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Adoptive Transfer of Tumor-Infiltrating Lymphocytes in Patients with Metastatic Melanoma: Intent-to-Treat Analysis and Efficacy after Failure to Prior Immunotherapies

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Abstract

Purpose: Adoptive cell transfer (ACT) using autologous tumor-infiltrating lymphocytes (TIL) was reported to yield objective responses in about 50% of metastatic patients with melanoma. Here, we present the intent-to-treat analysis of TIL ACT and analyze parameters predictive to response as well as the impact of other immunotherapies.

Experimental Design: Eighty patients with stage IV melanoma were enrolled, of which 57 were treated with unselected/young TIL and high-dose interleukin-2 (IL-2) following nonmyeloablative lymphodepleting conditioning.

Results: TIL cultures were established from 72 of 80 enrolled patients. Altogether 23 patients were withdrawn from the study mainly due to clinical deterioration during TIL preparation. The overall response rate and median survival was 29% and 9.8 months for enrolled patients and 40% and 15.2 months for treated patients. Five patients achieved complete and 18 partial remission. All complete responders are on unmaintained remission after a median follow-up of 28 months and the 3-year survival of responding patients was 78%. Multivariate analysis revealed blood lactate-dehydrogenase levels, gender, days of TIL in culture, and the total number of infused $CD8^+$ cells as independent predictive markers for clinical outcome. Thirty-two patients received the CTLA-4-blocking antibody ipilimumab prior or post TIL infusion. Retrospective analysis revealed that nonresponders to ipilimumab or IL-2 based therapy had the same overall response rate to ACT as other patients receiving TIL. No additional toxicities to TIL therapy occurred following ipilimumab treatment.

Conclusion: Adoptive transfer of TIL can yield durable and complete responses in patients with refractory melanoma, even when other immunotherapies have failed. *Clin Cancer Res; 19(17); 4792–800. ©2013 AACR.*

Introduction

Treatment options for patients with metastatic melanoma have improved in the past few years. Targeted therapy inhibiting mutant BRAF yields high response rates, but responses are of short duration and the durable complete response rate below 1% (1). Immunotherapy with the CTLA-4-blocking antibody ipilimumab (Yervoy®, Bristol-Myers Squibb) can lead to long-lasting responses, but only in a small number of patients (2). Six to 7% complete response rates were reported in phase I/II studies (3) and less than 2% in multicenter phase III trials (2, 4).

In recent years, it has been shown that adoptive cell therapy (ACT) with autologous tumor infiltrating lymphocytes (TIL) in combination with recombinant interleukin-2 (IL-2; Proleukin, Prometheus Laboratories) and nonmyeloablative lympho-depleting chemotherapy (NMA) is a powerful form of immunotherapy in patients with refractory melanoma (5, 6).

TIL, obtained from the patient's tumor can recognize tumor-associated antigens and thereby destroy malignant cells. Isolated TIL are *ex vivo* activated and expanded to 1×10^{10} and 1×10^{11} cells (7, 8). Before reinfusion,

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Translational Relevance

Adoptive cell transfer (ACT) of tumor infiltrating lymphocytes (TIL) has been reported to yield response rates around 50% in metastatic melanoma.

Here we present for the first time the intent-to-treat analysis of TIL ACT and give a broad perspective on its feasibility and efficacy. In addition, special focus will be given to the impact of U.S. Food and Drug Administration (FDA)–approved immunotherapies, such as ipilimumab, on TIL ACT.

Our data on 80 enrolled patients show that adoptive transfer of TIL can result in durable and complete remission of refractory melanoma. There was no correlation between responses to prior interleukin-2 (IL-2)–based or prior ipilimumab therapies and the response to TIL, and no additional toxicities were observed in patients previously treated with ipilimumab. We show that TIL ACT is an effective treatment, also for patients who had progressed on FDA-approved immunotherapies. This phase II study may have a significant impact towards placing TIL therapy into the mainstream of melanoma care.

patients undergo NMA conditioning, transiently generating a more favorable host immunologic environment, by eliminating endogenous T-regulatory cells and lymphocytes that could compete with the infused cells for homeostatic cytokines, thus improving the persistence of the transferred TIL (9–11). IL-2 is coadministered to further support the survival and proliferation of the transferred cells.

