Phase Ib/II trial testing combined radiofrequency ablation and ipilimumab in uveal melanoma (SECIRA-UM)

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Approximately, 50% of patients with uveal melanoma develop distant metastasis for which no standard therapy is established. In contrast to cutaneous melanoma, the anti-CTLA-4 antibody ipilimumab showed no clinical activity in uveal melanoma. Liver directed therapies improve local control, but fail to show overall survival (OS) benefit. Preclinical experiments demonstrated that radiofrequency ablation (RFA) induced durable responses in combination with anti-CTLA-4. The aim of this phase Ib/II study was to assess safety and efficacy of RFA plus ipilimumab in uveal melanoma. Patients underwent RFA of one liver lesion and subsequently received four courses ipilimumab 0.3, 3 or 10 mg/kg every 3 weeks in a 3+3 design. Primary endpoints were safety in terms of dose limiting toxicities per cohort to define the recommended phase II dose (RP2D) in the phase Ib part and confirmed the objective response rate and disease control rate (DCR) of non-RFA lesions in the phase II part. Secondary endpoints were progression-free survival (PFS) and OS. Ipilimumab 10 mg/kg + RFA was initially defined as the RP2D. However, after 19 patients, the study was amended to adjust the RP2D to ipilimumab 3 mg/kg + RFA, because 47% of patients treated with 10 mg/kg had developed grade 3 colitis. In the 3 mg/kg cohort, also 19 patients have been treated. Immunotherapy-related grade ≥3 adverse events were observed in 53% of patients in the 10 mg/kg cohort versus 32% in the 3 mg/kg cohort. No confirmed objective responses were observed; the confirmed DCR was 5% in the 10 mg/kg cohort and 11% in the 3 mg/kg cohort. Median PFS was 3 months and comparable for both cohorts, median OS was 14.2 months for the 10 mg/kg cohort versus 9.7 months for the 3 mg/kg cohort. Combining RFA with ipilimumab 3 mg/kg was well tolerated, but showed very limited clinical activity in uveal melanoma. *Melanoma Res* **30: 252–260**

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Introduction

Uveal melanoma is an infrequent malignancy (0.6–0.7 cases/100 000/year), representing only 3–5% of all melanomas but the majority of primary ocular malignancies [1]. Although local disease control after enucleation or radiotherapy of the primary tumor is high, dissemination occurs within 5 years in 25–30% (and within 15 years in approximately 50%) of patients [2–4]. The pattern of dissemination is predominantly hematogenic, most commonly to the liver (89%), lung (29%) and bone (17%) [2]. The median survival of patients with metastatic uveal melanoma is poor and was only 13 months according to a recently published large cohort study and an extensive meta-analysis [5,6].

Despite striking genomic difference with cutaneous melanoma (known driver mutations are different and mutational load is much lower), systemic management

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strategies for uveal melanoma have been adopted from cutaneous melanoma. Chemotherapies, like dacarbazine (DTIC), temozolomide, fotemustine and various combinations, have been described to induce responses in a minority of patients, but all failed to convincingly improve overall survival (OS) rates which is not attributable to patient selection [6,7]. The striking results of checkpoint inhibition in cutaneous melanoma [8–10] have led physicians using this drug for uveal melanoma patients, but the results are extremely disappointing. Both CTLA-4 and PD-1 inhibitors failed to induce promising response signals in retrospective analyses and single-arm studies [11–17].

As the liver is the most commonly affected organ in metastatic disease, liver-directed approaches are common in uveal melanoma management. Evidence is limited, but the data suggest that selected patients can benefit from hepatic resection, regional chemotherapy such as hepatic intra-arterial chemotherapy or hepatic arterial chemoembolization [18–21]. Current approaches using percutaneous hepatic perfusion with melphalan result in response rates of 35–60% in the liver [22–25]. Although the liver-directed therapies can improve local control, they failed to improve overall survival [19,23]. The majority of these patients develops subsequently extra hepatic disease undermining the remaining urgent need for an effective systemic therapy in uveal melanoma.

In preclinical analyses using a murine melanoma model, the addition of radiofrequency ablation (RFA) to CTLA-4 blockade induced enhanced antigen-loading of dendritic cells, resulting in long-lasting antitumor immune responses superior to CTLA-4 blockade alone [26,27]. Based on these data, we have set up in 2011 a phase Ib/II study with the aim to explore the safety and efficacy of the combination of RFA and ipilimumab in uveal melanoma patients that are ineligible for surgery. Here, we report the final results from this trial (SECIRA-UM, EudraCT: 2011-004200-38).

Methods

Study population

Eligible patients were aged 18 years or older and had histological or cytological confirmed unresectable metastatic uveal melanoma (as confirmed by multidisciplinary tumor board). All patients needed to have at least two liver metastasis (both >1 cm) and one of them should be feasible for RFA. Patients were not allowed to have been treated with previous systemic therapy for metastatic disease. A WHO performance status of 0 or 1, normal organ function and lactate dehydrogenase (LDH) levels less than two times the upper limit of normal were required. Exclusion criteria included cerebral metastasis and history of autoimmune disease requiring immunosuppressive medication. All participating patients provided written informed consent before enrollment.

