Research Article

Cancer Immunology Research

Local Tumor Treatment in Combination with Systemic Ipilimumab Immunotherapy Prolongs Overall Survival in Patients with Advanced Malignant Melanoma

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Abstract

Immune checkpoint inhibition with ipilimumab has revolutionized cancer immunotherapy and significantly improved outcomes of patients with advanced malignant melanoma. Local peripheral treatments (LPT), such as radiotherapy or electrochemotherapy, have been shown to modulate systemic immune responses, and preliminary data have raised the hypothesis that the combination of LPT with systemic immune checkpoint blockade might be beneficial. Clinical data from 127 consecutively treated melanoma patients at four cancer centers in Germany and Switzerland were analyzed. Patients received either ipilimumab ($n = 82$) or ipilimumab and additional LPT ($n = 45$) if indicated for local tumor control. The addition of LPT to ipilimumab significantly prolonged overall survival (OS; median OS 93 vs. 42 weeks, unadjusted HR, 0.46; $P = 0.0028$). Adverse immune-related events were not increased by the combination treatment, and LPT-induced local toxicities were in most cases mild. In a multivariable Cox regression analysis, we show that the effect of added LPT on OS remained statistically significant after adjusting for BRAF status, tumor stage, tumor burden, and central nervous system metastases (adjusted HR, 0.56; 95% confidence interval, 0.31–1.01, $P = 0.05$). Our data suggest that the addition of LPT to ipilimumab is safe and effective in patients with metastatic melanoma irrespective of clinical disease characteristics and known risk factors. Induction of antitumor immune responses is most likely the underlying mechanism and warrants prospective validation. *Cancer Immunol Res; 4(9); 744–54. ©2016 AACR.*

Introduction

The concept of immune checkpoint inhibition has revolutionized cancer immunotherapy (1). This progress has been pioneered by the characterization of the cytotoxic T-lymphocyte–associated-antigen-4 (CTLA-4) as an immune checkpoint receptor on T cells and by the development of the antibody to CTLA-4 ipilimumab as the first-in-class immune checkpoint inhibitor (2, 3). Activation of CTLA-4 physiologically downmodulates T-cell activation to limit overshooting immune responses. On the other hand, cancer cells can escape immune recognition through the upregulation of CTLA-4 on T cells. In this situation, ipilimumab can activate immune responses by CTLA-4 inhibition (4).

Two randomized trials demonstrated significant overall survival (OS) benefits of ipilimumab in advanced melanoma patients leading to its clinical approval in 2011 (5, 6). Pooled data from 1,861 ipilimumab-treated patients from several clinical trials showed a median OS of 11.4 months. Interestingly, a survival plateau was reached after 3 years by 20% to 25% of the patients that extended through at least 10 years, suggesting that profound immune responses can control even metastatic melanoma (7). Intensive research is aiming to increase the rates of long-term responders and strategies comprise the identification of new immune checkpoints or the combination of checkpoint blockers.

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For example, inhibition of the PD-1/PD-L1 axis by pembrolizumab has recently demonstrated high efficacy and might be superior to ipilimumab in certain patients (8). Also, the combined blockade of CTLA-4 and PD-1 signaling resulted in higher efficacy than anti–CTLA-4 monotherapy. However, this advantage was accompanied by increased higher-grade toxicities (9). Therefore, approaches that increase tumor immunogenicity with less systemic toxicity are required.

Here, we examined an alternative strategy, the combination of local tumor treatments with systemic immune checkpoint blockade. Classic radiotherapy has regained the attention of tumor immunologists because of the so-called "abscopal effect" (AE), a radiotherapy-induced tumor regression in lesions distant from the treated site (10, 11). A number of underlying mechanisms have been suggested for this clinical observation. First, radiotherapy can induce an immunogenic type of cancer cell death associated with antigen release, cytokine production, and complement activation finally generating an *in situ* tumor vaccine (12–14). Second, radiotherapy increases MHC class I expression and attracts immune cell migration into the tumor microenvironment (15–17). Third, radiotherapy enhances the diversity of the T-cell receptor repertoire of intratumoral T cells (18). All these mechanisms can contribute to enhanced systemic immune responses after local radiotherapy (19, 20).

Potentially beneficial interactions of radiotherapy and immunotherapeutic approaches have been seen in animal models (18, 21–23) and have been reported in patients with melanoma or other solid tumors (24–27). In a single-institution retrospective analysis of melanoma patients treated with ipilimumab in two different regimens (3 mg/kg or 10 mg/kg), 29 patients concomitantly received local radiotherapy (28). The combination of ipilimumab and radiotherapy was not associated with higher than expected toxicity rates. Further clinical interpretation was limited. More importantly, Victor and colleagues reported on nonredundant mechanisms of action of radiotherapy and dual checkpoint inhibition (anti–CTLA-4 and anti–PD-1) in a prospective phase I pilot trial comprising 22 patients (18).

Based on the available literature, the combination of local radiotherapy with ipilimumab seems feasible and preclinical data suggest that this approach could enhance antitumor immune responses. To test this hypothesis clinically, we performed a retrospective multicenter analysis of treatment outcomes of 127 consecutively treated melanoma patients who received either ipilimumab or ipilimumab and local peripheral treatments (LPT) if clinically indicated for local tumor control.

Materials and Methods

Patients

Clinical data of 127 consecutively ipilimumab-treated patients with American Joint Committee on Cancer (AJCC) stage IIIC/IV (29) malignant melanoma were analyzed at four clinical centers in Germany (University Hospital Cologne) and Switzerland (University Hospital Basel, Cantonal Hospital Aarau, St. Claraspital Basel). Patients received ipilimumab in line with intended labeling. The study was approved by institutional review boards. Additionally, cellular immune responses were prospectively analyzed in one patient during the treatment course at the University Hospital Cologne after written informed consent and review board approval (No. 08-144).

Systemic ipilimumab treatment

Ipilimumab (3 mg/kg) was administered intravenously in a 3-week cycle. Therapy was interrupted in case of immune-related adverse events (irAE) $\geq$ grade 3 (CTCAE version 4.03).