A recent report by Rosenberg and colleagues summarizes the long-term follow-up of 93 melanoma patients, treated in 3 sequential TIL trials, which differed in their preparative lymphodepleting regimens and included either NMA conditioning alone ($n = 43$) or in conjunction with 2 Gray (Gy) total body irradiation (TBI; $n = 25$) or myeloablative 12 Gy TBI ($n = 25$; ref. 5). Response rates were 49%, 52%, and 72%, respectively. Although response rates were impressive and comparable with those reported for the $BRAF^{V600}$ inhibitor vemurafenib (Zelboraf, Hoffmann-La Roche), the major advantage of TIL therapy was the long response durability in many patients. Twenty of 93 patients experienced complete remission, and all but one, are still in remission 4 to over 8 years after treatment, indicating the curative potential of TIL therapy (5, 6).

Patients treated with the intensive 12 Gy TBI preconditioning had the highest response rate (72%), but the myeloablative conditioning is toxic and patients need stem cell transplant, which limits the number of patients with advanced melanoma that can be treated (12). Thus, TIL therapy using the NMA conditioning alone is clinically the most applicable approach to date. Using this therapeutic modality, multiple independent centers confirmed objective response rates of 40% to 50% in patients with refractory melanoma, including 10% to 15% complete remissions, with many being durable (13–15).

In the current report, we summarize our clinical and laboratory results on 80 patients with metastatic melanoma, of whom 57 were treated with unselected TIL following NMA preparative regimen and high-dose IL-2. To date, this is the only study that reports the intent-to-treat analysis of TIL ACT and furthermore takes the impact of other immunotherapies into account.

Patients and Methods

Treatment protocol

Patients with stage IV melanoma were treated with TIL ACT as second line therapy as previously described (13). Patients signed an informed consent approved by the Israeli Ministry of Health (Helsinki approval no. 3518/2004, NCT00287131). Amendment for the enrollment of patients with asymptomatic brain metastasis was approved in December 2010. Patients received NMA conditioning regimen with cyclophosphamide (60 mg/kg for 2 days) and fludarabine (25 mg/m^2 for 5 days) before cell infusion. TIL were administered intravenously, followed by bolus high-dose IL-2 (720,000 IU/kg every 8 hours for 5 days or to tolerance).

Response was assessed using the Response Evaluation Criteria In Solid Tumors (RECIST v1.1) guidelines (16) 4 weeks following TIL administration and every 3 months thereafter or as clinically needed. Objective responders (OR) were defined as complete (CR) and partial responders (PR) and nonresponders (NR) as patients with stable disease (SD) or progressive disease (PD) by determining the best overall response using RECIST.

Generation and expansion of unselected/young TIL

The generation of unselected/young TIL was previously described in great detail (7). In short, fragmentation, enzymatic digestion, and cell remnants technique were used to isolate TIL from surgically resected metastatic lesions. TIL cultures were *ex vivo* expanded in 2 major steps: (i) Pre-Rapid Expansion Procedure (Pre-REP); Purified TIL cultures were established in approximately 2 weeks. Those short-term cultured ("young") TIL were neither assayed nor selected ("unselected") on the basis of their interferon-gamma secretion after antigenic stimulation, as this assay was previously shown to be insufficient in predicting clinical efficacy (14, 17, 18). The obtained TIL cultures were cryopreserved or entered directly into the second step of expansion. The cut-off for an established TIL culture was defined as $80 \times 10e6$ cells, because about $50 \times 10e6$ TIL are required to initiate REP, the next expansion step, and another $30 \times 10e6$ TIL to compensate for the loss of cells during cryopreservation and thawing. (ii) REP; approximately 50×10^6 unselected TIL were expanded to treatment levels in a large-scale expansion procedure using anti-CD3 antibody, rhIL-2 and irradiated feeder cells of healthy donors in gas-permeable bags (7) or GRex flasks (19). Within 14 days, cultures expanded by about 1,000 to

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2,000 fold. On day 14, cells were harvested and administered intravenously (20).

Flow cytometry

Flow cytometry was conducted using a Per-CP conjugated mouse antihuman $CD8^+$ antibody (7).

Statistical analysis

Logistic regression was applied to quantify the impact of variables on the prediction of objective response. The results are presented by *P* values and OR. The 2-sided *P* values were computed with Wald test and the OR was given with a 95% confidence interval (CI).

Significance of variation between groups was evaluated using a nonparametric 2-tailed Student *t* test. The differences between proportions were tested using 2-sided Fisher exact test. Survival and progression-free survival (PFS) rates were based on Kaplan–Meier estimation. All statistical analysis was conducted using the "R" software environment.