Study design and endpoints

This study was a single-center nonrandomized phase Ib/II study to determine the recommended phase II dose (RP2D) of the combination of RFA and ipilimumab and to evaluate its safety and efficacy in uveal melanoma patients. Primary endpoint of the phase Ib part was safety in terms of dose limiting toxicities (DLTs) per cohort to define the RP2D. Primary endpoints of the phase II part were confirmed objective response rate (ORR) and disease control rate (DCR) of only non-RFA lesions according to RECIST 1.1; secondary endpoints were progression-free survival (PFS) and OS.

In the phase Ib part, patients underwent RFA of one liver lesion and subsequently received four cycles of ipilimumab in a dose of 0.3, 3 or 10 mg/kg every 3 weeks in a standard 3 + 3 dose-escalation design (Fig. 1a and b). The DLT observation period ranged from day 1 (the day that the patient underwent RFA) until 12 weeks after the first infusion of ipilimumab. DLT toxicities were defined as any unexpected serious adverse event (SAE) and adverse event deemed related to the investigational treatment combination. After the determination of the maximum tolerated dose (MTD), which was considered as the RP2D, patients were included in the phase II part of the study. The patients treated at the MTD within the phase Ib part of the study were included into the efficacy and safety analysis of the phase II extension part.

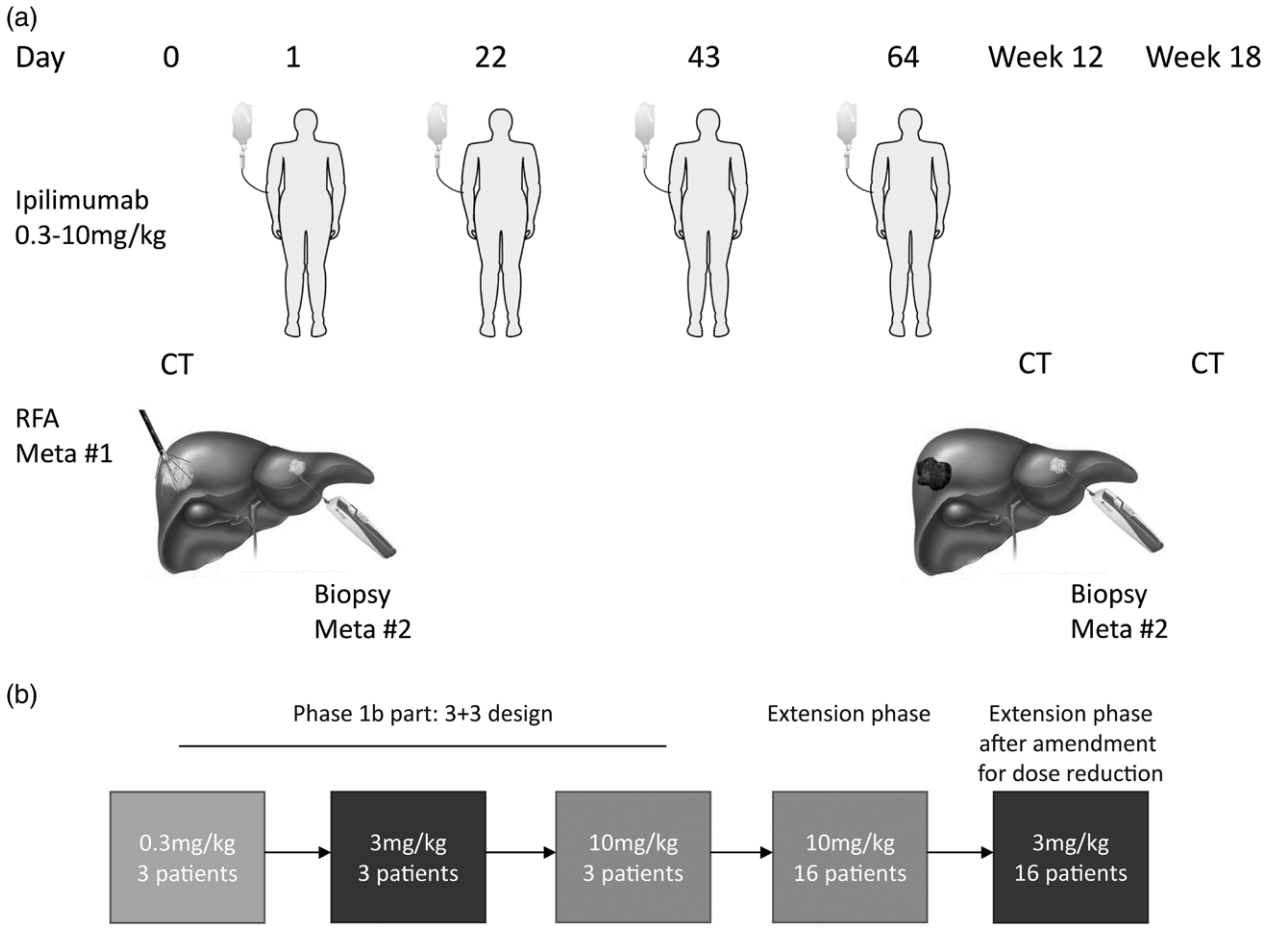
The study was performed according to the protocol and good clinical practice guidelines as defined by the International conference on harmonization and the declaration of Helsinki. The study protocol was approved by the medical ethical committee of the Netherlands Cancer Institute.

