementary Methods

Patients

Patients selected for treatment had histologically confirmed advanced cutaneous melanoma with confirmed evidence of progressive metastatic disease. Cardiac function was assessed by electrocardiogram and, for patients ≥60 years old or with a history of cardiac problems, stress echocardiogram. Patients had a metastatic site that could be excised to obtain a specimen of ≥1 cm^3 (>2 cm^3 for lymph nodes) and had measurable/evaluable disease after surgical resection. Patients were confirmed free from blood borne testable communicable viral diseases at the time of surgery.

Product assessments

The cell composition and T-cell subset phenotype of the final product, and expression of activation and exhaustion markers were assessed by flow cytometry. For phenotype analysis, samples were incubated with a murine serum block and a fragment crystallizable (Fc) block followed by labeling with fluorescently conjugated antibodies to CD3, CD4, CD8, CD95, CCR7, and CD45RO to distinguish between naive, effector, effector memory, and central memory subsets within the T-cell phenotype; samples were assessed using a fit-for-purpose flow cytometric method. For cell composition analysis, cells were incubated with a murine serum block and an Fc block prior to staining for CD3, CD14 (monocytes), CD19 (B cells), CD146, melanoma cell adhesion molecule (MCAM), melanoma-associated chondroitin sulfate proteoglycan (MCSP), and CD228 (melanotransferrin [melanoma marker]); cells were assessed using a fit-for-purpose flow cytometric method. Exhaustion/activation state was assessed by expression of programmed cell death protein 1 (PD-1), T-cell immunoglobulin and mucin-domain containing protein 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and 4-1BB on CD4+ and CD8+ T cells using flow cytometry. TCR beta-chain repertoire was profiled as previously described [1, 2]. Single-cell RNA sequencing libraries were prepared using the 10X Chromium controller and V.1 V(D)J and 5'GEX reagents and protocols (10X Genomics) and sequenced on an Illumina HiSeq 2500. TCR clones within the infusion product and in blood at specified timepoints after infusion were identified using Loupe V(D)J Browser (10X Genomics).

Supplementary references

[1] Liu Y, He S, Wang XL, Peng W, Chen QY, Chi DM, Chen JR, Han BW, Lin GW, Li YQ, Wang QY, Peng RJ, Wei PP, Guo X, Li B, Xia X, Mai HQ, Hu XD, Zhang Z, Zeng YX and Bei JX. Tumour heterogeneity and intercellular networks of nasopharyngeal carcinoma at single cell resolution. Nat Commun 2021; 12: 741.

[2] Krishna C, DiNatale RG, Kuo F, Srivastava RM, Vuong L, Chowell D, Gupta S, Vanderbilt C, Purohit TA, Liu M, Kansler E, Nixon BG, Chen YB, Makarov V, Blum KA, Attalia K, Weng S, Salmans ML, Golkaram M, Liu L, Zhang S, Vijayaraghavan R, Pawlowski T, Reuter V, Carlo MI, Voss MH, Coleman J, Russo P, Motzer RJ, Li MO, Leslie CS, Chan TA and Hakimi AA. Single-cell sequencing links multiregional immune landscapes and tissue-resident T cells in ccRCC to tumor topology and therapy efficacy. Cancer Cell 2021; 39: 662-677.