Results

Intent-to-treat analysis

Between January 2006 and September 2012, 80 patients with metastatic melanoma were enrolled. Baseline characteristics are presented in Table 1. Clinical results on the first

Table 1. Baseline characteristics

	Enrolled patients ($N = 80$)	Treated patients ($N = 57$)	All dismissed patients ($N = 22$)	Dismissed due to unavailable TIL or refusal ($N = 11$)
Mean age in years	54	54	57	47
Female, *n* (%)	30 (38%)	20 (35%)	9 (41%)	6 (55%)
M stage, *n* (%)[a]				
M1a	4 (5%)	4 (7%)	0	0
M1b	12 (15%)	8 (14%)	4 (18%)	1 (9%)
M1c	64 (80%)	45 (79%)	18 (82%)	10 (91%)

	Treated patients ($N = 57$)	Responders OR ($N = 23$)	Nonresponders NR ($N = 34$)	*P* value OR vs. NR
Treated and evaluated patients ($N = 57$)[b]				
Mean age in years	54	53	54	.360
Female, *n* (%)	20 (35%)	3 (13%)	17 (50%)	.007
M stage, *n* (%)				
M1a	4 (7%)	3 (13%)	1 (3%)	.292
M1b	8 (14%)	5 (22%)	3 (9%)	.247
M1c	45 (79%)	15 (65%)	30 (88%)	.051
ECOG performance status, *n* (%)[c]				
0	37 (65%)	17 (74%)	20 (59%)	.278
1	18 (32%)	6 (26%)	12 (35%)	.569
2	2 (4%)	0	2 (6%)	.516
LDH level above top limit of the normal range, *n* (%)	23 (40%)	4 (17%)	19 (56%)	.013
CNS metastases at baseline, *n* (%)	11 (19%)	6 (26%)	5 (15%)	.322
More than 5 metastasis, *n* (%)	45 (79%)	20 (87%)	25 (74%)	.231
BRAF V600E mutation, *n* (%)	22 of 50 (44%)	12 of 21 (57%)	10 of 29 (35%)	.209
HLA-A0201 positive, *n* (%)	13 of 56 (23%)	4 (17%)	9 of 33 (27%)	.525
Previous therapy for metastatic disease, *n* (%)	57 (100%)	23 (100%)	34 (100%)	1.0
Previous IL-2 therapy, *n* (%)	54 (95%)	21 (91%)	33 (97%)	.362
Previous ipilimumab therapy, *n* (%)	13 (23%)	5 (22%)	8 (24%)	.826

Abbreviations: M stage, metastasis stage; CNS, central nervous system; HLA, human leukocyte antigen; IL-2, interleukin-2, LDH, lactate dehydrogenase.

[a]The metastasis (M) stage was classified according to the tumor-node-metastasis (TNM) categorization for melanoma of the American Joint Committee on Cancer.

[b]One unevaluated patient died of cardiac arrest before cell infusion.

[c]Eastern Cooperative Oncology Group (ECOG) status ranges from 0 to 5, with higher scores indicating greater impairment (5 indicates death).

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27 enrolled patients, of whom 20 were finally treated, have been published previously (13).

Seventy-four percent (59 of 80), 19% (15 of 80), and 6% (5 of 80) of the patients had 1, 2, or 3 metastatic lesions resected, respectively, for the purpose of TIL generation, resulting in an overall of 105 specimens. Multilesion resection was mostly clinically indicated (e.g., removal of 2 infiltrated lymph nodes simultaneously). The lesions were derived from: lung ($n = 23$; 22%), subcutaneous ($n = 37$; 35%), lymph node ($n = 29$; 28%), brain ($n = 3$; 3%), or visceral tissues ($n = 13$; 12%). Four patients underwent a second surgery due to unsuccessful TIL cultivation, enabling 3 of them to be finally treated.

In 8 of 80 (10%) enrolled patients, we were unable to grow a melanoma-free TIL culture. Another 3 patients (4%) refused to enter the study despite available TIL cultures and 11 patients (14%) could not be treated due to clinical deterioration during cell preparation. Notably, 8 of 11 clinical deteriorations were due to the appearance of new brain metastases. A protocol amendment from December 2010 enabled the treatment of patients with CNS involvement. One patient developed fatal cardiac failure following cyclophosphamide administration before receiving TIL infusion and could not be evaluated.

