

Original Article

Clinical feasibility and treatment outcomes with nonselected autologous tumor-infiltrating lymphocyte therapy in patients with advanced cutaneous melanoma

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Abstract: Nonselected autologous tumor-infiltrating lymphocytes (TILs) may provide advantages over other treatments for solid tumors, including checkpoint inhibitor-refractory melanoma. This retrospective analysis reports a single-center experience of nonselected autologous TILs derived from digested tumors for compassionate use treatment of advanced cutaneous melanoma, including after programmed cell death protein 1 (PD-1) inhibition. Patients with histologically confirmed metastatic cutaneous melanoma and no standard-of-care treatment options underwent tumor resection for TIL product manufacturing. Patients received lymphodepleting chemotherapy with cyclophosphamide for 2 days and fludarabine for 5 days, followed by a single TIL infusion and post-TIL high-dose interleukin (IL)-2. Safety assessments included clinically significant adverse events (AEs). Efficacy assessments included overall response rate (ORR), complete response (CR) rate, disease control rate (DCR), and overall survival. Between October 2011 and August 2019, 21 patients underwent treatment (median follow-up time, 52.2 months from TIL infusion). Among all treated patients, median age was 45 years, median number of disease sites was 4, 100% had M1c or M1d disease, and 90% received prior checkpoint inhibitor. Twelve patients received TILs after prior PD-1 inhibition. The safety profile among all treated patients and the prior PD-1 inhibitor subgroup was generally consistent with lymphodepletion and high-dose IL-2. No treatment-related deaths occurred. Among all patients, the ORR was 67%, CR rate was 19%, and the DCR was 86%, which was consistent with that observed in the prior PD-1 inhibitor subgroup (58%, 8%, and 75%, respectively). Median overall survival in all treated patients and the prior PD-1 inhibitor subgroup was 21.3 months. In total, 5 patients (24%) had durable ongoing responses (>30 months post-TIL infusion) at data cutoff, and all patients who achieved CR remained alive and disease free. To further illustrate how TIL therapy may integrate into established treatment paradigms, several case studies of patients treated in this series were included. Overall, these data demonstrate that manufacturing of nonselected autologous TILs from tumor digests is feasible and resulted in high rates of durable response in poor-risk patient populations, which may address significant unmet medical need.

Keywords: Tumor-infiltrating lymphocytes, melanoma, immunotherapy, immunology, interleukin-2, T cell, checkpoint inhibition, PD-1 inhibitor, compassionate use

Introduction

In the last decade, immunotherapy, including checkpoint inhibition (eg, programmed cell death protein 1 [PD-1] inhibitors), has revolutionized treatment of advanced melanoma and markedly improved patient outcomes [1-4]. Additional advances resulted from identification and targeted inhibition of driver mutations (eg, *B-raf proto-oncogene [BRAF]*; *mitogen-activated protein kinase kinase [MEK]*) [5-8].

Despite these advances, patients frequently develop treatment resistance and relapse, with 5-year overall survival (OS) between 34-52%. Those who relapse following PD-1 inhibition and, if appropriate, BRAF and/or MEK inhibition, have limited treatment options [9], highlighting significant unmet medical need for new therapeutic approaches.

The intrinsic antitumor activity and broad T-cell receptor (TCR) repertoire of nonselected tumor-

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infiltrating lymphocytes (TILs) make them uniquely suited to address the marked clonal heterogeneity characteristic of solid tumors [9-14]. The promise of TIL therapy has been repeatedly demonstrated over many years in the academic setting. Owing to recent advances in manufacturing, TIL production can be scaled up to treat more patients [15, 16], as evidenced by several ongoing studies of TILs as monotherapy or in combination [4, 17]. In a meta-analysis of 13 studies (n=410), TILs demonstrated durable complete responses (CRs) in patients with advanced melanoma, with an estimated 41% objective response rate [17]. A large phase 2 trial of TIL therapy in patients whose disease had progressed following PD-1 inhibition and, if appropriate, BRAF inhibition, demonstrated clinical utility in this area of high unmet medical need [12]. Toxicity following TIL infusion is largely related to the preparative chemotherapy regimen and post-infusion interleukin (IL)-2 [4, 17-19]. Following the resolution of these anticipated and generally manageable adverse events (AEs) that occur immediately before and after the single TIL infusion, side effects related to TIL are rare [4, 17-19].

Collectively, these findings provide strong rationale for further clinical development of TIL therapy for advanced melanoma. Here, we present a retrospective analysis of a clinical series of nonselected autologous TILs made from digested tumors for the compassionate use treatment of heavily pretreated patients with advanced cutaneous melanoma, including those with prior PD-1 inhibitor exposure. To supplement these findings, clinical narratives from key patient cases are described.

Patients and methods

Compassionate use program design and conduct

This clinical series reflects a single-center experience of TILs administered at The Christie Hospital, Manchester, United Kingdom. As a compassionate use clinical series, the treatment was approved by institutional review board and National Health Service commissioning; post-treatment blood samples were collected and analyzed under a study approved by South Manchester Research Ethics Committee (Ref: 09/H1003/75). Written in-

formed consent was obtained in accordance with the Declaration of Helsinki.

Patients

Although strict eligibility criteria for treatment were not employed, patient selection guidelines were applied in determining suitability of patients to receive TILs. Patients had advanced, progressive cutaneous melanoma, no standard-of-care treatment options, satisfactory performance status and hematological/biochemical indices, and adequate organ function. Patients should not have had prior allogeneic transplant, extensive skeletal irradiation, symptomatic brain metastasis measuring ≥ 10 mm in diameter (treated and stable brain metastases were permitted), prior lymphotoxic therapy within 4 weeks of TIL harvest, concurrent serious infection within 28 days prior to treatment, or steroid use ≤ 3 weeks before treatment, except for physiological replacement doses of steroids. Additional guidelines are provided within [Supplementary Methods](#).

Treatment

Nonselected autologous TILs derived from digested tumor tissue were manufactured under a Medicines and Healthcare Products Regulatory Agency Manufacturing Specials license ([Figure S1](#)). Suitable patients underwent resection of ≥ 1 cm³ of tumor tissue for TIL production. Resected tumor tissue was trimmed to remove necrotic tissue, blood/hemorrhage, fat, and healthy tissue and was subsequently digested enzymatically and mechanically. Digested material was then washed and placed in culture for activation and expansion as previously described [20-22]. Specifically, cells were activated and expanded to a minimum dose of 1×10^9 T cells using anti-CD3 antibodies, irradiated allogeneic peripheral blood mononuclear cells, and IL-2 [20-22]. Expanded TILs were harvested, washed, and concentrated prior to final formulation and release. Quality control review of the final formulation product was performed using assays to identify T-cell dose, viability, phenotype, and functional activity, as well as screen for microbial contamination. Over the course of this clinical series, tumor procurement was modified to include a controlled-rate cryopreservation step immediately following tumor digestion. Seven days prior to TIL infusion,

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patients received intravenous lymphodepleting chemotherapy (cyclophosphamide 60 mg/kg/day on Days -7 and -6 and fludarabine 25 mg/m²/day on Days -5 through -1). On Day 0, TILs were infused followed by high-dose IL-2 (600,000-720,000 IU/kg every 8 hours for ≤12 doses). Patients were hospitalized for treatment and discharged when clinically well and following resolution of any AEs and recovery of cell counts to the satisfaction of the care team.

Patients received prophylactic and supportive medications (Table S1). Other concomitant medication could be given as medically indicated. Steroid use was avoided post-TIL infusion but, if necessary, given at the lowest possible dose and shortest duration.

Outcome assessments and analyses

All outcomes were analyzed retrospectively and descriptively. Safety and efficacy data were extracted from primary source documents and placed into a database for quality control and analysis. AEs and changes in laboratory values were collected during hospitalization. Clinically significant AEs were reported using verbatim terms in clinic/hospital notes; similar terms were summarized together per AE lookup table (Table S2). AEs were tabulated by AE terms and defined as those with onset during the lymphodepletion period or post-TIL infusion. Severity and relatedness of AEs are not presented as this was not systematically documented due to the nature of the compassionate use program, precluding definitive attribution of AEs with any particular treatment component. Deaths that occurred post-TIL infusion were tabulated by time windows in relation to the infusion date (i.e., within 30 days, after 30 days and within 3 months, and >3 months). Laboratory results collected during hospitalization and sporadically during outpatient follow-up were summarized through boxplots by time-points around TIL infusion.

Efficacy outcomes included overall response rate (ORR; combined CR and partial response [PR] rates); disease control rate (DCR; combined CR, PR, and stable disease [SD] rates); OS; change in tumor burden (change from baseline to post-baseline nadir); and time to response (time from TIL infusion to first CR or PR). Disease assessments were conducted per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (v1.1) [23] when possible. Otherwise, responses were assessed per stan-

dard clinical disease assessment methods, including positron-emission tomography (PET), computed tomography (CT) without detailed tumor volume measurements, or clinical monitoring (e.g., history and physical examination; laboratory assessments). The standard assessment methods prevented post hoc RECIST v1.1 response determinations. Rigorous confirmation of disease response required by RECIST v1.1 was not applied due to the inconsistent schedule and manner of longitudinal disease assessment utilized in this compassionate use program. Response rates were tabulated, and 95% exact confidence intervals (CIs) were calculated using the Clopper-Pearson method. Tumor burden was measured by the sum of target lesion diameters. Kaplan-Meier (KM) plots, estimates, and 2-sided 95% CIs were generated for OS (derived as the time from TIL infusion date to the date of death from any cause). Patients who were alive at the time of analysis were censored at the last date known to be alive.

Three separate outcome analyses were performed. The first was a safety and efficacy analysis performed on all treated patients. Safety and efficacy analysis was also performed on patients with prior PD-1 inhibitor exposure. Finally, efficacy was assessed on the subgroup of patients with RECIST-evaluable disease. The RECIST-evaluable subgroup comprised all patients with pre- and post-treatment disease assessments collected using CT/magnetic resonance imaging scans, which included quantitative tumor burden measurements in accordance with RECIST version 1.1.