Local peripheral treatments

Patients received LPT as clinically indicated for palliation of symptomatic metastases according to local standard protocols. As we collected data from consecutively treated, advanced-stage patients, we included all LPT types that were applied during the data collection time (March 2011 to November 2014) into our analysis. Radiotherapy of the central nervous system (CNS) was not defined as a "peripheral" treatment, i.e., LPT, in this study and analyzed separately. CNS treatments comprised whole-brain irradiation (WBI) or local (stereotactic) brain irradiation (LBI).

Outcome evaluation

Responses were classified according to response evaluation criteria in solid tumors (RECIST, version 1.1; ref. 30). Immune-related response criteria (31) were taken into account when analyzing overall responses and clinical benefits. Clinical benefit consisted of complete or partial remission or durable stable disease. OS was defined as time from ipilimumab initiation to death of any cause. Progression-free survival (PFS) was defined as the time from ipilimumab initiation to disease progression or disease-related death, whichever occurred first. Non-event cases were censored in PFS and OS analyses and defined as patients who are alive and documented progression free at the last visit.

Measures of immune response

Neutrophil to lymphocyte ratios (NLR) were calculated from absolute cell numbers of peripheral blood leukocytes, retrospectively, at the time prior to ipilimumab treatment initiation. A cutoff NLR value of 4, as previously established, was set in order to separate prognostic groups (32).

The occurrence of abscopal effects (AE) was analyzed retrospectively from the patients' records by an independent radiologist (J. Borggrefe, University of Cologne, Germany) if sufficient and comparable radiologic data were available at the appropriate time points.

In addition, we prospectively analyzed cellular immune responses in 1 patient (a 26-year-old male; ID 6) during the treatment with ipilimumab and LPT. We set up an *in vitro* system in which patient-derived peripheral blood mononuclear cells (PBMC) were stimulated with lysates of melanoma and nonmelanoma cell lines. Blood samples were taken at three time points: before ipilimumab, after two ipilimumab cycles before LPT, and after ipilimumab + LPT. PBMCs were isolated by Ficoll density gradient centrifugation and cryopreserved until analysis. Total tumor cell lysates were prepared as described by Thumann and colleagues (33) from four melanoma cell lines (A375, BLM, MeWo, and SKmel28) and two control lines (L428, from a Hodgkin lymphoma, and SW480, a colon carcinoma). Total protein content was determined with the Pierce 660-nm Protein Assay (Thermo-Scientific). For the immune assay, PBMCs were labeled with carboxyfluorescein succinimidyl ester (CFSE). Triplicates of 1.5×10^5 PBMCs/200 μL were stimulated with 10 μg/mL protein of each lysate or tetanus toxoid (TT) as a control in supplemented RPMI-1640 medium. CD3/28 dynabead (Life Technologies) stimulated or medium only cultured PBMCs served

as further controls. After a 6-day incubation (37°C, 5% CO_2) T-cell activation and proliferation were determined by flow cytometry (MACSquant, Miltenyi Biotech) using fluorochrome-conjugated monoclonal antibodies: CD3-APC (OKT3), CD4-PB (SK3), CD8-PerCP-Cy5.5 (RPA-T8), CD25-APC-Cy7 (BC96), and CD137-PE-Cy7 (4B4-1) (Biolegend).

Statistical analysis

Patients and disease characteristics were analyzed using descriptive measures and comparison of proportions between two groups was performed with Fisher exact (two-sided). Normally distributed data of two groups were compared using the Student unpaired *t* test (two-sided). For the analysis of non-normally distributed data, the Wilcoxon–Mann–Whitney test (two-sided) was applied. Comparisons of more than two groups were performed with ANOVA. PFS and OS were estimated by the Kaplan–Meier method and curve comparisons were calculated using the log-rank test (Graphpad Prism 5 software). Multivariable Cox proportional hazards regression was performed to evaluate the effect of multiple covariates (i.e., BRAF mutation status, stage, tumor burden, and CNS metastases) simultaneously on OS. Predicted OS assuming referent values of all four covariates was calculated and plotted using MedCalc Statistical Software version 15.11.4 (MedCalc Software). In all analyses, *P* values ≤ 0.05 were considered statistically significant.

Results

Patient characteristics

A total of 127 consecutively ipilimumab-treated patients were identified at the four study centers between March 2011 and November 2014. Patient baseline characteristics are summarized in Table 1. The cohort comprised 69 (54.3%) men and 58 (45.7%) women and the mean age was 61.7 years (range, 23–89). Most of the patients were diagnosed with AJCC stage IV disease ($n = 113$; 89%), and 49 (38.6%) individuals had CNS metastases at ipilimumab initiation. Activating mutations in the BRAF gene were detected in 42 (33.1%) patients. BRAF-targeted therapy was not combined with ipilimumab treatment because of known increased toxicity and BRAF-mutated patients received ipilimumab in general as first-line treatment. Patient data were separated into two groups depending on whether LPT was administered (LPT group, $n = 45$) or not (ipilimumab group, $n = 82$). In these two groups, distribution of patient characteristics and clinical risk factors did not show statistically significant imbalances except of CNS metastasis treatments, which were significantly overrepresented in the ipilimumab group (8/45 vs. 41/82; $P = 0.00004$; Table 2).