The median overall survival (OS) of all 80 enrolled patients calculated from the date of surgery was 9.8 months. Treated and evaluated patients ($n = 57$) had a significantly higher OS (OS = 16.4 months) than untreated patients ($n = 22$; OS = 3.6 months; $P < 0.001$; Fig. 1) or than untreated patients that were dismissed from the study due to unavailability of TIL cultures or refusal, but not due to clinical deterioration ($n = 11$; OS = 4.4 months; $P < 0.001$).

Objective clinical responses according to RECIST criteria were observed in 23 of 57 (40%) evaluated patients or 23 of 80 (29%) enrolled patents. Responses included 5 complete remissions (9% of evaluated patients and 6% of enrolled patients) and 18 partial responses (32% of evaluated patients and 23% of enrolled patients). Thirty-four nonresponders (60% of evaluated patients and 43% of enrolled patients) experienced disease stabilization ($n = 14$) or progression ($n = 20$).

Clinical outcome and baseline characteristics of patients

TIL was only offered as second-line treatment for metastatic disease and 24 of 57 patients had even multiple prior lines of systemic treatments (Table 1). The majority of the patients (45 of 57; 79%) had multiple metastatic lesions in visceral organs (stage M1c). Eleven patients had CNS involvement, 6 of which achieved a partial ($n = 5$) or complete response ($n = 1$), including remission in their brain lesions (Table 1).

Baseline characteristics such as age, performance status, number of metastasis, HLA-A0201, existence of the $BRAF^{V600E}$ mutation or previous therapy with the anti-CTLA4 antibody ipilimumab did not correlate with response (Table 1).

Only 3 of 20 female patients responded to TIL ACT ($P = 0.007$; OR 0.15; 95% CI: 0.031–0.54). This could not be explained by worse performance status, staging, or unfavorable cell characteristics among females (Supplementary Fig. S1). Thus, gender stands currently as an independent prognosis factor, which could not be further clarified. Other published TIL ACT trials did not find this correlation (12, 14, 15). Elevated serum level of lactate dehydrogenase (LDH), a marker of disease activity and tumor burden (21, 22) was negatively correlated with clinical response ($P = 0.013$; 0.219 OR; 95% CI: 0.061–0.992).

TIL expansion and characteristics

We have previously suggested that TIL cultures of responding patients were established in a shorter period of time compared with nonresponding patients (13). This finding was confirmed in the current cohort as well, as the average culture time for TIL generation was 13.5 ± 2.8 days for responders versus 17.5 ± 5.1 days for nonresponders ($P = 0.005$; OR 0.767; 95% CI: 0.623–0.905; Table 2).

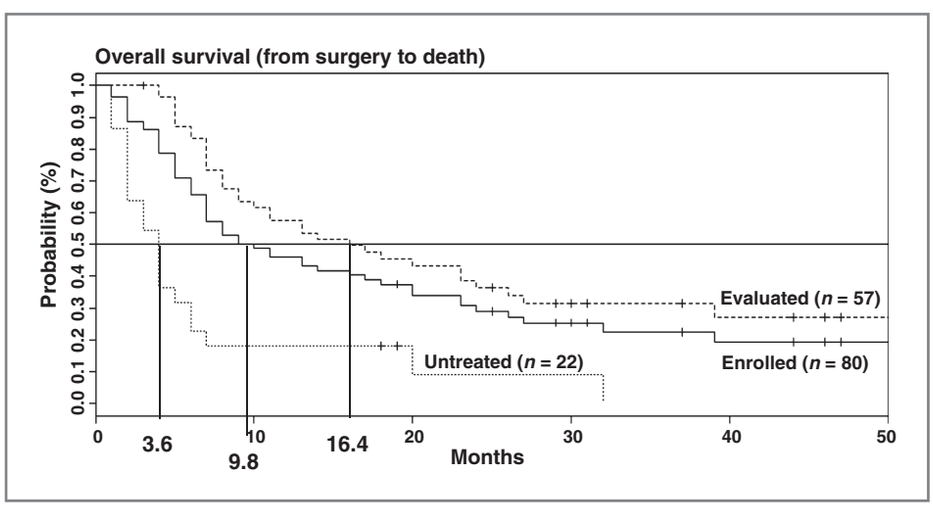

Figure 1. OS in the enrolled patient population. Kaplan–Meier estimation; OS was measured from the day of surgery to death in months.