Product assessments

The cell composition, T-cell phenotype, and activation/exhaustion state of the TIL products were assessed. Additional analyses were performed on the final product to profile the CD4/CD8 T-cell ratio and TCR repertoire and clonality. All methods are described within the [Supplementary Methods](#). Assessments were performed using available patient samples for validated analysis; as such, the sample sizes vary by assay type.

Results

All treated patients

Between October 2011 and August 2019, 21 patients with advanced cutaneous melanoma

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Table 1. Demographics and baseline characteristics among all treated patients (N=21) and the prior PD-1 inhibitor subgroup (n=12)

	Prior PD-1 Inhibitor Subgroup (n=12)	All Treated Patients (N=21)
Age (years), median (range)	55 (33-64)	45 (16-68)
Male, n (%)	7 (58)	15 (71)
Stage IV, n (%)	12 (100)	21 (100)
Time (months) from original diagnosis to TIL treatment, median (range)	36 (8-177)	39 (8-177)
Disease sites, median (range)	4 (2-10)	4 (2-10)
M1c disease, n (%)	9 (75)	14 (67)
M1d disease, n (%)	3 (25)	7 (33)
History of brain metastasis, n (%)	4 (33)	8 (38)
Brain metastasis at baseline, n (%)	3 (25)	7 (33)
Baseline brain metastasis irradiated, n (%)	3 (25)	7 (33)
Tumor burden (mm) ^a , median (range)	123 (51-169)	100 (13-281)
LDH, n (%)		
>ULN to ≤2× ULN	4 (33)	7 (33)
>2× ULN	2 (17)	3 (14)
Prior no. of systemic regimens, median (range)	3 (1-9)	2 (1-9)
Checkpoint inhibitor, n (%)	12 (100)	19 (90)
PD-1 inhibitor	12 (100)	12 (57)
CTLA-4 inhibitor	12 (100)	19 (90)
Dual PD-1/CTLA-4 inhibitor relapsed/refractory	12 (100)	12 (57)
Cytotoxic therapy, n (%)	2 (17)	6 (29)
Radiotherapy, n (%)	6 (50)	11 (52)
Outcome to last prior melanoma therapy ^b , n (%)		
Refractory	2 (17)	4 (19)
Relapsed	3 (25)	6 (29)
Progressed with unknown best response	6 (50)	8 (38)
Intolerant	0	2 (10)
Unknown	1 (8)	1 (5)
<i>BRAF</i> mutation positive, n (%)	6 (50)	11 (52)
<i>BRAF</i> inhibitor ± MEK inhibitor	6 (50)	11 (52)

^aEleven of 12 and 20 of 21 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). ^bRefractory 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; PD-1, programmed cell death protein 1; RECIST, Response Evaluation Criteria in Solid Tumors; TIL, tumor-infiltrating lymphocyte; ULN, upper limit of normal.

underwent treatment. TILs were manufactured for 4 patients using cryopreserved tumor digests. All patients completed lymphodepleting chemotherapy; the median number of TILs infused was 32×10^9 cells (range, 8×10^9 - 63×10^9), and the median number of IL-2 doses was 8 (range, 4-11). Patients were hospitalized for a median of 10 days (range, 7-15) following TIL treatment. By the analysis cutoff date (December 31, 2019), patients had a median

follow-up time of 52.2 months (range, 4.6-98.8) from the TIL infusion date.

Among all treated patients, 67% had M1c disease, and 33% had M1d disease (**Table 1**). Median baseline tumor burden was 100 mm (n=20; range, 13-281). Serum lactate dehydrogenase (LDH) levels were elevated above upper limit of normal in 48% of patients and by >2× upper limit of normal in 14%. The median num-

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Table 2. Adverse events with onset after TIL infusion among all treated patients (N=21)

Adverse Event Term, n (%)	All Treated Patients (N=21)
Thrombocytopenia	13 (62)
Pyrexia	12 (57)
Rigors	9 (43)
Neutropenia	6 (29)
Tachycardia	6 (29)
Pulmonary edema	5 (24)
Vascular leak	5 (24)
Rash	4 (19)
Atrial fibrillation	3 (14)
Cardiovascular instability	3 (14)
Chest infection	3 (14)
Edema	3 (14)
Confusion	2 (10)
Hypokalemia	2 (10)
Hypotension	2 (10)
Neurological deficit	2 (10)
Renal impairment	2 (10)
Respiratory sepsis	2 (10)
Seizure	2 (10)
Sepsis	2 (10)
Vitiligo	2 (10)
Weight gain	2 (10)
Wheezing	2 (10)
Cough	1 (5)
Diarrhea	1 (5)
Dysphasia	1 (5)
Engraftment syndrome	1 (5)
Hallucinations	1 (5)
Lethargy	1 (5)
PICC line infection	1 (5)
Pleural effusion	1 (5)
Pneumonia	1 (5)
Pneumonitis	1 (5)
Respiratory problems	1 (5)
Tachypnea	1 (5)

PICC, peripherally inserted central catheter; TIL, tumor-infiltrating lymphocyte.

ber of prior systemic regimens was 2 (range, 1-9; mean, 3); 90% of patients had prior checkpoint inhibitor therapy (cytotoxic T lymphocyte-associated protein 4 [CTLA-4] inhibitor, 90%; PD-1 inhibitor, 57%; both PD-1 inhibitor and CTLA-4 inhibitor, 57% [38% sequentially and 19% concurrently]). Eleven patients (52%) had tumors with *BRAF* mutations; of these patients,

all had failed prior BRAF inhibitor alone or in combination with a MEK inhibitor.

The most commonly reported AEs (>1 patient) during the lymphodepletion period were neutropenia (43%) and nausea (19%; [Table S3](#)). The most frequently reported AEs ($\geq 25\%$ of patients) post-TIL infusion were thrombocytopenia (62%), pyrexia (57%), rigors (43%), neutropenia (29%), and tachycardia (29%; [Table 2](#)), and were primarily lymphodepleting chemotherapy- and IL-2-related toxicities [19]. Two patients experienced vitiligo. Similar AEs were observed among patients with TILs manufactured from cryopreserved tumor digests ([Table S4](#)). No unexpected serious AEs were reported. AEs were generally managed supportively and by IL-2 discontinuation.

Seizures occurred in 2 patients, both with brain metastasis at time of treatment. For 1 patient, seizure occurred 16 days post-infusion. A CT scan confirmed the presence of pre-existing brain metastasis and a new finding of increased edema around the metastasis following recent stereotactic radiotherapy to the site. The other patient, Patient 2, a 16-year-old further described below, had a seizure associated with fever 2 days post-TIL infusion. Both cases of seizure were managed supportively with levetiracetam and resolved without sequelae or recurrence.

A trend towards reduced blood counts in all lineages was observed during the lymphodepletion period ([Figure S2](#)). Cell counts and hemoglobin levels generally reached nadirs approximately 1-4 days post-TIL infusion, with recovery to baseline levels approximately 7 days post-infusion.

No treatment-related deaths occurred. Ten patients (48%) died prior to data cutoff (1 death on Day 90 shortly after progressive disease and 9 deaths >3 months after infusion). Of these, 4 deaths were attributed to progressive disease and 1 was possibly attributed, in part, to AEs caused by subsequent therapy given for progressive disease. The remaining 5 patients had documented progressive disease prior to death but the specific causes of death were not reported.

Among all treated patients, the ORR was 67% (95% CI, 43-85), including a CR rate of 19%

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Table 3. Best response rates among all treated patients (N=21) and the prior PD-1 inhibitor subgroup (n=12)

	Prior PD-1 Inhibitor Subgroup (n=12)		All Treated Patients ^a (N=21)	
	n (%)	95% CI	n (%)	95% CI
Overall response rate (CR+PR)	7 (58)	28-85	14 (67)	43-85
CR	1 (8)	0-38	4 (19)	5-42
PR	6 (50)	21-79	10 (48)	26-70
SD	2 (17)	2-48	4 (19)	5-42
PD	3 (25)	5-57	3 (14)	3-36
Disease control rate (CR+PR+SD)	9 (75)	43-95	18 (86)	64-97

^aIncludes 2 patients counted as responders who had dabrafenib and MEK inhibitor-refractory disease whose disease was unequivocally progressing on combination therapy prior to TIL therapy and who received post-infusion dabrafenib to prevent tumor flare. CR, complete response; MEK, mitogen-activated protein kinase kinase; PD, progressive disease; PD-1, programmed cell death protein 1; PR, partial response; SD, stable disease.

(95% CI, 5-42), and a DCR of 86% (95% CI, 64-97; **Table 3**). The median time from TIL infusion to first response was 1.7 months (range, 1-12). In total, 6 patients were not part of the RECIST-evaluable subgroup and were instead followed by PET, CT without detailed tumor burden measurements, and clinical monitoring. Responses were also observed in these patients, including best responses of CR and PR in 2 and 3 patients, respectively. Fluorodeoxyglucose-PET imaging pre- and post-treatment is shown for 1 of the complete responders not followed in the RECIST-evaluable subgroup in [Figure S3](#).

Notwithstanding small sample sizes for certain subgroups, responses were generally consistent across covariates, including age, number of disease sites, number of prior lines of therapy, prior BRAF, MEK, or PD-1 inhibitor, baseline brain metastasis, and baseline tumor burden (**Figure 1**). Patients with baseline brain metastases (n=7) and those with TILs manufactured from cryopreserved tumor digests (n=4) achieved ORRs of 71% (29% CR) and 75% (25% CR), respectively, which were generally consistent with those observed in all treated patients (**Figure 1**).

As of the data cutoff date, 5 of 21 patients (24%) had durable ongoing responses of >30 months post-TIL infusion. All 4 patients who achieved a CR remained alive and disease free (range of 30-85 months of follow-up; **Figure 2**). Median OS for all treated patients was 21.3 months (95% CI, 6.8-not estimable) and the KM estimate of the 24-month OS rate was 48% (95% CI, 24-68; **Figure 3**).

