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Long-term follow-up of anti-PD-1 naïve patients with metastatic melanoma treated with IDO/PD-L1 targeting peptide vaccine and nivolumab

Cathrine Lund Lorentzen ,[1] Julie Westerlin Kjeldsen,[1] Eva Ehrnrooth,[2] Mads Hald Andersen ,[1] Inge Marie Svane[1]

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[1]Department of Oncology, CCIT-DK, Herlev, Denmark
[2]IO Biotech ApS, Copenhagen, Denmark

Correspondence to
Dr Inge Marie Svane;
Inge.Marie.Svane@regionh.dk

ABSTRACT

Background We have previously published initial efficacy of the indoleamine 2,3-dioxygenase (IDO)/anti-programmed death ligand 1 (PD-L1) vaccine in combination with nivolumab in 30 anti-PD-1 therapy naïve patients with metastatic melanoma (cohort A). We now report long-term follow-up of patients in cohort A. Further, we report results from cohort B, where the peptide vaccine was added to anti-PD-1 therapy for patients with progressive disease during anti-PD-1 treatment.

Methods All patients were treated with a therapeutic peptide vaccine in Montanide targeting IDO and PD-L1 combined with nivolumab (NCT03047928). A long-term follow-up of safety, response rates, and survival rates were performed in cohort A including patient subgroup analyses. Safety and clinical responses were analyzed for cohort B.

Results Cohort A: At data cut-off, January 5, 2023, the overall response rate (ORR) was 80%, and 50% of the 30 patients obtained a complete response (CR). The median progression-free survival (mPFS) was 25.5 months (95% CI 8.8 to 39), and median overall survival (mOS) was not reached (NR) (95% CI 36.4 to NR). The minimum follow-up time was 29.8 months, and the median follow-up was 45.3 months (IQR 34.8–59.2). A subgroup evaluation further revealed that cohort A patients with unfavorable baseline characteristics, including either PD-L1 negative tumors (n=13), elevated lactate dehydrogenase (LDH) levels (n=11), or M1c (n=17) obtained both favorable response rates and durable responses. The ORR was 61.5%, 79%, and 88% for patients with PD-L1$^-$ tumors, elevated LDH, and M1c, respectively. The mPFS was 7.1 months for patients with PD-L1$^-$ tumors, 30.9 months for patients with elevated LDH, and 27.9 months for M1c patients. Cohort B: At data cut-off, the best overall response was stable disease for 2 of the 10 evaluable patients. The mPFS was 2.4 months (95% CI 1.38 to 2.52), and the mOS was 16.7 months (95% CI 4.13 to NR).

Conclusion This long-term follow-up confirms the promising and durable responses in cohort A. Subgroup analyses of patients with unfavorable baseline characteristics revealed that high response rates and survival rates were also found in patients with either PD-L1 negative tumors, elevated LDH levels, or M1c. No meaningful clinical effect was demonstrated in cohort B patients.

Trial registration number NCT03047928.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Therapeutic peptide vaccines targeting immune regulation are safe.
⇒ The indoleamine 2,3-dioxygenase (IDO) and anti-programmed death ligand 1 (PD-L1) vaccine in combination with nivolumab previously induced favorable response and survival rates in patients with metastatic melanoma.

WHAT THIS STUDY ADDS

⇒ This study reports the long-term follow-up on safety and efficacy data in anti-PD-1 therapy naïve patients with metastatic melanoma treated with an IDO/PD-L1 vaccine in combination with nivolumab.
⇒ The study further reports the safety and efficacy data from patients with progressive disease during anti-PD-1 treatment.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The promising follow-up data support further development of the dual vaccine in combination with anti-PD-1 therapy.
⇒ An ongoing phase III trial is now examining the IDO/PD-L1 vaccine in combination with the anti-PD-1 treatment pembrolizumab in patients with advanced melanoma (NCT05155254).

