Immunotherapy for metastatic melanoma and beyond = immunotherapie voor gemetastaseerd melanoom en verder

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Tumor-infiltrating lymphocyte therapy or ipilimumab in advanced melanoma

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ABSTRACT

Background

Immune checkpoint inhibitors and targeted therapies have dramatically improved outcomes in patients with advanced melanoma, but approximately half of these patients will not have a durable benefit. Phase 1-2 trials of adoptive cell therapy with tumor-infiltrating lymphocytes (TILs) have shown promising responses, but data from phase 3 trials are lacking to determine the role of TILs treating advanced melanoma.

Methods

In this phase 3, multicenter, open-label trial, we randomly assigned patients with unresectable stage IIIC or IV melanoma in a 1:1 ratio to receive TIL or anti–cytotoxic T-lymphocyte antigen 4 therapy (ipilimumab at 3 mg per kilogram of body weight). Infusion of at least $5x10^9$ TILs was preceded by non-myeloablative, lymphodepleting chemotherapy (cyclophosphamide plus fludarabine) and followed by high-dose interleukin-2. The primary end point was progression-free survival.

Results

A total of 168 patients (86% with disease refractory to anti–programmed death 1 treatment) were assigned to receive TILs (84 patients) or ipilimumab (84 patients). In the intention-to-treat population, median progression-free survival was 7.2 months (95% confidence interval [CI], 4.2 to 13.1) in the TIL group and 3.1 months (95% CI, 3.0 to 4.3) in the ipilimumab group (hazard ratio for progression or death, 0.50; 95% CI, 0.35 to 0.72; P<0.001); 49% (95% CI, 38 to 60) and 21% (95% CI, 13 to 32) of the patients, respectively, had an objective response. Median overall survival was 25.8 months (95% CI, 18.2 to not reached) in the TIL group and 18.9 months (95% CI, 13.8 to 32.6) in the ipilimumab group. Treatment-related adverse events of grade 3 of higher occurred in all patients who received TILs and in 57% of those who received ipilimumab; in the TIL group, these were mainly chemotherapy-related myelosuppression.

Conclusions

In patients with advanced melanoma, progression-free survival was significantly longer among those who received TIL therapy than among those who received ipilimumab. (Funded by the Dutch Cancer Society and others; ClinicalTrials.gov number: NCT02278887)

INTRODUCTION

Programmed death 1 (PD-1) protein blockade with nivolumab or pembrolizumab is a frequently used first-line treatment in patients with metastatic melanoma.[1-4] Combination immunotherapy with ipilimumab (an anti–cytotoxic T-lymphocyte antigen 4 antibody) and nivolumab induces responses in a higher percentage of patients(58% vs. 45%)[5] but is associated with a high incidence of severe adverse events and is currently recommended primarily for a subgroup of patients with poor prognostic factors such as a high serum lactate dehydrogenase (LDH) level or liver or brain metastases.

Approximately 50% of melanomas harbor a mutation in BRAF; thus, an additional treatment option is combined BRAF and MEK inhibition. Although this therapy is associated with high a response, resistance develops in most patients over time.[6, 7] Ipilimumab (with or without nivolumab) has become a second-line treatment option, but objective responses and durable benefits occur in only 15 to 30% of patients.[8-12] Combination treatment with nivolumab and anti–lymphocyte-activation gene 3 (LAG-3) has also been associated with objective responses in 16% of patients with disease that was refractory to anti-PD-1 therapy, but data on progression-free survival are lacking.[13] Although these new treatment options have substantially improved the prognosis in patients with metastatic melanoma, approximately 50% still die from the disease within 5 years after diagnosis of stage IV disease.[14]

Adoptive cell therapy with tumor-infiltrating lymphocytes (TILs) is a personalized autologous treatment that involves the ex vivo outgrowth and expansion of tumor-resident T cells and subsequent intravenous adoptive transfer of the cells after preparative lymphodepleting chemotherapy, which is supported by the administration of interleukin-2 to enhance the in vivo expansion of the cells and augment antitumor responses.[15-17] Evidence of clinical activity of TIL therapy in patients with advanced melanoma was reported by Rosenberg and colleagues in the 1990s.[18] Subsequent phase 1-2 trials showed responses in 36% and 70% of patients, with durable complete responses in up to 20% of patients.[19-26] More recently, objective responses were observed in 36% of patients who received LN-144 TIL therapy, even among those who had disease progression while receiving anti-PD-1 treatment, findings that illustrate the potential of this treatment after failure of previous immune checkpoint inhibition.[27] Despite these promising results, the role of TIL in the current treatment landscape remains undefined because data on a direct comparison of TILs with standard treatment are lacking. In this multicenter, open-label, phase 3, randomized trial, we compared TILs with ipilimumab as first- or second-line treatment in patients with advanced melanoma.

METHODS

Patients

Patients were eligible for inclusion in the trial if they were 18 to75 years of age and had histologically confirmed, unresectable or metastatic stage IIIC or IV cutaneous melanoma (hereafter "advanced melanoma") (as defined in the seventh edition of the *Cancer Staging Manual* of the American Joint Committee on Cancer) with one or more lesions (collectively 2 to 3 cm in diameter) that could be surgically removed for generation of TILs. In addition, patients were required to have residual measurable disease after resection as defined by the following: Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1[28]; a World Health Organization performance-status score of 0 or 1 (on a scale of 0 to 5, with higher numbers indicating greater disability); and a serum LDH level that was less than or equal to 2 times the upper limit of the normal range. One previous line of systemic treatment for this disease stage, excluding ipilimumab, was allowed. A full overview of eligibility criteria is provided in the Supplementary Appendix.

Trial design and treatment

In this multicenter, open-label, phase 3 trial, patients were randomly assigned in a 1:1 ratio to receive either TILs or ipilimumab. Randomization was stratified according to $BRAF^{V600}$-mutation status, line of treatment, and treatment center. Patients who were assigned to receive TILs underwent a metastasectomy for the retrieval and expansion of TILs, followed by hospital admission for administration of non-myeloablative, lymphodepleting chemotherapy (cyclophosphamide at a dose of 60 mg per kilogram of body weight per day for 2 days intravenously and fludarabine at a dose of 25 mg per square meter of body-surface area per day for 5 days intravenously), single intravenous adoptive transfer of $5x10^9$ to $2x10^{11}$ TILs, and subsequent high-dose interleukin-2 (600,000 IU per kilogram per dose) every 8 hours, for a maximum of 15 doses per protocol (Figure S1 in the Supplementary Appendix). Patients in the ipilimumab group received 3 mg of ipilimumab per kilogram intravenously every 3 weeks, for a maximum of 4 doses. Administration of ipilimumab could be delayed or discontinued if adverse events occurred, in accordance with the protocol. No dose reductions were allowed.

End points and assessments

The primary end point was progression-free survival assessed by the investigator with the use of RECIST, version 1.1. Progression-free survival was defined as the time from randomization to first disease progression (either radiologic progression or subsequent anticancer therapy, including systemic therapy, radiotherapy, or surgery) or death. The secondary end points were the following: progression-free survival assessed according to immune-related response criteria;[29] objective response assessed according to RECIST, version 1.1, and immune-related response criteria; complete response; overall survival; health-related quality of life; and safety.

Health-related quality of life was measured with the use of the European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire Core 15 Palliative care (EORTC QLQ-C15-PAL), a 15-item questionnaire on which higher scores on the global quality-of-life and functioning scales indicate better functioning and higher scores on the symptom scales indicate higher levels of symptom burden.[30] Adverse events were evaluated by the treating physician in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03. Efficacy analyses included all patients who underwent randomization (the intention-to-treat population), and safety analyses included all patients who had received chemotherapy and TIL or at least on dose of ipilimumab. Additional information on end-point assessment is provided in the Supplementary Methods section of the Supplementary Appendix.

Trial oversight

The trial was designed at one of the two participating clinical sites (the Netherlands Cancer Institute, Amsterdam) and was approved by the Central Committee on Research Involving Human Subjects in the Netherlands and the institutional review board and independent ethics committee at each trial center. The other participating clinical site was the National Center for Cancer Immune Therapy, Copenhagen University Hospital, Herlev, Denmark. The trial was conducted in accordance with the principles of the Declaration of Helsinki, the Harmonized Tripartite Guideline for Good Clinical Practice from the International Council for Harmonization, and the ethical principles underlying European Union Directive 2001/20/EC. All the patients provided written informed consent and received treatment at one of the two primary clinical sites. An independent data and safety monitoring board reviewed progress and safety.

Data were collected at each participating site, and raw data were seen only by the trial team from each participating site in accordance with the clinical trial agreement; a master data and sample transfer contract was signed by both sites. The data were analyzed at the Netherlands Cancer Institute. Authors who were not employees of the two participating clinical sites did not have access to the raw data. The authors agreed to maintain confidentiality of the data until publication and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. All the authors contributed to drafting the manuscript, provided critical revision, or did both, and all approved the decision to submit the final manuscript for publication. No one who is not an author contributed to writing the manuscript.

Generation of tumor-infiltrating lymphocytes

The manufacturing of TILs was based on established techniques.[19, 24, 31] TILs were manufactured at each trial center with the use of harmonized standard operating procedures according to the Good Manufacturing Practice guidelines of the European Union and EudraLex volume

4, which is specific to advanced therapy medicinal products. The TILs were classified as advanced therapy medicinal products under European Commission regulation 1394/2007. Further details are provided in the Supplementary Methods section of the Supplementary Appendix.

Statistical analysis

The sample size was calculated on the basis of a comparison of the percentage of patients with progression-free survival at 6 months. On the basis of a study by Hodi et al.,[32] it was expected that the percentage of patients with progression-free survival at 6 months in the ipilimumab group would be 20 to 25%. We estimated that at least 80 patients would have to undergo randomization in each group (160 patients in total) for the trial to have 90% to detect an increase in progression-free survival at 6 months from 20% in the ipilimumab group to 45% in the TIL (odds ratio, 3.27), using a two-group continuity corrected chi-squared test with a two-sided significance level of 0.05. With this level of accrual, an absolute increase from 25 precentage points with ipilimumab to 50 percentage points with TIL therapy (odds ratio, 3.0) in progression-free survival could be detected with 88% power. Considering the possibility that 5 to 10% of the patients randomly assigned to the TIL group would not receive the intended treatment, the required sample size was calculated to be 168 to 176 patients. Although the trial was powered to compare progression-free survival at 6 months, during the course of the trial it was considered statistically more efficient to analyze the complete progression-free survival curve with the use of survival methods, and this was included in a protocol amendment. Considering that the power calculation reflected a conservative approach, analysis of complete progression-free survival would yield sufficient power.

Progression-free and overall survival curves were constructed with the use of the Kaplan-Meier method, and treatment groups were compared with the use of the stratified (unweighted) log-rank test and the stratified Cox regression model. The trial was considered to be positive if the progression-free survival among patients who received TILs was significantly longer than that among those who received ipilimumab, on the basis of the log-rank test with a two-sided P value below 0.05. In addition, a prespecified per-protocol analysis of the primary end point with the use of a landmark approach was performed, including patients who received the trial treatment without rapid clinical progression within 5 weeks after randomization. As exploratory post hoc analyses, comparisons of progression-free and overall survival across subgroups of interest were performed. Data are presented in a forest plot, and survival curves were constructed with the use of the Kaplan-Meier method.

Responses after TIL and ipilimumab treatment were reported with their associated 95% binomial confidence intervals. Health-related quality-of-life outcomes were evaluated with the use of a generalized-estimating-equations model for longitudinal data.[33, 34] The widths

of the confidence intervals for the secondary end points and exploratory post hoc analyses have not been adjusted for multiplicity and cannot be used in place of a hypothesis test. Details are provided in the Statistical Analyses section of the Supplementary Appendix, protocol, and statistical analysis plan.

RESULTS

Patients and treatment

Between September 2014 and March 2022, a total of 168 patients were randomly assigned to receive either TILs (84 patients) or ipilimumab (84 patients) (the intention-to-treat population) (Figure S2). Baseline characteristics were balanced between the two treatment groups (Table 1). A total of 149 of 168 patients (89%) had disease progression after receiving previous systemic therapy - mostly adjuvant anti-PD-1 therapy (40 patients [24%]) or first-line anti-PD-1 therapy (105 patients [62%]). Details regarding these systemic therapies are provided in Table S1.

At the time of data cut-off on June 9, 2022, the overall median follow-up was 33.0 months. A total of 80 patients had received TILs and 82 patients had received at least one infusion of ipilimumab . The reasons for nonreceipt of TILs were patient decision (in 1 patient), late response to previous therapy (in 1 patient), insufficient TIL outgrowth (in 1 patient), and rapid clinical progression (in 1 patient). Patients who received TILs received a median of 40.9×10^9 cells (range, 4.9 to 110.4) and a median of 4 doses of high-dose interleukin-2 (range, 0 to 10). The median duration of hospital admission was 17 days (range, 12 to 38). Two patients did not receive ipilimumab owing to patients' decision or rapidly progressive disease that warranted the immediate initiation of combined BRAF and MEK inhibition. Patients who received ipilimumab received a median of 3 infusions (range, 1 to 4) and 26 of the 42 (62%) patients who discontinued treatment prematurely did so because of adverse events (Table S2).