TIL products were primarily composed of T cells with undetectable quantities of monocytes, B cells, or residual melanoma cells (**Figure 4A**). Products tended to comprise predominantly CD8+ T cells with a primarily effector memory phenotype (**Figure 4B, 4C**). The differential expression of cell surface activation and exhaustion markers on both CD4+ and CD8+ cells supported an activated rather than an exhausted T-cell phenotype [24, 25], with very few cells exhibiting coexpression of PD-1, TIM-3, and LAG-3 (**Figure 4D-F**). Single-cell RNA sequencing on a limited number of patient samples revealed product-associated clones that were recoverable at up to 4% frequency from peripheral circulation 7 days following infusion ([Figure S4](#)). In 1 complete responder, several product-associated clones were persistent in circulation 6 months post-infusion.

Prior PD-1 inhibitor subgroup

Twelve patients received TILs after prior PD-1 inhibition, all of whom had disease that was also relapsed after or primarily refractory to CTLA-4 inhibition either in combination or in sequence with PD-1 blockade. The median number of TILs infused was 32×10^9 cells (range, 8×10^9 - 53×10^9), and the median number of IL-2 doses was 8 (range, 6-9). Median follow-up time was 45.5 months (range, 4.6-59.3) from the TIL infusion date for this subgroup. Patients had higher baseline tumor burden (median, 123 [range, 51-169]) and were more heavily pretreated (**Table 1**).

Generally, the safety profile of the prior PD-1 inhibitor subgroup was consistent with that of

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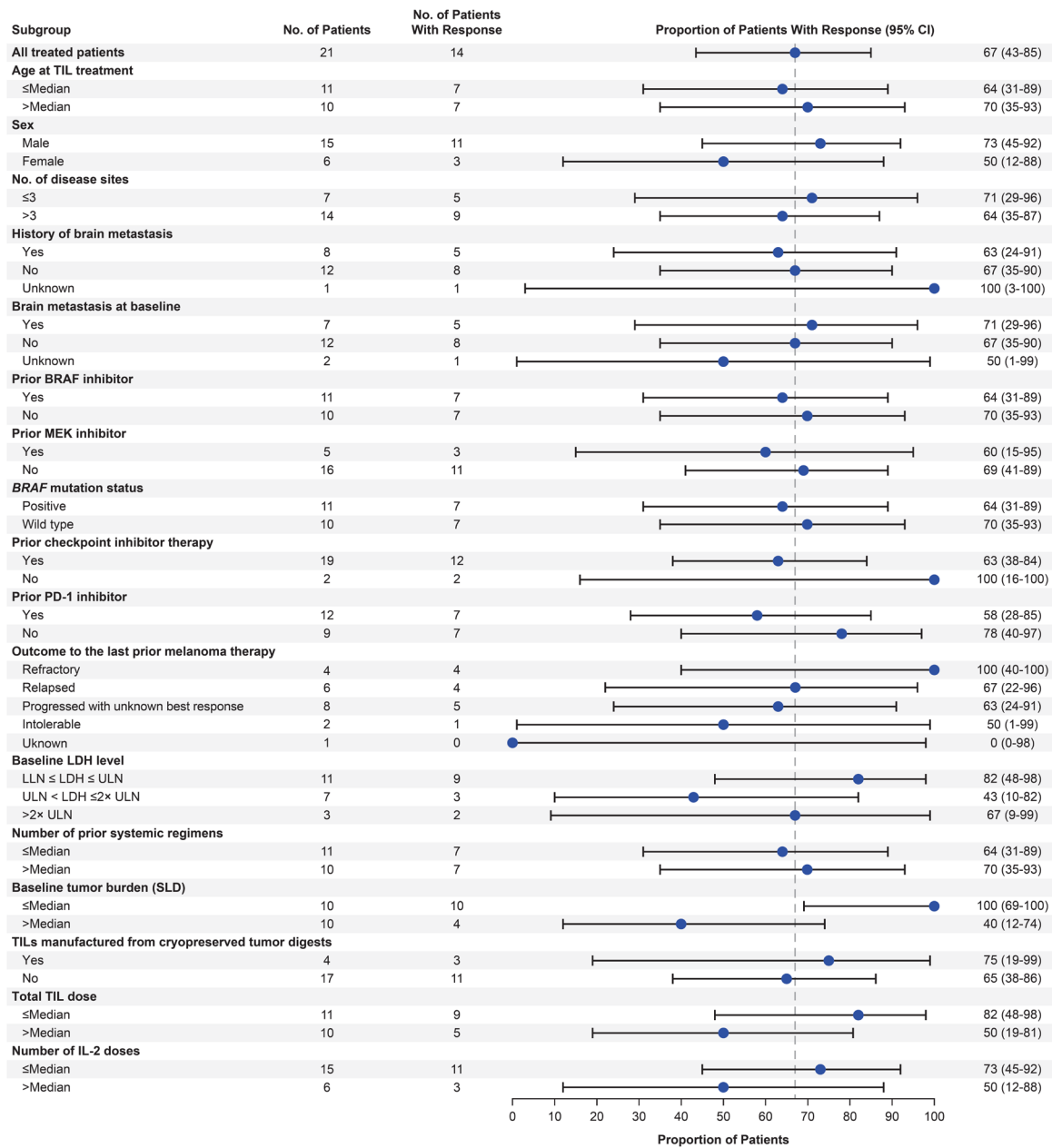


Figure 1. Response rates by subgroup in all treated patients (N=21). Data include 2 patients counted as responders who had dabrafenib and MEK inhibitor-refractory disease whose disease was unequivocally progressing on combination therapy prior to TIL therapy and who received post-infusion dabrafenib to prevent tumor flare. BRAF, B-raf proto-oncogene; IL-2, interleukin-2; LDH, lactate dehydrogenase; LLN, lower limit of normal; MEK, mitogen-activated protein kinase kinase; ORR, overall response rate; PD-1, programmed cell death protein 1; SLD, sum of target lesion diameters; TIL, tumor-infiltrating lymphocyte; ULN, upper limit of normal.

the all-treated population. Neutropenia was the most commonly reported AE during the lymphodepletion period (5 [42%]), though nausea, peripherally inserted central catheter infection, and sepsis were also observed (each in 1 patient [8%]). The most frequently reported AEs ($\geq 25\%$ of patients) post-TIL infusion were thrombocytopenia (75%), pyrexia (50%), rigors

(50%), vascular leak (33%), chest infection (25%), and neutropenia (25%; [Table S5](#)). AEs were managed supportively and were mostly toxicities related to lymphodepleting chemotherapy and IL-2 [19].

Among those who received prior PD-1 inhibitor, the ORR was 58% (95% CI, 28-85), including an

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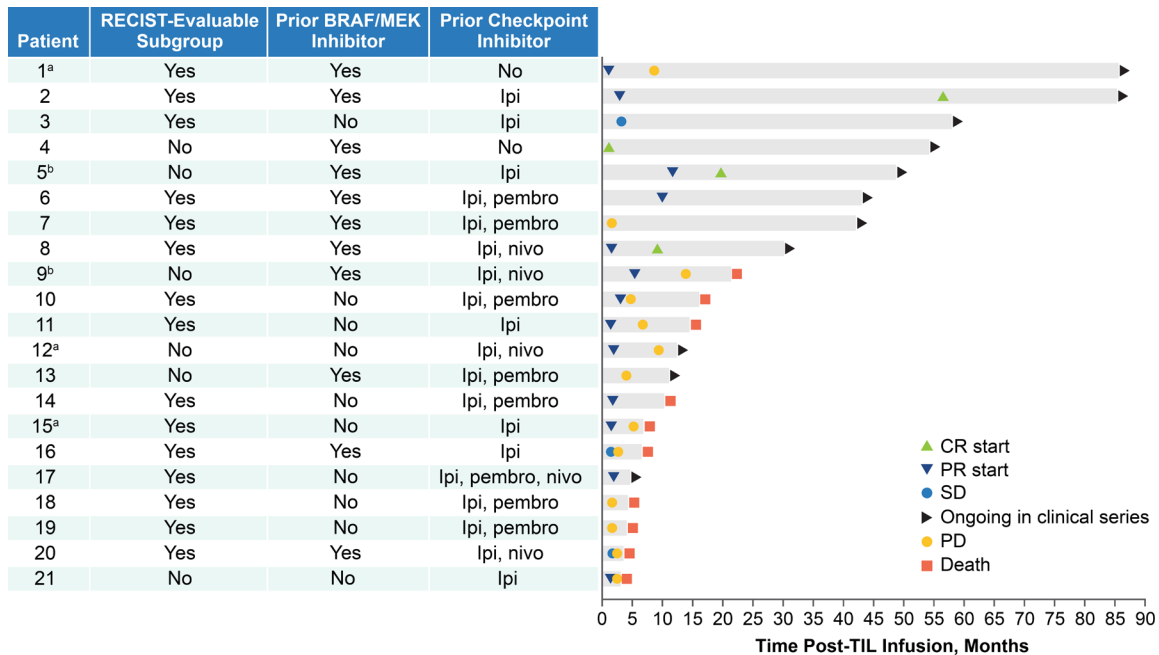


Figure 2. Time to response and survival status by patient. ^aPatient 1 received a checkpoint inhibitor at the time of disease progression; patients 15 and 12 received high-dose IL-2 and checkpoint inhibitor, respectively, prior to documented disease progression. ^bPatients 5 and 9 had unequivocally BRAF+MEK-refractory melanoma immediately prior to TIL treatment but were continued on dabrafenib, with brief interruptions for tumor harvest and TIL infusion, to prevent tumor flare upon discontinuation. Patient 5 was treated with dabrafenib for 3 months following TIL infusion, at which point the dabrafenib was stopped. Patient 9 achieved a PR that lasted approximately 14 months from TIL infusion during which time dabrafenib was continued. BRAF, B-raf proto-oncogene; CR, complete response; IL-2, interleukin-2; ipi, ipilimumab; MEK, mitogen-activated protein kinase kinase; nivo, nivolumab; PD, progressive disease; pembro, pembrolizumab; PR, partial response; TIL, tumor-infiltrating lymphocyte.