BACKGROUND

Patients with metastatic melanoma often respond well to checkpoint inhibitor treatment. The response rates increase when checkpoint inhibitors are combined, but the risk of severe immune-related adverse events is pronounced with a dual checkpoint blockade.[1 2] Consequently, there is a need for effective and more tolerable regimens for patients with metastatic melanoma, and therapeutic peptide vaccines plus checkpoint inhibitors is a promising novel combination.[3]

At our institution, we have conducted preclinical experiments and clinical trials to examine the immunogenicity, efficacy,

and safety of various peptide vaccines targeting immune regulation.[4–9] The therapeutic vaccine principle is based on the discovery of antiregulatory T cells (anti-Tregs) in cancer patients.[10] Anti-Tregs are T cells that specifically react to regulatory immune cells, including myeloid-derived suppressor cells and regulatory T cells (Tregs). Anti-Tregs comprise both CD4+ and CD8+ T cells. The cells recognize antigens such as PD-L1, IDO, and arginase-1 fragments expressed on major histocompatibility complex molecules by various cells in the tumor microenvironment (TME).[4 6 11–14] The aim of immune modulatory vaccines is to activate anti-Tregs that may infiltrate and reverse the immunosuppressive TME.[15]

Our clinical trial data have shown that the therapeutic peptide vaccines targeting immune regulation were safe and could induce immune responses in the blood of vaccinated patients.[7 9] In a pilot study, a monotherapeutic IDO-targeting vaccine induced long-lasting disease control in patients with metastatic lung cancer.[16]

Immune modulatory vaccines induce novel T cell activation and target immunosuppressive cells in the TME. Specific anti-Tregs are, however, correspondingly inhibited by their cognate targets. Thus, if the inflammatory effects of the vaccines are combined with immune checkpoint inhibitors, the number of responding patients will presumably increase.[10 17] Additionally, the T cell-induced inflammation in the TME could result in an upregulation of PD-L1 and thereby enhance the effect of anti-PD-1 therapy. The synergistic effect was recently demonstrated in an in vivo tumor model.[18]

Consequently, we initiated a clinical trial to examine the efficacy and safety of a peptide vaccine in combination with a checkpoint inhibitor. In this phase I/II trial, 30 anti-PD-1 therapy naïve patients with metastatic melanoma (cohort A) were treated with a therapeutic peptide vaccine targeting the immune suppressive proteins IDO and PD-L1 with Montanide as an adjuvant in combination with the anti-PD-1 checkpoint inhibitor nivolumab. The trial results were published in 2021,[3] and follow-up data were presented in 2022.[19] Here, we report a long-term follow-up on safety and efficacy. We further describe a subgroup analysis of patients with unfavorable baseline characteristics. Finally, we report the safety and efficacy data of the combination treatment for patients with progressive disease (PD) (on anti-PD-1 therapy (cohort B).

MATERIALS AND METHODS

Trial design

The clinical trial was an investigator-initiated, single-center, non-randomized phase I/II trial. Initially, the trial was designed to include 30 anti-PD-1 naïve patients (cohort A) with metastatic melanoma. The primary endpoint was to evaluate the treatment feasibility and safety in accordance with Common Terminology Criteria for Adverse Events (CTCAE) V.4.0. The secondary endpoint was to assess the immunomodulatory changes, and the tertiary endpoint was to evaluate the clinical efficacy by Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1. To evaluate the combination treatment in anti-PD-1 therapy-resistant patients, we amended the protocol to include 10 patients with metastatic melanoma and PD on anti-PD-1 therapy (cohort B). All patients have been included in cohorts A and B at the data cut-off: January 5, 2023.