Efficacy

In the intention-to-treat population, TILs were associated with a significant benefit with respect to progression-free survival assessed according to RECIST, version 1.1, with a median progression-free survival of 7.2 months (95% confidence interval [CI], 4.2 to 13.1), as compared with 3.1 months (95% CI, 3.0 to 4.3) with ipilimumab (hazard ratio for progression or death, 0.50; 95% CI, 0.35 to 0.72; $P<0.001$ by an unweighted stratified log-rank test) (Figure 1). The percentage of patients with progression-free survival at 6 months was 52.7% (95% CI, 42.9 to 64.7) in the TIL group and 21.4% (95% CI, 14.2 to 32.2) in the ipilimumab group. This benefit of TILs over ipilimumab was confirmed in a prespecified per-protocol analysis

(see the Supplementary Results section in the Supplementary Appendix and Figure S3). With assessment according to immune-related response criteria, median progression-free survival was 6.0 months (95% CI, 4.6 to 12.0) in the TIL group, as compared with 3.2 months (95% CI, 3.0 to 4.4) in the ipilimumab group (hazard ratio, 0.56; 95% CI, 0.39 to 0.79) (Figure S4). Results of a post hoc analysis of progression-free survival in key subgroups are shown in Figures S5 and S6.

Table 1. Baseline characteristics of the patients*

Characteristic	TIL (n=84)	Ipilimumab (n=84)	Total (n=168)
Sex – no. (%)			
Male	47 (56)	53 (63)	100 (60)
Female	37 (44)	31 (37)	68 (40)
Median age (range) - yr	59 (26 - 74)	59 (30 - 77)†	59 (26-77)
WHO performance-status score – no. (%)‡			
0	69 (82)	70 (83)	139 (83)
1	15 (18)	14 (17)	29 (17)
***BRAF* mutation status – no. (%)**			
V600 mutation	37 (44)	36 (43)	73 (43)
Wild-type	47 (56)	48 (57)	95 (56)
Treatment Center – no. (%)			
NKI	66 (79)	66 (79)	132 (79)
CCIT-DK	18 (21)	18 (21)	36 (21)
Disease stage at trial entry – no. (%)§			
Unresectable stage IIIC	2 (2)	2 (2)	4 (2)
Stage IV	82 (98)	82 (98)	164 (98)
M1a	13 (15)	18 (21)	31 (18)
M1b	7 (8)	17 (20)	24 (14)
M1c	56 (67)	40 (48)	96 (57)
Liver metastases	20 (24)	9 (11)	29 (17)
M1d	6 (7)	7 (8)	13 (8)
Lactate dehydrogenase level – no. (%)			
≤ ULN	67 (80)	70 (83)	137 (82)
1-2 x ULN	17 (20)	14 (17)	31 (18)

Table 1. Continued

Characteristic	TIL (n=84)	Ipilimumab (n=84)	Total (n=168)
Smoking status – no. (%)			
Yes	9 (11)	11 (13)	20 (12)
No	46 (55)	49 (58)	95 (56)
Prior systemic therapy – no. (%)			
Yes	75 (89)	74 (88)	149 (89)
No	9 (11)	10 (12)	19 (11)
Type of previous systemic therapy – no. (%)			
Adjuvant anti-PD-1 therapy	17 (20)	23 (27)	40 (24)
First-line anti-PD-1 therapy	56 (67)	49 (58)	105 (62)
Other	2 (2)	2 (2)	4 (2)

*Data shown are for the intention-to-treat population, which consisted of all patients who underwent randomization. Percentages may not total 100 because of rounding. *CCIT-DK denotes National Center for Cancer Immune Therapy, NKI Netherlands Cancer Institute, PD-1 programmed cell death protein 1, TIL tumor-infiltrating lymphocyte, and ULN upper limit of the normal range.*
†Two patients who were older than 75 years of age were included in the trial because these patients were deemed to be in excellent clinical condition by the principal investigator.
‡The World Health Organization (WHO) performance-status score is based on a five-step grading system, with 0 indicating no performance restrictions and higher scores indicating increased restrictions.
§Disease stages are defined according to the seventh edition of the *Cancer Staging Manual* of the American Joint Committee on Cancer.

The percentage of patients with an objective response according to RECIST, version 1.1, was 49% (95% CI, 38 to 60) in the TIL group and 21% (95% CI, 13 to 32) in the ipilimumab group. Complete responses were observed in 20% (95% CI, 12 to 30) of the patients in the TIL group and 7% (95% CI, 3 to 15) of those in the ipilimumab group (Table 2 and Figure 2), with durable complete responses in both treatment groups (Figure S7). With assessment according to immune-related response criteria, objective responses were seen in 50% (95% CI, 39 to 61) of patients in the TIL group and 20% (95% CI, 12 to 30), of those in the ipilimumab group. Table S3, which shows an overview of systemic treatments administered after disease progression, indicates that more patients in the TIL group who had not had a response received ipilimumab or the combination of ipilimumab and nivolumab than those in the ipilimumab group who had not had a response.

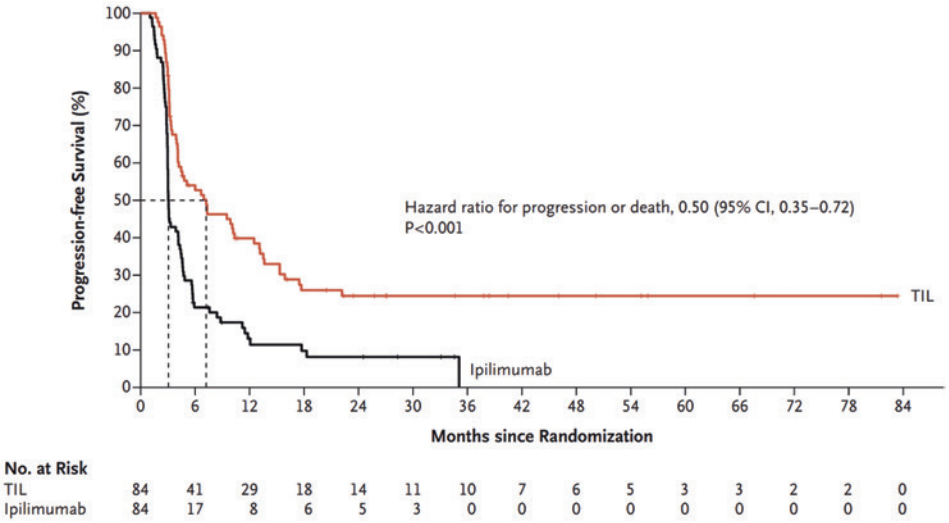

Figure 1. Progression-free survival. Progression-free survival assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, is shown for all patients who were randomly assigned to receive tumor-infiltrating lymphocyte (TIL) therapy or ipilimumab (the intention-to-treat population). The patients were stratified according to *BRAF* V600-mutation status, line of treatment, and treatment center. Hazard ratios were estimated with the use of the stratified Cox regression model. The P value was calculated with use of the stratified log-rank test with a two-sided 95% confidence interval. Tick marks indicate censored data. CI denotes confidence interval.

Overall survival

Median overall survival among patients in the TIL group was 25.8 months (95% CI, 18.2 to not reached), as compared with 18.9 months (95% CI, 13.8 to 32.6) among those in the ipilimumab group (hazard ratio for death, 0.83; 95% CI, 0.54 to 1.27). The 2-year overall survival was 54.3% (95% CI, 43.9 to 67.2) in the TIL group and 44.1% (95% CI, 33.6 to 57.8) in the ipilimumab group (Figure S8). Overall survival in key subgroups is shown in Figures S9 through S11.

Safety

Adverse events that were assessed by the investigator as being related to treatment occurred in all patients in the TIL group and in 96% of those in the ipilimumab group. The most common adverse events of any grade related to TILs and ipilimumab are presented in Table 3. All patients in the TIL group had grade 3 or 4 neutropenia owing to preparative lymphodepleting chemotherapy, with a median duration of neutropenia of 7 days (range, 2 to 58 days). Capillary leak syndrome (of any grade) associated with interleukin-2 occurred in 30% of the patients who received TILs and interleukin-2 (Table 3). In the TIL group, autoimmune toxic effects leading to skin hypopigmentation occurred in 9 patients (11%) (Table 3); uveitis occurred in 6 patients (8%), and hearing impairment occurred in 3 patients (4%) (Table S4).

Table 2. Best response*

	TIL (n=84)	Ipilimumab (n=84)
Best response		
Complete response		
No. of patients	17	6
Percentage of patients (95% CI)	20 (12-30)	7 (3-15)
Partial response		
No. of patients	24	12
Percentage of patients (95% CI)	29 (19-40)	14 (8-24)
Stable disease		
No. of patients	16	15
Percentage of patients (95% CI)	19 (11-29)	18 (10-28)
Progressive disease		
No. of patients	24	40
Percentage of patients (95% CI)	29 (19-40)	48 (37-59)
Could not be determined – no. (%)†	3 (4)	11 (13)
Objective response‡		
No. of patients	41	18
Percentage of patients (95% CI)	49 (38-60)	21 (13-32)
Clinical benefit§		
No. of patients	57	33
Percentage of patients (95% CI)	68 (57-78)	39 (29-51)

*The best objective response was assessed according to RECIST, version 1.1, and according to investigator review in the intention-to-treat population.
†In 3 of the patients in the TIL group (4%) and 11 of those in the ipilimumab group (13%), the best radiologic response could not be evaluated or was not evaluated because of an event (death or rapid clinical progression that warranted the initiation of subsequent anticancer therapy) before the first response evaluation. One of the 3 patients in the TIL group had target lesions that could not be evaluated during follow-up. In the other 2 patients in the TIL group and all 11 patients in the ipilimumab group, the best radiologic response could not be evaluated because of an event.
‡Objective response was defined according to RECIST, version 1.1, as a complete response or partial response.
§Clinical benefit was defined as a complete response, a partial response, or stable disease. Responses are reported with their associated 95% binomial confidence intervals. The widths of the confidence intervals have not been adjusted for multiplicity and cannot be used in place of a hypothesis test.

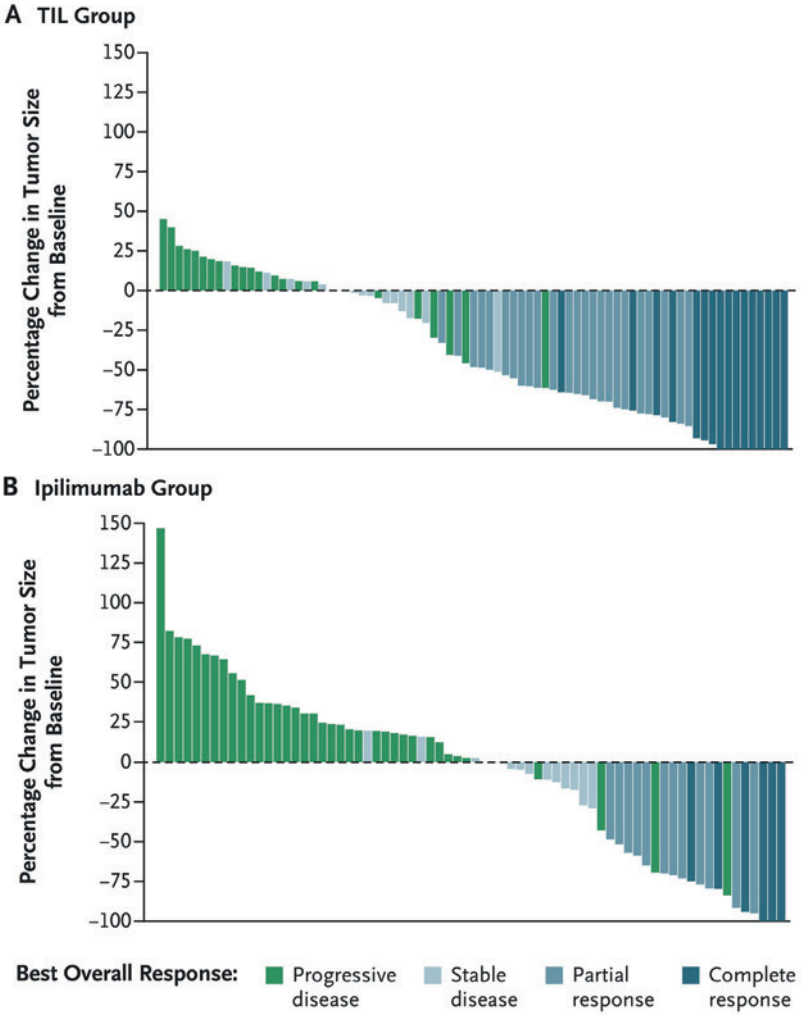

Figure 2. Clinical activity of treatment. The waterfall plot shows the maximum percentage of change in tumor burden from baseline (on computed tomographic imaging closest to the start of treatment in both groups) in the intention-to-treat population in patients who were assigned to receive TIL therapy (Panel A) or ipilimumab (Panel B). The tumor size was calculated as the sum of the diameters of all target lesions in each patient. In 14 patients (3 patients [4%] in the TIL group and 11 [13%] in the ipilimumab group), the best radiologic change in tumor size could not be evaluated or evaluation was not performed because of an event (death or rapid clinical progression for which the initiation of subsequent anticancer therapy was warranted) that occurred before the response evaluation. Data from these patients were excluded from this figure. In 6 patients (3 patients [4%] in the TIL group and 3 [4%] in the ipilimumab group), the best change in tumor size from baseline was 0.0%. Each bar represents 1 patient, and bar colors indicate the best objective response category according to RECIST, version 1.1, in evaluable patients. The change in tumor size was calculated as the maximum percentage change in the size of target lesions from baseline to the time of progression. Patients who had a complete response without a 100% decrease in tumor size had residual lymph nodes smaller than 10 mm in shortest diameter or residual lesions smaller than 5mm in diameter.