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Table 2. Cell and treatment characteristics of treated patients

	All patients ($N = 57$)	Responders OR ($N = 23$)	Nonresponders NR ($N = 34$)	*P*-value OR vs. NR
TIL and infusion product characteristics				
Days to TIL culture generation, average	15.9 ± 4.8	13.5 ± 2.8	17.5 ± 5.1	.005
Fold expansion of TIL during REP, average	1145 ± 455	1280 ± 342	1053 ± 503	.074
Infusion product, average				
Total cell number (×10e9)	52 ± 24	60 ± 19	46 ± 25	.040
$CD8^+$ frequency (%)[a]	62 ± 25	71 ± 17	55 ± 27	.019
Total $CD8^+$ number (×10e9)	35 ± 24	44 ± 20	29 ± 25	.026
Treatment characteristics				
Days of hospitalization[b], median (range)	19 (14–44)	20 (16–36)	19 (14–44)	.472
No. of IL-2 doses, average	6.7 ± 3.0	6.8 ± 2.9	6.6 ± 3.2	.824
Patients receiving G-CSF mobilized cells, *n* (%)	7 (12%)	1 (4%)	6 (17%)	.222
Units RBC transfusion, median (range)	4 (0–40)	3 (0–13)	4 (0–40)	.618
Units PLT transfusion, median (range)	18 (0–300)	16 (0–48)	21 (0–300)	.483
Absolute lymphocyte count, average				
1 month before TIL (K/μL)	1.96 ± 0.71	1.96 ± 0.74	1.96 ± 0.70	.859
2 weeks after TIL (K/μL)	0.90 ± 0.82	1.16 ± 0.86	0.69 ± 0.73	.066
1 month after TIL (K/μL)	1.39 ± 0.98	1.49 ± 0.69	1.31 ± 1.16	.989

Abbreviations: IL-2, interleukin-2; REP, rapid expansion procedure; TIL, tumor infiltrating lymphocyte; NMA, nonmyeloablative lymphodepletion; GCSF, granulocyte colony stimulating factor; RBC, red blood cells; PLT, platelets.

[a]Percent $CD8^+$ cytotoxic T cells, all other cells are $CD4^+$ T helper cells.

[b]Days of hospitalization include 7 days of NMA preconditioning.

During the subsequent large-scale expansion, TIL expanded by 1,145 ± 455 fold and reached a final cell number of 52 ± 24 × 10e9 (responders 60 ± 19 × 10e9; nonresponders 46 ± 25 × 10e9; $P = 0.04$; OR 1.306; 95% CI: 1.027–1.724). Fluorescence-activated cell sorting (FACS) analysis conducted on cell samples from infusion bags revealed that the percentage of $CD8^+$ T cells was significantly higher in responding patients ($P = 0.019$; OR 1.033; 95% CI: 1.007–1.064; Table 2). Consequently, higher numbers of $CD8^+$ cells were administered to objective responders (44 ± 20 × 10e9 vs. nonresponder 29 ± 25 × 10e9; $P = 0.026$; OR 1.327; 95% CI: 1.049–1.724). These results are in line with previous publications (13, 14). FACS analysis conducted on 8 different TIL cultures revealed that the percentage of CD8 cells before and after REP was similar (pre-REP 61 ± 17%; post-REP 56 ± 21%; $P = 0.654$). Other TIL surface markers such as CD45RA, CD62L, CD27, CD28, PD-1, CTLA-4, CD69, CD57, and CD25 or chemokine receptors did not reveal conclusive results and will require further investigations (data not shown).

In conclusion, gender, blood LDH levels, number of days required to generate a TIL culture and the percent of $CD8^+$ cytotoxic T cells in the infusion product were found to be independent prognostic markers in a multivariate analysis. Supplementary Fig. S1 shows a correlation matrix presented as a bidimensional table of Kendall τ coefficient and their associated *P* values in which all variables are shown in correlation to one another.

Treatment characteristics and toxicities

Patients received cyclophosphamide and fludarabine before cell infusion. Thrombocytopenia, anemia, neutropenia, lymphopenia, and extended depression of $CD4^+$ lymphocytes were observed in all patients. One patient died of cardiac failure caused by cyclophosphamide before receiving his TIL.

Six patients were suspected of a slow recovery of their peripheral blood counts and received therefore autologous G-CSF mobilized peripheral blood stem cells (≥1.5 × 10e6 $CD34^+$ hematopoietic stem cells/kg) 1 or 2 days following TIL infusion (Table 2). Those patients had at least 2 lines of chemotherapy prior TIL ACT, chemotherapy and large-field radiotherapy, or were above 70 years of age. One additional patient received his G-CSF mobilized cells 3 weeks posttreatment, as his blood counts did not reconstitute by then.