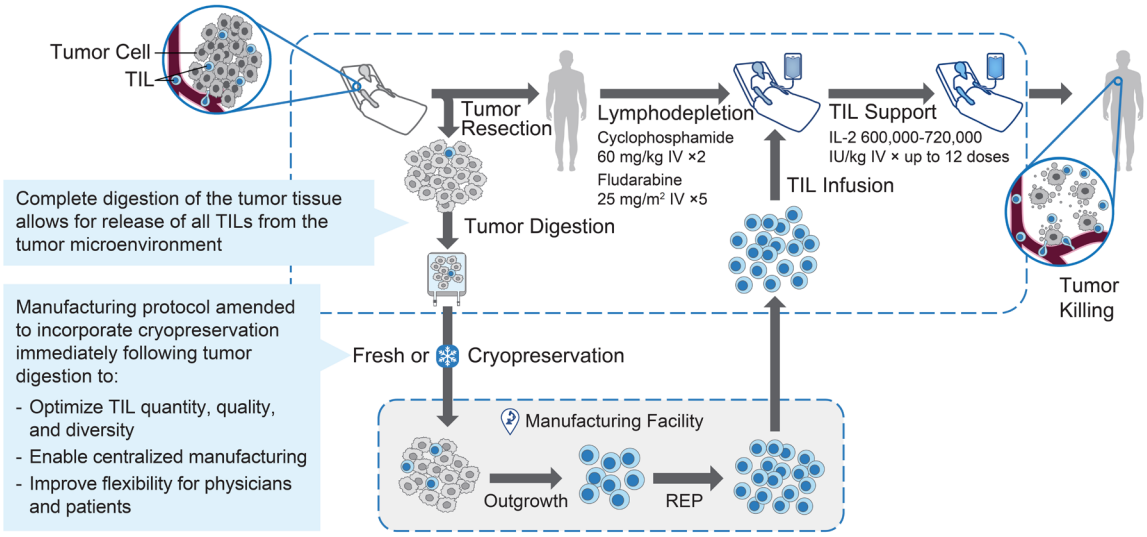

Figure S1. Tissue procurement and manufacturing. IL-2, interleukin-2; IV, intravenous; REP, rapid expansion protocol; TIL, tumor-infiltrating lymphocyte.