Treatment-related adverse events of grade 3 or higher occurred in all patients in the TIL group and 57% of those in the ipilimumab group. Treatment-related serious adverse events occurred in 15% of the patients in the TIL group and 27% of those in the ipilimumab group (Table S5). All treatment-related serious adverse events are presented in Table S6. New TIL-related adverse events of grade 3 or higher occurred typically during hospital admission (in 99% of cases) and were handled according to protocol on the oncology ward; short-term stabilization in an intensive care unit was warranted in eight patients (10%). One patient in the TIL group died from an arterial thromboembolism on day 22 after treatment; this death was not considered by the investigators to be related to treatment.

Table 3. Most common treatment-related adverse events*

Adverse Event	TIL Group (n=80)				Ipilimumab Group (n=82)	
	Chemotherapy		TILs and Interleukin-2		Ipilimumab	
	Any Grade	≥Grade 3	Any Grade	≥Grade 3	Any Grade	≥Grade 3
	number of patients (percent)					
Neutrophil count decreased	80 (100)	80 (100)	-	-	-	-
Platelet count decreased	73 (91)	71 (89)	-	-	-	-
Anemia	73 (91)	16 (20)	-	-	-	-
Nausea	69 (86)	2 (2)	41 (51)	0	30 (37)	2 (2)
Febrile neutropenia	69 (86)	69 (86)	59 (74)	59 (74)	-	-
White-cell count decreased	57 (71)	57 (71)	-	-	-	-
Fatigue	49 (61)	4 (5)	54 (68)	7 (9)	37 (45)	1 (1)
Hypophosphatemia	49 (61)	20 (25)	57 (71)	48 (60)	-	-
Alopecia†	37 (46)	0	-	-	-	-
Diarrhea	36 (45)	2 (2)	36 (45)	2 (2)	37 (45)	12 (15)
Hypocalcemia	36 (45)	1 (1)	29 (36)	0	-	-
Hypoalbuminemia	27 (34)	0	31 (39)	0	-	-
Vomiting	26 (32)	2 (2)	15 (19)	0	11 (13)	1 (1)
Headache	20 (25)	0	19 (24)	0	22 (27)	1 (1)
Hypokalemia	20 (25)	2 (2)	12 (15)	0	-	-
Elevated AST level	18 (22)	4 (5)	26 (32)	8 (10)	18 (22)	7 (9)
Rash	18 (22)	2 (2)	37 (46)	9 (11)	28 (34)	4 (5)
Weight gain	17 (21)	0	28 (35)	0	-	-
Elevated ALT level	14 (18)	7 (9)	25 (31)	8 (10)	22 (27)	8 (10)
Elevated alkaline phosphatase level	14 (18)	3 (4)	17 (21)	3 (4)	12 (15)	4 (5)
Anorexia	13 (16)	1 (1)	-	-	14 (17)	1 (1)
Dizziness	12 (15)	0	-	-	-	-

Table 3. Continued

Adverse Event	TIL Group (n=80)				Ipilimumab Group (n=82)	
	Chemotherapy		TILs and Interleukin-2		Ipilimumab	
	Any Grade	≥Grade 3	Any Grade	≥Grade 3	Any Grade	≥Grade 3
	number of patients (percent)					
Increased γ-glutamyltransferase level	11 (14)	6 (8)	12 (15)	6 (8)	-	-
Fever	11 (14)	1 (1)	74 (92)	36 (45)	11 (13)	2 (2)
Dysgeusia	11 (14)	0	-	-	-	-
Hypomagnesemia	11 (14)	0	-	-	-	-
Dyspnea	10 (12)	2 (2)	63 (79)	15 (19)	-	-
Constipation	9 (11)	0	-	-	-	-
Edema limbs	8 (10)	0	23 (29)	0	-	-
Chills	-	-	67 (84)	6 (8)	-	-
Pruritus	-	-	-	-	34 (41)	0
Sinus tachycardia	-	-	40 (50)	1 (1)	-	-
Colitis	-	-	-	-	20 (24)	16 (20)
Abdominal pain	-	-	-	-	19 (23)	1 (1)
Hypotension	-	-	33 (41)	6 (8)	-	-
Malaise	-	-	-	-	13 (16)	0
Creatine kinase level increased	-	-	29 (36)	9 (11)	-	-
Dry mouth	-	-	-	-	9 (11)	0
Pulmonary edema	-	-	26 (32)	1 (1)	-	-
Capillary leak syndrome	-	-	24 (30)	1 (1)	-	-
Hypoxia	-	-	19 (24)	5 (6)	-	-
Hypertension	-	-	15 (19)	11 (14)	-	-
Myalgia	-	-	12 (15)	1 (1)	-	-
Blurred vision	-	-	9 (11)	0	-	-
Skin hypopigmentation	-	-	9 (11)	0	-	-

*Included are the most common treatment-related adverse events of any grade and those of grade 3 or higher, as defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03, that occurred in at least 10% of the patients who received chemotherapy and TILs or at least on dose of ipilimumab (the safety analysis population). Dashes indicate that the adverse events did not occur in at least 10% of the patients All the patients had more than one adverse event. ALT denotes alanine aminotransferase, and AST aspartate aminotransferase.
†Transient alopecia totalis occurred in all patients in the TIL group after chemotherapy. However, this event was not systematically reported in medical records and thus cannot be reported.

Health-related quality of life

Patients in the TIL group had higher mean scores on the global health-related quality-of-life, physical functioning, and emotional functioning domains after treatment than those in the ipilimumab group (Table 4). Patients in the TIL group reported a lower symptom burden of fatigue, pain and insomnia than those in the ipilimumab group, with differences still observed at week 60 (Table S8). However, patients in the TIL group reported a higher symptom burden of nausea and vomiting than those in the ipilimumab group, with a mean difference in symptom scores of 1.6 at week 24.

Table 4. Health-related quality-of-life scores at 6 months

Scores on the EORTC QLQ-C15 PAL quality-of-life a and functioning scales†	**TIL group (mean)**	**Ipilimumab group (mean)**	**Difference (95%CI)***	
Global quality of life	77.4	69.6	7.7	(5.1 to 10.4)
Physical functioning	82.0	79.1	2.9	(1.4 to 4.5)
Emotional functioning	85.4	75.7	9.7	(7.5 to 11.9)
Scores on the EORTC QLQ-C15 PAL symptom scales‡	**TIL (mean)**	**Ipilimumab (mean)**	**Difference (95%CI)**	
Fatigue	25.9	33.8	-7.9	(-11.2 to -4.6)
Nausea and vomiting	7.5	5.9	1.6	(0.7 to 2.5)
Pain	14.3	20.7	-6.4	(-9.3 to -3.5)
Dyspnea	10.0	12.4	-2.4	(-5.0 to 0.1)
Insomnia	23.6	28.1	-4.5	(-7.2 to -1.9)
Appetite loss	12.4	13.5	-1.1	(-2.9 to 0.7)
Constipation	6.7	7.1	-0.4	(-1.3 to 0.5)

* The widths of the confidence intervals have not been adjusted for multiplicity and cannot be used in place of a hypothesis test.

† Scores on the European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire Core 15 palliative Care (EORTC QLQ-C15 PAL) global quality-of-life and functioning scales range from 0 to 100, with higher scores indicating better functioning.

‡ Scores on the EORTC QLQ-C15 PAL symptom scales range from 0 to 100, with higher scores indicating higher levels of symptom burden.

DISCUSSION

This multicenter, phase 3, randomized trial involving patients with advanced melanoma compared TIL T-cell therapy as first- or second-line treatment with ipilimumab, which has previously been used as a second-line option in metastatic melanoma.[4] Progression-free survival was more than twice as long in the TIL group as in the ipilimumab group, and the hazard of disease progression or death was 50% lower. Separation of the progression-free survival curves occurred within 6 months after randomization, with a 30 percentage-point difference between the groups at 6 months and a continued benefit for patients in the TIL group.

Previous phase 1-2 trials have shown the potential clinical benefit of TILs in patients with metastatic melanoma, although most involved patients who had not received anti-PD-1 therapy.[19-24, 27] In the current trial, although 86% of the patients had disease progression after they received previous anti-PD-1 treatment either as adjuvant or first-line agents, 49% of the patients in the TIL group had an objective response, and of these patients, 20% had a complete response. These percentages are higher than those seen in the recent trial of LN-144 TIL,[27] possibly because most patients who received LN-144 TIL therapy had had disease progression after multiple previous lines of systemic treatment, including anti-PD-1 therapy, ipilimumab, and – in patients with $BRAF^{V600}$-mutated melanoma – BRAF and MEK inhibition. In our trial, no major differences in progression-free survival were observed according to the stratification factors of BRAF mutation status, line of treatment, or treatment center.

First-line treatment options for advanced melanoma have rapidly evolved over the past 5 years. In addition to anti-PD-1 therapy, currently approved treatment options are the following: combination therapy with ipilimumab and nivolumab, combined BRAF and MEK inhibitors, and relatlimab (anti-LAG-3 antibody).[6, 14, 35] In our trial, nine patients (11%) received TILs as first-line treatment, and no major difference was seen in progression-free survival among patients who had received no previous therapy, those who had received adjuvant therapy, and those who received previous first-line anti-PD-1 therapy. This finding suggests that TIL therapy can also be effective as first-line treatment; however, patient and disease characteristics (e.g., brain metastases, a high serum LDH level, or poor performance status), potential toxic effects and the availability of the treatment play important roles in the choice of treatment. Our trial primarily included patients who had received previous adjuvant or first-line anti-PD-1 monotherapy. For these patients, TIL therapy could be a possible first- or second-line treatment option for metastatic disease, as shown in this trial, whereas the data on LN-144 TIL therapy in patients with more refractory disease clearly suggest a broader indication for TILs.

The antitumor activity of ipilimumab monotherapy after failure of anti-PD-1 inhibition is well known, with objective responsesof 4 to 56%,[9-12] results that were confirmed in this trial. In a retrospective, multicenter, cohort trial involving 355 patients with advanced melanoma that was refractory to anti-PD-1 therapy, 31% of the patients who received a combination of ipilimumab plus nivolumab had an objective response, as compared with 13% of those who received ipilimumab alone.[9] Similar objective responses were observed in a recent prospective trial involving patients with advanced melanoma that was refractory to anti-PD-1 therapy. That trial showed objective responses in 19 of 69 patients (28%) who received a second-line combination of ipilimumab and nivolumab and in 2 of 23 patients (9%) who received second-line ipilimumab monotherapy.[8] The estimates of 6-month progression-free survival were 34% (90% CI, 25 to 44) in the combination-treatment group and 13% (90% CI, 4 to 27) for ipilimumab-monotherapy group. In our trial, the percentage of patients with progression-free survival at 6 months was 52.7% (95% CI, 42.9 to 64.7) in the TIL group and 21.4% (95% CI, 14.2 to 32.2) in the ipilimumab group. The results of these two trials cannot be directly compared, but they suggest a benefit of TILs over the combination of ipilimumab plus nivolumab. The difference between the two ipilimumab groups could be explained by differences in the baseline characteristics of the patients, especially the serum LDH level. In addition to immunotherapies, combined BRAF and MEK inhibition remains a second-line treatment option for patients with $BRAF^{V600}$-mutated melanoma. Although this treatment has been associated with high objective responses in up to 57% of patients,[11, 36] treatment resistance remains a problem for the majority of patients.

In our trial, treatment-related adverse events were more frequently seen with TILs than with ipilimumab, owing predominantly to chemotherapy, interleukin-2, or both, , and these events were in line with those in previous studies.[19, 24] Despite the increased frequency of adverse events, the global health-related quality-of-life scores were higher in patients who received TILs. In this trial, treatment with ipilimumab resulted in a high incidence of adverse events of grade 3 or higher (57%).

This phase 3, multicenter, open-label, randomized trial involving patients with advanced melanoma (the majority of whom had disease that was refractory to anti-PD-1 therapy) showed that TILs can be successfully generated from resected melanoma metastases in patients with advanced melanoma. Treatment with TILs was associated with significantly longer progression-free survival than treatment with ipilimumab.

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SUPPLEMENTARY MATERIAL

Supplementary methods

Inclusion Criteria

- Histologically confirmed unresectable AJCC (7^{th} edition) stage IIIc or stage IV melanoma
- Patients must have metastatic melanoma with a resectable metastatic lesion(s) of sufficient size (≥2-3 cm in total) and must be willing to undergo such a resection for experimental purposes. Resected metastases during stage IV disease that were removed at earlier time points, but were used to grow clinical grade TIL up to Rapid Expansion Protocol, may be used as well with informed consent of the patient.
- Patients should have received a maximum of one line of prior systemic therapy, excluding ipilimumab, for unresectable or metastatic melanoma. Note that prior adjuvant or neoadjuvant melanoma therapy is permitted if it was completed at least six weeks prior to randomization, and all related adverse events have either returned to baseline or stabilized.
- Patients must be ≥18 years and ≤75 years of age and must have measurable disease by CT or MRI per RECIST, version 1.1, criteria next to the resected lesion.
- Patients must have a clinical performance status of ECOG/WHO 0 or 1.
- Patients of both genders must be willing to practice a highly effective method of birth control during treatment and for four months after receiving the preparative chemotherapy regimen.
- Patients must be able to understand and sign the Informed Consent document.
- Laboratory Parameters (Note: patients may undergo resection with lab values outside of the parameters listed below if it is anticipated that the resection will correct the abnormality).
    - Hematology:
        - Absolute neutrophil count greater than $1.5x10^9$/L without support of filgrastim.
        - Platelet count greater than $100x10^9$/L.
        - Hemoglobin greater than 5 mmol/L, or 80 g/L.
    - Chemistry
        - Serum ALAT/ASAT less than 3 times the upper limit of normal, or less than 5 times ULN for patients with liver metastases.
        - Serum creatinine clearance 50 mL/min or higher.
        - Total bilirubin less than or equal to 20 micromol/L, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 50 micromol/L.
        - LDH ≤ 2x ULN

- Serology:
  - Seronegative for anti-HIV antibodies. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune-competence and thus be less responsive to the experimental treatment and more susceptible to its toxicities).
  - Seronegative for anti-hepatitis B antigen, and anti-hepatitis C antibodies. Seronegative for syphilis antibodies.
  - HSV, EBV, and CMV (positivity for HSV, EBV, or CMV is not an exclusion criterion for participation, but prophylactic medication can be started when deemed necessary prior to chemotherapy treatment in case patients randomize for the TIL treatment arm).