Table 1. Patient characteristics

Parameters	
Age at diagnosis (mean)	61.7 (range, 23–89)
Sex (*n*, patients)	
Males	69 (54.3%)
Females	58 (45.7%)
Stages (*n*, patients)	
IIIc	14 (11.0%)
IV	113 (89.0%)
M_{1a}	9 (7.1%)
M_{1b}	23 (18.1%)
M_{1c}	81 (63.8%)
Sites of metastases[a] (*n*, patients)	
CNS	49 (38.6%)
Bone or soft tissue	36 (23.8%)
Visceral	95 (74.8%)
Lymph nodes	96 (75.6%)
Skin	59 (46.5%)
BRAF mutation status (*n*, patients)	
Mutated	42 (33.1%)
Wild-type	85 (66.9%)
Ipilimumab cycles (mean)	3.34 (range, 1–8)
$\leq$2 cycles (*n*, patients)	31 (24.4%)
$\geq$3 cycles (*n*, patients)	95 (74.8%)
n.a.[b]	1 (0.8%)
Treatments during time of study (*n*, patients)	
Ipilimumab + LPT	39 (30.7%)
Ipilimumab + LPT + brain irradiation	6 (4.7%)
Ipilimumab only	42 (33.1%)
Ipilimumab + LBI	17 (13.4%)
Ipilimumab + WBI	15 (11.8%)
Ipilimumab + combination of LBI and WBI	8 (7.1%)
Timing of LPT to ipilimumab (*n*, patients)	
Pre	9 (20.0%)
During	19 (42.2%)
Post	17 (37.8%)

NOTE: Local brain irradiation (LBI) included stereotactic radiosurgery and cyberknife irradiation. WBI was conventionally performed. LPTs were defined as non-CNS-directed therapies and included the following: local irradiation (of lymph node, bone, visceral, or skin metastases) or skin-directed ECT or SIRT of liver metastases.

[a]Patients with more than one metastatic site were counted in every respective group. Timing of LPT referred to the entire ipilimumab treatment.

[b]n.a. refers to one patient who was retreated with 4 additional ipilimumab cycles following the initial 4-cycle treatment.

Ipilimumab treatment

The majority of all patients ($n = 95$; 74.8%) received at least three ipilimumab cycles (Table 1). One patient (University Hospital Basel) was reexposed to ipilimumab after relapse and received four additional cycles. Median ipilimumab cycle numbers were equally distributed between the two study groups (Table 2). A total of 23 (18.1%) patients developed irAEs (CTCAE $\geq 3°$) with balanced occurrence in both treatment groups (Table 2).

Local peripheral treatments

LPT comprised radiotherapy, electrochemotherapy (ECT), or selective internal radiotherapy (SIRT) and were applied depending on the metastatic site and treatment availability at the respective clinical center. Surgical excision was not performed in these advanced-stage patients. Most of the patients received LPT during ($n = 19$; 42.2%) or after ($n = 17$; 37.8%) ipilimumab therapy and only 9 patients (20%) had LPT prior to ipilimumab initiation (Table 1).

In summary, additional LPT was applied to 45 of all 127 patients (35.4%) merely as radiotherapy ($n = 40$). In 4 cases, LPT consisted of ECT and one patient underwent SIRT. ECT was applied to skin metastases only. The different LPT types and treated sites are summarized in Table 3. The ipilimumab group without additional LPT comprised 82 (64.6%) patients. Both treatment groups included patients with CNS metastases (LPT group: 8/45 patients; ipilimumab group: 41/82 patients), which represented a statistically significant difference ($P < 0.001$). From those patients with CNS metastases, 6 of 8 (LPT group) and 40 of 41 (ipilimumab group) received CNS irradiation during the period of study (Table 2).

Table 2. Treatment group characteristics

Parameters	Ipilimumab + LPT (*n* = 45)	Ipilimumab (*n* = 82)	*P*
Age (y, mean)	62.1 (25–87)	57.3 (22–83)	0.10
Sex (*n*)			
Males	25 (55.5%)	44 (53.7%)	
Females	20 (44.5%)	38 (46.3%)	0.85
Stage AJCC (*n*)			
III_C	8 (17.7%)	6 (7.3%)	
IV	37 (82.2%)	76 (92.6%)	0.08 (III_C vs. IV)
M_{1a}	6 (13.3%)	3 (3.6%)	
M_{1b}	9 (20.0%)	14 (17.0%)	0.24 (M_{1a} vs. M_{1b})
M_{1c}	22 (48.8%)	59 (71.9%)	0.30 (M_{1b} vs. M_{1c})
BRAF status (*n*)			
Mutated	11 (24.4%)	31 (37.8%)	
Wild-type	34 (75.6%)	51 (62.2%)	0.16
LDH level (U/L) (*n*, patients)			
Normal (≤250 U/L)	28 (62.2%)	47 (57.3%)	
(mean, SEM)	*(192.9, 5.57)*	*(186.7, 4.15)*	
Elevated (>250 U/L)	16 (35.6%)	35 (42.7%)	0.57 (normal vs. elevated)
(mean, SEM)	*(424.0, 68.64)*	*(454.4, 52.13)*	
Not available (*n*, percent)	1 (2.2%)		
CNS metastases (*n*, patients)	8 (17.8%)	41 (50%)	<0.001
CNS radiotherapy applied[a] (*n*, patients)	6 (13.3%)	40 (48.8%)	<0.001
Ipilimumab cycles (median, range)	4 (2–4)	4 (1–8)[b]	0.23
IrAE ≥ CTCAE grade 3 (*n*)	8 (17.8%)	15 (18.3%)	1.0

NOTE: All patients were allocated to two groups depending on the application of LPT. LPT included one of the following: local radiotherapy (lymph node, bone, visceral, or skin metastases) or ECT or SIRT. Statistical analyses of contingency tables were performed with the two-sided Fisher exact test. Differences of the mean numbers of ipilimumab cycles in both groups were statistically analyzed with the two-sided Mann–Whitney *U* test.

Abbreviation: LDH, serum lactate dehydrogenase.

[a]Of the total of 49 patients with CNS metastases, 2 patients of the LPT group and one from the non-LPT group did not undergo CNS radiotherapy based on clinical decision or the patient's wish.

[b]One patient received two treatment cycles of ipilimumab (one cycle = four infusions).

LPT radiation doses were calculated for each treated site separately. Depending on the treated body site, single conventional radiation doses ranged between 1.8 and 4 Gray (Gy) and cumulative conventional radiation doses ranged from 20 to 60 Gy (Table 3). In case of stereotactic radiotherapy, single doses were higher and ranged from 17 to 25 Gy. Some patients received radiotherapy at different sites consecutively. Therefore, we also analyzed the "personal cumulative radiation doses" for each patient. The mean personal radiation dose was 53.3 Gy for all radiotherapies. Separate analysis of LPT, CNS, or LPT + CNS radiotherapy revealed mean personal cumulative doses of 59.8 Gy, 45.4 Gy, and 67.8 Gy, respectively, which did not represent a statistically significant difference (Supplementary Fig. S1A).