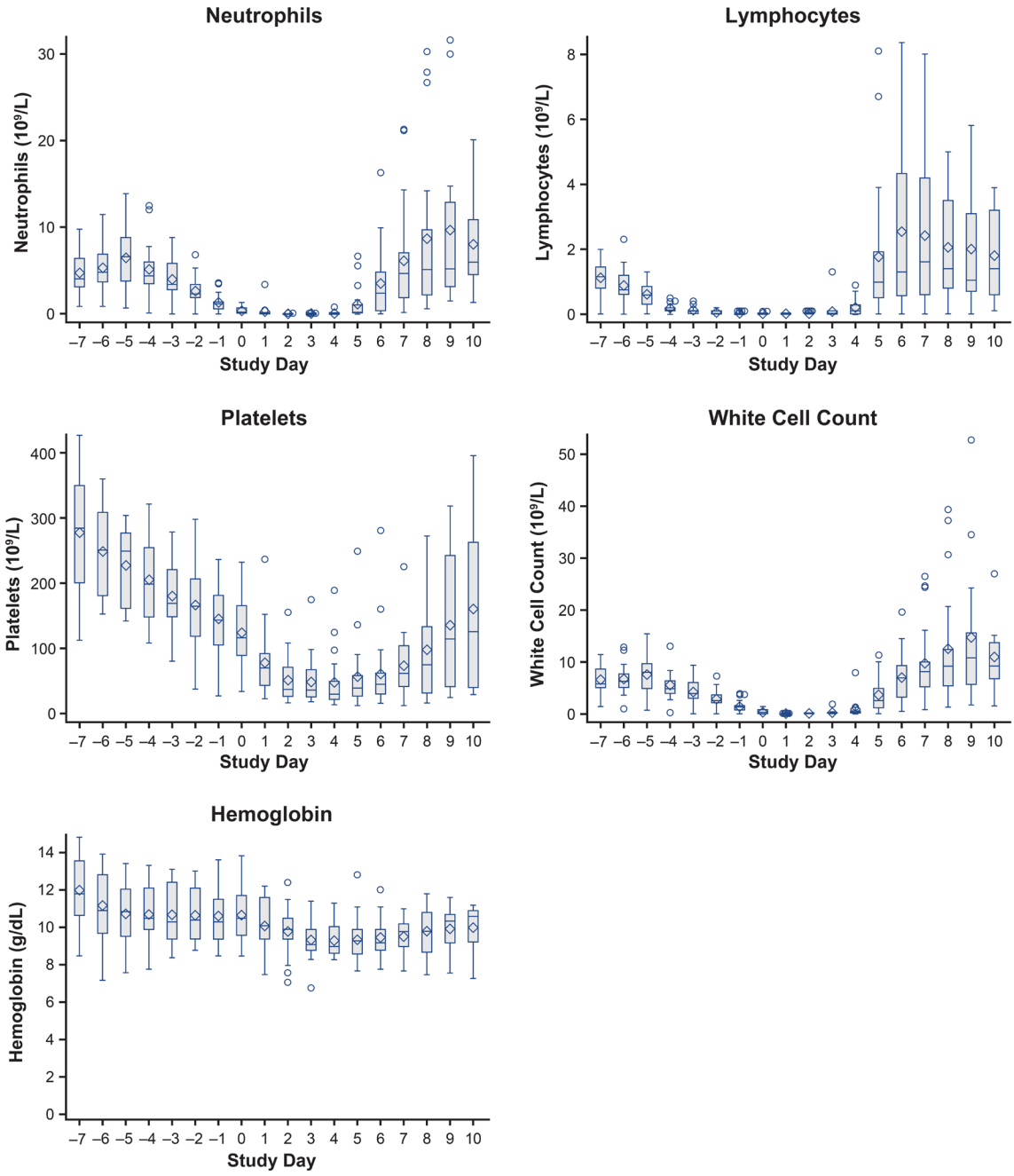

Figure S2. Blood counts and hemoglobin levels over time. Data are shown for all treated patients (N=21). The bar and diamond represent the median and mean values, respectively. Outliers are shown with circles.