Exclusion Criteria

- Life expectancy of less than three months.
- Patients with metastatic ocular/ mucosal or other non-cutaneous melanoma.
- Adjuvant treatment with ipilimumab within 6 months prior to randomization.
- Requirement for immunosuppressive doses of systemic corticosteroids (>10 mg/day prednisone or equivalent) or other immunosuppressive drugs within the last 3 weeks prior to randomization.
- Presence of more than two CNS metastases.
- Patients who have any CNS lesion that is symptomatic, greater than 1 cm in diameter or shows significant surrounding edema on MRI scan will not be eligible until they have been treated and demonstrated no clinical or radiologic CNS progression for at least 2 months.
- Patients with the following factors will be excluded because of inability to receive high dose interleukin-2:
  - History of coronary revascularization
  - Documented LVEF of less than 45% in patients with:
    - Clinically significant atrial and/or ventricular arrhythmias, including but not limited to: atrial fibrillation, ventricular tachycardia, 2° or 3° heart block.
  - Documented FEV1 less than or equal to 60% predicted for patients with:
    - A prolonged history of cigarette smoking (greater than 20 pack/year within the past 2 years).
    - Symptoms of respiratory distress.
- Patients with toxicities from prior non-systemic treatment that have not recovered to grade 1 or less. Patients may have undergone minor surgical procedures or focal palliative radiotherapy (to non-target lesions) within the past 4 weeks, as long as all toxicities have recovered to grade 1 or less.

- Pregnancy or breastfeeding women, because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant.
- Any active systemic infections, coagulation disorders or other active major medical illnesses.
- Any autoimmune disease: patients with a documented history of inflammatory bowel disease, including ulcerative colitis and Crohn's disease are excluded from this study, as are patients with a history of symptomatic autoimmune disease including rheumatoid arthritis, autoimmune thyroiditis (e.g. Hashimoto's disease), autoimmune hepatitis, systemic progressive sclerosis (scleroderma), Systemic Lupus Erythematosus, and autoimmune vasculitis (e.g., Wegener's Granulomatosis). Subjects with motor neuropathy considered of autoimmune origin (e.g., Guillain-Barré Syndrome) are excluded from this study. Patients with vitiligo are eligible to enter the study.

End points

Disease progression was defined as radiologic progression according to RECIST, version 1.1, or need of subsequent anticancer therapy (including systemic therapy, radiotherapy, and surgery). The CT scan closest to start of treatment was used as baseline in both treatment groups.

As pseudo-progression has been described in patients treated with ipilimumab, progression-free survival was also evaluated according to immune-related response criteria (irRC).[1]

Adverse events (AEs) and serious adverse events (SAE) were continuously assessed by the treating physician according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.03 from the moment of signing informed consent until 28 days after the last protocol treatment and were followed until resolution or stabilization until 100 days after the last protocol treatment.

Health Related Quality of Life (HRQoL) scores of patients were assessed by means of the European Organisation for the Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire-Core 15 Palliative care (QLQ-C15 PAL).[2] Other HRQoL instruments used were EuroQol 5D-3L (EQ5D) and Impact of Event Scale (IES), and results will be reported seperately.[3, 4] HRQoL assessments took place prior to treatment (either first ipilimumab dose or at hospitalization for TIL treatment [baseline]). The second evaluation took place at fourth ipilimumab dose (if patients could not receive a fourth dose due to AEs, the evaluation took place on the date of the initially scheduled fourth dose), or at discharge after hospital admission for TIL treatment. Follow-up evaluations took place at 12, 18, 24 weeks after treatment in both treatment arms, thereafter every three months for two years and every four months in the following year. Patients were evaluated for HRQoL up to three years after treatment. Missing items from the QLQ-C15 PAL were imputed according to EORTC guidelines.[2, 5]

Generation of TIL

One or more melanoma lesions (primary or metastasis) of at least 2-3 cm in total were surgically resected and enzymatically digested using an enzyme mixture of Pulmozyme (containing rhDNase) and collagenase, or mechanically fragmented for the harvest of autologous TIL. Subsequently, TIL were expanded *ex vivo* for approximately two to four weeks by addition of interleukin-2 (6,000 IU/mL) to generate ≥5x10^7 T cells, with at least 10% $CD3^+$ (or $CD4^+$ / $CD8^+$) T cells, as determined by flow cytometry (initial outgrowth phase or pre-Rapid Expansion Protocol [pre-REP]). This TIL product generated in the pre-REP could be frozen when clinically indicated.

Subsequently, pre-REP TILs were expanded with the REP, TIL were cultured in the presence of irradiated allogeneic peripheral blood mononuclear cells ("feeder cells") in a 200:1 feeder cell: TIL ratio, soluble anti-CD3 antibody (OKT-3) and interleukin-2 (3,000 IU/mL) in a WAVE Base20/50EHT-L and KIT20EHT (GE Healthcare) or Xuri Cell Expansion System W25 (Cytiva) for 14 days. Release specifications were sterility, cell number (≥ 5x10^9 cells), and viability (>70%). Cell number and viability was determined by visual counting using a hemacytometer under a microscope, according Ph.Eur. 2.7.29. Sterility was confirmed using rapid sterility testing, according Ph. Eur. 2.6.27. The administered "young" TIL product was dosed with a maximum of 2x10^{11} cells. The fresh, non-cryopreserved, TIL drug product was administered to the patients intravenously in a volume of 200-300 mL in 20-30 minutes, within two hours after arrival at the clinical ward.

Clinical grade pre-REP TIL that were available through metastasectomy for stage IV disease prior to enrollment of this study, could be used upon written informed consent from the patient.

Statistical analyses

The required sample size was based on an initial six-month comparison of PFS rates between the treatment groups. Hodi et al. (2010)[6] demonstrated a six-month PFS of 20-25% in patients with unresectable stage III-IV melanoma treated with ipilimumab. Since the trial was designed, several new insights and methods have been proposed for the analysis of complete PFS in immunotherapy trials, considering a delayed treatment effect often observed in immunotherapy. The original primary end point was the comparison of the PFS rate between treatment arms at six months. However, since the analysis of the complete PFS using survival methods is considered crucial for a convincing analysis, complete PFS was selected as primary end point, as included in a protocol amendment.

For comparison of progression-free survival (PFS; according to RECIST, version, 1.1, [primary end point]) curves between ipilimumab and TIL, the stratified (unweighted) log-rank test, Fleming-Harrington weighted log-rank test (putting more weight on events that occur later in time) and the Restricted Mean Survival Time (RMST) analyses were performed. The latter two methods were used as sensitivity analyses to estimate the treatment effect in the possible presence of non-proportional hazards, induced by an expected delayed treatment effect.[7] For PFS and OS, in addition to the (unweighted) log-rank test, the hazard ratio (HR), median survival times, six-month PFS rates, and 2-year OS rates (together with their 95% confidence intervals [CI]) were used to assess the effect of TIL compared to ipilimumab. The HR was derived using the stratified Cox regression model. Median overall survival was calculated according to the reverse Kaplan-Meier method. Stratification factors were those that had been used for randomization ($BRAF^{V600}$ mutation, treatment line, and treatment center).

As progression due to failure of treatment with need for subsequent anti-cancer therapy is not per definition radiologic progression according to RECIST, version 1.1, or irRC, a sensitivity analysis was performed. In this sensitivity analysis, progression events defined as the need for start of subsequent therapy instead of radiologic progression were disregarded, meaning that patients having such progression events were either censored or had death as event for PFS.

In a pre-specified per-protocol analysis of the primary end point using a landmark approach, patients receiving at least one dose of treatment, without rapid clinical progression within five weeks after randomization, were evaluated for PFS according to RECIST, version 1.1. As TIL production generally takes five weeks, this analysis excluded patients for whom TIL treatment was not feasible. The starting point for this PFS analysis was time since randomization plus an additional five weeks (landmark approach).

A power analysis for OS was performed a-priori using the following assumptions. A median follow-up of at least two years would be required as the estimated historical median OS after ipilimumab is ten months.[6] Assuming that TIL would be able to decrease the hazard of death by 33%, approximately 138 deaths would need to be observed to provide 80% power for a one-sided test at 5% level.

Safety was evaluated in all patients receiving at least one dose of treatment (one dose of ipilimumab or one dose of chemotherapy in patients treated with TIL [Safety Analysis Set]). Highest grade of treatment-related AEs and SAEs are reported per treatment arm. AEs for both arms are additionally presented as occurring during the entire study period (Table S5), and for patients receiving TIL also by specific time periods (before and after TIL administration) (Table S7).

HRQoL scores were calculated by means of the European Organization for the Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire-Core 15 Palliative care (QLQ-C15 PAL).[2] Missing items from the EORTC QLQ-C15 PAL were imputed according to EORTC guidelines. [2, 5] Outcomes were evaluated using a generalized estimating equations model for longitudinal data.[8, 9] All models were adjusted for center (NKI versus CCIT-DK), $BRAF^{V600}$ mutation (yes/no), first-line treatment (yes/no), age (in years), gender (female/male), progression status (yes/no; measured as a time-dependent variable), and time. Coefficients of all covariates were considered as fixed effects. Results were interpreted with a p-value below 0.05. All analyses were conducted using R statistical computing version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Supplementary results

Efficacy

For PFS according to RECIST, version 1.1 (primary end point), the proportional hazards assumption was not violated, according to the statistical test based on the Schoenfeld residuals (χ^2 = 0.91, degrees of freedom=1, p=0.34). Nevertheless, the Fleming-Harrington weighted log-rank test and the RMST analyses also showed TIL treatment outperformed ipilimumab treatment for PFS (P<0.001 for both the Fleming-Harrington log-rank test and the RMST analysis). According to the RMST analysis, the restricted mean survival time for PFS was 25.4 months for TIL treatment and 7.0 months for ipilimumab.

A sensitivity analysis evaluating the influence of progression due to the need for start of subsequent anticancer therapy as opposed to radiologic progression according to RECIST, version 1.1, or irRC, confirmed the benefit of TIL compared to ipilimumab according to both RECIST, version 1.1 (HR: 0.53 [95% CI, 0.37 to 0.76] according to the unweighted stratified log-rank test) and irRC (HR: 0.69 [95% CI, 0.48 to 0.99] according to the unweighted stratified log-rank test).

According to the per-protocol analysis of the primary end point in the per-protocol analysis set, including patients who started treatment and who did not experience rapid clinical progression within five weeks after randomization (landmark approach), PFS was also significantly longer in patients treated with TIL compared to ipilimumab, with a median PFS of 7.2 months (95% CI, 4.2 to 13.1) and 3.0 months (95% CI, 3.0 to 4.2), respectively (HR: 0.47 [95% CI, 0.33 to 0.69] according to the unweighted stratified log-rank test) (Figure S3).

Although the median follow-up is more than two years, there was no significant OS difference between the arms. This inconclusive finding can be explained by the fact that the trial is underpowered for the secondary end point OS. OS was powered on an anticipated 33% decrease in the hazard of death for TIL patients (HR of 0.67), which required 138 deaths

to achieve 80% power. In this trial, 90 deaths have been observed to date resulting in a HR of 0.83 (95% CI, 0.5 to 1.3), meaning too few events to show a significant treatment benefit on OS, even if such an effect exists. Note that the anticipated 33% decrease in hazard of death is within the 95% CI reported in this trial.