Table 3. Local treatment characteristics and toxicities

Type of treatment	Site	Patients (*n*)	Single dose (Gy) median (range)	Total dose (Gy) median (range)	Local toxicity (patients, *n*)					
					None	Grade 1	Grade 2	Grade 3	Grade 4	ND
LPT (convent. radiation)	Skin	12	2.75 (1.8–3)	41,25 (30–60)	0	4 (33.3%)	2 (16.7%)	2 (16.7%)	0	4 (33.3%)
	Bone	17	2 (1.8–4)	44 (20–60)	0	10 (58.8%)	4 (23.5%)	0	0	3 (17.6%)
	LN (sf)	14	2 (1.8–3)	50.4 (30–60)	3 (21.4%)	4 (28.6%)	3 (21.4%)	3 (21.4%)	0	1 (7.1%)
	LN (deep)	6	2 (1.8–3)	50 (30–54)	2 (33.3%)	1 (16.7%)	1 (16.7%)	0	0	2 (33.3%)
	Lung	1	7 (na)	35 (na)	0	0	0	0	0	1
	Mediastinum	3	2.2 (1.8–2.5)	51 (36–56)	0	1 (33.3%)	1 (33.3%)	0	0	1 (33.3%)
	Liver	1	2.5 (na)	40 (na)	1	0	0	0	0	0
LPT (stereotactic radiation)	Lung	1	25 (na)	25	1	0	0	0	0	0
	Mediastinum	1	17 (na)	51	1	0	0	0	0	0
LPT (other)	Liver (SIRT)	1	na	na	0	0	0	0	0	1
	Skin (ECT)	4	na	na	4	0	0	0	0	0
CNS (radiation)	Whole brain, conventional	28	2 (2–4)	30 (20–90)	3 (10.7%)	7 (25%)	4 (14.3%)	0	0	14 (50%)
	Local brain, stereotactic or cyberknife	27	20 (3–22)	20 (18–60)	26 (96.3%)	0	0	0	0	1 (3.7%)

NOTE: All local treatment types, including CNS radiation as non-LPT, that were applied to the patients during the study period are shown. Each treatment per body site and patient was analyzed separately. The patient numbers in this table do not sum up to the total count of the study population because some patients received more than one LPT during the period of analysis. Also, some patients treated with stereotactic CNS radiation received this treatment more than one time. Local radiotherapy toxicities were assessed according to the CTCAE version 4.03 and affected only the skin or mucosa.

Abbreviations: LN, lymph node; ND, no data available; sf, superficial.

Table 4. Overall responses and abscopal effects per treatment group

Parameters	Ipilimumab + LPT ($n = 45$)	Ipilimumab ($n = 82$) (67)[a]	*P*
Best systemic response (all patients)			
Complete remission (CR)	3 (6.7%)	0	
Partial remission (PR)	14 (31.1%)	12 (17.9%)	
Stable disease (SD)	9 (20.0%)	14 (20.9%)	
Progressive disease (PD)	19 (42.2%)	41 (61.2%)	
Clinical benefit (CR + PR + SD)	26 (57.7%)	26 (38.8%)	0.05 (CR+PR+SD vs. PD)
Abscopal effects (AE)[b]			
LPT ($n = 19$)	AE: $n = 4$ (21%)	n.a.	
LPT + CNS radiotherapy ($n = 6$)	AE: $n = 0$	n.a.	
CNS radiotherapy ($n = 15$)	n.a.	AE: $n = 3$ (20%)	

NOTE: The best systemic response was evaluated and classified according to RECIST (version 1.1).

[a]In the ipilimumab group, response data on 15 patients were unavailable. Therefore, the relative numbers for responses in this group were calculated for a total of 67 patients.

[b]The occurrence of abscopal effects (AE) could be retrospectively analyzed from 40 patient records with appropriate available data.

Local treatment toxicities were mild to moderate in the majority, and none of the patients developed grade 4 toxicities (Table 3). Local affections of the skin or local mucosa were the only toxicities observed.

Outcomes

Median potential follow-up time (from ipilimumab treatment initiation to data lock in November 2014) was calculated with the reverse survival method (Schemper and Smith 1996) and resulted in 39 and 87 weeks for the ipilimumab and LPT group, respectively. During the period of analysis, 42 (51.2%) patients of the ipilimumab group died versus 19 (42.2%) patients in the LPT group. Median OS of the entire cohort was 55 weeks. Analysis of the best systemic treatment response revealed that patients in the LPT group had a significantly higher rate of clinical benefits as compared with patients in the ipilimumab group (57.8% vs. 38.8%, $P = 0.05$). The relative number of patients who reached a complete or partial remission was also increased in the LPT group (Table 4). Around 25% of all 127 patients showed long-term responses (OS $\geq$ 120 weeks; data not shown). Patients who did not receive additional LPT reached a median OS of 42 weeks. The addition of LPT significantly prolonged median OS to 93 weeks (HR, 0.46; 95% CI, 0.27–0.73, $P = 0.0028$; Fig. 1A). The survival benefit was also statistically significant if patients with CNS metastases (both treated and untreated) were excluded from the analysis. In the remaining patients, the addition of LPT led to a median OS of 117 weeks compared with 46 weeks in patients without LPT (HR, 0.41; 95% CI, 0.17–0.78, $P = 0.0116$; Fig. 1B). Because a higher (although not significant) proportion of M1c patients were treated in the ipilimumab group, we performed OS analysis separately in stage IV M1c patients (including CNS metastases). Here, median OS was significantly prolonged in the LPT group (68 vs. 31 weeks; HR, 0.55; 95% CI, 0.30–0.97, $P = 0.05$; Supplementary Fig. S1B). Of the 49 patients with CNS metastases (ipilimumab $n = 41$; LPT $n = 8$), 46 patients received brain-directed radiotherapy during the time of our analysis. In addition, for these 46 patients, those treated with LPT had a nonsignificant prolongation of OS as compared with those treated with ipilimumab alone (82 vs. 34 weeks, HR, 0.49; 95% CI, 0.19–0.90, $P = 0.06$; Fig. 1C).