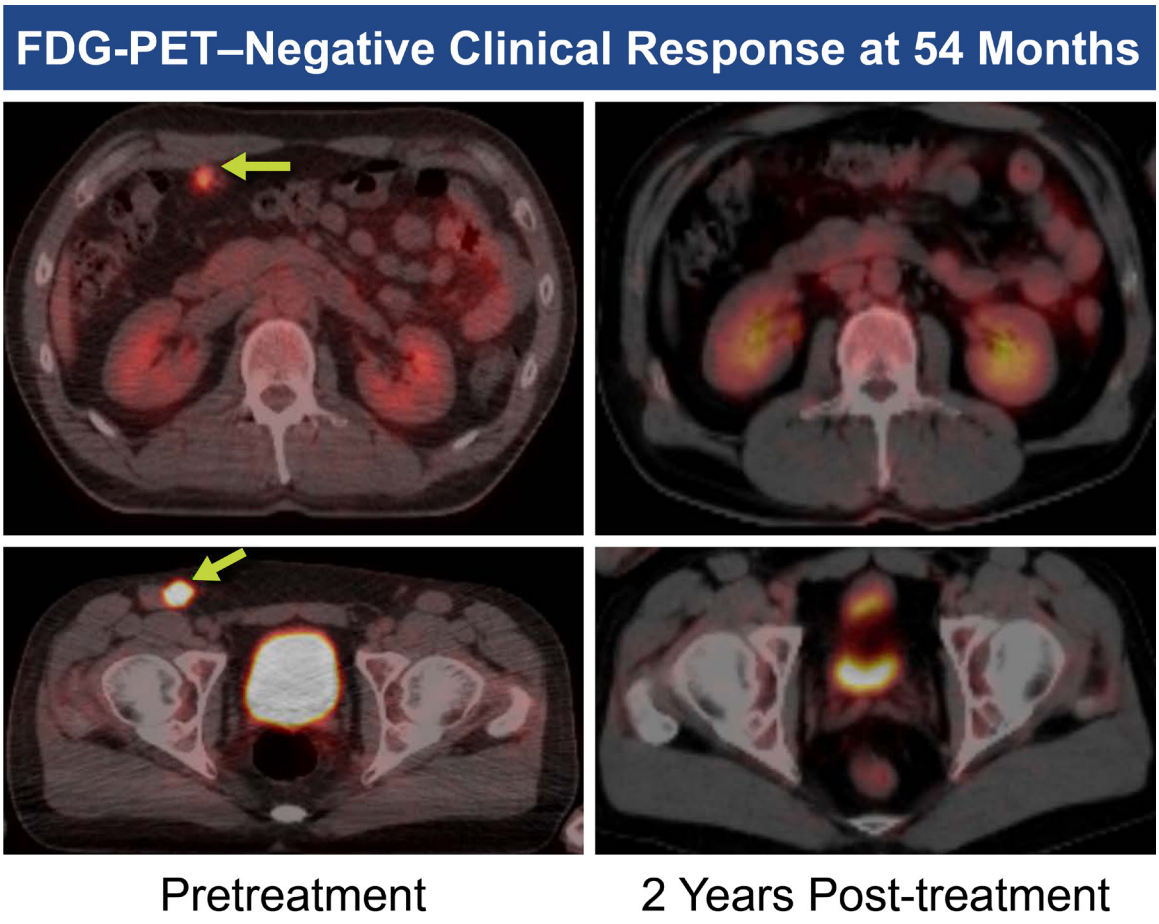

Figure S3. FDG-PET imaging in a complete responder (Patient 4). This 43-year-old male had right groin disease and a right upper quadrant mass prior to TIL treatment. Responses for this patient were identified using methods that prevented RECIST version 1.1 assessments. First complete response was observed 1 month post-treatment; the image depicts FDG-PET-negative clinical response observed 2 years post-treatment. By data cutoff, the complete response was ongoing 54 months post-treatment. FDG-PET, fluorodeoxyglucose-positron emission tomography; RECIST, Response Evaluation Criteria in Solid Tumors; TIL, tumor-infiltrating lymphocyte.

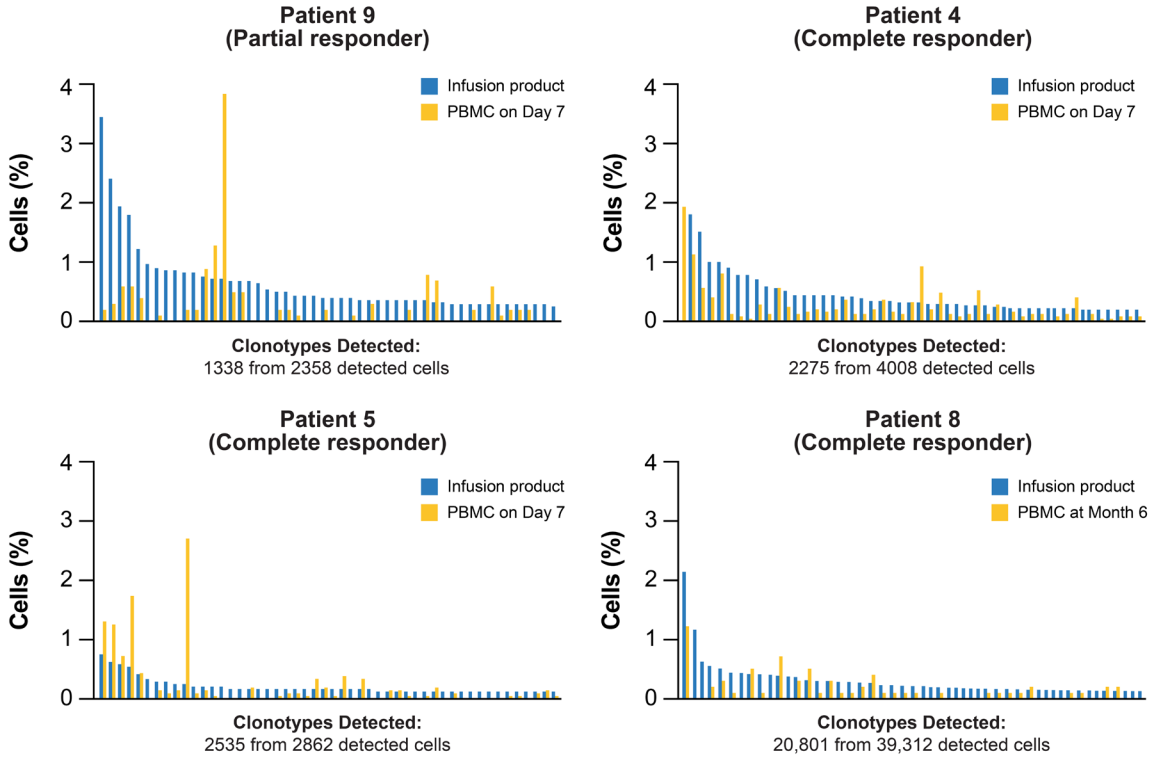

Figure S4. Clonal TCR distribution over time (n=4). Clonotypes were measured by scRNA-Seq. PMBC, peripheral blood mononuclear cell; RNA-Seq, ribonucleic acid sequencing; scRNA-Seq, single-cell ribonucleic acid sequencing; TCR, T-cell receptor.