SUPPLEMENTARY FIGURES

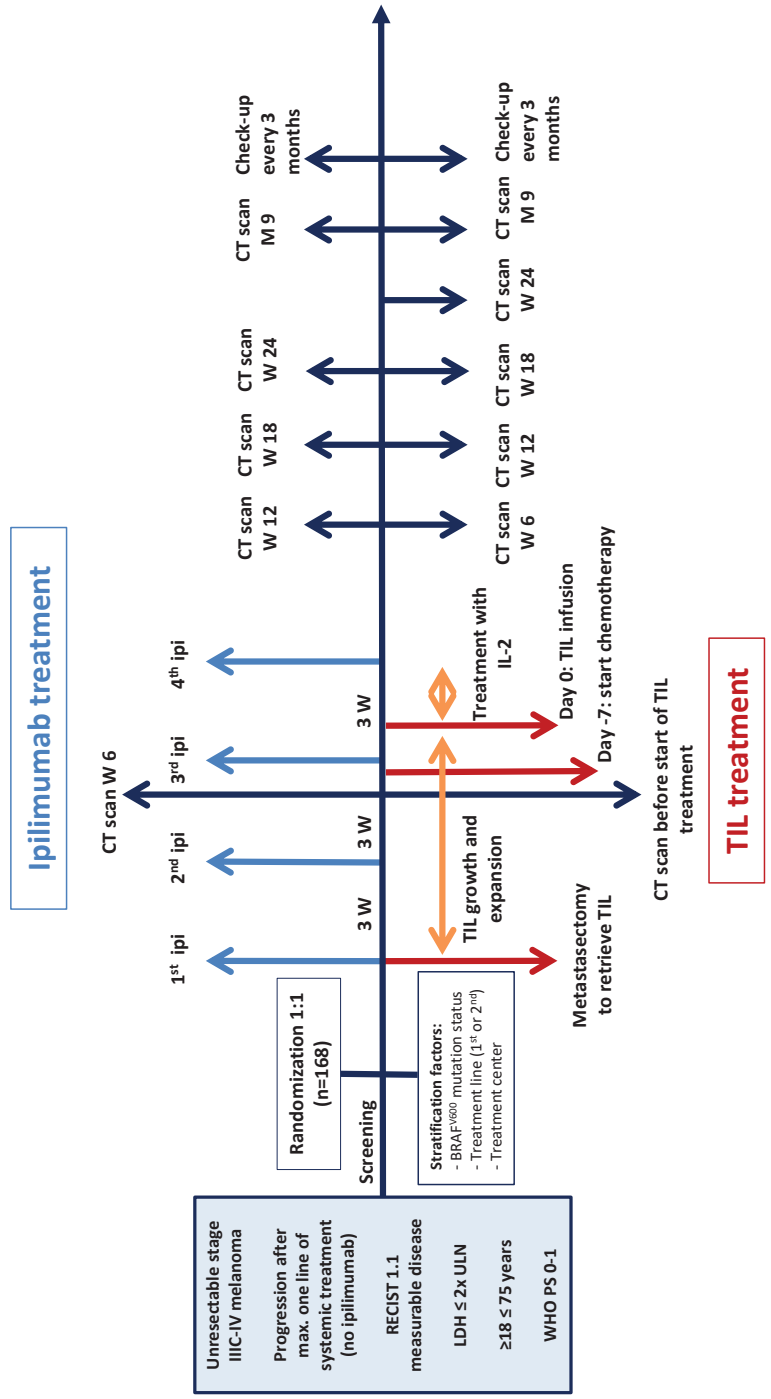

Figure S1. Trial design. Evaluation of response was performed six weeks after TIL infusion or twelve weeks after first dose of ipilimumab to align response evaluation relative to time of randomization, and periodically thereafter until disease progression. *LDH, lactate dehydrogenase; ULN, upper limit of normal; WHO PS, World Health Organization Performance Score.*

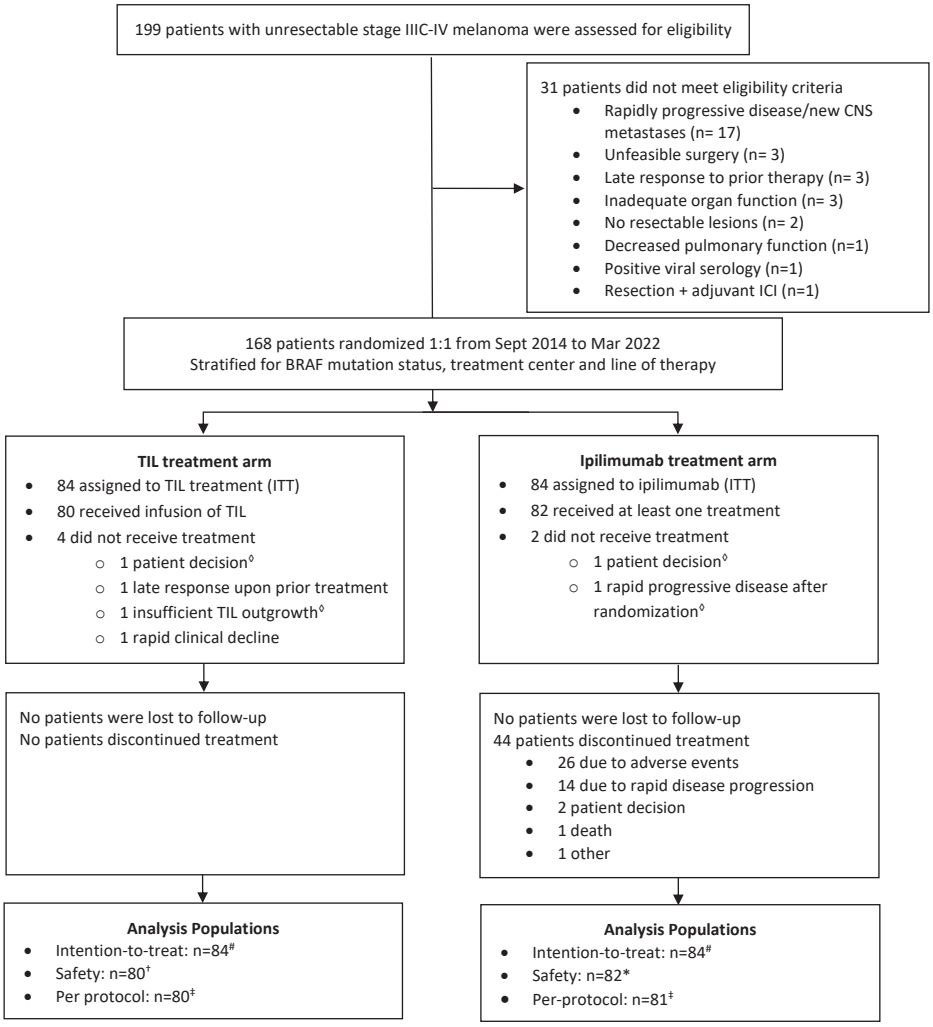

Figure S2. CONSORT diagram. Study design, treatment allocation and patient disposition. ◊Patients started subsequent immune checkpoint inhibition or targeted therapy as standard of care. #Includes all patients randomized to a treatment, regardless whether they received any treatment. †Includes patients receiving chemotherapy and TIL. *Includes patients receiving at least one dose of ipilimumab. ‡Includes patients receiving at least one dose of treatment (chemotherapy and TIL or ipilimumab) and who did not rapidly progress within 5 weeks. *ICI, immune checkpoint inhibition.*

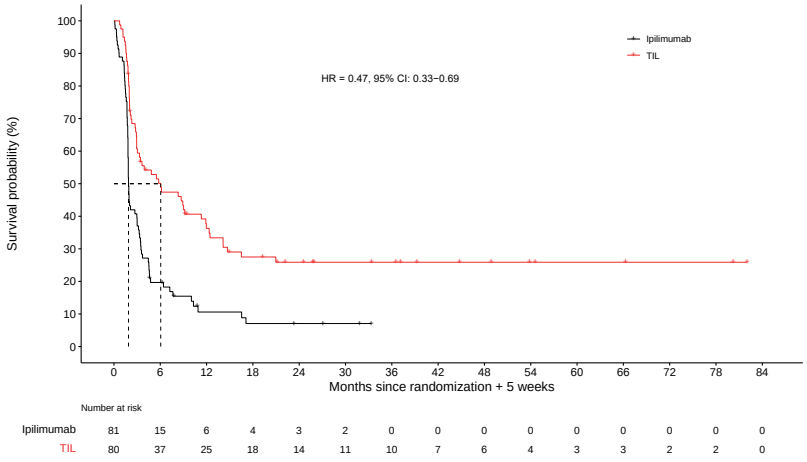

Figure S3. Progression-free survival in the per-protocol analysis set. Data depict progression-free survival according to RECIST, version 1.1, in all patients randomized to a treatment arm who received the allocated treatment (per-protocol). Starting point was five weeks after randomization, excluding patients that did not start treatment or showed disease progression within five weeks after randomization (landmark approach). Hazard ratios were estimated using the stratified Cox regression model. The widths of the confidence interval has not been adjusted for multiplicity and cannot be used in place of a hypothesis test. Censored patients are indicated with tick marks.

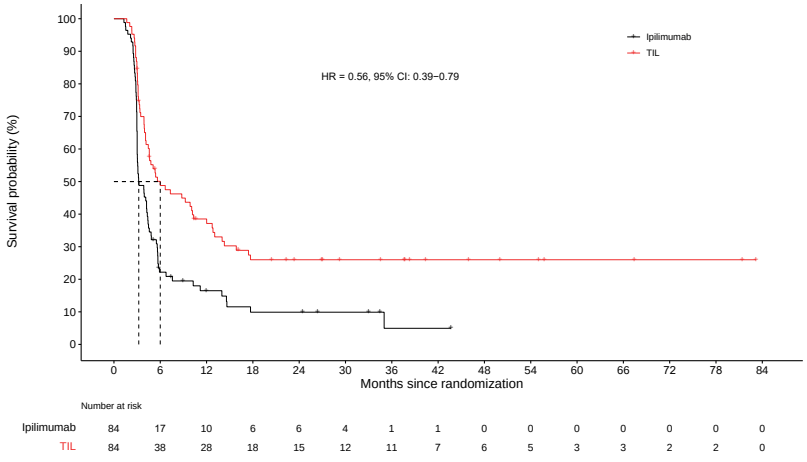

Figure S4. Progression-free survival according to immune-related response criteria. Data shown depict progression-free survival according to immune-related response criteria in all randomized patients (intention-to-treat population). Hazard ratios were estimated using the stratified Cox regression model. The widths of the confidence interval have not been adjusted for multiplicity and cannot be used in place of a hypothesis test. Censored patients are indicated with tick marks.

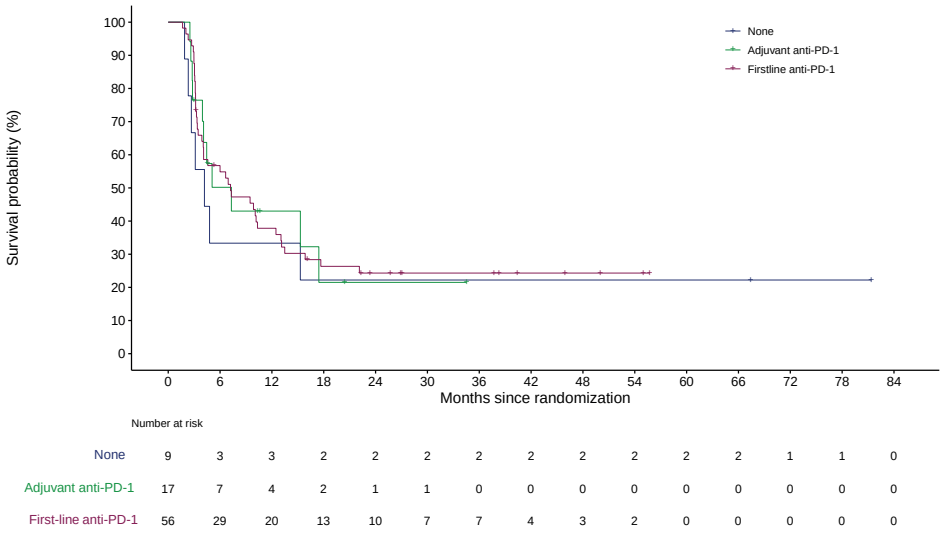

Figure S5. Progression-free survival in TIL treated patients by prior systemic therapy. Data depict an exploratory post hoc analysis of progression-free survival according to RECIST, version 1.1, in patients treated with TIL based on prior systemic therapy, having received either no prior therapy, adjuvant anti-PD-1 or first-line anti-PD-1. Hazard ratios were estimated using the stratified Cox regression model. Censored data is indicated with tick marks.