Patients harboring tumor cell–activating BRAF mutations ($n = 42$) also took advantage from additional LPT. In these patients, median PFS was significantly prolonged from 11 to 15 weeks (HR, 0.45; 95% CI, 0.19–0.73, $P = 0.0138$; Fig. 1D). In BRAF wild-type patients, LPT prolonged median OS from 42 to 88 weeks (HR, 0.45; 95% CI, 0.21–0.79, $P = 0.0076$) comparable with the entire patient cohort (Fig. 1E). Patients with a druggable BRAF mutation received BRAF-targeted treatment usually first at the time of progression following ipilimumab therapy. In order to distinguish immunotherapeutic effects from BRAF-targeted therapy effects, we analyzed only PFS here.

The impact of timing of LPT administration was analyzed in three subgroups (before, during, or after ipilimumab). Patients who received LPT during ($n = 19$) or prior to ipilimumab treatment ($n = 9$) reached a median OS of 117 or 96 weeks, respectively, versus 86 weeks in patients who had LPT after ipilimumab treatment ($n = 17$). This difference was not statistically significant ($P = 0.72$; Fig. 1F).

Due to different covariate profiles between the two groups, a multivariable Cox model was constructed that included BRAF status (mutated vs. wild-type), AJCC stage (IIIC/IVM1a vs. IVM1b+c), tumor burden [high vs. low; approximated by the number of metastatic sites (organs) (low $\leq$ 2 sites; high > 2 sites)] and the occurrence of CNS metastases as covariates. LPT was found to be an independent factor, after adjustment for these covariates (adjusted HR, 0.56; 95% CI, 0.31–1.01, $P = 0.05$; Table 5). A high tumor burden and the diagnosis of CNS metastases represented significant adverse prognostic factors in this analysis. Based on the Cox model including the above-mentioned covariates, the predicted median OS was almost doubled in the LPT group (Fig. 1G).

Measures of immune responses

Abscopal effects (AE) are defined as treatment responses of metastatic lesions distant from locally treated sites. In our study cohort, we identified a total of 40 cases (LPT $n = 19$; LPT + CNS radiotherapy $n = 6$; CNS radiotherapy $n = 15$) with radiologic measures at appropriate time points, enabling us to analyze the occurrence of AE. Four (21%) out of the 19 LPT cases had AE, whereas in none of the 6 LPT + CNS radiotherapy cases were AE detectable. In contrast, 3 (20%) out of 15 patients who received CNS radiotherapy without LPT had measurable AE (Table 4). AE were merely located to pulmonary metastatic sites (not shown).

Baseline NLR values as prognostic markers for cancer immunotherapy could be analyzed from a total of 107 patient records. The mean baseline NLR [ipilimumab: 4.0 $\pm$ 0.29

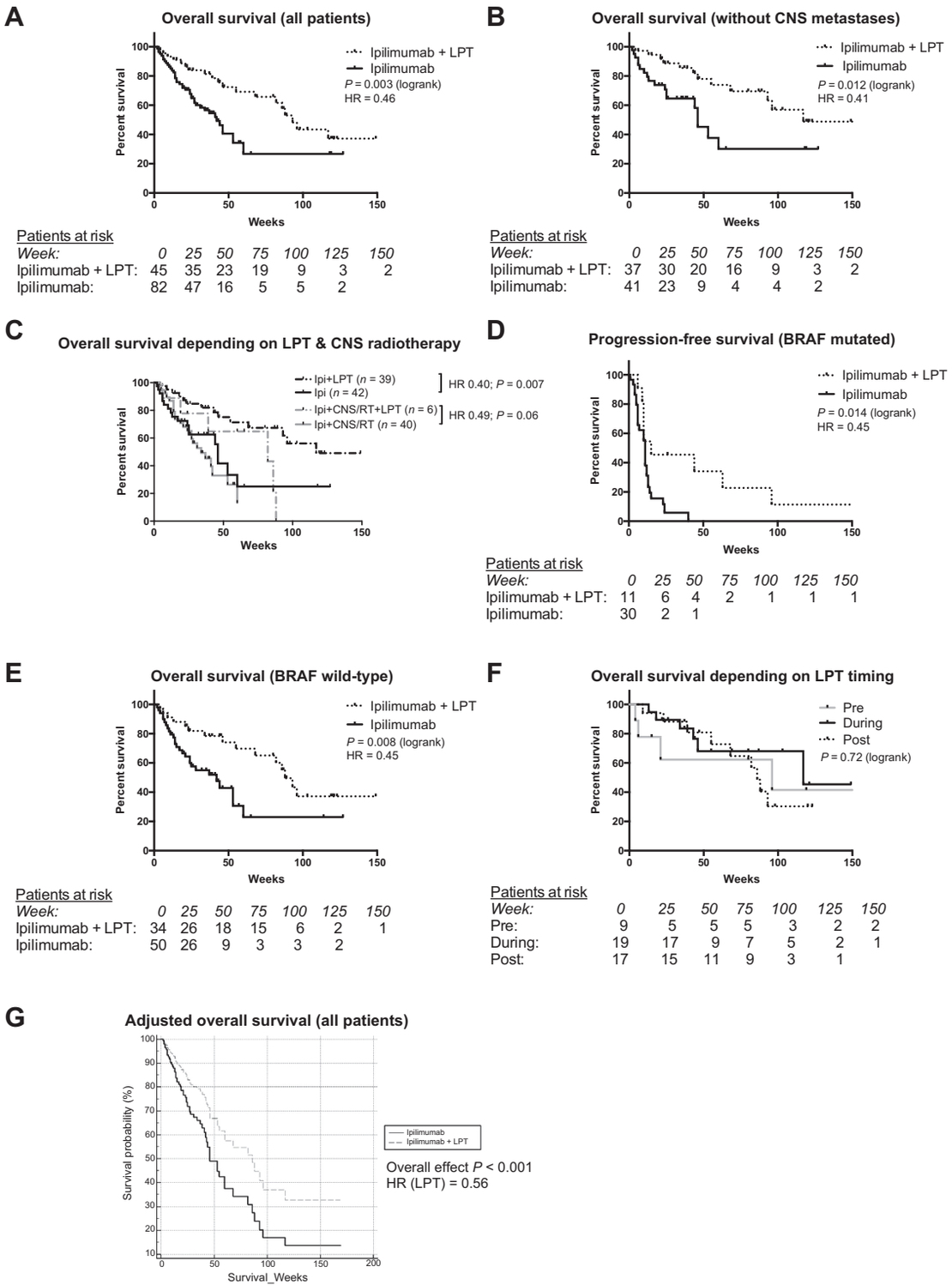

Figure 1.