A

	RECIST-Evaluable Subgroup (n=15)	
	n (%)	95% CI
Overall response rate (CR+PR)	8 (53)	27-79
CR	2 (13)	2-40
PR	6 (40)	16-68
SD	3 (20)	4-48
PD	4 (27)	8-55
Disease control rate (CR+PR+SD)	11 (73)	45-92

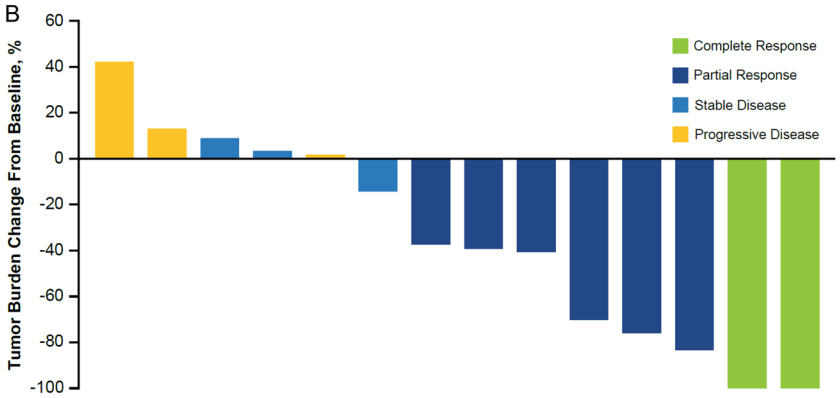

Figure S5. Response outcomes among the RECIST-evaluable subgroup. (A) Best response rates. (B) Depth of responses. Data are presented for 14 patients with detailed tumor measurements; 1 patient with a best overall response of progressive disease did not have any post-treatment target lesion measurements reported (progression determined by observation of new lesions) and hence was not presented in the plot. CR, complete response; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