Within 1 hour following TIL infusion, patients received their first dose of IL-2. If clinically possible, bolus high-dose (HD) IL-2 was administered every 8 hours to a maximum of 15 doses. Only 1 patient received the complete IL-2 schedule, whereas 53 (93%) patients developed at least one grade 3/4 toxicity causing discontinuation. Three patients refused to the administration of 15 doses. All adverse events were transient and patients were released from hospital about 12 days after TIL infusion. The number of IL-2 doses (Table 2) or the type/severity of toxicity (Table 3) did not correlate with clinical response.

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Table 3. Nonhematologic grade 3/4 toxicities of treated patients

n (%)	All patients (*N* = 57)	Responders OR (*N* = 23)	Nonresponders NR (*N* = 34)	*P*-value OR vs. NR
Pulmonary congestion	27 (47%)	10 (43%)	17 (50%)	.629
Renal failure	11 (19%)	5 (22%)	6 (18%)	.701
Prolonged hypotension	13 (23%)	5 (22%)	8 (24%)	.874
Hyperbilirubinemia	8 (14%)	5 (22%)	3 (9%)	.181
Diarrhea	7 (12%)	3 (13%)	4 (12%)	.885
Cardiac toxicity	1 (2%)	1 (4%)	0	.404
Confusion	4 (7%)	3 (13%)	1 (3%)	.289
Skin rash	2 (4%)	2 (9%)	0	.158
Autoimmunity (vitiligo)	1 (2%)	1 (4%)	0	.404
Mortality (cardiac arrest; one additional patient died during preconditioning)	1	na	na	na

NOTE: Adverse events were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0.

Abbreviation: na, not applicable.

Response duration and overall survival

Patients were followed for up to 52 months, with a median follow-up time of 28 months. Survival was measured from TIL infusion to death and the PFS from infusion to documented disease progression. The median OS of all 57 treated patients was 15.2 months (Fig. 2A and B). Responders did not reach the median OS time by the end of the study and nonresponders had a median OS of 6.1 months ($P < 0.001$). The 1-, 2-, and 3-year survival rates of all treated patients, the responding and nonresponding group, are summarized in Table 4. Seventy-eight percent (7 of 9) of the responding patients were alive 3 years after treatment. Survival benefit was significantly associated with objective response. The median PFS of all treated patients was 4.0 months (responders 7.0 months and nonresponders 2.0 months; $P < 0.001$; Fig. 2C and D). The OS of individual patients is summarized in Fig. 2B. Noteworthy, all 5 complete responders are on continuing remission 13, 15, 37, 42, and 52 months after TIL therapy. None of them received any additional treatment. The 1-year PFS of responding patients was 42% (8 of 19).

Twelve and 24-months survival rates, measured from the day of surgery, of all enrolled patients were 46% (33 of 71) and 33% (18 of 54), respectively, and included patients not receiving TIL due to clinical deterioration during cell preparation.

Impact of other immunotherapies

Prior IL-2–based therapy. All, but 3 patients received IL-2–based therapy before TIL treatment. IL-2–based therapies included mainly chemobiotherapy ($n = 40$) or high-dose IL-2 ($n = 7$). There was no correlation between the clinical outcome to TIL ACT and the type of response to prior IL-2–based therapy, and 31% (17 of 54) of the patients failing IL-2 therapy responded to TIL (5, 13). Supplementary Table S1 shows the clinical outcome to different types of immunotherapy in individual patients.

Ipilimumab therapy. To assess whether ipilimumab therapy affects the response to TIL ACT and vice versa, we retrospectively divided the patient cohort into 3 groups: Patients receiving ipilimumab (3 mg/kg) prior TIL ACT ($n = 13$; "IPI-TIL patients"); Patients receiving ipilimumab post TIL ACT ($n = 19$; "TIL-IPI patients"); and patients not receiving ipilimumab at any time ($n = 25$; "TIL only"). Notably, all IPI-TIL patients were nonresponders to ipilimumab therapy and progressed immediately (11 of 13) or relapsed after stabilization (2 of 13). The time between the first course of ipilimumab and TIL infusion was 26 ± 14 weeks. IPI-TIL patients had slightly worse baseline characteristics compared with the rest of the patients treated with TIL (100% stage M1c disease, 69.2% CNS involvement), which might explain why they received fewer doses of IL-2 (4.1 ± 1.9 vs. 7.4 ± 2.9 doses; $P < 0.001$; Supplementary Table S2). All other treatment characteristics and most importantly, the frequencies of grade 3/4 toxicities were utterly comparable (Supplementary Table S2). The overall response rate (38%) and median OS after TIL ACT (13.4 months) in IPI-TIL patients was almost identical to all other patients receiving TIL ($P = 1.0$ and $P = 0.59$, retrospectively; Supplementary Table S2). Despite the small cohort, the results indicate that failure to previous ipilimumab treatment does not affect the efficacy or safety of TIL.