Kaplan–Meier survival curves comparing ipilimumab with ipilimumab + LPT in advanced melanoma patients. **A,** survival analysis of all 127 patients shows that OS is significantly prolonged in patients who additionally received LPT. **B,** the OS advantage remained significant also in the analysis of patients without CNS metastases. **C,** OS analysis of all 127 patients divided into four treatment groups depending on CNS radiotherapy and LPT revealed that the addition of LPT prolongs OS in each respective subgroup. **D,** statistically significant prolongation of PFS in BRAF-mutant patients receiving ipilimumab + LPT. **E,** significantly improved OS for BRAF wild-type patients treated with ipilimumab + LPT. **F,** timing effects of the addition of LPT to ipilimumab. **G,** predicted survival based on multivariable Cox proportional hazard regression. Curves are compared with model-based χ^2 test.

Table 5. Overall survival, univariable (Kaplan–Meier), and multivariable (Cox proportional hazard regression) analysis

	Univariable		Multivariable	
Covariate	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
LPT (yes vs. no)	0.46 (0.26–0.76)	0.0028	0.56 (0.31–1.01)	0.05
BRAF (mutated vs. wild-type)	0.88 (0.51–1.52)	0.65	0.61 (0.34–1.10)	0.1
Stage (IIIC + IVM1a vs. IVM1b+c)	0.45 (0.25–0.83)	0.01	0.57 (0.23–1.41)	0.23
Tumor burden (high vs. low)	2.18 (1.30–3.66)	0.003	2.79 (1.43–5.43)	0.003
CNS metastases (yes vs. no)	2.98 (1.62–5.49)	0.0005	1.90 (1.07–3.38)	0.03

NOTE: For Cox proportional hazard regression, five covariates were defined: administration of LPT, BRAF mutation status, stage of the disease, tumor burden, and the occurrence of CNS metastases. For the factor tumor stage, AJCC stages IIIC and IVM1a (which comprise skin and lymph node metastases) were opposed to stages IVM1b and IVM1c (which include distant organ metastases). Tumor burden was approximated by the number of metastatic sites (organs; low $\leq$ 2 sites; high > 2 sites).

(SEM); LPT: 4.1 ± 0.44 (SEM)] was not different between the two treatment groups (Fig. 2A) even when patients with CNS metastases were excluded from this analysis (Fig. 2B). Based on a NLR cutoff value of 4, patients of each treatment group were further divided into two subgroups and survival analyses were performed. Here, patients who had additional LPT reached longer survival regardless of a high (>4) or low (<4) NLR value (Fig. 2C and D). However, this difference did not reach statistical significance.

Finally, we also had the chance to prospectively follow cellular immune responses during ipilimumab and LPT treatment in an exemplary case. The patient, a 26-year-old male (ID6), was diagnosed with stage IIIA nodular malignant melanoma at the neck in May 2013. Tumorgenetic analysis revealed BRAF, c-KIT, and NRAS wild-types. The patient progressed to stage IV, M1b (lung and lymph node metastases) and received dacarbacine chemotherapy in the setting of a clinical trial. Due to further progression (stage IV, M1c) with new, asymptomatic bone metastases, the patient was excluded from the trial and treatment was changed to ipilimumab (3 mg/kg). After the second ipilimumab cycle, LPT (radiotherapy ad 56 Gy) was applied to symptomatic cervical lymph node metastases. Symptomatic inguinal lymph node metastases were irradiated (at 36 Gy) after completion of ipilimumab therapy. Both radiotherapies were tolerated well. Four ipilimumab cycles were given without signs of severe immune-related side effects. Response evaluations demonstrated partial remission under ipilimumab and further regression over time. At the time of submission of this article, the patient was still in complete remission almost 3 years after initial diagnosis.

In order to monitor T-cell responses in this patient, PBMCs were collected at three different time points during the entire treatment (Fig. 2E) and stimulated over 6 days with cell lysates of melanoma and nonmelanoma cell lines as well as antigen-dependent and -independent controls. Proliferation, measured by CFSE staining, and CD25 and CD137 expression, as markers of activation, were the read outs. Prior to ipilimumab, specific T-cell responses were not detectable. Also following two cycles of ipilimumab, specific T-cell responses were undetectable. In contrast, after four cycles of ipilimumab and additional LPT, the patient's PBMCs contained $CD4^+$ T cells that specifically responded to melanoma cell lysate stimulation but not to the control lysates (Fig. 2F and G). Here, proliferated (CFSE low) and activated ($CD25^+$) $CD4^+$ T cells were significantly increased (Fig. 2F) as well as CD137-positive $CD4^+CD25^+$ T cells (Fig. 2G). Melanoma-specific T-cell responses were exclusively detectable in $CD4^+$ but not in $CD8^+$ T cells (Supplementary Fig. S1D and S1E). Experimental controls confirmed general responsiveness (CD3/CD28 stimulation) of the used cells as well as the ability of the assay to detect antigen-specific T-cell responses (tetanus toxin stimulation).

Discussion

Immune checkpoint inhibition has significantly improved outcomes of patients with metastatic melanoma (5, 6). Here, we present the data of our multicenter retrospective study showing that the addition of LPT to ipilimumab can significantly further improve OS of patients with metastatic melanoma. Our data strongly support the hypothesis of immunologic synergy of these two treatment modalities.