Translational analysis

Programme death-ligand-1 (PD-L1) immunohistochemistry staining of pretreatment tumor biopsies was performed with the 28-8 clone on a Dako platform. The percentage of PD-L1 positive tumor cells was scored by the pathologist.

Digital Spatial Profiling (DSP) analyses were performed on pretreatment and posttreatment formalin fixed paraffin embedded (FFPE) tumor biopsies. The GeoMx Digital Spatial Profiler simultaneously characterizes regional and quantitative protein expression related to immune cell activation and tumor cell classification on a single FFPE tissue section. It uses a cocktail of primary antibodies conjugated to unique oligonucleotide tags with a ultraviolet (UV) photocleavable linker. The GeoMx DSP procedure implements five nondestructive steps: a standard FFPE tissue preparation step, a tissue incubation step with a mixture of morphology markers and GeoMx DSP probes, an imaging and region of interest (ROI) selection step, a UV exposure and oligo collection step and a quantification step on NanoString's nCounter

Fig. 1



Study design. (a) Schematic overview of the SECIRA-UM study design. (b) Schematic overview of the dosing of the ipilimumab per cohort, displayed in the chronological order of inclusion.

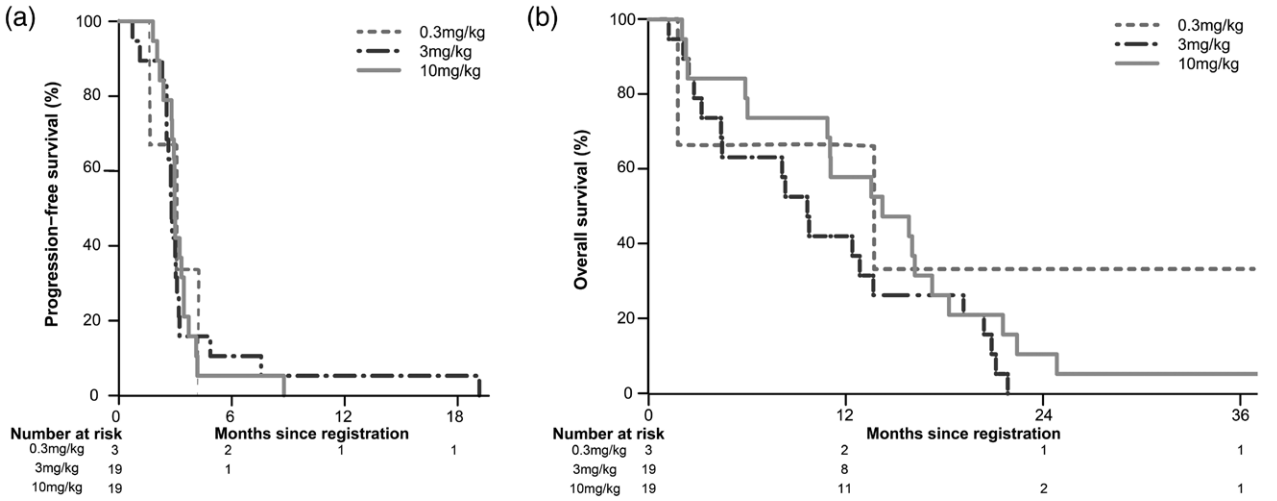
system (Supplementary Figure 1, Supplemental digital content 1, *http://links.lww.com/MR/A195*). The 4-μm-thick FFPE slides were incubated with an antibody cocktail of up to 44 unique oligonucleotide-labeled antibodies (Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/MR/A195*). The compartments were defined by fluorescent imaging with antibodies for syto13 for detection of nuclei, S100B/Pmel17 for melanoma, CD45 for leukocyte and CD3 for T cell detection (Supplementary Table 2, Supplemental digital content 1, *http://links.lww.com/MR/A195*). Based on the fluorescent image, the most representative ROIs within the tumor-enriched tissue areas were chosen for multiplex profiling (Fig. 3a). Photocleaved oligos were transferred into a microwell and quantified using optical barcodes in the nCounter platform. For analysis, digital counts were first normalized with internal spike-in controls (ERCCs) to account for technical variations, then normalized to the area of their defined regions of interest and consequently the housekeeping controls. Details about the statistical analysis of the DSP data are described in the Supplementary Methods, Supplemental digital content 1, *http://links.lww.com/MR/A195*.

Statistical analysis

A true response rate of 5% would be considered insufficient and 20% was considered desirable. Based on a Fleming one-stage design, a sample size of 38 patients would be needed to have a power of 95%, given a one-sided type I error of 0.09. After amendment of the trial, a new power calculation was performed (Supplementary Methods, Supplemental digital content 1, *http://links.lww.com/MR/A195*).