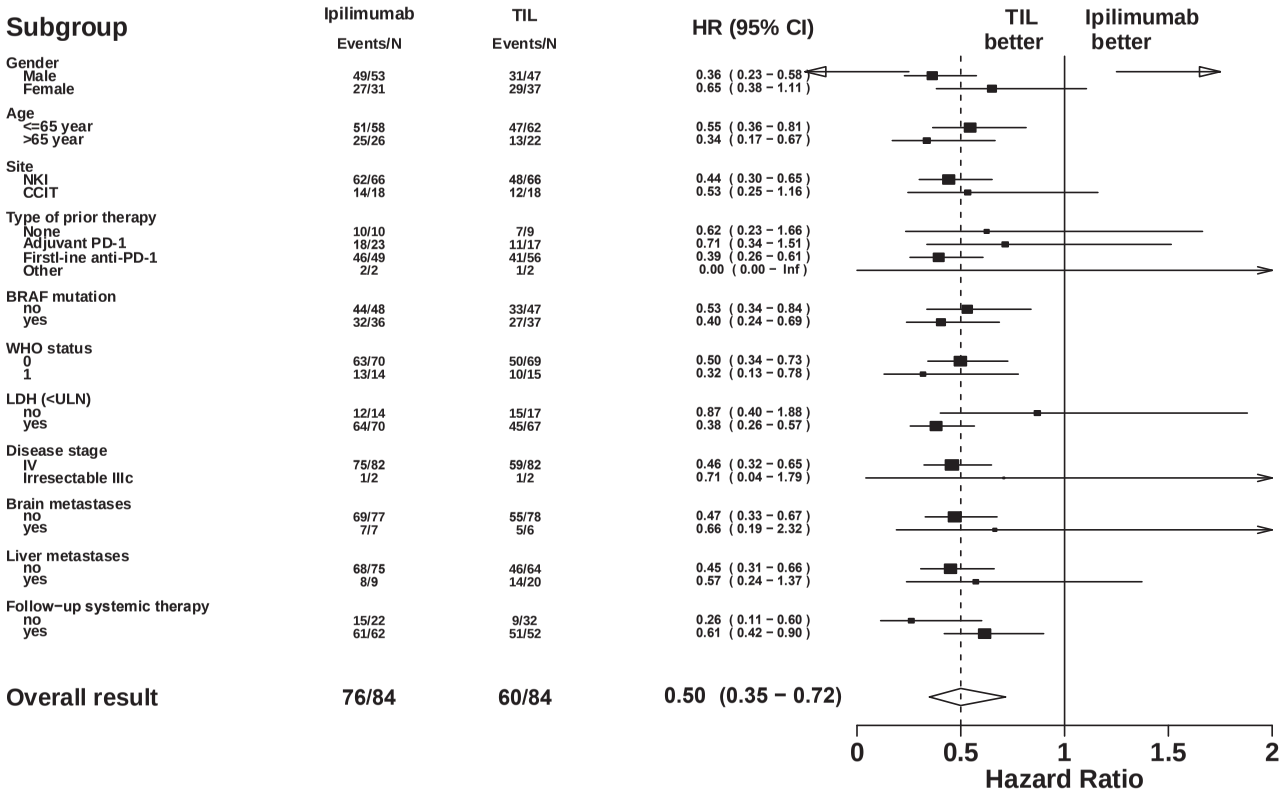

Figure S6. Progression-free survival, subgroup analysis. Forest plot depicting an exploratory post hoc analysis of progression-free survival according to key subgroups. The widths of the confidence intervals have not been adjusted for multiplicity and cannot be used in place of a hypothesis test. *CCIT, National Center for Cancer Immune Therapy; CI, confidence interval; HR hazard ratio; LDH, lactate dehydrogenase; NKI, Netherlands Cancer Institute; PD-1, programmed cell death protein-1; WHO status, World Health Organization performance status.*

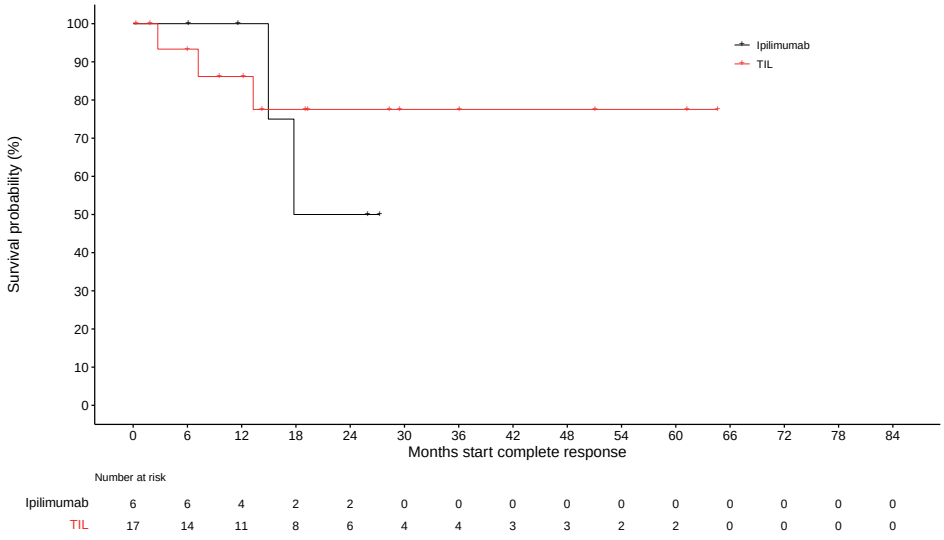

Figure S7. Duration of complete response. Data depict an exploratory post hoc survival analysis with Kaplan-Meier estimates of the duration of a complete response until progression or death in TIL and ipilimumab treated patients, respectively (intention-to-treat-population). Censored data are indicated with tick marks.

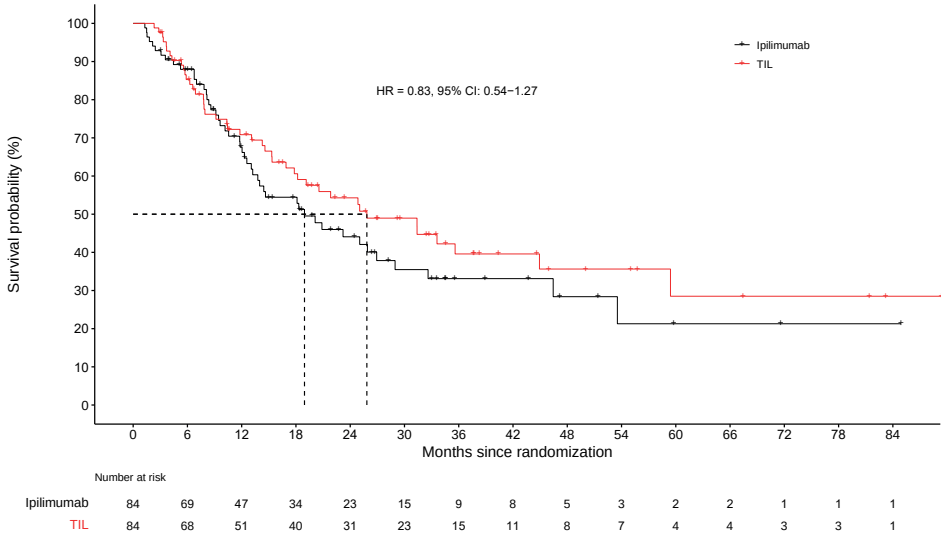

Figure S8. Overall survival. Data depict Kaplan-Meier estimates of overall survival in all randomized patients (intention-to-treat population). Hazard ratios were estimated using the stratified Cox regression model. The widths of the confidence interval have not been adjusted for multiplicity and cannot be used in place of a hypothesis test. Censored data are indicated with tick marks.

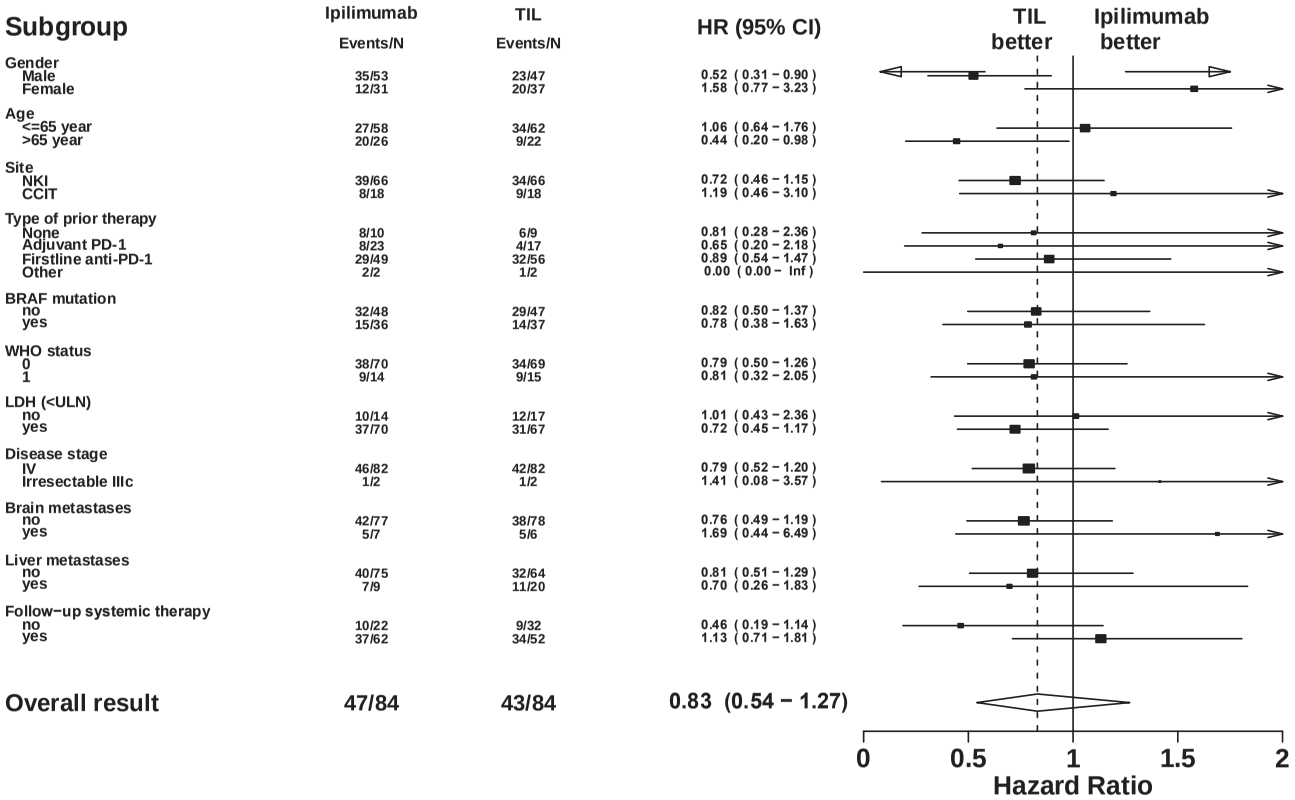

Figure S9. Overall survival, subgroup analysis. Forest plot depicting an exploratory post hoc analysis of overall survival according to key subgroups. The widths of the confidence intervals have not been adjusted for multiplicity and cannot be used in place of a hypothesis test. *CCIT, National Center for Cancer Immune Therapy; CI, confidence interval; HR hazard ratio; LDH, lactate dehydrogenase; NKI, Netherlands Cancer Institute; PD-1, programmed cell death protein-1; WHO status, World Health Organization performance status.*

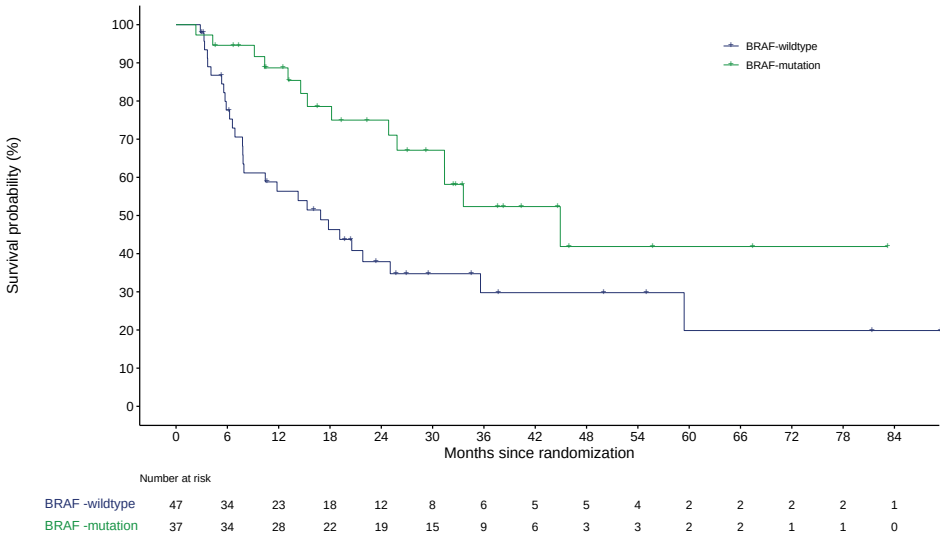

Figure S10. Overall survival in patients treated with TIL by *BRAF*-mutation status. Data depict Kaplan-Meier estimates of an exploratory post hoc analysis of overall survival in all patients randomized for TIL (intention-to-treat population) for *BRAF*-mutation. Censored data are indicated with tick marks.

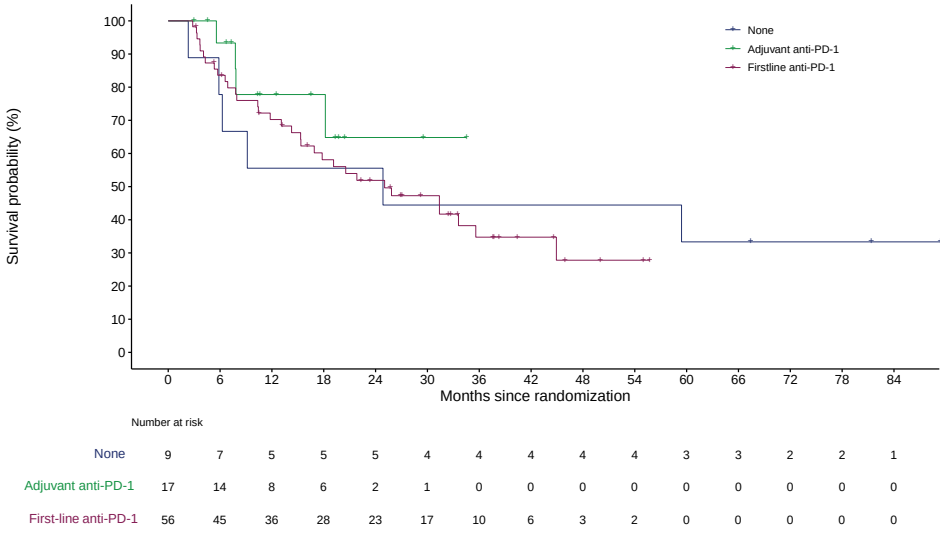

Figure S11. Overall survival in TIL treated patients by prior systemic therapy. Data depict an exploratory post hoc analysis of overall survival in patients randomized for TIL (intention-to-treat population) based on prior systemic therapy, having received either no prior therapy, adjuvant anti-PD-1 or first-line anti-PD-1. Censored data is indicated with tick marks.