The study treatment counted a maximum of 15 IDO/PD-L1 vaccines with the adjuvant Montanide ISA in combination with nivolumab. The first six IDO/PD-L1 vaccines were administered biweekly, and the remaining nine vaccines were administered monthly. Nivolumab (3 mg/kg) was administered biweekly for 24 cycles. Patients with ongoing responses could continue nivolumab (6 mg/kg, monthly) for 2 years or until PD. Patients with ongoing responses were scheduled for follow-up evaluations three and 6 months subsequent to the last vaccine. Patients who received fewer than three peptide vaccines were replaced with new participants. No patients in cohort A were replaced. Four patients were replaced in cohort B. The reasons for exclusion are described in online supplemental table 6. The trial candidates were screened and treated at the Department of Oncology, Copenhagen University Hospital, Herlev, Denmark, from December 2017. The clinical trial was conducted as specified in the Good Clinical Practice (GCP) guidance and the Declaration of Helsinki. The GCP unit, Copenhagen, monitored the trial and the study was registered at www.clinicaltrials.gov, ID: NCT03047928.

Patients

Cohort A: Thirty anti-PD-1 therapy naïve patients with metastatic melanoma were enrolled in the trial between December 2017 and June 2020.

Cohort B: Ten patients with metastatic melanoma with PD on anti-PD-1 therapy were enrolled from May 2019 to September 2022.

Patients in both cohorts were enrolled following written informed consent. Patients with Eastern Cooperative Oncology Group performance status (PS) of 0 or 1 with metastatic or locally advanced melanoma belonging to patient cohort A or B were enrolled. All patients had a minimum of one target lesion, according to RECIST V.1.1. The main exclusion criteria were active autoimmune diseases and treatment with systemic steroids or other experimental drugs. Patients with over four central nervous system metastases were excluded if the lesions measured more than 1 cm.

Vaccine

The vaccines were administered subcutaneously. Each IDO/PD-L1 vaccine consisted of 100 µg of IDO peptides with the sequence: DTLLKALLEIASCLEKALQVF and 100 µg of PD-L1 peptides with the sequence: FMTYWHLLNAFTVTVPKDL, PolyPeptide, France. The IDO and PD-L1 peptides were dissolved in DMSO, filtered, and frozen in NUNC CyroTubes, CryoLine System Internal Thread, Sigma-Aldrich, at −20°C. The peptides were

thawed within 24 hours prior to administration. Sterile water (400 µL) was added to the dissolved PD-L1 peptides, and the solution was mixed with the IDO peptides and the adjuvant Montanide ISA (500 µL) immediately prior to injection.

Clinical evaluation

Adverse events were evaluated according to CTCAE V.4.0 and laboratory analyses. Toxicity evaluation was performed bi-weekly for the first 12 weeks and then every 4 weeks until week 47 and prior to the 3-month and 6-month evaluations. Clinical tumor responses were assessed every 12 weeks up until PD using PET-CT scans. The clinical responses were registered as complete response (CR), partial responses (PR), or stable disease (SD) The clinical data were listed in the electronic case report form (eCRF) program OpenClinica V.1.0.

Statistical analysis

The survival curves were created in GraphPad Prism V.9.0.0 with the Kaplan-Meier method. The median follow-up time was calculated by the reverse Kaplan-Meier method (GraphPad Prism V.9.0.0). Statistical analyses were not applied to the evaluation of safety.

RESULTS

Long-term follow-up results for patients in cohort A

Treatment and safety

At data cut-off, all 30 patients were off trial treatment and had finished the 6 months follow-up evaluation. The patient baseline characteristics are listed in online supplemental table 1 (previously in 2021).[3] At that time, six patients were still on trial treatment. The reason to stop treatment for these patients was: CR (33.3%), immune-related toxicity (33.3%), and completion of a total of >1.5 years of nivolumab treatment (33.3%) (online supplemental table 2).

The most common toxicities were CTCAE grades 1–2 rash (47%), fatigue (47%), diarrhea (30%), and arthralgia (30%). Twenty-three (77%) experienced local vaccination site reactions. Five patients (17%) experienced grades 3–4 adverse events, and 1 patient experienced a grade 5 adverse event. The patient died due to multiorgan failure with symptoms of myocarditis. At the time of death, the patient had highly elevated cardiac troponin I levels, and a bedside echocardiography showed that the ejection fraction had decreased to 15% from 60% at baseline. However, an autopsy was not conducted, and myocarditis was not confirmed pathologically. In the months prior to her death, the patient had multiple immune-related adverse events including grade 3 arthralgia, grade 3 colitis, grade 2 pneumonitis, and grade 2 vasculitis. This patient case was described in the publication from 2021.[3] The possible treatment-related adverse events are listed in online supplemental table 3.