Analyses were performed in all patients that received RFA and at least one dose of ipilimumab. Descriptive statistics were used to report baseline characteristics of the study population and efficacy. The ORR was defined as a confirmed complete response (CR) or partial response (PR) according to RECIST 1.1. DCR was defined as the

Fig. 2



Progression- free survival and overall survival. Kaplan Meyer analysis of (a) progression-free and (b) overall survival per dose cohort.

percentage of patients with a confirmed CR, PR or stable disease. Adverse events were scored on the basis of the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE v4.03) by the investigators whom also defined whether the adverse event was related to immunotherapy or RFA. PFS was measured from date of registration until date of progression or death whichever occurs first. OS was defined as the time from the date of registration to the date of death by any cause. Patients without progression and whom were still alive at time of data cutoff were censored at last date of follow-up. Analyses were done using R (version 3.3.1).

Results

Determination of recommended phase II dose

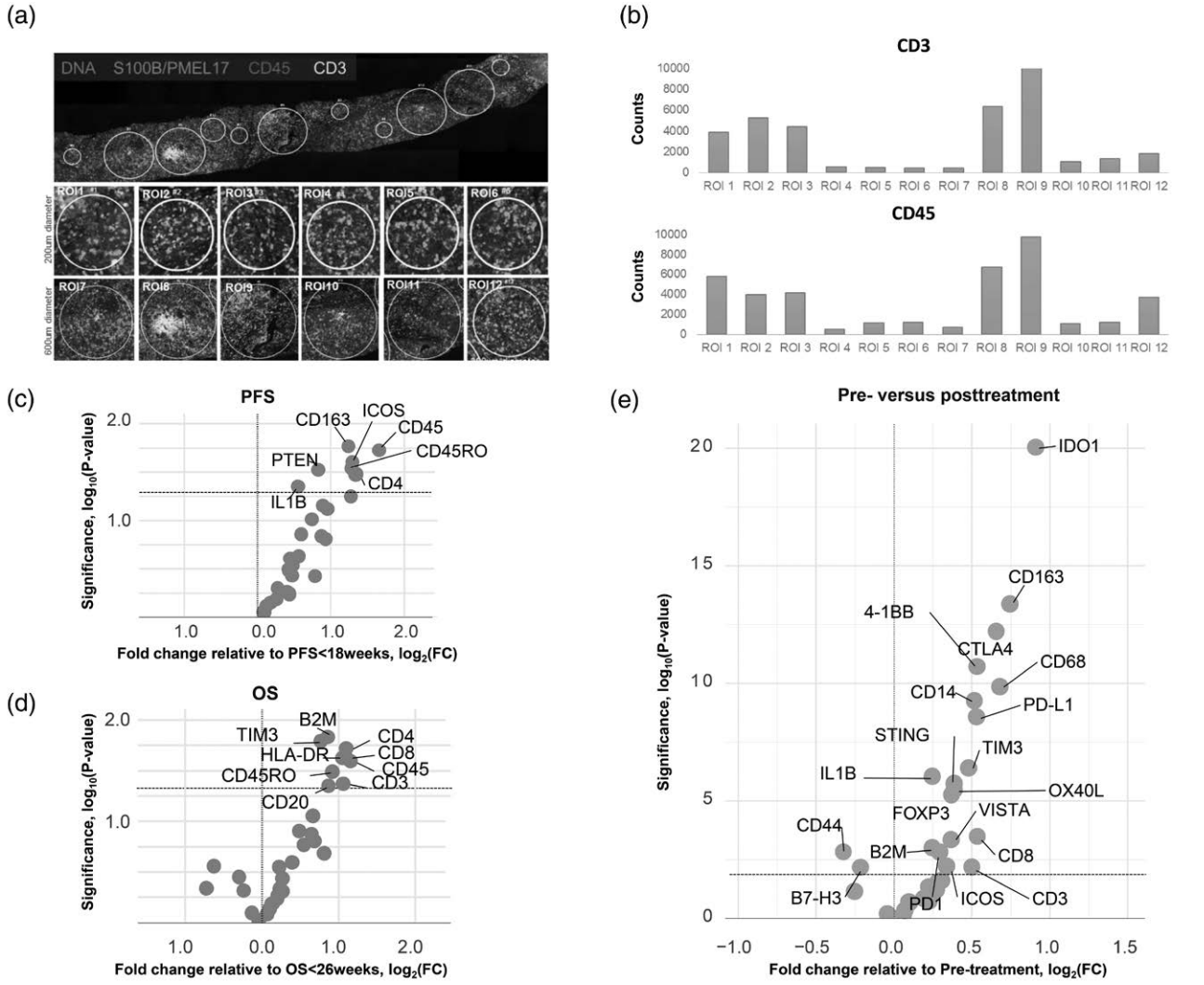
In the first dosing cohort in which three patients were treated with ipilimumab 0.3 mg/kg + RFA, no DLTs were observed. The only grade 3 adverse events that were observed were hepatic pain not related to therapy and short-term elevated transaminases due to and after RFA. None of the patients had to stop therapy due to treatment related toxicities. In the second dosing cohort (ipilimumab 3 mg/kg + RFA) also, no unexpected SAE or adverse events were observed and none of the patients had to stop ipilimumab due to toxicity. Also, within this dosing cohort, the only reported high grade adverse events were a temporary elevation of liver enzymes related to RFA and hepatic pain and cholestasis due to disease progression. Within the following three patients treated with ipilimumab 10 mg/kg + RFA, one grade 3 immunotherapy-related adverse event was observed. After two cycles of ipilimumab, the patient developed a colitis which was treated with steroids and the immunotherapy was discontinued. The colitis was not considered as a DLT as it is a rather frequent and not-unexpected adverse event known from ipilimumab therapy in cutaneous melanoma. One of the other patients developed a nontreatment-related pneumonia, and in the third patient, a temporary elevation of liver enzymes related to RFA was observed. Therefore, in the phase Ib part, ipilimumab 10 mg/kg + RFA was defined as the RP2D. All adverse advents that were observed in the phase Ib part of the study are displayed in Supplementary Table 3, Supplemental digital content 1, *http://links.lww.com/MR/A195*.