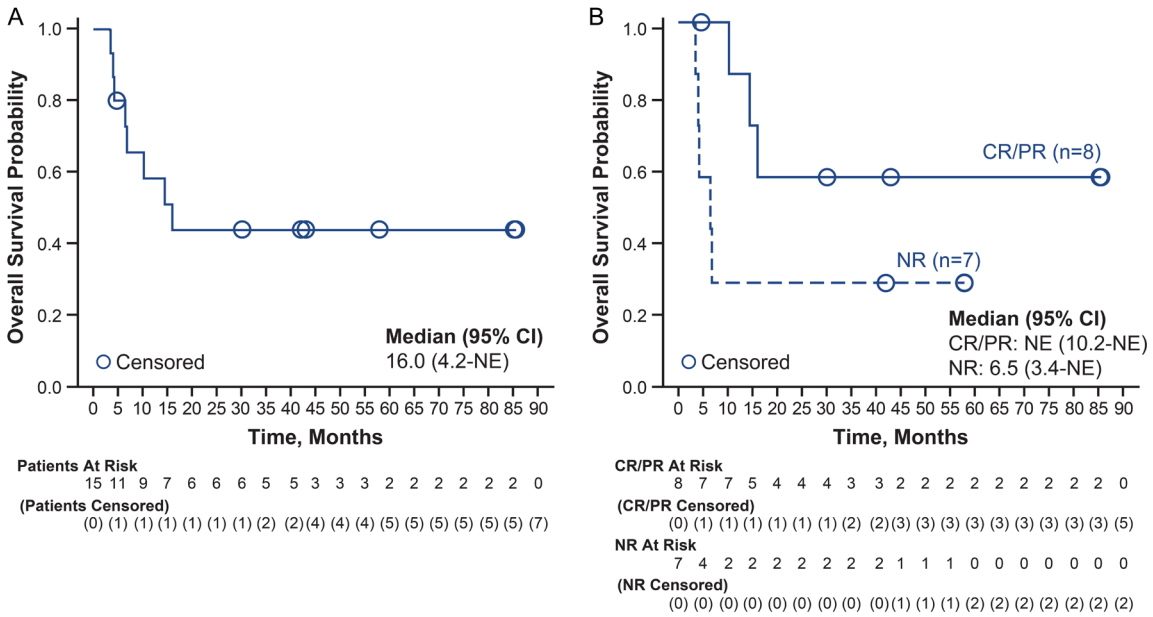

Figure S6. Overall survival among the RECIST-evaluable subgroup. (A) All RECIST-evaluable patients. (B) Overall survival by best response. CR, complete response; NE, not estimable; NR, nonresponder; OS, overall survival; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors.

Table S1. Prophylactic and supportive medication

Drug	Days Required (Inclusive)	Dose Regimen
Dexamethasone	Days -7 to -6[a]	4 mg orally/IV twice daily
Ondansetron	Days -7 to 4	8 mg orally twice daily
GCSF	Day 0 until neutrophil count >1.0×10^9/L	263 µg subcutaneously once daily
Chlorpheniramine	Day 0, 15 minutes prior to TIL infusion	10 mg IV bolus once
Ibuprofen	Days 0 to 4	400 mg orally 3 times/day PRN
Acetaminophen	Days 0 to 4	1 g orally 4 times/day PRN
Ranitidine	Days 0 to 4	150 mg orally 2 times/day
Levofloxacin	Day 0 until neutrophil count >1.0×10^9/L	500 mg orally/IV once daily
Trimethoprim/sulfamethoxazole	Day 0 for 3 months or until T-cell count >1.0×10^9/L	160 mg/800 mg orally twice daily 3 times/week
Acyclovir	Day 0 for 3 months or until T-cell count >1.0×10^9/L	400 mg orally twice daily
Fluconazole	Day 0 for 3 months or until T-cell count >1.0×10^9/L	100 mg orally once daily

[a]If necessary, dexamethasone was also given for 2 days after cyclophosphamide as an antiemetic but had to be discontinued no later than Day -3. GCSF, granulocyte colony stimulating factor; IV, intravenous(ly); PRN, as needed; TIL, tumor-infiltrating lymphocyte.

Table S2. Adverse event lookup table

Adverse Event	Adverse Event Term
Abnormal liver function test	Abnormal liver function test
Acute renal failure	Renal impairment
Atrial fibrillation	Atrial fibrillation
Cardiovascular instability	Cardiovascular instability
Chest infection	Chest infection
Confusion	Confusion
Cough	Cough
Diarrhea	Diarrhea
Dysphasia	Dysphasia
Engraftment syndrome	Engraftment syndrome
Febrile reaction	Pyrexia
Fever	Pyrexia
Fluid accumulation	Edema
Hallucinations	Hallucinations
Hypokalemia	Hypokalemia
Hypotension	Hypotension
Lethargy	Lethargy
Lymphopenia	Lymphopenia
Nausea	Nausea
Neurological deficit	Neurological deficit
Neutropenia	Neutropenia
Edema	Edema
Peripheral edema	Edema
PICC line infection	PICC line infection
PICC line infection-removed	PICC line infection
Pleural effusion	Pleural effusion
Pneumonia	Pneumonia
Pneumonitis	Pneumonitis
Pulmonary congestion	Pulmonary edema
Pulmonary edema	Pulmonary edema
Pyrexia	Pyrexia
Rash	Rash
Renal impairment	Renal impairment
Respiratory problems	Respiratory problems
Respiratory sepsis	Respiratory sepsis
Rigors	Rigors
Seizure	Seizure
Sepsis	Sepsis
Tachycardia	Tachycardia
Tachypnea	Tachypnea
Thrombocytopenia	Thrombocytopenia
Vascular leak	Vascular leak
Vitiligo	Vitiligo
Vomiting	Vomiting
Weight gain	Weight gain
Wheeze	Wheezing
Wheeziness	Wheezing

PICC, peripherally inserted central catheter.