Clinical responses and survival

Median follow-up time was 45.3 months (IQR 34.8–59.2) and ranged from 29.8 months to 61.9 months. The overall response rate (ORR) was 80%, and 50% of patients obtained CR. PR was seen in 30%, and 20% had PD as best overall response (BOR). Nine (60%) of 15 patients with CR as BOR still have ongoing CRs (figure 1A,B). The reason to stop treatment for the 6 patients with CR that later developed PD was: CR (33.3%), immune-related toxicity (50%), and PD (16.7%). The reason to stop treatment for the 9 patients with ongoing CR was: CR (55%) and immune-related toxicity (44%) (online supplemental table 2). At the first evaluation scan, 73.3% of patients had obtained clinical responses, and at data cut-off, 40% had ongoing responses (figures 1A and 2A). The median overall survival (mOS) was not reached (NR) (95% CI 36.4 to NR), and the median progression-free survival (mPFS) was 25.5 months (95% CI 8.8 to 39). Median duration of response (mDoR) was: 27 months (95% CI 14.2 to NR) (figure 2B). For patients achieving CR as BOR, 66% still had ongoing CR after 2 years. For patients with PR as BOR, 33.3% still had a response after 2 years had received no other subsequent anticancer therapy (figure 2B). Three patients have died since the data was published in 2021.[19] All deaths were due to PD.

We further evaluated patients with unfavorable baseline characteristics such as PD-L1 negative ($PD\text{-}L1^-$) tumors, elevated LDH, and disease stage M1c in a subgroup analysis. The ORR for $PD\text{-}L1^-$ and $PD\text{-}L1^+$ patients was 61.5% and 94%, respectively. The ORR for patients with elevated LDH was 79%, and patients with normal LDH had an ORR of 82%. The ORR for patients with M1c and M1a+b tumors was 88% and 69%, respectively (figure 3A). The mPFS for $PD\text{-}L1^-$ patients was 7.1 months (95% CI 2.0 to 25.6), and mPFS for $PD\text{-}L1^+$ patients was 30.9 months (95% CI 16.8-NR). Patients with elevated baseline LDH and normal LDH had a mPFS of 30.9 (95% CI 2.6 to NR) and 19.2 months (95% CI 4.4 to 39), respectively. The mPFS for patients with disease stage M1c was 27.9 months (95% CI 7.2 to NR), and the mPFS for patients with stage M1a+b was 14.8 months (95% CI 2.6 to NR) (figure 3B).

Results for patients in cohort B

Baseline patient characteristics

Ten patients were enrolled between May 2019 and September 2022. Prior to inclusion, all patients had PD during anti-PD-1 therapy. The baseline characteristics are registered in table 1 and in online supplemental table 4. All patients had BRAF wild-type tumors, and 50% of patients with available PD-L1 status (n=8) were PD-L1<1%. Nine (90%) patients had normal LDH levels, and 2 (20%) patients had disease stage M1c. The mean age was 68.5 years.

Treatment

At data cut-off, all patients were off trial treatment. The mean number of vaccines was 6.1 (4–9 vaccines). The reason for exclusion was PD for all patients. Prior to inclusion, 8 of 10 patients received anti-PD-1 therapy as the most recent therapy. One patient received ipilimumab and one patient received