8% CR rate (95% CI, 0-38), and the DCR was 75% (95% CI, 43-95; **Table 3**). The median time from TIL infusion to first response was 1.8 months (range, 1.5-10.0). Nine of the patients who received prior PD-1 inhibitor had RECIST-based response assessments, and their outcomes were similar to the overall prior PD-1 inhibitor subgroup (ORR, 56% [95% CI, 21-86]; CR rate, 11% [95% CI, 0-48]). At data cutoff, 2 of 12 patients (17%) had durable ongoing responses (>30 months after TIL infusion; **Figure 2**). Median OS in the prior PD-1 inhibitor subgroup was 21.3 months (95% CI, 4.0-not estimable; **Figure 3**), and the 24-month OS rate was 39% (95% CI, 10-68).

RECIST-evaluable subgroup

The RECIST-evaluable subgroup comprised 15 patients who underwent quantitative disease assessment pre- and post-treatment, which included detailed tumor volume measurements, per RECIST v1.1. Patient characteristics for the RECIST-evaluable subgroup were similar to those observed in all treated patients (**Table S6**).

Among the RECIST-evaluable subgroup, the ORR was 53% (95% CI, 27-79), including a CR rate of 13% (95% CI, 2-40); the DCR was 73% (95% CI, 45-92; **Figure S5A**). Of 14 patients with detailed post-treatment tumor measurements, 8 demonstrated marked tumor regression (**Figure S5B**). The median time from TIL infusion to first response was 1.7 months (range, 1-10). Median OS was 16.0 months (95% CI, 4.2-not estimable), and the 24-month OS rate was 44% (95% CI, 18-67; **Figure S6A**). Median OS was not reached among patients in the RECIST-evaluable subgroup who achieved a response (n=8; 95% CI, 10.2-not estimable) and was 6.5 months among nonresponders (n=7; 95% CI, 3.4-not estimable; **Figure S6B**).

TIL therapy in context of standard clinical management: hypothesis-generating case studies

Tumor resection for TIL manufacture before PD-1 inhibitor treatment with TIL product administration after PD-1 inhibitor failure (n=2): Two patients (Patients 6 and 7) had tumors resected for TIL harvest prior to receiving PD-1 inhibitor therapy (**Figure 5**). Following resection,

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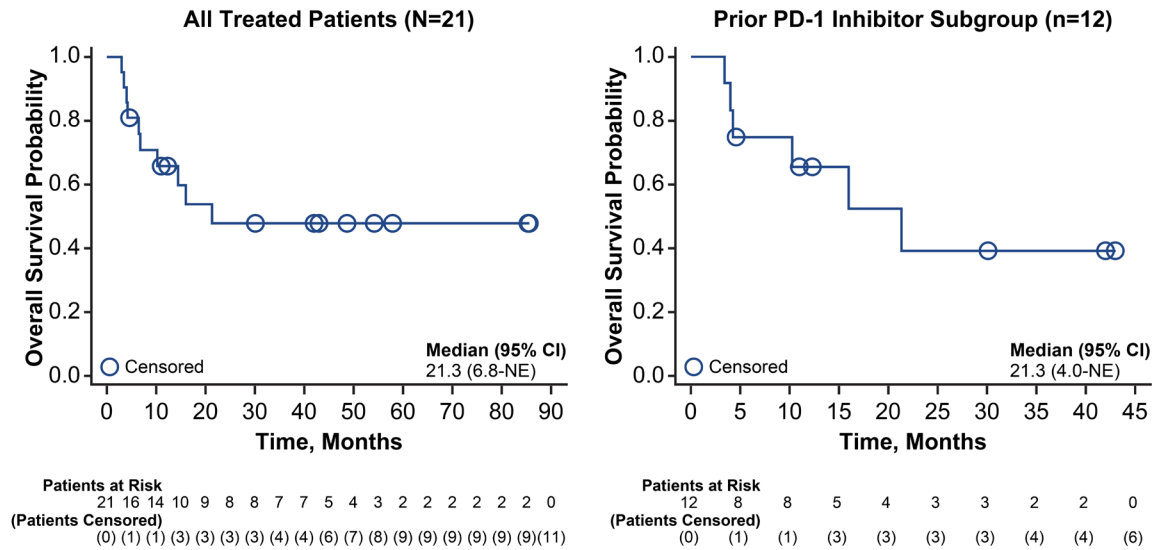


Figure 3. Overall survival among all treated patients (N=21) and the prior PD-1 inhibitor subgroup (n=12). NE, not estimable; OS, overall survival; PD-1, programmed cell death protein 1.

TIL manufacturing was initiated, and cells were washed and frozen after outgrowth (Figure S1) as the patient underwent treatment with PD-1 inhibitor. Upon disease progression, the rapid expansion process was initiated, product manufacture was completed, and the product was infused.

Patient 6, a 45-year-old female with *BRAF*-mutated melanoma, received vemurafenib followed by ipilimumab. While on ipilimumab, the patient presented with hypophysitis, requiring hormone replacement with glucocorticoids. After no response to ipilimumab, the patient had tumor resection for potential TIL manufacture and treatment. Following tumor harvest, the patient went on to receive dabrafenib with trametinib, and later pembrolizumab. Approximately 34 months after tumor harvest and cryopreservation of the TIL manufacturing intermediate, the cells were thawed, rapid expansion was performed, and the patient received the TIL infusion. During the TIL treatment period, the patient experienced short-term cytopenias, confusion, and pneumonia, and recovered with supportive care. Despite tumor harvest for product manufacture occurring prior to PD-1 administration, the product retained antitumor activity. The patient achieved a durable PR to the TIL product administered and remained in an ongoing PR for 42 months at the time of the data cutoff, with no further therapy.

Patient 7, a 34-year-old male, received vemurafenib and achieved a best response of stable disease. The patient then underwent resection for TIL manufacture followed by sequential treatment with ipilimumab, pembrolizumab, and dabrafenib, with disease progression following each therapy. Approximately 22 months after tumor harvest and cryopreservation of the manufacturing intermediate, rapid expansion was performed and the patient received the TIL infusion that was accompanied by low-grade, short-term pneumonia. After no clinical response to TILs, the patient received dabrafenib, followed by nivolumab, then radiation therapy of the neck while continuing nivolumab. Notably, the patient presented with colitis and pneumonitis while on nivolumab, which was managed by reducing dosing frequency to every 6 weeks. At the time of data cutoff, the patient continued to receive nivolumab every 6 weeks and had experienced neither disease progression nor recurrence of colitis or pneumonitis.

These cases illustrate that TILs may retain anti-tumor activity despite being collected early in the course of treatment and before administration of immunomodulatory agents, such as PD-1 and CTLA-4 inhibitors. This opens the possibility of harvesting and banking tumors for patients who have not yet failed standard therapies. Such a strategy would significantly reduce complexity and urgency associated with