In 19 TIL-IPI patients the average time between TIL infusion and the first course of ipilimumab was 46 ± 36 weeks. Only patients that progressed or relapsed after TIL therapy were treated with ipilimumab. The baseline characteristics of TIL-IPI patients (Supplementary Table S3) were comparable with the study population of a phase III trial with 3 mg/kg ipilimumab (2). The overall response rate

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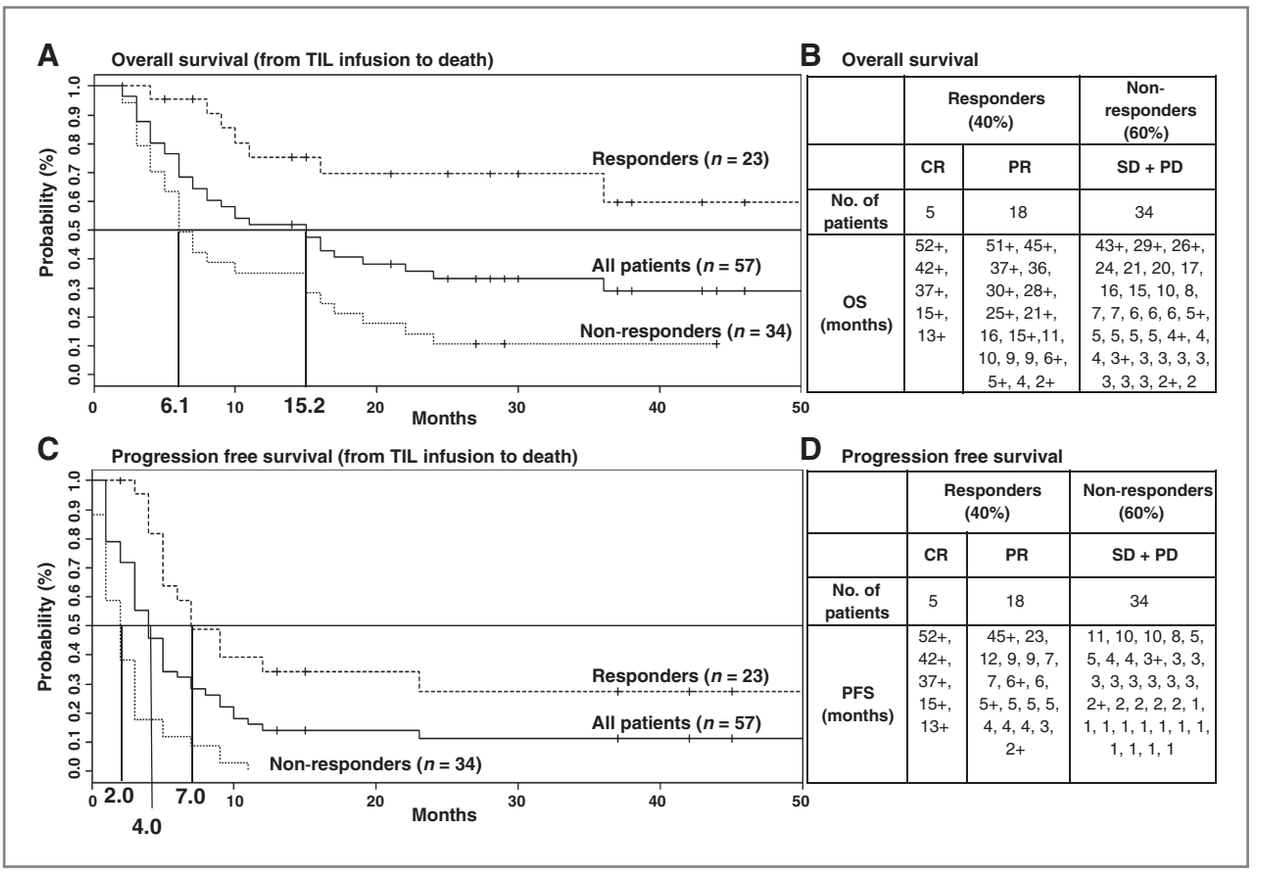

Figure 2. OS and PFS in the treated patient population. OS and PFS are measured from the day of TIL administration to death in months (A and B) or documented disease progression (C and D). A and C, Kaplan–Meier estimation. B and D, OS and PFS of individual patients according to their best overall response. Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; +, ongoing.

of ipilimumab in TIL-IPI patients was 16% (3 of 19). Notably, all 3 TIL-IPI responders experienced complete remission and are progression-free for 25, 23, and 17 months following ipilimumab.