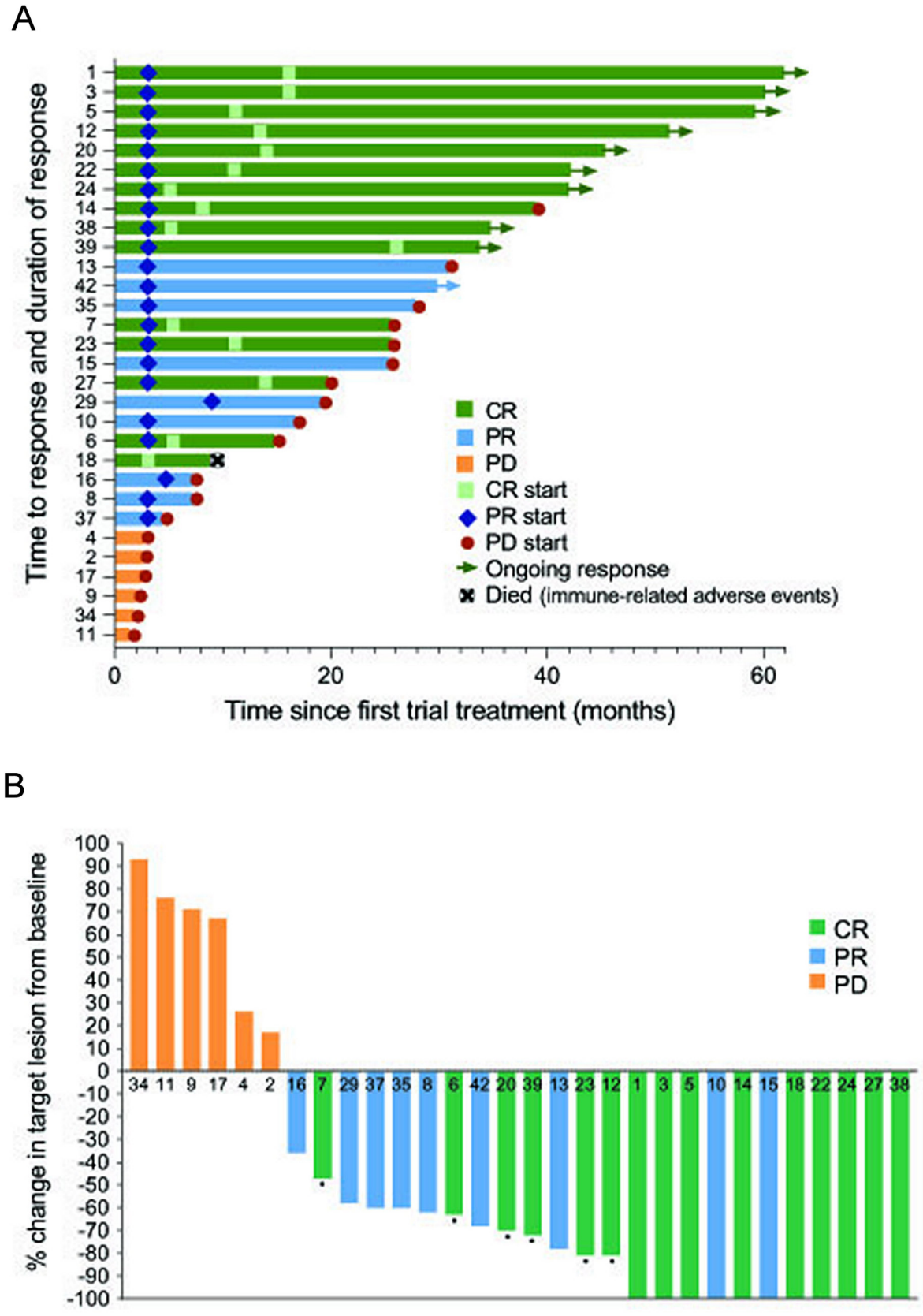

Figure 1 (A) Swimmer plot illustrating the duration of responses and time to responses (n=30). The numbers on the y-axis represent patient IDs. (B) Waterfall plot showing the BOR and the best change in target lesion size compared with baseline according to RECIST V.1.1. The black squares show patients with lymph nodes target lesions >1.5 cm at baseline that shrank to normal size on treatment (n=30). BOR, best overall response; CR, complete response; ID, indentification number; mOS, median overall survival; mPFS, median progression-free survival; PD, progressive disease; PD, progressive disease; PR, partial response.