Nonselected TIL therapy in patients with advanced melanoma

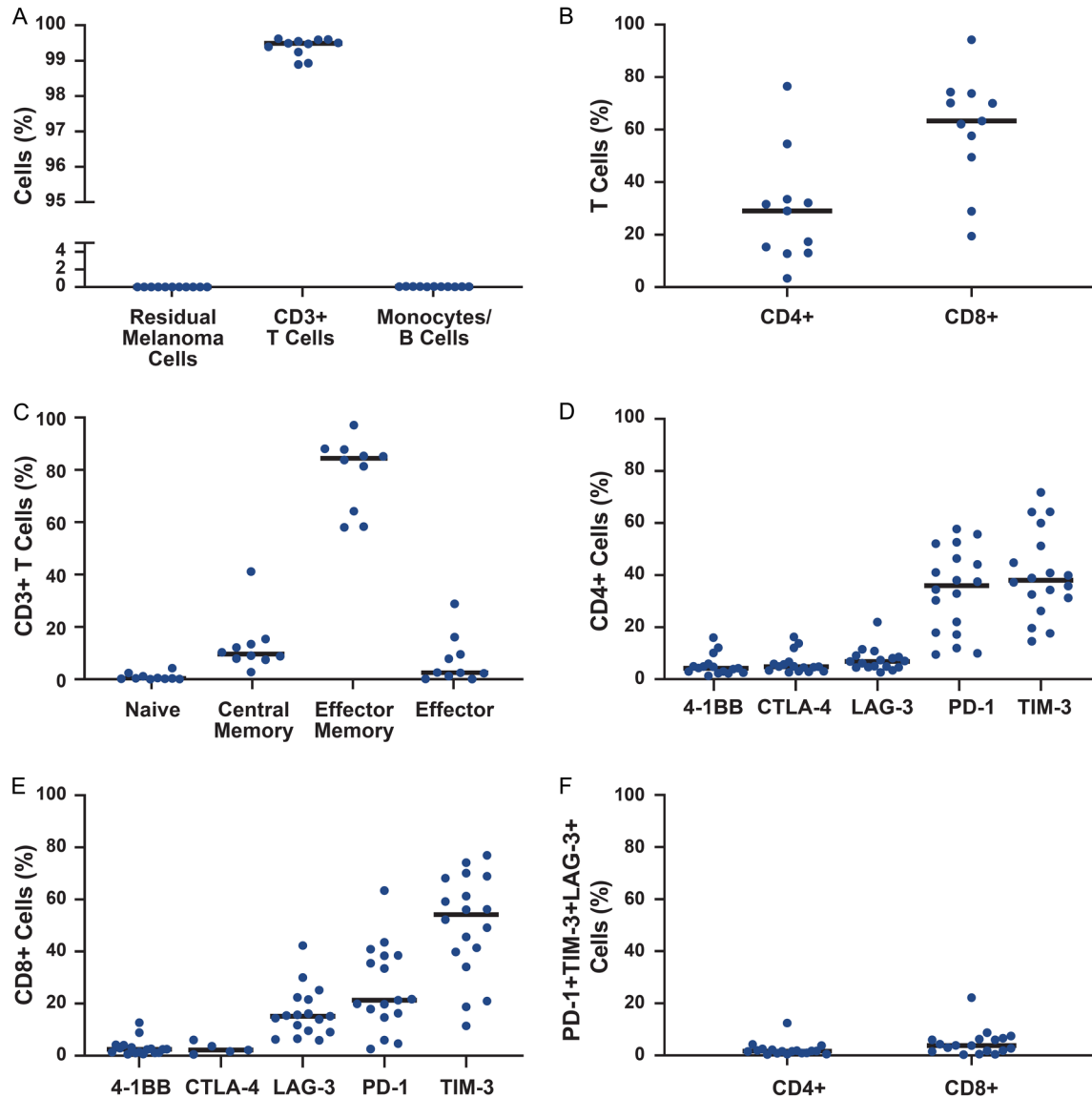


Figure 4. Final TIL product characteristics. (A) Analysis of cell composition in final product (n=11). Cell subsets were defined as follows: monocytes, CD14+; B cells, CD19+; residual melanoma cells, melanoma markers (CD146, MCAM, MCSF, CD228). (B) Distribution of CD4+ and CD8+ T cells in final product (n=11). (C) Phenotype of T cells in final product (n=10). Cell subsets were defined as follows: naive, CD62L+CD45RO-; central memory, CD62L+CD45RO+; effector memory, CD62L-CD45RO+; and effector, CD62L-CD45RO-. (D) Expression of activation and exhaustion markers on final product (n=18) CD4+ T cells and (E) CD8+ T cells. (F) Exhausted T-cell phenotype, defined as PD-1+TIM-3+LAG-3+ cells. CTLA-4, cytotoxic T-lymphocyte-associated protein 4; LAG-3, lymphocyte activation gene 3; MCAM, melanoma cell adhesion molecule; MCSF, melanoma-associated chondroitin sulfate proteoglycan; PD-1, programmed cell death protein 1; TIM-3, T-cell immunoglobulin and mucin-domain containing protein 3; TIL, tumor-infiltrating lymphocyte.

scheduling and performing tumor harvest, TIL product manufacturing, and administration once the patient's disease has progressed on all approved drugs. Tumor harvests could even occur at the time of standard-of-care procedures, like at the time of definitive surgical resection for localized disease, thus eliminat-

ing the need for an additional surgery only for TIL production.

Coadministration of TILs with dabrafenib (n=2): Treatment of *BRAF*-mutated melanoma with targeted *BRAF* ± *MEK* inhibitors is a mainstay of melanoma therapy [5, 7]. Development of

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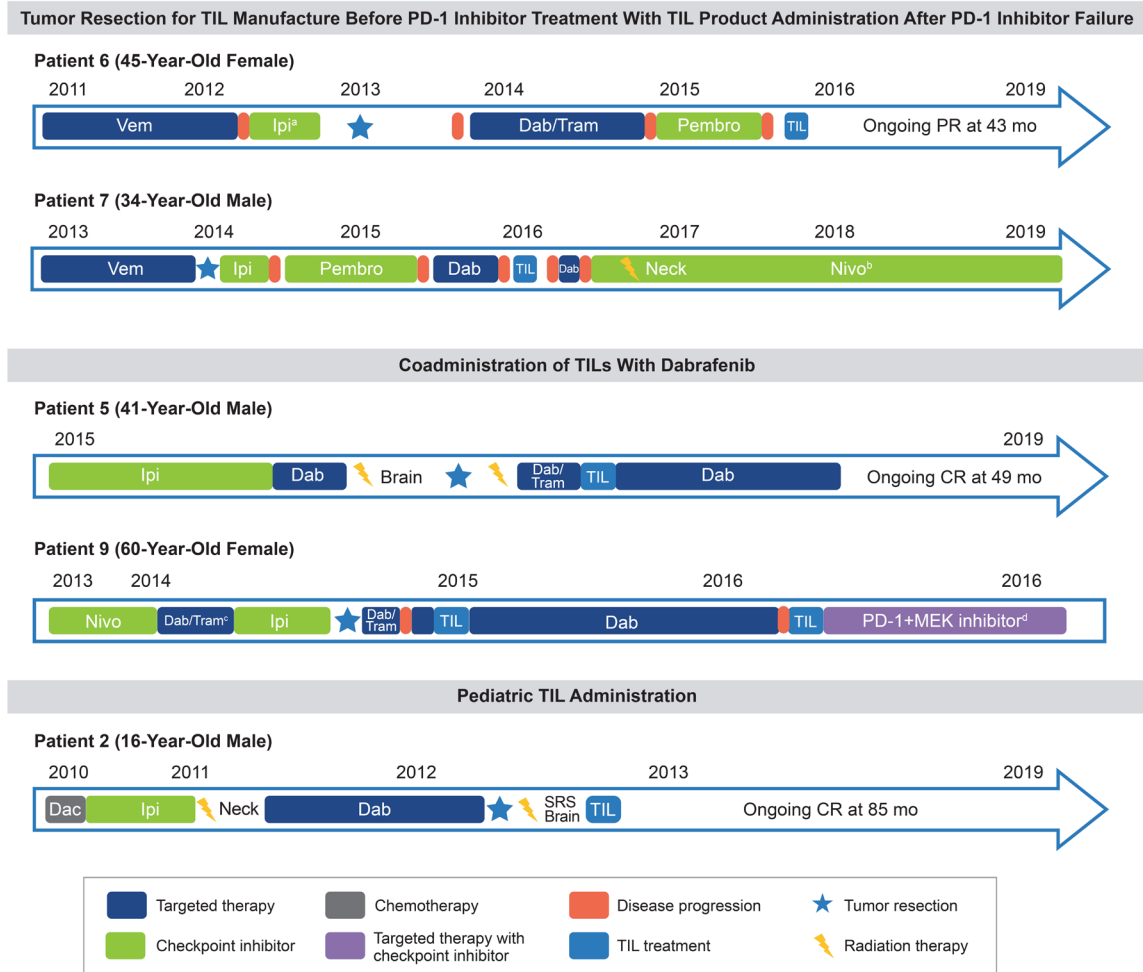


Figure 5. Hypothesis-generating patient cases. The starting event for each patient's timeline is the initiation of first-line therapy. ^aPatient presented with hypophysitis while on ipilimumab, requiring hormone replacement with glucocorticoids; no pembrolizumab-associated toxicity was observed. ^bPatient presented with colitis/pneumonitis while on nivolumab, which was managed by reducing nivolumab frequency (given every 6 weeks). ^cCombination dabrafenib/trametinib required 50% dose reduction after 3 weeks due to fever and cholecystitis. ^dPatient went on to receive PD-1 inhibitor with MEK inhibitor. The patient died 21 months after initial TIL infusion, with a combination of progressive disease and possible pneumonitis caused by PD-1 and MEK inhibitors. Dac, dacarbazine; Dab, dabrafenib; Ipi, ipilimumab; MEK, mitogen-activated protein kinase; Nivo, nivolumab; PD-1, programmed cell death protein 1; Pembro, pembrolizumab; SRS, stereotactic radiosurgery; TIL, tumor-infiltrating lymphocyte; Tram, trametinib; Vem, vemurafenib.

resistance to such therapy, however, is nearly universal and can sometimes be associated with aggressive tumor growth at the time of treatment discontinuation [26]. To prevent such tumor flare, 2 patients in this clinical series who had developed progressive disease while on combination dabrafenib and MEK inhibitor therapy were continued on dabrafenib during TIL treatment, with brief interruptions for tumor harvest for TIL product manufacture and subsequent infusion (**Figure 5**).

Patient 5, a 41-year-old male, had progressed on 3 prior lines of therapy, including ipilimum-

ab, and was progressing on dabrafenib with metastases to the liver, brain, and lung at the time of TIL treatment. Dabrafenib was interrupted approximately 5 weeks prior to liver resection for TIL harvest. The patient restarted dabrafenib, and trametinib was added after surgery while the product was being manufactured. Both agents were held prior to lymphodepleting chemotherapy and TIL administration. Following TIL infusion, the patient received high-dose IL-2 and experienced increasingly severe fevers (>40°C) and rigors, necessitating cessation after 7 doses. Thereafter, the patient had rapid and sustained lymphocyte engraftment.

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Dabrafenib monotherapy was restarted approximately 2 weeks after TIL infusion to prevent rapid tumor growth and was continued for 3 months, at which point it was stopped given the patient was responding post TIL therapy. There were no new or unusual safety signals observed during coadministration of TILs with dabrafenib. Off all therapy, the patient achieved a CR at 20 months after TIL infusion, and remained in durable CR at time of data cutoff.

Patient 9, a 60-year-old female, had progressed on nivolumab, combination dabrafenib/trametinib (which required 50% dose reduction due to fever and cholecystitis), and ipilimumab. At the time of TIL administration, she had metastases to lung, pleura, and bone. Dabrafenib and trametinib were stopped for tumor resection and were resumed to prevent tumor flare during TIL manufacture. While awaiting product generation, the patient's disease continued to progress. Dabrafenib and trametinib were briefly held again for TIL treatment, which was accompanied by fever and rigors and fluid retention requiring diuresis. Despite unequivocal resistance to dabrafenib, it was resumed to mitigate tumor flare. The patient achieved a PR that lasted approximately 14 months from TIL infusion, during which time dabrafenib was continued. Similar to Patient 5, there were no new or unusual safety signals observed during coadministration of TILs with dabrafenib. At 14 months post-TIL infusion, the patient had disease progression of pleural metastasis, prompting discontinuation of dabrafenib and TIL retreatment. Despite an initial response, the patient went on to receive PD-1 inhibitor with MEK inhibitor. The patient died 21 months after initial TIL infusion, with a combination of progressive disease and possible pneumonitis caused by PD-1 and MEK inhibitors.

These 2 cases provide evidence that targeted BRAF/MEK inhibition may continue with brief interruptions for tumor harvest and TIL administration without interfering with TIL activity. As emerging clinical data suggest sequencing targeted therapy after failure of frontline immunotherapy in *BRAF*-mutated melanoma [27], such patients will likely be considered for TIL therapy while their disease is progressing on BRAF/MEK-targeted agents. Administration of TILs followed by a period of BRAF inhibition did not lead to increased toxicity and may have prevented aggressive tumor growth during the

period of TIL engraftment. Discontinuation of treatment with BRAF inhibitors may be possible once tumor flare is no longer a concern.