With regard to the entire cohort, the addition of LPT resulted in a more than doubled median OS (93 vs. 42 weeks, $P = 0.0028$). In BRAF-mutated cases, additional LPT significantly prolonged median PFS from 11 to 15 weeks ($P = 0.0138$). The clinical benefit was consistent even when patients with CNS metastases were excluded from analysis (median OS 117 vs. 46 weeks, $P = 0.0116$) or if only patients with the most advanced stage (IV M1c) were analyzed (68 vs. 31 weeks, $P = 0.05$). Separate analysis of patients without CNS metastases was important in this study because the two treatment groups were imbalanced concerning this risk factor. Besides CNS metastases, other risk factors also contribute to reduced survival rates in melanoma patients and could influence the impact of additional LPT. Therefore, we performed a multivariable Cox regression analysis consisting of four risk factors as covariates: BRAF mutation status, tumor stage, tumor burden, and CNS metastases. Here, the addition of LPT to ipilimumab treatment turned out to be an independent factor for improved survival. Although this finding needs further prospective validation, our data suggest that the addition of LPT to ipilimumab seems effective irrespective of established adverse prognostic factors.

Our data are consistent with preclinical evidence demonstrating irradiation-induced immune responses and with the clinical observation of abscopal effects. In early attempts, the combination of radiotherapy with immunotherapy agents showed some activity, where growth factors recruited dendritic cells (DC) to an irradiated tumor, or direct DC injection into a tumor showed synergistic effects with irradiation (34–36).

However, in the clinical setting, detection of abscopal effects is rare. This situation might now change in the new therapeutic era of immune checkpoint blockade. A number of preclinical studies and clinical cases have been published which present further evidence for abscopal effects of LPT and a synergy with systemic checkpoint inhibition (24, 28, 34, 37–40). For example, Postow and colleagues reported that a 33-year-old patient with metastatic melanoma, who had received ipilimumab treatment and local radiotherapy, showed immunologic

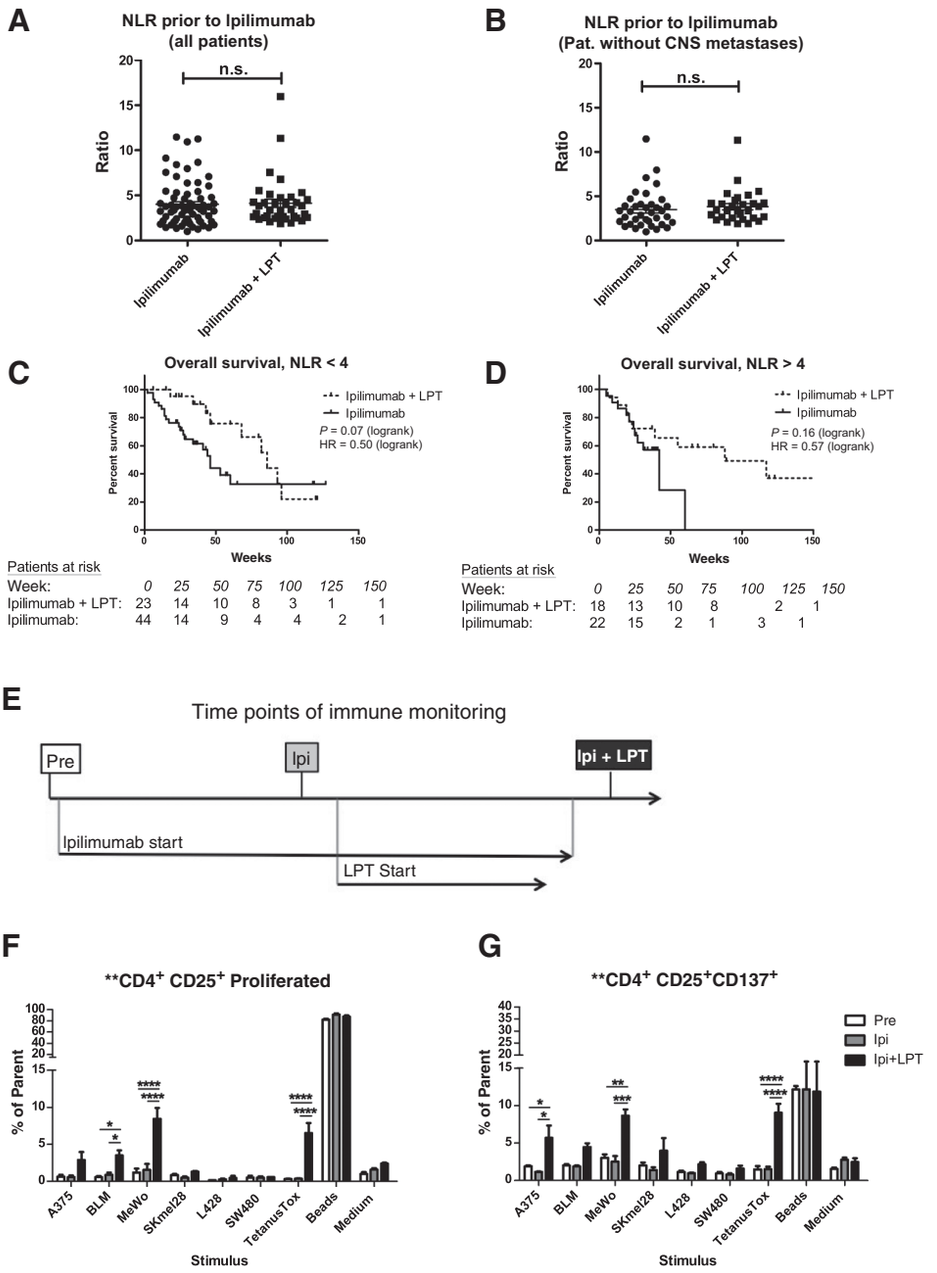

Figure 2.