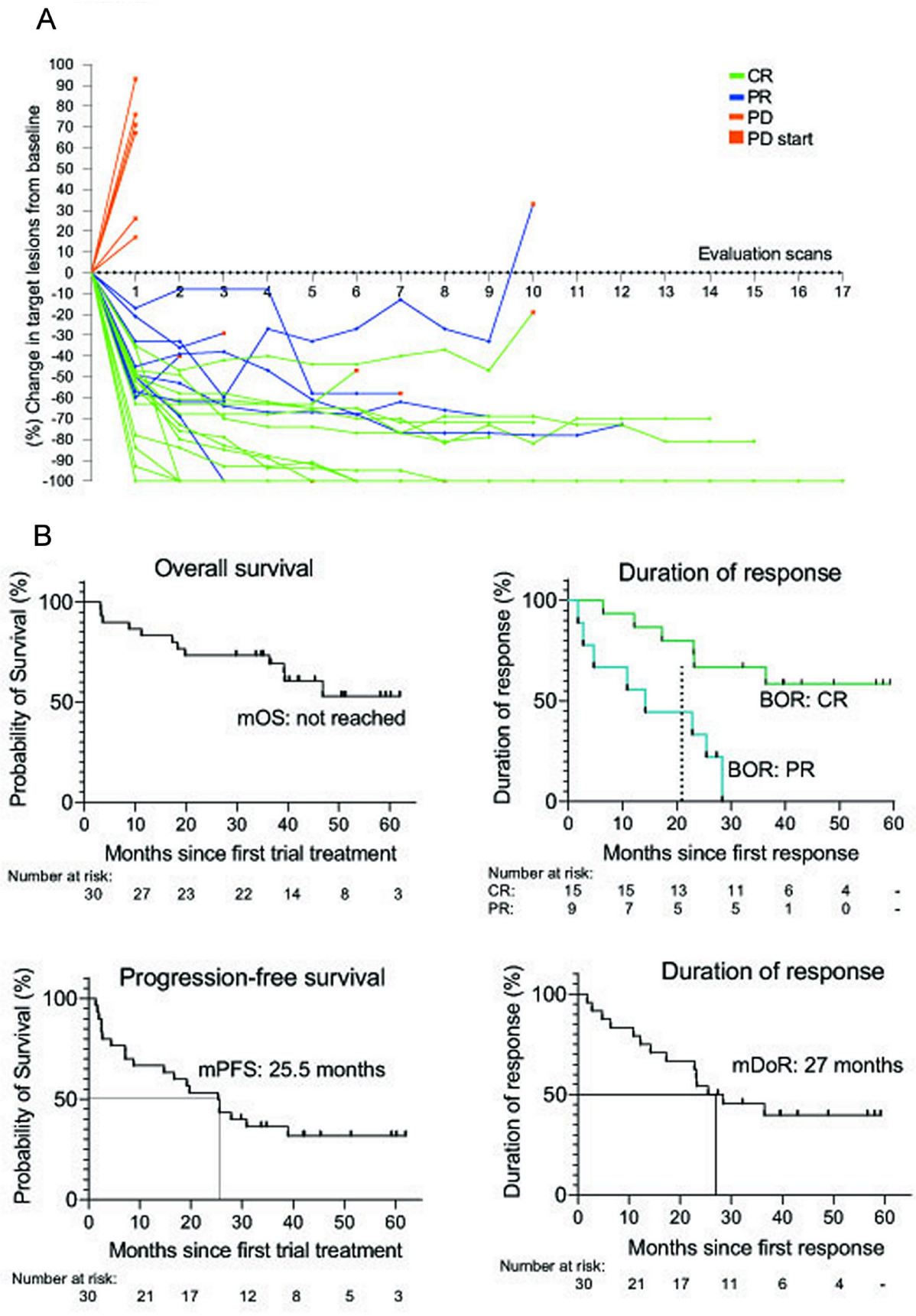

Figure 2 (A) Spider plot illustrating the target lesion size (sum) over time in all treated patients. (B) Kaplan-Meier curves of OS, PFS (n=30), and DoR for the responding patients (n=24) and for the responding patients divided into patients with BOR: CR and BOR: PR. BOR, best overall response; CR, complete response; mDoR, median duration of response; mOS, median overall survival; mPFS, median progression-free survival; PD, progressive disease; PR, partial response.