After 19 patients had been treated with 10 mg/kg ipilimumab, the study was amended to adjust the RP2D to ipilimumab 3 mg/kg + RFA, because an unplanned analysis requested by the principal investigator revealed that 9 (47%) of the 19 patients that were treated with 10 mg/kg had developed grade 3 colitis.

Patients and treatment

Between May 2012 and September 2016 in total, 41 patients were enrolled in the trial. As described above, three patients were treated with 0.3 mg/kg ipilimumab + RFA and in both the 3 mg/kg ipilimumab + RFA cohort and the 10 mg/kg ipilimumab + RFA cohort, 19 patients were treated (Fig. 1b). All patients underwent RFA on day one and received the first cycle of ipilimumab on the next day. Baseline characteristics for each cohort are displayed in Table 1. Off all patients included in the trial, 59% were male and the median age was 62 years (range: 38–79). Extrahepatic disease was found in 54% of patients, most commonly in the lungs (41%). Patient characteristics were generally the same for 3 and 10 mg cohort except that in the 3-mg/kg cohort, more patients had extrahepatic disease and elevated LDH levels. Of the 19 patients that were treated with ipilimumab 3 mg/kg, 11 (58%) received all four cycles ipilimumab

Fig. 3



Digital spatial profiling analysis of pretreatment and posttreatment biopsies. Digital spatial profiling analysis of pretreatment tumor biopsies. S100B, CD45 and CD3 visualization markers were used to identify tumor rich and immune rich regions of interest (ROI). Per patient 12 ROIs were selected. ROIs were selected in high tumor-infiltrating lymphocyte (TIL) tumor area ($n = 3$) or in low TIL tumor areas ($n = 3$) and another six geometric ROIs were used with 200 μm in diameter and were placed randomly in the tumour area. Data were ERCC and Housekeeping normalized and log2 transformed. (a) Example of ROI selection. Protein profiling of ROI was achieved using an oligoconjugated antibody panel and read-out with NanoString barcodes. (b) CD3 and CD45 counts per ROI of the same biopsy as in (a) showing that the counts match the intensity of the visualization markers. (c)–(e) Volcanoplots displaying differentially expressed immune-related between patients with PFS > 18 weeks and patients with a PFS < 18 weeks (c), patients with OS > 26 weeks and those with an OS < 26 weeks (d) and between matched pretreatment and posttreatment biopsies (e). Dotted lines represent adjusted *P* value cutoffs.

(Table 2). Six patients had to discontinue due to immunotherapy-related adverse events and three patients have stopped therapy because of progressive disease. Within the 10 mg/kg ipilimumab patient cohort, seven (47%) patients completed four cycles, nine patients had to stop due to immunotherapy related adverse events and three because of disease progression or death. Detailed information about the immune-related adverse events leading to discontinuation of ipilimumab is displayed in Supplementary Table 4, Supplemental digital content 1, *http://links.lww.com/MR/A195*.

Safety

Grade 3 or 4 adverse events were observed in six (32%) patients treated with ipilimumab 3 mg/kg and in 10 (52%) patients treated with 10 mg/kg ipilimumab (Table 3). The most frequent grade 3–4 adverse event was colitis which was observed in five patients treated with 3 mg/kg (26%), and nine patients treated with 10 mg/kg ipilimumab (47%). Other high-grade immunotherapy-related adverse events were fatigue, rash adrenal insufficiency, hypophysitis and mucositis which were all observed only in one patient. Any treatment related adverse events were observed in 18 out

Table 1 Baseline characteristics

Characteristic	Dose cohort 0.3 mg/kg (*N* = 3)	3 mg/kg (*N* = 19)	10 mg/kg (*N* = 19)
Gender			
Male	2 (67%)	12 (63%)	10 (53%)
Female	1 (33%)	7 (37%)	9 (47%)
Age			
Median (range)	65 (55–65)	63 (48–79)	61 (38–69)
WHO performance status			
0	2 (67%)	18 (95%)	19 (100%)
1	1 (33%)	1 (5%)	
Site of disease			
Liver only	2 (67%)	7 (37%)	10 (53%)
Liver + Lung	1 (33%)	4 (21%)	4 (21%)
Liver + Lung + other	0	6 (32%)	2 (11%)
Liver + other	0	2 (11%)	3 (16%)
LDH	260 (134–504)	224 (139–607)	205 (134–471)
<ULN	1 (33%)	11 (58%)	13 (68%)
>ULN–2×ULN	1 (33%)	4 (21%)	6 (32%)
>2×ULN	1 (33%)	4 (21)	0
ALT	18 (9–57)	32 (16–110)	34 (12–198)
<ULN	2 (67%)	12 (63%)	12 (63%)
>ULN–3×ULN	1 (33%)	7 (37%)	6 (32%)
>3×ULN	0	0	1 (5%)
PD-L1			
<1%	2 (67%)	15 (79%)	12 (53%)
1–50%	0	2 (11%)	0
>50%	0	0	0
Unknown[a]	1 (33%)	2 (11%)	7 (47%)

Data are median (range) or *n* (%).
ALT, alanine aminotransferase; LDH, lactate dehydrogenase; PD-L1, programmed death ligand 1; ULN, upper limit of normal.
[a]Not enough tumor in pretreatment biopsy.