SUPPLEMENTARY TABLES

Table S1. Details on anti-PD-1 treatment prior to trial participation

	TIL (n=84)	Ipilimumab (n=84)	Total (n=168)
Prior adjuvant anti-PD-1 – n (%)	17 (20.2)	23 (27.4)	40 (23.8)
Time (days) from last cycle to randomization, median (range)	83 (21 - 449)	77 (27 - 715)	80 (21 – 715)
Number of cycles, median (range)	6 (3 - 18)	5 (2 - 18)	6 (2 - 18)
Off-treatment >6 months prior to enrollment – n (%)	3 (17.7)	7 (30.4)	10 (25.0)
Prior first-line anti-PD-1 – n (%)	56 (66.7)	49 (58.3)	105 (62.5)
Time (days) from last cycle to randomization, median (range)	49 (8 – 449)	46 (4 – 572)	47 (4 – 572)
Number of cycles, median (range)	8 (1 - 44)	9 (3 - 52)	9 (1 - 52)
Off-treatment >6 months prior to enrollment – n (%)	4 (7.1)	5 (10.2)	9 (8.6)

Details on anti-PD-1 treatment in either adjuvant or first-line setting prior to trial participation in the intention-to-treat population.

Table S2. Treatment characteristics

TIL (n=80)	
Time in days between randomization and metastasectomy for TIL production, days (median, range)	10.3 (-14.5 – 22.5)*
Site of metastasectomy for TIL – n (%)	
Lymph node metastasis	36 (45)
(sub)cutaneous metastasis	33 (41.3)
Visceral organ	11 (13.8)
Lung	6
Liver	1
Spleen	1
Adrenal gland	1
Small intestine	1
Peritoneal	1
Number of lesions resected – n (%)	
One lesion ≥ 2-3 cm	50 (62.5)
Multiple lesions together ≥ 2-3 cm	30 (37.5)

Table S2. Continued

TIL (n=80)	
Second metastasectomy required – n (%)	10 (12)
Reason second metastasectomy	
Insufficient TIL outgrowth	9
Contaminated TIL outgrowth	1
Successful TIL outgrowth – n (%)	82 (98.8)†
Time in days between randomization and TIL infusion in patients receiving TIL (n=80) – median (range)	48.6 (27.5 – 356.4)‡
Total number of infused cells ($x10^9$) – median (range)	40.9 (4.9 – 110.4)
Duration of hospitalization (days) – median (range)	17 (12 – 38)
Number of doses of HD-IL-2 – median (range)	4 (0 – 10)
ICU admittance – n (%)	8 (10)
Duration in days – median (range)	4 (1 – 24)
Ipilimumab (n=82)	
Time in days between randomization and first cycle – median (range)	6.4 (0.4 – 22.7)
Number of cycles - median, (range)	3 (1 – 4)
Discontinuation of treatment - n (%)	42 (51.2)
Reason discontinuation treatment - n (%)	
Adverse events	26 (61.9)
Quick disease progression	14 (33.3)
Patient decision	1 (2.4)
Death	1 (2.4)

Treatment characteristics of patients who received at least one dose of treatment (per-protocol analysis set). *One patient had a metastasectomy for palliative reasons prior to randomization. TIL production for this patient was initiated independent of the randomization result, as allowed by the study protocol. †In patients for whom a TIL manufacturing was initiated (n=83). Of these, one TIL production was not feasible. One patient had a late response upon prior therapy and did not receive TIL. Another showed rapid clinical progression thus treatment with TIL was not feasible. As these patients did not receive TIL, they are not included in the per-protocol analysis set. ‡Treatment of two patients was delayed for approximately one year after randomization. In one patient this was due to a late response to prior treatment and received TIL upon subsequent progression. In the second patient, the initial TIL production was unsuccessful, after which the patient had no suitable lesions for a second metastasectomy. Per patient request, disease progression was monitored until a new TIL production was feasible. *HD-IL-2, high-dose interleukin-2.*

Table S3. Systemic treatments given after disease progression

	TIL (n=80)					Ipilimumab (n=82)						
	Systemic follow-up treatment line					Systemic follow-up treatment line						
Systemic treatment	1	2	3	4	5	1	2	3	4	5	6	7
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Targeted therapy	16 (20.0)	5 (6.3)	5 (6.3)	0	0	24 (29.3)	5 (6.1)	1 (1.2)	1 (1.2)	0	1 (1.2)	0
Trial	0	6 (7.5)	3 (3.8)	2 (2.5)	0	16 (19.5)	7 (8.5)	1 (1.2)	0	0	0	0
Ipilimumab	16 (20.0)	2 (2.5)	2 (2.5)	0	0	2 (2.4)*	0	0	0	0	0	0
Anti-PD-1	1 (1.3)	1 (1.3)	0	1 (1.3)	0	8 (9.8)	0	0	0	0	0	0
Ipilimumab + anti-PD-1	12 (15)	8 (10.0)	0	0	0	0	3 (3.7)	1 (1.2)	0	0	0	0
Dacarbazine	0	2(2.5)	0	0	0	1 (1.2)	0	0	0	0	0	0
Temozolomide	1 (1.3)		0	1 (1.3)	1 (1.3)	1 (1.2)		2 (2.4)		1 (1.2)	0	0
Other												
Imatinib	0	0	0	0	0	0	2 (2.4)	0	0	0	0	0
HDM inhibitor	0	0	0	0	0	0	0	1 (1.2)	0	0	0	0
Olaparib	0	0	0	0	0	0	0	1 (1.2)	0	0	0	0
IMCgp100 + Durvalumab/ tremelimumab	0	0	0	0	0	0	0	0	1 (1.2)	0	0	0
Arginase-1 peptide vaccination	0	0	0	0	0	0	0	0	0	0	0	1 (1.2)

Overview of all systemic treatments given after disease progression in patients treated with either TIL or ipilimumab (safety analysis set). Patients could have received more than one line of subsequent systemic therapy after study treatment. *Rechallenge upon prior response after ipilimumab treatment.

Table S4. Less common treatment-related adverse events

TIL treatment arm (n=80)						Ipilimumab treatment arm (n=82)		
Chemotherapy			TIL and IL-2			Ipilimumab		
Adverse event	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**
Blood bilirubin increased	7 (8.8)	1 (1.3)	**Anorexia**	7 (8.8)	1 (1.3)	**Dyspnea**	8 (9.8)	2 (2.4)
Malaise	7 (8.8)	0	**Cough**	7 (8.8)	0	**GGT increased**	8 (9.8)	7 (8.5)
Mucositis oral	7 (8.8)	0	**Malaise**	7 (8.8)	0	**Lipase increased**	7 (8.5)	3 (3.7)
Pulmonary edema	6 (7.5)	0	**Pruritus**	7 (8.8)	0	**Myalgia**	7 (8.5)	0
Sinus tachycardia	6 (7.5)	0	**Blood bilirubin increased**	6 (7.5)	1 (1.3)	**Cough**	5 (6.1)	0
Abdominal pain	5 (6.3)	0	**Dysgeusia**	6 (7.5)	0	**Hypothyroidism**	5 (6.1)	1 (1.2)
Dry mouth	5 (6.3)	0	**Edema face**	6 (7.5)	0	**Serum amylase increased**	5 (6.1)	1 (1.2)
Hypertension	5 (6.3)	2 (2.5)	**Pleural effusion**	6 (7.5)	2 (2.5)	**Skin hypopigmentation**	5 (6.1)	0
Hyponatremia	5 (6.3)	2 (2.5)	**Uveitis**	6 (7.5)	1 (1.3)	**Adrenal insufficiency**	4 (4.9)	0
Allergic reaction	4 (5)	0	**Dizziness**	5 (6.3)	0	**Chills**	4 (4.9)	0
Back pain	4 (5)	0	**Acute kidney injury**	4 (5)	0	**Hypophosphatemia**	4 (4.9)	2 (2.4)
Cardiac troponin T increased	4 (5)	1 (1.25)	**Cardiac troponin T increased**	4 (5)	3 (3.8)	**Hypophysitis**	4 (4.9)	3 (3.7)
Hematuria	4 (5)	0	**Non-cardiac chest pain**	4 (5)	0	**Anemia**	3 (3.7)	0
Hiccups	4 (5)	0	**Skin hyperpigmentation**	4 (5)	0	**Arthralgia**	3 (3.7)	0
Stomach pain	4 (5)	0	**Arthralgia**	3 (3.8)	0	**Blood corticotrophin decreased**	3 (3.7)	0

Table S4. Continued

TIL treatment arm (n=80)						Ipilimumab treatment arm (n=82)		
Chemotherapy			TIL and IL-2			Ipilimumab		
Adverse event	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**
Urinary frequency	4 (5)	0	**Cardiac chest pain**	3 (3.8)	1 (1.3)	**Dysgeusia**	3 (3.7)	0
Adult respiratory distress syndrome	3 (3.8)	3 (3.8)	**Hallucinations**	3 (3.8)	0	**Hepatitis**	3 (3.7)	2 (2.4)
Cystitis noninfective	3 (3.8)	0	**Hearing impaired**	3 (3.8)	1 (1.3)	**Hyperglycemia**	3 (3.7)	2 (2.4)
Non-cardiac chest pain	3 (3.8)	0	**Hypomagnesemia**	3 (3.8)	0	**Pneumonitis**	3 (3.7)	1 (1.2)
Pleural effusion	3 (3.8)	1 (1.3)	**Localized edema**	3 (3.8)	0	**Weight loss**	3 (3.7)	0
Pruritus	3 (3.8)	0	**Myocarditis**	3 (3.8)	2 (2.5)	**Abnormal gonadotrophin**	2 (2.4)	0
Sinus pain	3 (3.8)	0	**Palpitations**	3 (3.8)	0	**Acute kidney injury**	2 (2.4)	1 (1.2)
Sore throat	3 (3.8)	0	**Platelet count decreased**	3 (3.8)	2 (2.5)	**Allergic reaction**	2 (2.4)	0
Weight loss	3 (3.8)	0	**Purpura**	3 (3.8)	0	**Blood bilirubin increased**	2 (2.4)	1 (1.2)
Acute kidney injury	2 (2.5)	1 (1.3)	**Weight loss**	3 (3.8)	0	**Blurred vision**	2 (2.4)	0
Agitation	2 (2.5)	0	**Abdominal pain**	2 (2.5)	0	**Dizziness**	2 (2.4)	0
Anal mucositis	2 (2.5)	0	**Adult respiratory distress syndrome**	0	2 (2.5)	**Dry eye**	2 (2.4)	0
Atrial fibrillation	2 (2.5)	0	**Allergic reaction**	2 (2.5)	0	**Hypokalemia**	2 (2.4)	0
Bone pain	2 (2.5)	0	**Anemia**	2 (2.5)	0	**Insomnia**	2 (2.4)	0
Cough	2 (2.5)	0	**Atrial fibrillation**	2 (2.5)	0	**Stomach pain**	2 (2.4)	1 (1.2)

Table S4. Continued

TIL treatment arm (n=80)						Ipilimumab treatment arm (n=82)		
Chemotherapy			TIL and IL-2			Ipilimumab		
Adverse event	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**
Edema face	2 (2.5)	0	**Dry eye**	2 (2.5)	0	**Tumor pain**	2 (2.4)	0
Epistaxis	2 (2.5)	0	**Dry skin**	2 (2.5)	0	**Uveitis**	2 (2.4)	0
Hypotension	2 (2.5)	0	**Hyponatremia**	2 (2.5)	0	**White blood cells decreased**	2 (2.4)	1 (1.2)
Localized edema	2 (2.5)	0	**Mucositis oral**	2 (2.5)	0	**Allergic rhinitis**	1 (1.2)	0
Lymphocyte count decreased	0	2 (2.5)	**Pain in extremity**	2 (2.5)	0	**Anal hemorrhage**	1 (1.2)	0
Mucosal infection	2 (2.5)	0	**Periorbital edema**	2 (2.5)	0	**Atrial fibrillation**	1 (1.2)	0
Myalgia	2 (2.5)		**Tinnitus**	2 (2.5)	0	**Bloating**	1 (1.2)	0
Myocarditis	0	2 (2.5)	**Decreased urinary output**	2 (2.5)	1 (1.3)	**Conjunctivitis**	1 (1.2)	0
Pericardial effusion	2 (2.5)	1 (1.3)	**Abdominal distension**	1 (1.3)	0	**Constipation**	1 (1.2)	0
Peripheral sensory neuropathy	2 (2.5)	0	**Agitation**	1 (1.3)	0	**Dysphagia**	1 (1.2)	0
Petechiae	2 (2.5)	0	**Atrioventricular block first degree**	1 (1.3)	0	**Edema face**	1 (1.2)	0
Sepsis	0	2 (2.5)	**Bronchospasm**	1 (1.3)	0	**Encephalitis**	1 (1.2)	0
Arthralgia	1 (1.3)	0	**Concentration impairment**	1 (1.3)	0	**Erythema multiforme**	1 (1.2)	0
Blurred vision	1 (1.3)	0	**Confusion**	1 (1.3)	0	**Flu-like symptoms**	1 (1.2)	0
Candida infection	1 (1.3)	0	**Conjunctivitis**	1 (1.3)	0	**Gastritis**	1 (1.2)	0