These findings build on preclinical data that suggest the BRAF inhibitor vemurafenib may induce higher levels of MAPK activation, intratumoral cytokine secretion, and cytotoxic activity of adoptively transferred T cells [28]. They are also supported by work from Sarnaik et al who documented the feasibility of combining vemurafenib with TIL therapy in 12 patients with *BRAF*-mutated melanoma. Those findings showed improved feasibility of TILs and comparable antitumor activity with continued vemurafenib prior to TIL infusion compared with patients in a previous study that did not allow BRAF inhibition during TIL manufacture in which 35% of patients enrolled were unable to proceed to TIL infusion due to disease progression [29, 30].

TIL administration in a pediatric patient (n=1):

Although rare, melanoma is known to occur in children [31, 32]. Patient 2, a 16-year-old male, had *BRAF*-mutated melanoma, bulky disease (sum of lesion diameters, 103 mm), and extensive mediastinal disease and brain metastases (**Figure 5**). The patient was relapsed/refractory to 3 prior lines of therapy, including ipilimumab and dabrafenib, before receiving TILs. Following TIL infusion, the patient received 8 doses of IL-2 and experienced expected IL-2-related toxicities, including tachycardia, fever, and cardiovascular instability. After the 6th dose of IL-2, seizure in the setting of high fever and tachycardia/shortness of breath occurred, all of which resolved spontaneously. The seventh dose of IL-2 was delayed and prophylactic levetiracetam was administered; no further seizure events were observed. The patient experienced neutropenia from Days 1-6 without need for platelet support. Rapid reduction in disease burden was achieved at 6 weeks, with onset of a PR at 3 months. By 60 months, the patient's response deepened to a radiographic CR (**Figure 6**). At time of data cutoff, the patient remained in ongoing CR for >7 years post-TIL therapy (follow-up time, 85 months), without any further anticancer therapy.

Discussion

Melanoma is a markedly immunogenic cancer with high mutational burden and inter- and

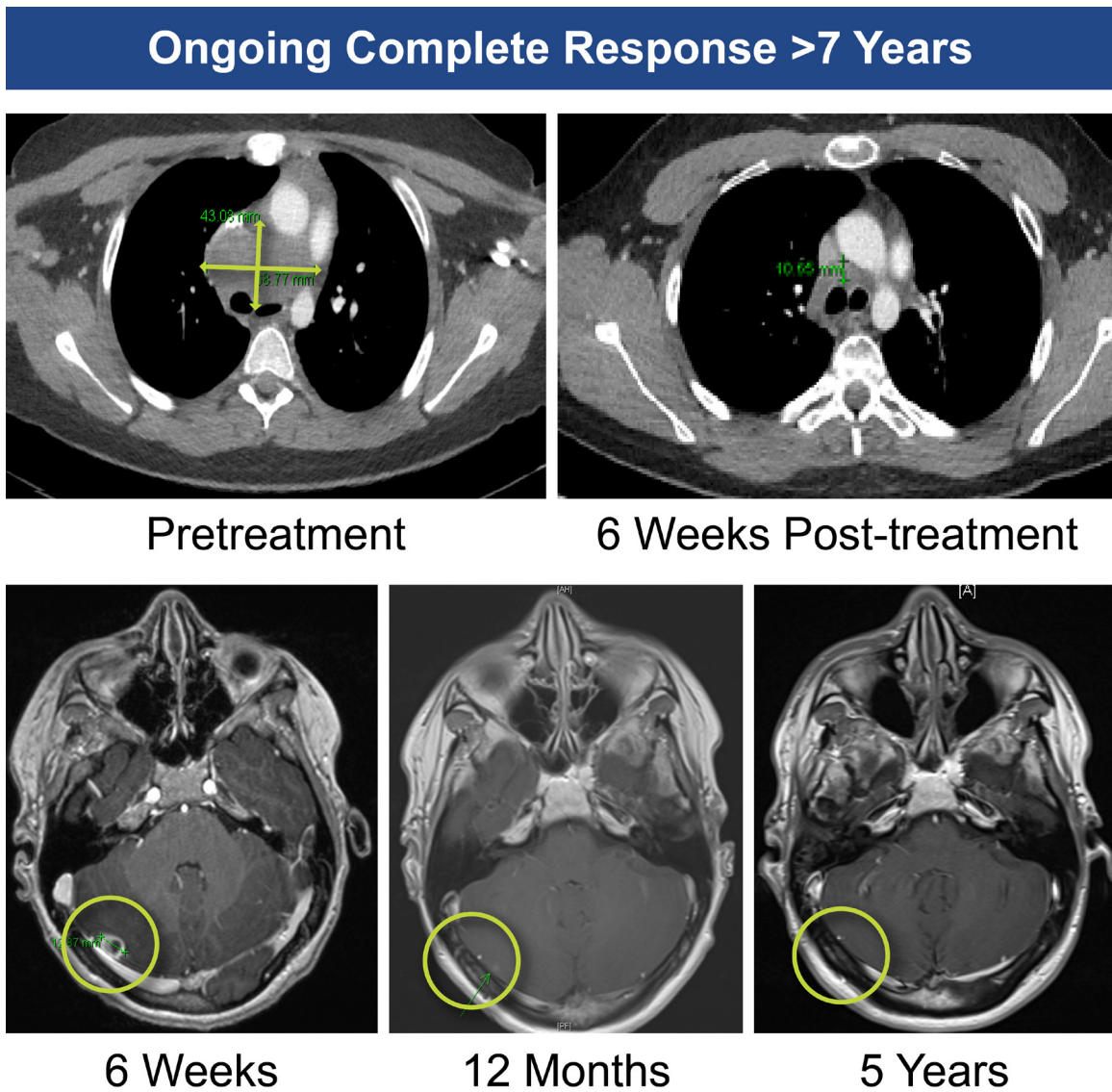


Figure 6. Successful treatment of brain metastases in a 16-year-old (Patient 2).

intratumoral heterogeneity. This immunogenicity has likely enabled its treatment with T-cell-based therapies like immune checkpoint inhibitors and TILs [9, 33, 34]. Targeted therapy and checkpoint inhibitors have indeed revolutionized treatment of advanced melanoma, but relapse is common and those who experience disease progression after these approaches have poor outcomes and limited treatment options [9, 26, 35]. This unmet medical need may be addressed by therapeutic approaches that are patient-specific and possess broad antigen specificity. Nonselected autologous TILs represent a highly personalized therapeutic approach that leverages each patient's

unique population of tumor-reactive T cells that encompass a broad panel of TCRs that can target patient-specific tumor-associated antigens, including neoantigens [4, 9, 13, 17, 36]. This retrospective compassionate use clinical series confirms that nonselected TIL products can be manufactured from digested tumors and deliver clinical benefit to patients with advanced cutaneous melanoma, including those patients who experience disease progression after checkpoint inhibition and, if applicable, targeted therapy.

The safety profile of the TIL treatment regimen in this clinical series was similar to that in other

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TIL studies. AEs were primarily attributable to lymphodepleting chemotherapy and/or high-dose IL-2 rather than the TIL product, and product-specific AEs were uncommon [17, 19]. With respect to the latter, 2 cases of vitiligo were observed, both in patients who achieved CRs. Generally, AEs were self-limited and managed by supportive care within the hospital setting. Of note, AE reporting in this series was limited to those documented within hospital notes. Accordingly, it is possible that AEs were underreported, particularly any that may have occurred outside the hospitalization window.

In this clinical series, patients had been heavily pretreated, had several high-risk features (e.g., advanced disease, high disease burden, and high LDH), and lacked standard-of-care treatment options. The majority (57%) of patients were relapsed/refractory to both PD-1 and CTLA-4 inhibitors. Nevertheless, TIL products generated high ORRs, with similar results among all treated patients and amongst subgroups, including those patients with PD-1 inhibitor-resistant disease (67% and 58%, respectively). The kinetics of response were rapid and durable with 24% of patients in ongoing response >30 months after TIL infusion. The majority of responding patients demonstrated >1 consecutive scan showing ongoing disease response. However, there was not a schedule of assessments that governed the schedule and manner of disease monitoring as is generally used in a clinical trial; thus, confirmation of response per RECIST v1.1 was not possible for all responders.

Notably, approximately 75% of patients with advanced melanoma develop brain metastases [37, 38]. Highlighting the unmet need in this population, treatments for brain metastases are limited, and although outcomes have improved with checkpoint inhibitor therapy, treatment is associated with notable morbidity [39, 40]. In this series, patients with a history of brain metastases achieved an ORR of 71% (29% CR), supporting existing literature [38, 41] and highlighting that TILs may be an effective treatment option for this difficult-to-treat patient population that is frequently excluded from clinical trials. Collectively, these results highlight the successful application of nonselected autologous TILs to address unmet medi-

cal need for patients with advanced melanoma, including those refractory to checkpoint inhibitor therapy and targeted therapy.

Unlike off-the-shelf treatments, TIL therapy is a complex multistep and multidisciplinary intervention requiring surgical resection of the tumor, an offsite manufacturing process, and inpatient administration sequenced with lymphodepletion and high-dose IL-2 [19]. Management of rapidly progressing, treatment-refractory melanoma in the midst of the preparation for TILs can be challenging. A number of case studies have been included in this report that illustrate several commonly encountered clinical scenarios and provide evidence that clinical implementation of TIL therapy may be further optimized. First, we detail 2 patients who underwent tumor harvest before PD-1 inhibitor therapy and then went on to receive PD-1 inhibition during TIL manufacturing. One of these patients developed a durable PR to TIL infusion 34 months after tumor resection. The other patient, whose TIL product was administered 22 months after surgical resection, did not have a response to TIL but surprisingly went on to develop durable disease control to repeat exposure to PD-1 blockade despite having previously failed PD-1 inhibition.

These cases suggest that early tumor collection and banking may offer additional options for patients with early or high-risk disease. Notably, digested tumors are freezer-stable long-term, which may enable tumor collection during routine surgery and the potential to manufacture and administer TIL therapy if a patient relapses. Additionally, these results suggest sensitivity to PD-1 inhibitor may be restored by TIL therapy, supporting combination treatment with TILs and PD-1/programmed death-ligand 1 axis blockade.