Table 2 Treatment disposition and response

	Dose cohort 0.3 mg/kg (*n* = 3)	3 mg/kg (*n* = 19)	10 mg/kg (*n* = 19)
Number of cycles			
1	0	2 (11%)	3 (16%)
2	1 (33%)	2 (11%)	6 (32%)
3	0	4 (21%)	3 (16%)
4	2 (67%)	11 (58%)	7 (37%)
Reason for treatment discontinuation			
Progression or death	1 (33%)	2 (11%)	3 (16%)
Adverse events	0	6 (32%)	9 (47%)
Best overall response[a]			
PR	0	0	0
Stable disease	0	2 (11%)	1 (5%)
PD	3 (100%)	16 (84%)	15 (79%)
Nonevaluable	0	0	1 (5%)
Death before evaluation	0	1	2 (11%)

Data are *n* (%) unless otherwise indicated.
PD, progressive disease; PR, partial response.
[a]PR and stable disease needed to be confirmed after at least 4 weeks.

of 19 patients in both dosing cohorts. The most common immunotherapy-related grade 1–2 adverse events were fatigue, pruritus, rash and thyroid disorders.

The most common RFA-related toxicities were transient elevation of liver enzymes and flank pain. The frequency of RFA-related side effects did not differ between the different dosing cohorts.

Efficacy

Radiologic responses according to RECIST 1.1. are described in Table 3. Thirty-seven patients were evaluable for response. Among the four patients who were not assessable, one patient had in retrospect no measurable disease and three patients died before the first planned response evaluation. None of the patients had a confirmed objective response (Table 2). The confirmed DCR was 7% [95% confidence interval (CI): 2–22%] in the total cohort, 11% in the 3 mg/kg and 5% in the 10 mg/kg cohort. The six-month PFS was 7% and the one- and two-year OS were 51% (95% CI: 38–69%) and 7% (95% CI: 2–22%), respectively. Median PFS was 3 months in the total cohort and did not differ per cohort (Fig. 2a). The median OS from date of registration was 12.4 months for the total cohort, 9.7 months for the 3 mg/kg cohort and 14.2 months in the 10 mg/kg cohort (Fig. 2b).

Subsequent therapies

There was no significant difference in the number of patients that were treated with systemic and local subsequent treatments between both cohorts. In the 3 mg/kg cohort, five patients (26%) received subsequent systemic therapy (two patients received a PKC inhibitor, one patient was treated with MART-1 directed TCR therapy, one with the combination of ipilimumab plus nivolumab, one with a PKC inhibitor followed by DTIC and Lurbinectidin) and radiotherapy and two patients were treated with palliative radiotherapy only. In the 10 mg/kg cohort, six patients (32%) received subsequent systemic therapy (two patients were treated with combination of a PKC inhibitor and an MEK inhibitor, one patient with nivolumab followed by a PKC inhibitor, one with nivolumab and radiotherapy, one with a PKC inhibitor followed by embolization with yttrium and one with a PKC inhibitor), two patients were treated with radiotherapy, and one patient had surgery and radiotherapy.

PD-L1 expression and digital spatial profiling

Only two out of the 31 patients of whom a PD-L1 staining was performed on the pretreatment biopsy had a PD-L1 positive liver metastasis (defined as >1% PD-L1 positive tumor cells). One of these patients died before the first radiologic evaluation and the other patient had progressive disease as best overall response.

Digital spatial profiling analysis revealed that CD163, ICOS, CD45, CD45RO and CD4 were higher in pretreatment tumor biopsies of patients with a PFS of >18 weeks (Fig. 3c) and CD3, CD4, CD8, CD20 CD45, B2M, TIM-3, HLA-DR, were upregulated in patients with an OS of >6 months compared to patients with an OS <6 months (Fig. 3d). When comparing the posttreatment samples with pretreatment samples of the same patient, we observed an increase in several proteins that are related to a more immunogenic tumor microenvironment including B2M, CD3, CD8, STING, and the immune-checkpoints CTLA-4, PD-L1, PD-1, 4-1BB, VISTA and TIM-3 (Fig. 3e). We also observed an increase in markers that are related to a more immune-suppressive microenvironment