Immune response parameters: survival according to baseline NLR and exemplary T-cell responses in a patient during the course of ipilimumab and LPT. **A,** baseline NLR values prior to ipilimumab initiation were calculated from absolute blood leucocyte counts from all patients ($n = 107$) or **B,** only from patients without CNS metastases ($n = 69$). **C** and **D,** based on an NLR cutoff value of 4, patients from each treatment group were further divided into two subgroups. **E,** schedule of blood sampling in one patient: before ipilimumab initiation, during ipilimumab, and after ipilimumab + LPT. **F** and **G,** PBMCs derived from all three time points were stimulated with lysates of each respective tumor cell line or controls over 6 days. $CD4^+$ T-cell responses were analyzed by flow cytometry with regard to proliferation and activation. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

synergy and had profound systemic responses to therapy (25). These systemic responses correlated with increasing antibody titers to the tumor antigen NY-ESO-1 and with enhanced activation of adoptive immune responses. A retrospective analysis of 21 melanoma patients who had progressive disease after ipilimumab treatment, and then received radiotherapy to the brain (62%) or to peripheral sites (48%), showed an abscopal response in 11 (52%) patients (41). Other authors have described abscopal effects induced by the combination of radiotherapy with GM-CSF (42) and also that CNS radiotherapy itself can induce peripheral abscopal effects (43).

We therefore sought to analyze the occurrence of abscopal effects in our cohort. However, our analysis was limited by the fact that appropriate, comparable radiologic data were only available in a limited number of cases, which underlines the importance of prospectively well-defined settings to detect abscopal effects. Nonetheless, we analyzed the data of a total of 40 patients who received either LPT or CNS irradiation in addition to ipilimumab. We found that 20–21% of these patients had abscopal effects after their treatment. This number is comparable with published data on abscopal effects during immune checkpoint inhibition (42, 44), but further conclusions cannot be drawn here due to the limitations mentioned above.

Since the introduction of ipilimumab into clinical practice, pooled study data show a survival curve plateau in about 20% of

the patients (7). In our study, approximately 40% to 45% of patients who received additional LPT achieved a survival plateau, compared with about 22% to 25% of the ipilimumab-only-treated patients. To find supporting data for this difference, we analyzed clinical benefit rates in the two treatment groups. Patients in our LPT group had a significantly better clinical benefit rates with a higher percentage of complete and partial remissions. Also, the separate analysis of patients without CNS metastases did not change these results.

Baseline NLR represents an independent prognostic factor for cancer immunotherapy outcomes, and high values (cutoff 4 or 5) correlate with worse treatment responses (32, 45). In our study, we did not find significant differences of baseline NLR values between the two treatment groups. OS was improved in LPT-treated patients regardless of high or low baseline NLR values (cutoff 4). However, this survival benefit was not statistically significant, most likely due to the low case numbers in each subgroup.

Various cellular immune responses are involved in cancer control, and ipilimumab induces melanoma-specific $CD4^+$ and $CD8^+$ T-cell responses (46–48). In an exemplary patient who developed long-term disease control, we had an opportunity to analyze T-cell responses longitudinally during the course of treatment. Instead of specific peptides, we used whole tumor cell lysates as T-cell stimuli because the majority of melanoma-specific tumor antigens remain unknown (49). In our assay, T-cell activation was most likely not due to the recognition of the five tested melanoma associated antigens (HMB45, MelanA, MiTF, S100, or tyrosinase), because T-cell responses did not mirror the antigen expression patterns of these proteins in the cell lines and the primary tumor (Supplementary Fig. S1C). We therefore assume that our assay contained unidentified tumor antigens that were recognized by *in vivo* primed T cells. In this patient, activation of $CD4^+$, but not $CD8^+$, T cells was detected at the last time point measured (following the addition of LPT), but not earlier (Fig. 2F and G and Supplementary Fig. S1D and S1E). However, control antigen-specific (tetanus toxin) T-cell responses were also detected only at this last time point, and we therefore cannot rule out that delayed general T-cell activation was the underlying cause for these kinetics. Future prospective analysis of immune responses during immunotherapy and LPT may provide insights into the biological mechanisms and determine if LPT can enhance tumor-specific T-cell responses.

irAEs and autoimmunity after ipilimumab treatment correlate with improved outcomes (50). In our analysis irAE were not significantly increased in patients who additionally received LPT, despite their survival benefit. This might further suggest that additional LPT can activate immune responses more specifically, which limits the risk of immune-mediated off-tumor tissue damage.

Timing of LPT administration, which was also analyzed in this study, did not show statistically significant differences if LPT were applied prior, during, or after ipilimumab. However, patient numbers in this subgroup analysis were limited, thus preventing firm conclusions. From a tumor-immunological perspective, induction of immunogenic cell death by LPT in an already activated immune system might induce specific immune responses more effectively. The results of our immune response analysis support this view.

Pembrolizumab, another immune checkpoint inhibitor targeting PD-1, has demonstrated high efficacy and might be superior to ipilimumab (8). Therefore, it would be interesting to study if the impact of additional LPT could also be detected after PD-1/PD-L1 blockade. Patients who acquire resistance to CTLA-4 blockade can respond to consecutive PD-1/PD-L1 inhibition (18). This approach could further improve immunotherapy and suggests that nonredundant functions of immune checkpoints should be taken into account in future trial designs.

To our knowledge, currently our study represents the largest analysis focusing on additional LPT in melanoma patients undergoing immune checkpoint blockade. Although the interpretation of retrospective data is naturally somewhat limited, the combination of LPT with ipilimumab is feasible and additional LPT can improve patient outcomes by enhancing antitumor immune responses. The combined concept of local therapy with systemic immune checkpoint inhibition is currently being studied in prospective clinical trials and has the potential to further improve the success of cancer immunotherapy (51).

Disclosure of Potential Conflicts of Interest

D. Koeberle is a consultant/advisory board member for Merck Darmstadt. A. Zippelius reports receiving speakers bureau honoraria and serves as a consultant/advisory board member for Bristol-Myers Squibb. M. Schlaak is a consultant/advisory board member for Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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