Tumor flare after BRAF-positive disease progression and discontinuation of BRAF inhibitors often poses a difficult clinical problem [42]. We detail 2 patients in whom TILs were coadministered with dabrafenib. No inherent limitations on efficacy were observed with the combination, and importantly, no added toxicities were seen. Both patients achieved response (1 CR, 1 PR) and subsequently discontinued dabrafenib. These observations are supported by

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findings observed in a pilot study of TILs in combination with vemurafenib in patients with metastatic melanoma, where no new safety signals emerged, and objective responses were observed [43].

These results of a compassionate use program at a single institution suggest that treatment with nonselected TILs manufactured from both fresh and frozen tumor digests is feasible and may offer significant clinical and logistical benefit to patients with advanced melanoma who otherwise lack standard-of-care treatment options, warranting future prospective clinical trials. Notably, the product herein was manufactured with an earlier version of the ITIL-168 manufacturing process [44]. A global, multicenter, pivotal phase 2 clinical trial of ITIL-168 in adults and pediatric patients aged 12 and above with advanced melanoma who received prior PD-1 inhibitor will build on the observations shared here (DELTA-1; NCT05050006; EudraCT, 2020-003862-37). DELTA-1 and other ongoing and future TIL studies may afford new therapeutic options for patients with significant unmet medical need.

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NovalGen, Ltd; and nonexecutive director for and stock or other ownership in Bivictrix Plc.

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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 $\geq 1 \text{ cm}^3$ ($>2 \text{ cm}^3$ 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

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Nonselected TIL therapy in patients with advanced melanoma

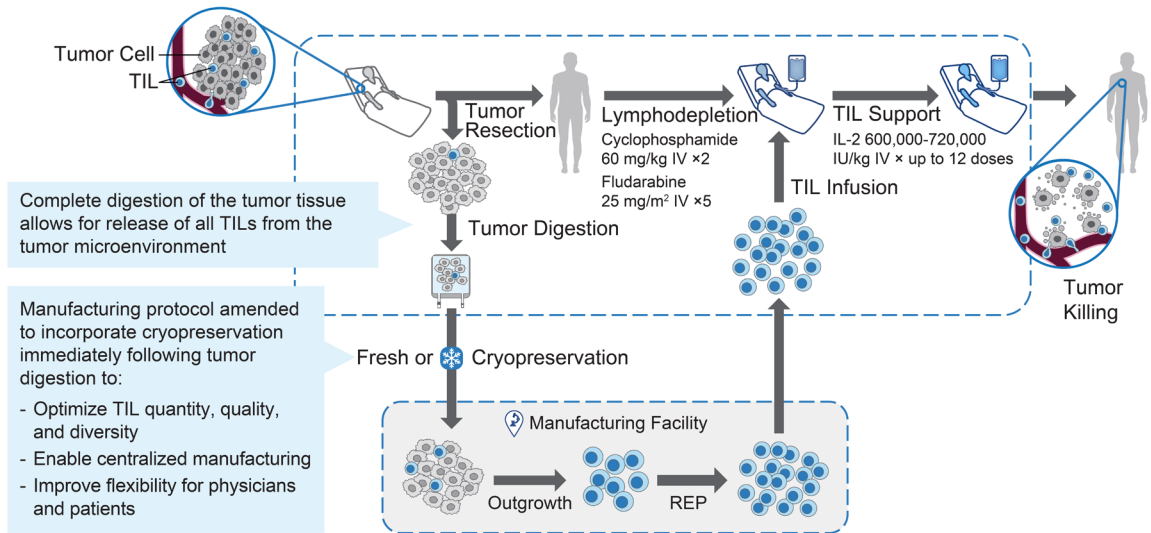


Figure S1. Tissue procurement and manufacturing. IL-2, interleukin-2; IV, intravenous; REP, rapid expansion protocol; TIL, tumor-infiltrating lymphocyte.

Nonselected TIL therapy in patients with advanced melanoma

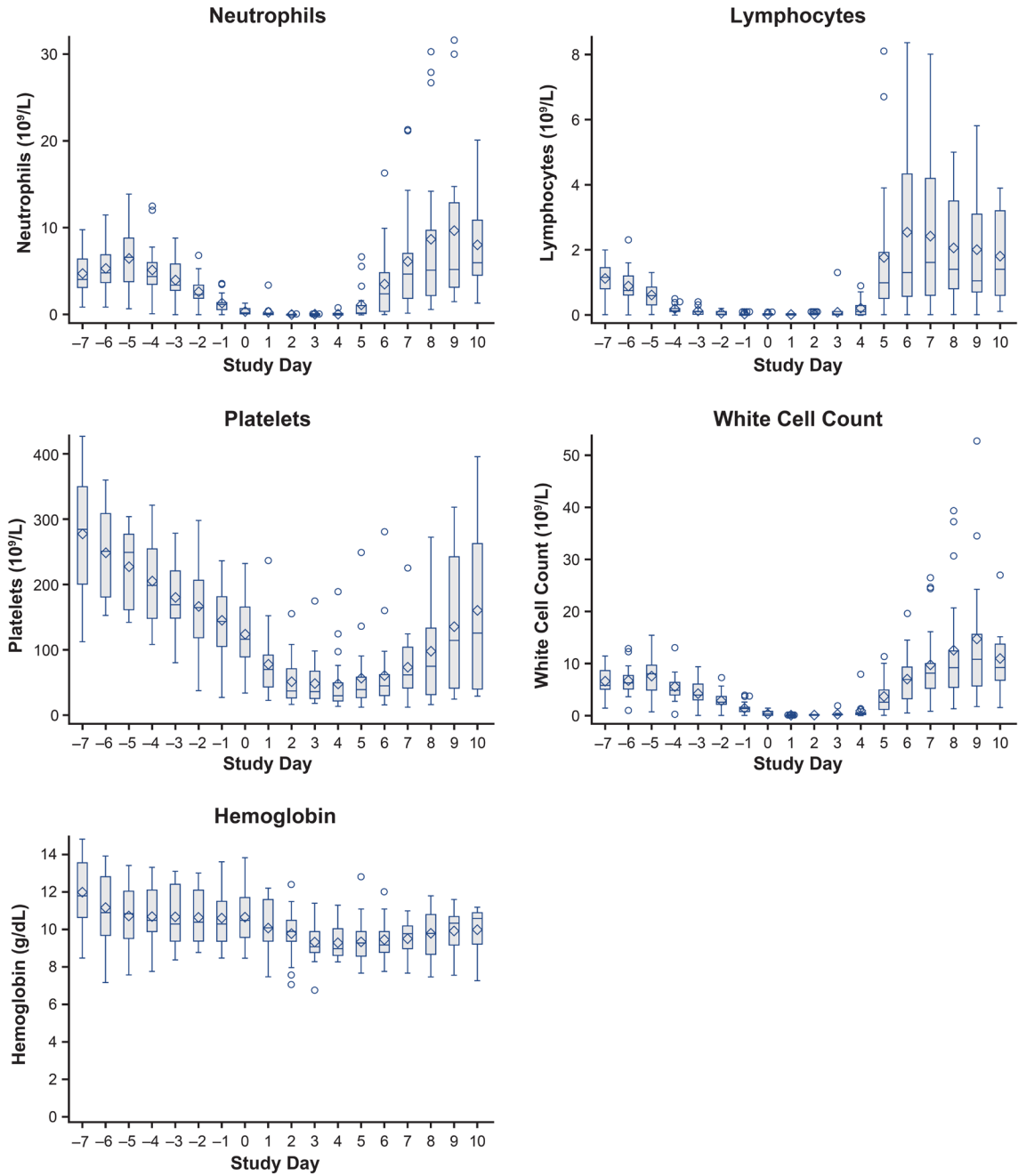


Figure S2. Blood counts and hemoglobin levels over time. Data are shown for all treated patients (N=21). The bar and diamond represent the median and mean values, respectively. Outliers are shown with circles.

FDG-PET–Negative Clinical Response at 54 Months

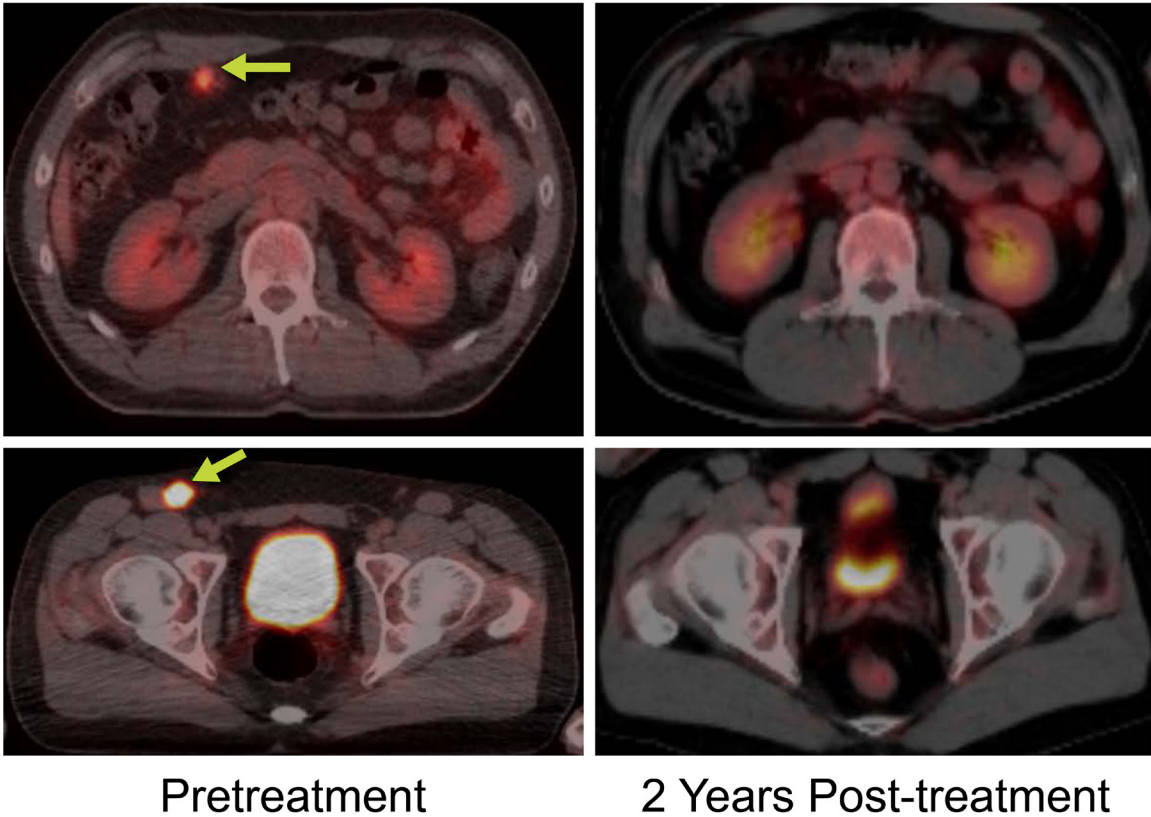


Figure S3. FDG-PET imaging in a complete responder (Patient 4). This 43-year-old male had right groin disease and a right upper quadrant mass prior to TIL treatment. Responses for this patient were identified using methods that prevented RECIST version 1.1 assessments. First complete response was observed 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.