Table 3 Treatment-related adverse events

Immunotherapy-related toxicity	Dose cohort			
	3 mg/kg ($n = 19$)		10 mg/kg ($n = 19$)	
Adverse event	All grades; *n* (%)	Grade 3/4; *n* (%)	All grades; *n* (%)	Grade 3/4; *n* (%)
Any adverse event[a]	18 (95)	6 (32)	18 (95)	10 (53)
Diarrhea	7 (37)	3 (16)	11 (58)	5 (26)
Colitis	5 (26)	5 (26)	9 (47)	9 (47)
Rash	4 (21)	1 (5)	9 (47?)	0 (0)
Fatigue	6 (32)	0	4 (21)	1 (5)
Pruritus	6 (32)	0	4 (21)	0
Nausea	6 (32)	0	2 (11)	0
Fever	1 (5)	1 (5)	5 (26)	0
Vomiting	1 (5)	0	3 (16)	0
Weight loss	1 (5)	0	3 (16)	0
Adrenal insufficiency	2 (11)	1 (5)	1 (5)	0
Hyperthyroidism	2 (11)	0	1 (5)	0
Abdominal pain	1 (5)	0	2 (11)	0
ALT increased	1 (5)	0	2 (11)	
Chills	1 (5)	0	1 (5)	0
Hypothyroidism	1 (5)	0	1 (5)	0
Anorexia	2 (11)	0	0	0
Headache	1 (5)	0	1 (5)	0
Dysgeusia	0	0	2 (11)	
Hypophysitis	1 (5)	0	0	0
Pneumonitis	0	0	1 (5)	0
Uveitis	0	0	1 (5)	0
Mucositis oral	0	0	1 (5)	1 (5)
Vasovagal reaction	0	0	1 (5)	1 (5)
RFA-related toxicity				
Adverse event	All grades; *n* (%)	Grade 3/4; *n* (%)	All grades; *n* (%)	Grade 3/4; *n* (%)
Any adverse event[a]	15 (79)	10 (53)	17 (89)	7 (37)
AST increased	15 (79)	9 (47)	14 (74)	7 (37)
ALT increased	14 (74)	7 (37)	15 (79)	5 (26)
Flank pain	5 (26)	0	0	0
Hepatic pain	1 (5)	0	4 (21)	0
Abdominal pain	2 (11)	0	1 (5)	0
Hematoma	1 (5)	0	0	0
Fever	0	0	1 (5)	0

Treatment-related adverse events that occurred in at least >5% of patients and all grade 3–4 and immune-related adverse events of interest are displayed in the table. Data are *n* (%).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; RFA, radiofrequency ablation.

[a]Some patients had more than one event.

like IDO1, the regulatory T cell (Treg) marker FOXP3 and the macrophage markers CD68 and CD163.

Discussion and conclusion

To date, all efforts establishing a systemic therapy improving the overall survival in uveal melanoma in randomized trials have failed. A randomized trial testing the MEK inhibitor selumetinib is the most recent failure [28]. Combining local induction of a 'danger signal' [29] with immunotherapy to achieve long-term benefit in uveal melanoma seemed to us a very promising approach when starting this trial in 2011, especially in the light of positive preclinical data. Unfortunately, our trial also failed to induce promising responses or long-term clinical benefit.

In the phase 1 dose-escalation part of the study, ipilimumab 10 mg/kg + RFA was defined as the RP2D because no unexpected or unexpectedly high frequencies of treatment-related adverse events were observed within the three dosing cohorts. However, the high toxicity rate observed in the dose-extension cohort (when 19 patients had been treated with 10 mg/kg ipilimumab + RFA) led to an amendment of the trial in which the dose of the ipilimumab was adjusted to 3 mg/kg. Our observation that the toxicity of ipilimumab was dose dependent is in line with recent results testing ipilimumab 3 versus 10 mg/kg in cutaneous melanoma patients [30]. These data became available after completion of patient enrollment in our trial. Otherwise, we would have switched to the lower dose earlier.

In comparison to the studies that assessed the activity of ipilimumab monotherapy, we observed no additional effect of RFA plus ipilimumab [11–13]. Notably, the median OS was longer in our cohort than the observed 7 months after ipilimumab monotherapy [6], but this is likely attributable to the fact that we only included patients naïve for systemic therapy. Indeed, a recent meta-analysis reported that differences in reported OS among uveal melanoma trials are largely attributed to the percentage of patient treated in the first line [6]. Because the clinical benefit of PD-1 monotherapy in uveal melanoma is also nihil [14–17], checkpoint inhibitor monotherapy or ipilimumab plus RFA are no viable treatment options for patients with uveal melanoma.

Data on the combination of CTLA-4 and PD-1 blockade are conflicting. Cases of objective responses have been described even after failure to PD-1 blockade, but in a prospective expanded access program, no responses were observed [17,31–34]. The first prospective phase II trial (NCT01585194) investigating this combination reported the first data recently at ASCO 2019. The ORR was 17% and OS was 19.1 months which seems promising in this population in which 43% of patients had received ≥1 line(s) of systemic therapy for metastatic disease [35].