Table S4. Continued

TIL treatment arm (n=80)						Ipilimumab treatment arm (n=82)		
Chemotherapy			TIL and IL-2			Ipilimumab		
Adverse event	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**
Central venous catheter infection	0	1 (1.3)	**Depression**	1 (1.3)	0	**Gastroesophageal reflux disease**	1 (1.2)	0
Cardiac chest pain	0	1 (1.3)	**Dry mouth**	1 (1.3)	0	**Glucose intolerance**	1 (1.2)	0
Chills	1 (1.3)	0	**Dysphasia**	1 (1.3)	0	**Hoarseness**	1 (1.2)	0
CPK increased	1 (1.3)	0	**Edema trunk**	1 (1.3)	0	**Hyperthyroidism**	1 (1.2)	0
Dry skin	1 (1.3)	0	**Fall**	1 (1.3)	0	**Hypoalbuminemia**	1 (1.2)	0
Edema trunk	1 (1.3)	0	**Flu-like symptoms**	1 (1.3)	0	**Hypocalcemia**	1 (1.2)	0
Facial pain	1 (1.3)	0	**Flushing**	1 (1.3)	0	**Hypotension**	1 (1.2)	0
Flashing lights	1 (1.3)	0	**Generalized muscle weakness**	1 (1.3)	0	**Mucositis oral**	0	1 (1.2)
Generalized muscle weakness	1 (1.3)	0	**Hematuria**	1 (1.3)	0	**Neutrophil count decreased**	0	1 (1.2)
Genital edema	1 (1.3)	0	**Hemorrhage**	1 (1.3)	0	**Pain in extremity**	1 (1.2)	0
Hemorrhage	1 (1.3)	0	**Hot flashes**	1 (1.3)	0	**Pain in skin**	1 (1.2)	
Hypernatremia	1 (1.3)	0	**Hypernatremia**	1 (1.3)	0	**Pancreatitis**	0	1 (1.2)
Infection	0	1 (1.3)	**Libido decreased**	1 (1.3)	0	**Papilledema**	1 (1.2)	0
Infusion related reaction	1 (1.3)	0	**Out of body sensation**	1 (1.3)	0	**Paresthesia**	1 (1.2)	0

Table S4. Continued

TIL treatment arm (n=80)						Ipilimumab treatment arm (n=82)		
Chemotherapy			TIL and IL-2			Ipilimumab		
Adverse event	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**
Insomnia	1 (1.3)	0	**Peripheral ischemia**	0	1 (1.3)	**Peripheral motor neuropathy**	1 (1.2)	0
Irregular menstruation	1 (1.3)	0	**Peripheral sensory neuropathy**	1 (1.3)	0	**Peripheral sensory neuropathy**	1 (1.2)	0
Laryngeal inflammation	1 (1.3)	0	**Photophobia**	1 (1.3)	0	**Sore throat**	1 (1.2)	0
Libido decreased	1 (1.3)	0	**Pneumonitis**	0	1 (1.3)	**Stevens-Johnson syndrome**	0	1 (1.2)
Oral dysesthesia	1 (1.3)	0	**Retinal detachment**	1 (1.3)	0	**Tinnitus**	1 (1.2)	0
Peripheral motor neuropathy	0	1 (1.3)	**Sinus bradycardia**	1 (1.3)	0	**Tremor**	1 (1.2)	0
Pharyngeal mucositis	1 (1.3)	0	**Syncope**	1 (1.3)	0	**Urinary tract infection**	1 (1.2)	0
Pneumonia	0	1 (1.3)	**Urinary frequency**	1 (1.3)	0	**Urticaria**	1 (1.2)	0
Small intestine mucositis	0	1 (1.3)	**Urticaria**	0	1 (1.3)	**Watering eyes**	1 (1.2)	0
Somnolence	1 (1.3)	0	**Watering eyes**	1 (1.3)	0	**Wound infection**	1 (1.2)	0
Tumor hemorrhage	1 (1.3)	0	**White blood cells decreased**	1 (1.3)	0			
Urinary tract pain	1 (1.3)	0						
Urticaria	1 (1.3)	0						
Vaginal infection	1 (1.3)	0						
Vasovagal reaction	0	1 (1.3)						

Table S4. Continued

TIL treatment arm (n=80)						Ipilimumab treatment arm (n=82)		
Chemotherapy			TIL and IL-2			Ipilimumab		
Adverse event	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Any grade n (%)**	**≥ grade 3 n (%)**
Vision loss	0	1 (1.3)						
Vitreous hemorrhage	1 (1.3)	0						
Wound infection	0	1 (1.3)						

Less common treatment-related adverse events of any grade and grade ≥3 by highest grade according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.03 that occurred in <10% of patients receiving at least one dose of treatment (safety analysis set), per treatment arm. For TIL treatment, adverse events related to chemotherapy and TIL plus interleukin-2 are presented separately.

Table S5. Treatment-related adverse events

	TIL (n=80), n (%)	Ipilimumab (n=82), n (%)
Any TEAE	80 (100)	82 (100)
Any TEAE grade ≥ 3	80 (100)	59 (72.0)
Any treatment-related TEAE	80 (100)	79 (96.3)
Any treatment-related TEAE grade ≥ 3	80 (100)	47 (57.3)
Any cyclophosphamide-related TEAE	80 (100)	-
Any cyclophosphamide-related TEAE grade ≥ 3	80 (100)	-
Any fludarabine-related TEAE	80 (100)	-
Any fludarabine-related TEAE grade ≥ 3	80 (100)	-
Any TIL/IL-2-related TEAE	80 (100)	-
Any TIL/IL-2-related TEAE grade ≥ 3	77 (96.3)	-
Any treatment-related SAE	12 (15.0)	22 (26.8)

All treatment emergent adverse events (TEAE) that are treatment-related by worst grade in patients receiving at least one dose of treatment (safety analysis set), per treatment arm during the entire study period. *SAE, serious adverse event.*

Table S6. All treatment-related serious adverse events

	TIL treatment arm (n=80)			Ipilimumab treatment arm (n=82)	
Adverse event	**Grade 1-2 n (%)**	**≥ grade 3 n (%)**	**Adverse event**	**Grade 1-2 n (%)**	**≥ grade 3 n (%)**
Adult respiratory distress syndrome	0	3 (3.8)	**Acute kidney injury**	1 (1.2)	2 (2.4)
Anemia	0	1 (1.3)	**Adrenal insufficiency**	1 (1.2)	0
Cardiac troponin T increased	0	2 (2.5)	**Anemia**	1 (1.2)	0
Intracranial hemorrhage	0	1 (1.3)	**Biliary tract infection**	0	1 (1.2)
Myocarditis	0	2 (2.5)	**Chills**	0	1 (1.2)
Peripheral motor neuropathy	0	1 (1.3)	**Colitis**	1 (1.2)	7 (8.5)
Platelet count decreased	0	1 (1.3)	**Constipation**	1 (1.2)	0
Pleural effusion	0	1 (1.3)	**Dehydration**	0	1 (1.2)
Pneumonia	0	1 (1.3)	**Diarrhea**	0	2 (2.4)
Pneumonitis	0	1 (1.3)	**Duodenal perforation**	0	1 (1.2)
Uveitis	1 (1.3)	1 (1.3)	**Dyspnea**	1 (1.2)	1 (1.2)
Venous thromboembolism	0	2 (2.5)	**Elevated ALT**	0	2 (2.4)
Vision loss	0	1 (1.3)	**Infectious enterocolitis**	0	1 (1.2)
Wound infection	0	1 (1.3)	**Fever**	1 (1.2)	0

Table S6. Continued

	Ipilimumab treatment arm (n=82)	
Adverse event	**Grade 1-2 n (%)**	**≥ grade 3 n (%)**
Flu-like symptoms	0	1 (1.2)
Gastric ulcer	1 (1.2)	0
Headache	0	1 (1.2)
Hemodynamic instability	0	1 (1.2)
Hepatitis	0	2 (2.4)
Hyperglycemia	0	2 (2.4)
Hypophysitis	0	2 (2.4)
Ileus	0	1 (1.2)
Intracranial hemorrhage	0	1 (1.2)
Malaise	2 (2.4)	0
Non-cardiac chest pain	0	1 (1.2)
Pneumonitis	1 (1.2)	1 (1.2)
Pulmonary thromboembolism	0	1 (1.2)
Rash	0	2 (2.4)
Sinus tachycardia	1 (1.2)	0
Skin infection	0	2 (2.4)
Small intestinal obstruction	1 (1.2)	1 (1.2)
Stevens-Johnson syndrome	0	1 (1.2)
Stomach pain	0	1 (1.2)
Tumor pain	2 (2.4)	0

All treatment-related serious adverse events (SAEs) by highest grade according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.03 in patients receiving at least one dose of treatment (safety analysis set), per treatment arm during the entire study period, independent of the relation to study treatment. *ALT, alanine aminotransferase.*

Table S7. Overview of all treatment-emergent adverse events by specific time in TIL patients

	Before TIL infusion, n (%)	After TIL infusion, n (%)
Any TEAE	79 (98.8)	80 (100)
Any treatment-related TEAE	78 (97.5)	80 (100)
Any cyclophosphamide-related TEAE	78 (97.5)	80 (100)
Any fludarabine-related TEAE	76 (95)	80 (100)
Any TIL/IL-2-related TEAE	-	80 (100)
Any treatment-related SAE	0 (0)	12 (15)

All treatment-emergent adverse events (TEAE) by highest grade in TIL treated patients receiving at least one dose of treatment (safety analysis set, n=80), by specific time period. *SAE, serious adverse event.*

Table S8. Adjusted health-related quality-of-life score differences

	QLQ-C15 PAL Global quality-of-life score difference (95%CI)		QLQ-C15 PAL Physical functioning score difference (95%CI)	
Baseline	3.9	(2.63-5.07)	0.8	(-0.07-1.61)
Week 9	3.9	(0.74-6.97)	1.0	(-0.56-2.63)
Week 12	3.8	(0.61-6.94)	0.4	(-2.15-2.90)
Week 18	7.9	(2.96-12.81)	3.0	(-0.42-6.52)
Week 24	7.7	(5.14-10.35)	2.9	(-1.40-4.47)
Week 48	9.4	(6.15-12.56)	4.0	(-0.41-8.55)
Week 60	5.1	(-3.95-14.16)	2.4	(-3.73-8.51)
	QLQ-C15 PAL Emotional functioning score difference (95%CI)		**QLQ-C15 PAL Fatigue difference (95%CI)**	
Baseline	6.0	(4.99-7.07)	-3.9	(-5.72- -2.09)
Week 9	6.0	(3.05-8.86)	-3.1	(-5.81- -0.31)
Week 12	5.4	(2.90-7.99)	-2.9	(-7.64-1.69)
Week 18	7.8	(4.32-11.27)	-9.6	(-16.08- -3.16)
Week 24	9.7	(7.52-11.88)	-7.9	(-11.24- -4.60)
Week 48	9.8	(7.11-12.4)	-5.9	(-14.34-2.58)
Week 60	5.6	(-2.43-13.59)	-6.4	(-11.44- -1.38)

Table S8. Continued

		QLQ-C15 PAL Nausea & vomiting difference (95%CI)		QLQ-C15 PAL Pain difference (95%CI)
Baseline	2.7	(2.02-3.45)	-4.1	(-5.81- -2.29)
Week 9	1.5	(0.26-2.82)	-3.0	(-5.29- -0.76)
Week 12	2.9	(0.96-4.80)	-3.4	(-7.62- -0.84)
Week 18	1.1	(-1.33-3.56)	-9.8	(-15.96- -3.54)
Week 24	1.6	(0.69-2.51)	-6.4	(-9.28- -3.51)
Week 48	2.0	(-3.08-7.16)	-5.2	(-11.91- -1.54)
Week 60	3.2	(-1.86-8.19)	-6.2	(-10.60- -1.76)
		QLQ-C15 PAL Dyspnea difference (95%CI)		**QLQ-C15 PAL Insomnia difference (95%CI)**
Baseline	1.1	(-0.32-2.52)	-2.5	(-4.48- -0.54)
Week 9	-1.5	(-5.33-2.33)	-1.6	(-4.43-1.25)
Week 12	1.5	(-1.96-4.93)	-0.8	(-5.64- 4.06)
Week 18	-0.5	(-6.16-5.10)	-5.1	(-11.19-1.08)
Week 24	-2.4	(-4.97-0.14)	-4.5	(-7.23- -1.86)
Week 48	-4.7	(-12.30-2.99)	-2.6	(-10.69-5.49)
Week 60	1.9	(-11.09-15.03)	-3.2	(-9.02-2.60)
		QLQ-C15 PAL Appetite loss difference (95%CI)		**QLQ-C15 PAL Constipation difference (95%CI)**
Baseline	-0.2	(-1.27-1.58)	0.1	(-0.62-0.75)
Week 9	-0.4	(-2.58-1.74)	-1.1	(-2.02- -0.15)
Week 12	1.0	(-2.85-4.90)	0.1	(-2.04-2.23)
Week 18	-1.5	(-6.05-3.1)	-1.6	(-3.83-0.55)
Week 24	-1.1	(-2.87-0.70)	-0.4	(-1.33-0.48)
Week 48	1.0	(-7.01-9.07)	-0.03	(-5.44-5.38)
Week 60	0.2	(-5.40-5.72)	0.04	(-5.14-5.22)

Scores on the European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire Core 15 palliative Care (EORTC QLQ-C15 PAL) global quality-of-life score and functioning scales range from 0 to 100, with higher scores indicating better functioning. Scores on the EORTC QLQ-C15 PAL symptom scales range from 0 to 100, with higher scores indicating higher levels of symptom burden. The widths of the confidence intervals have not been adjusted for multiplicity and cannot be used in place of a hypothesis test.

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