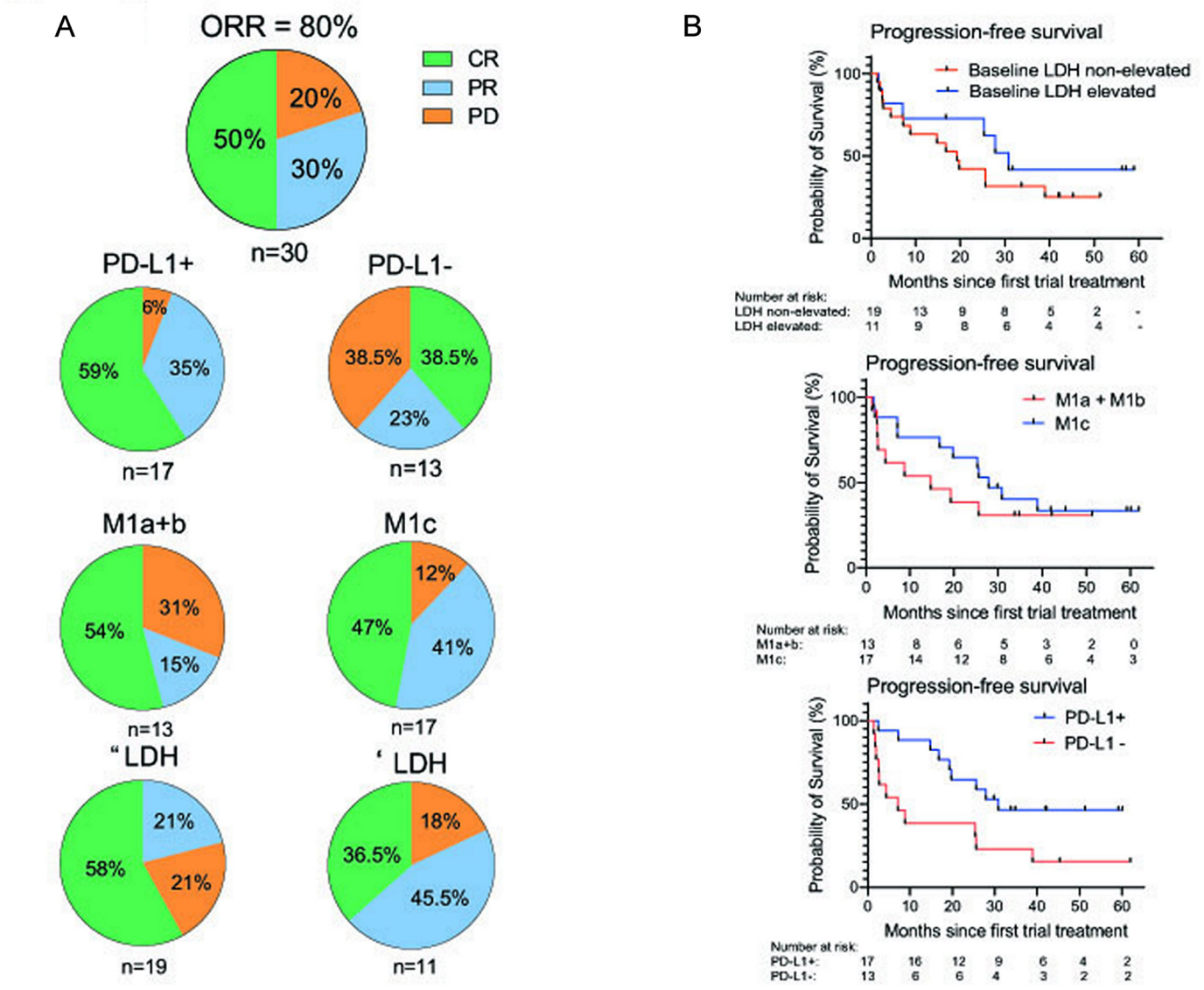

Figure 3 (A) Pie charts showing the ORR of all patients in cohort A and the ORR of the patients when divided into PD-L1 status, M-stage, and LDH status at baseline. (B) PFS for the patients in cohort A according to PD-L1 status, M-stage, and LDH status at baseline. CR, complete response; LDH, lactate dehydrogenase; mDoR, median duration of response; mOS, median overall survival; mPFS, median progression-free survival; ORR, overall response rate; PD, progressive disease; PR, partial response.

temozolomide as the most recent therapy. Following trial exclusion, eight patients received subsequent therapy; seven received ipilimumab (online supplemental table 5).

Four patients were treated without being included in the data analyses. Reasons for exclusion are mentioned in online supplemental table 6. One patient left the trial after one treatment cycle due to inclusion in another clinical trial (NCT03296137). Two patients were excluded before the third treatment cycle because of vaccine-related adverse events (see safety). One patient was excluded after a baseline scan reevaluation showed PR to the first-line treatment. The four participants were replaced with new trial patients according to protocol.

Safety

The most frequently reported adverse events as considered related to nivolumab were CTCAE grades 1–2 fatigue (30%), nausea (30%), and dry skin (30%). Local injection site reactions were seen in 40% of the patients (online supplemental table 7). Patient MM1636.49 experienced grade 3 adverse events with hypophysitis, adrenal gland insufficiency, and hyponatremia. Before inclusion, the patient had not experienced grades 3–4 events on anti-PD-1 therapy. For the patients excluded before the third combination treatment cycle, one patient experienced a systemic allergic reaction with flushing and dizziness following the second IDO/PD-L1 vaccine. The symptoms were remitted without the use of antihistamine