In line with previous studies, we found very low PD-L1 expression in uveal melanoma liver metastases [36]. Because most uveal melanoma metastases are well infiltrated with leukocytes and T cells, this suggests that either these cells do not recognize the tumor cells, are exhausted or are strongly inhibited. The low activation status of the immune infiltrate is reflected in the low PD-1 expression on tumor infiltrating lymphocytes (TIL) of uveal melanoma lesions when compared to cutaneous melanoma [37]. This argues for the lack of antigen-specific TIL in uveal melanoma metastases, and might explain the absence of efficacy from checkpoint inhibition. The lack of any clinical response in our trial suggests that this likely absence of tumor-specific T cells cannot be overcome by the RFA-induced antigen release and inflammatory signal induction or that RFA of only one lesion was not sufficient to induce this.

Indeed, we observed a significant but only modest increase in the expression of PD1 and PD-L1 in biopsies taken post RFA plus ipilimumab suggesting the

induction of an interferon producing activated T-cell infiltrate in the tumor. This weak induction of inflammation might have been counteracted by the parallel induction of IDO, FOXP3 (expressed by T-regulatory cells, Tregs) and CD68 and CD163 (expressed on tumor-associated macrophages, TAMs). TAMs and Tregs have been shown to impair antitumor immune responses [38,39]. We also observed a higher expression of markers related to immune infiltration like CD45, CD4 and CD8 in patients with a longer PFS and OS which points out that immune infiltration seems to be related to a better prognosis and might advocate for therapies that result in increased and stronger activated immune infiltration.

The low tumor mutational burden (and thus less likelihood of neoantigen presentation) in uveal melanoma might be the cause of a too small repertoire of tumor-specific T cells that could be modulated by immunotherapeutic approaches [40]. However, recent preclinical data on adoptive cell therapy in a uveal melanoma mouse model [41], the identification of tumor-specific T-cell responses in a subgroup of uveal melanoma patients [42], and the first study data testing adoptive cell transfer of autologous TIL in the late stage uveal melanoma patients argue against this idea [43]. In this trial, seven (35%) out of the 20 evaluable patients achieved objective responses [43]. This clearly indicates that T cells that can recognize uveal melanoma are present despite the low mutational load, and that the lack of an activated immune infiltrate might be more likely related to a strong local immune suppression, or the low frequency of antigen-specific T cells. For the latter argue recent promising data from an immune-mobilizing monoclonal T-cell receptor against cancer. This bispecific IMCgp100 (tebentafusp) contains two functional ends: a soluble affinity enhanced TCR for the melanoma-associated antigen gp100 and an anti-CD3 fragment. The phase 1 study showed very encouraging results with a DCR at 24 weeks of 32 and 73% one-year OS rate in patients with pretreated uveal melanoma.

These promising data from cellular therapies and the T-cell mobilizing approach indicate that immunotherapy might also work in mutational load low tumors, but requires innovative and stronger approaches, most likely in combinatorial therapies. Whether local therapies like RFA or chemoembolization will still play a role in uveal melanoma combination therapy, needs to be evaluated. Our trial showed that ipilimumab monotherapy is not the right combination partner, and underlines once more that uveal melanoma remains one of the few solid cancers for which no proven therapy for the metastatic setting exists. In that way, as many as possible patients should be treated within trials to solve this unmet clinical need.

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C.U.B. designed the study. E.A.R. and C.U.B. interpreted the data and wrote the manuscript. W.P. and M.A.J.M. performed the RFA. W.P. and F.L. assessed radiologic response. K.S. performed the statistical analysis of the clinical data. T.M.V. did the Digital Spatial Profiling analysis. B.A.v.d.W. and J.v.d.W. assessed the biopsies and scored PD-L1 staining. H.A.M., J.V.v.T., J.B.A.G.H., E.K. and C.U.B. were responsible for the clinical care of the patients. L.G.G.-O. performed the data management. A.B. was responsible for storing and processing the tumor samples. J.R. performed the statistical analysis of the DSP data. S.W. was responsible for the DSP analysis. All authors critically revised the manuscript.

Conflicts of interest

E.A.R. received travel support from NanoString and MSD. T.M.V. received travel support from BMS. J.R. is an employee of and stockholder of NanoString Technologies. S.W. is an employee and stockholder of NanoString Technologies, and has served as a consultant advisor for Roche. J.V.v.T. has served as a consultant adviser for Pfizer and Novartis, for which the institution (Netherlands Cancer Institute) received funding. J.B.A.G.H. has served as a consultant advisor for BMS, MSD, Pfizer, AstraZeneca–MedImmune, Roche–Genentech, Ipsen, Bayer, Immunocore, Novartis, Seattle Genetics, Neon Therapeutics, Celsius Therapeutics, Gadet, and GlaxoSmithKline (GSK), for which the institution (Netherlands Cancer Institute) received funding, and has received grant support from BMS, MSD, Novartis and Neon Therapeutics all paid to the institution. E.K. has served as a consultant advisor for BMS, Novartis, Roche, Merck, Amgen, Pierre-Fabre, EISAI, Bayer, Genzyme-Sanofi for which the institution (LUMC) received funding, and received research grants from BMS. C.U.B. reports personal fees as a consultant advisor for BMS, MSD, Roche, Novartis, Lilly, Pfizer, GSK, GenMab and Pierre Fabre for which the institution (Netherlands Cancer Institute) received funding, and has received research grants from BMS, Novartis, and NanoString all paid to the institution (Netherlands

Cancer Institute). There are no conflicts of interest for the remaining authors.

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