Nonselected TIL therapy in patients with advanced melanoma

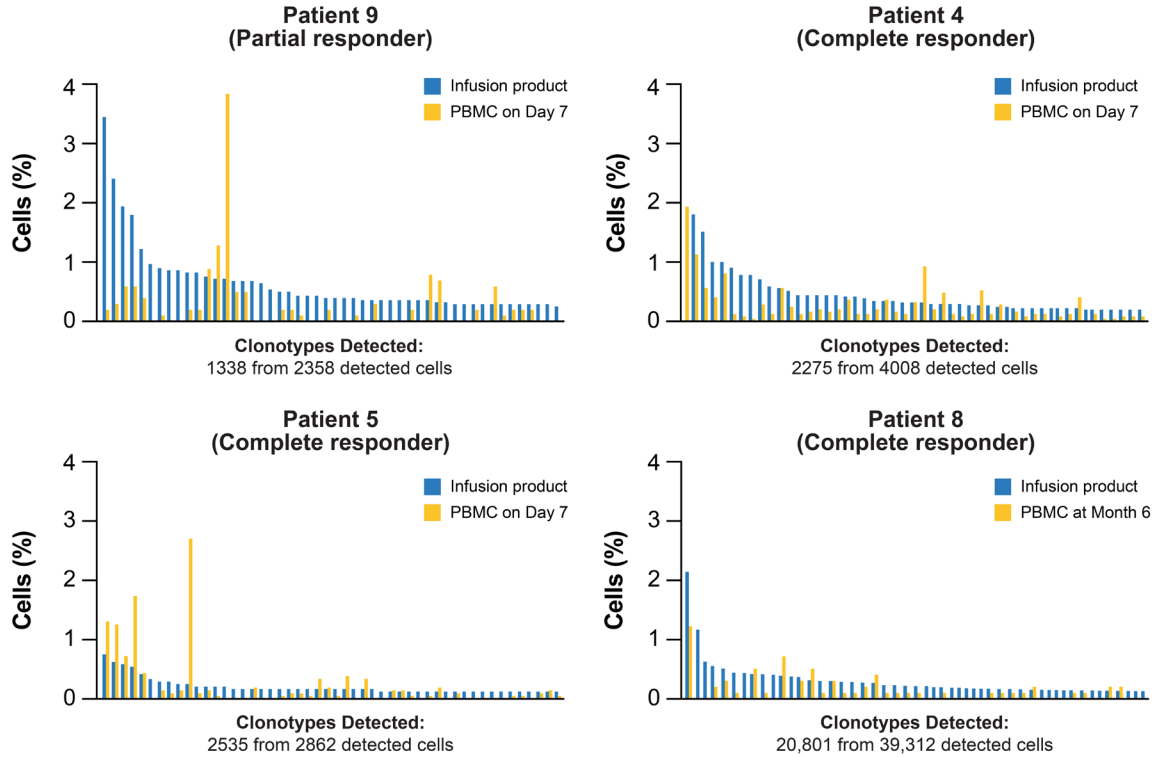


Figure S4. Clonal TCR distribution over time (n=4). Clonotypes were measured by scRNA-Seq. PMBC, peripheral blood mononuclear cell; RNA-Seq, ribonucleic acid sequencing; scRNA-Seq, single-cell ribonucleic acid sequencing; TCR, T-cell receptor.

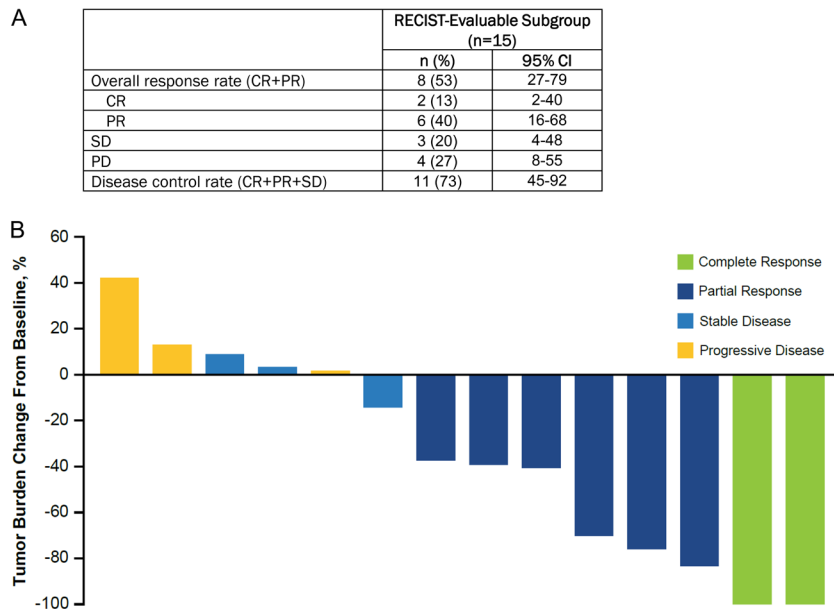


Figure S5. Response outcomes among the RECIST-evaluable subgroup. (A) Best response rates. (B) Depth of responses. Data are presented for 14 patients with detailed tumor measurements; 1 patient with a best overall response of progressive disease did not have any post-treatment target lesion measurements 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.

Nonselected TIL therapy in patients with advanced melanoma

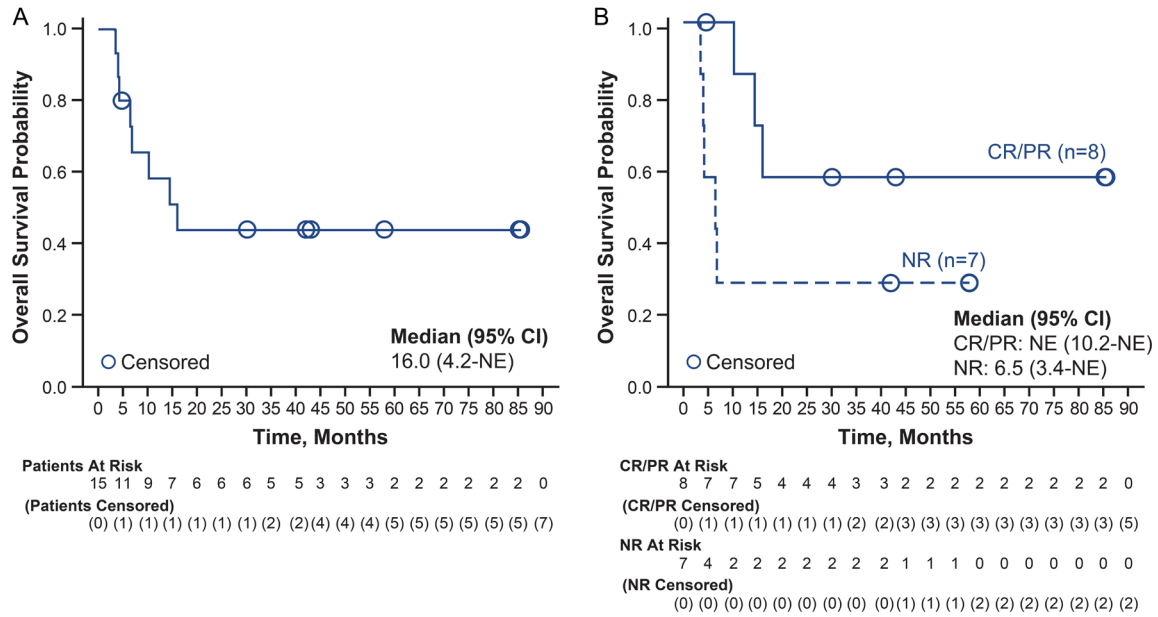


Figure S6. Overall survival among the RECIST-evaluable subgroup. (A) All RECIST-evaluable patients. (B) Overall survival by best response. CR, complete response; NE, not estimable; NR, nonresponder; OS, overall survival; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors.

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 $>1.0 \times 10^9/L$	263 μ g 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 $>1.0 \times 10^9/L$	500 mg orally/IV once daily
Trimethoprim/sulfamethoxazole	Day 0 for 3 months or until T-cell count $>1.0 \times 10^9/L$	160 mg/800 mg orally twice daily 3 times/week
Acyclovir	Day 0 for 3 months or until T-cell count $>1.0 \times 10^9/L$	400 mg orally twice daily
Fluconazole	Day 0 for 3 months or until T-cell count $>1.0 \times 10^9/L$	100 mg orally once daily

^aIf 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.

Nonselected TIL therapy in patients with advanced melanoma

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.

Nonselected TIL therapy in patients with advanced melanoma

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.

Nonselected TIL therapy in patients with advanced melanoma

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)
<i>BRAF</i> mutation positive, n (%)	7 (47)
<i>BRAF</i> inhibitor ± MEK inhibitor	7 (47)

^aAll 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). ^bRefractory 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.