Table S3. Adverse events with onset during the lymphodepleting chemotherapy period

Adverse Event Term, n (%)	All Treated Patients (N=21)
Neutropenia	9 (43)
Nausea	4 (19)
Cardiovascular instability	1 (5)
Chest infection	1 (5)
Lymphopenia	1 (5)
PICC line infection	1 (5)
Pleural effusion	1 (5)
Sepsis	1 (5)
Vomiting	1 (5)

PICC, peripherally inserted central catheter.

Table S4. Adverse events with onset after TIL infusion among patients treated with TILs manufactured from cryopreserved tumor digests

Adverse Event Term, n (%)	Patients (n=4)
Thrombocytopenia	4 (100)
Pyrexia	2 (50)
Rash	2 (50)
Rigors	2 (50)
Hypotension	1 (25)
Renal impairment	1 (25)
Vascular leak	1 (25)
Vitiligo	1 (25)

TIL, tumor-infiltrating lymphocyte.

Table S5. Adverse events with onset after TIL infusion among the prior PD-1i subgroup

Adverse Event Term, n (%)	Prior PD-1i Subgroup (n=12)
Thrombocytopenia	9 (75)
Pyrexia	6 (50)
Rigors	6 (50)
Vascular leak	4 (33)
Chest infection	3 (25)
Neutropenia	3 (25)
Atrial fibrillation	2 (17)
Hypokalemia	2 (17)
Pulmonary edema	2 (17)
Rash	2 (17)
Respiratory sepsis	2 (17)
Tachycardia	2 (17)
Weight gain	2 (17)
Confusion	1 (8)
Hallucinations	1 (8)
Hypotension	1 (8)
Edema	1 (8)
Pleural effusion	1 (8)
Pneumonia	1 (8)
Renal impairment	1 (8)
Tachypnea	1 (8)
Vitiligo	1 (8)
Wheezing	1 (8)

PD-1i, programmed cell death protein 1 inhibitor; TIL, tumor-infiltrating lymphocyte.

Table S6. Demographics and baseline characteristics among the RECIST-evaluable subgroup

	RECIST-Evaluable Subgroup (n=15)
Age (years), median (range)	54 (16-68)
Male, n (%)	11 (73)
Stage IV, n (%)	15 (100)
Time (months) from original diagnosis to TIL treatment, median (range)	29 (8-117)
Disease sites, median (range)	4 (2-10)
M1c disease, n (%)	10 (67)
M1d disease, n (%)	5 (33)
History of brain metastasis, n (%)	6 (40)
Brain metastasis at baseline, n (%)	5 (33)
Baseline brain metastasis irradiated, n (%)	5 (33)
Tumor burden (mm)[a], median (range)	123 (29-281)
LDH, n (%)	
>ULN to ≤2× ULN	3 (20)
>2× ULN	3 (20)
Prior no. of systemic regimens, median (range)	2 (1-5)
Checkpoint inhibitor, n (%)	14 (93)
PD-1 inhibitor	9 (60)
CTLA-4 inhibitor	14 (93)
Dual PD-1/CTLA-4 inhibitor relapsed/refractory	9 (60)
Cytotoxic therapy, n (%)	4 (27)
Radiotherapy, n (%)	7 (47)
Outcome to last prior melanoma therapy[b], n (%)	
Refractory	3 (20)
Relapsed	4 (27)
Progressed with unknown best response	5 (33)
Intolerant	2 (13)
Unknown	1 (7)
BRAF mutation positive, n (%)	7 (47)
BRAF inhibitor ± MEK inhibitor	7 (47)

[a]All 15 patients had tumor burden data available at baseline, respectively, as measured by the sum of diameters of all target lesions (local assessment per RECIST v1.1). [b]Refractory was defined as no response to the prior therapy; relapsed was defined as a response was achieved but the patient's disease subsequently progressed. BRAF, B-raf proto-oncogene; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; LDH, lactate dehydrogenase; MEK, mitogen-activated protein kinase kinase; PD-1, programmed cell death protein 1; RECIST, Response Evaluation Criteria in Solid Tumors; TIL, tumor-infiltrating lymphocyte; ULN, upper limit of normal.