Discussion

In the past few years, an increasing number of cancer centers including ours have reported that TIL ACT immunotherapy in combination with high-dose IL-2 and NMA conditioning is an effective therapeutic tool in patients with metastatic melanoma, yielding 40% to 50% objective responses (13–15, 18).

Here we report our clinical results on 80 enrolled patients with unselected TIL focusing on the intent-to-treat scenario and the impact of other immunotherapies administered pre and post TIL.

TIL cultures were established for 90% of the patients. Only 23 (28.8%) enrolled patients discontinued the study,

Table 4. Survival data of treated patients

	All patients ($N = 57$)	Responders OR ($N = 23$)	Nonresponders NR ($N = 34$)	P value OR vs. NR
Median OS from surgery to DOD (months)	16.4	not reached	8.1	<0.001
Median OS from TIL to DOD (months)	15.2	not reached	6.1	<0.001
1-year survival[a]	50% (24 of 48)	79% (15 of 19)	31% (9 of 29)	0.003
2-year survival	45% (14 of 31)	71% (10 of 14)	24% (4 of 17)	0.012
3-year survival	42% (8 of 19)	78% (7 of 9)	10% (1 of 10)	0.005

Abbreviations: OS, overall survival; DOD, died of disease.

[a]Survival rates were measured from the day of TIL infusion to death.

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which emphasizes the feasibility and applicability of TIL ACT. Treated patients had significantly improved survival compared with untreated patients.

Furthermore, we applied TIL immunotherapy to a highly advanced patient group, as 80% of the enrolled or treated patients had M1c disease. Notably, 30% of the treated patients with M1c disease responded to therapy, including patients with brain metastases, indicating that TIL ACT is an effective treatment for this unfavorable patient group (23, 24). The overall objective response rate was 29% among enrolled patients and 40% in the evaluated patient population. The median OS of all 57 treated patients, measured from TIL infusion to death, was 15.2 months and 16.4 months, when measured from surgery to death. Objective responders did not reach the median OS after a median follow-up time of 28 months and almost 80% are alive 3 years later. Five patients experienced ongoing complete remission (follow-up: 1– 4 years). Of note, patients with CR, responding to immunotherapy with high-dose IL-2 for over 30 months, have never progressed even 25 years later (25) and are potentially cured, as stated previously by Rosenberg (26). Randomized trials and long-term follow-ups are still required to prove this statement, but TIL ACT currently provides the hope to cure patients achieving complete responses.

Currently, information regarding the interaction of TIL ACT with ipilimumab includes only a single report, which shows durable complete remissions in 5 of 11 patients receiving the anti-CTLA4 antibody prior TIL ACT (5). However those patients were treated with TIL ACT in combination with 12 Gy TBI, which anyhow results in 40% CR (27). An important aspect of our study is therefore the impact of other U.S. Food and Drug Administration-approved immunotherapies on TIL ACT and vice versa, as well as the efficacy of TIL in patients that failed prior immunotherapies. Here, we show that there is no correlation between response to prior ipilimumab or IL-2–based therapy and the overall response to TIL. Furthermore, no additional toxicities occurred in patients previously treated with ipilimumab. Nonresponders to prior ipilimumab or IL-2 based therapy had the same probability to response as all other patients receiving TIL. TIL ACT is therefore also an effective treatment for patients failing other immunotherapies.

Interestingly, 3 of 19 (16%) patients that received ipilimumab after TIL therapy, experienced ongoing CRs, in contrast to only 0.7% CR rates reported in a phase III trial conducted with the same dose of ipilimumab alone (2). Although our patient cohort was small and the study was conducted retrospectively, this difference is noteworthy. To verify this observation, a controlled prospective study combining TIL ACT and ipilimumab will be conducted in the near future.

In conclusion, adoptive transfer of TIL is an effective treatment, which yields objective response rates of 29% in intent-to-treat patients and 40% in treated patients. TIL ACT can mediate durable objective responses in patients with highly advanced melanoma, including patients who had progressed on other immunotherapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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