Table 1 Baseline characteristics of the analyzed patients in cohort B (n=10)

Cohort B patient baseline characteristics (n=10)	
Mean age, years	68.5
Performance status: 0no (%)	6 (60)
Sex—male no (%)	5 (50)
M-stage—no (%)	
M1a	4 (40)
M1b	4 (40)
M1c	2 (20)
Metastatic sites (no)	
1	3 (30)
2–3	5 (50)
>3	2 (20)
Patients with liver metastases	1 (10)
Lactate dehydrogenaselevels	
≤Upper limit of normal	9 (90)
>Upper limit of normal	1 (10)
PD-L1 (%)	
<1%	5 (50)
>1%	3 (30)
Unknown	2 (20)
BRAF-status (%)	
Mutant	0 (0)
Wild-type	10 (100)
Previous systemic treatment	
Ipilimumab	2 (20)
Pembrolizumab	7 (70)
Adjuvant nivolumab	3 (30)
Temozolomide	1 (10)
IL-2	1 (10)

or epinephrine treatment and were interpreted as probably related to the adjuvant Montanide. A second patient developed considerable and painful granulomas (CTCAE grade 2) at the vaccination sites.

Clinical responses and survival

At data cut-off, 2 of 10 patients had SD, and 8 had PD as BOR (figure 4A). Four patients were still alive. The mOS was 16.7 months (95% CI 4.13 to NR), and the mPFS was 2.4 months (95% CI 1.38 to 2.52) (figure 4B). Patients were followed for up to 5.3 months. The PFS for the two patients with SD (MM1636.33 and MM1636.47) was 5.3 and 5.0 months, respectively. Both patients received nine peptide vaccines before exclusion due to PD (online supplemental table 5).

DISCUSSION

The long-term follow-up data demonstrate that the majority of anti-PD-1 naïve patients with metastatic melanoma that were treated with nivolumab in combination with IDO/PDL1 targeting vaccine have durable responses translating into a mOS that was NR (95% CI 36.4 to NR) at a median follow-up of 45.3 months (IQR 34.8–59.2). The survival data compare favorably with the survival data from a recent phase II/III trial RELATIVITY-047. Here, mOS was 34 months in the group with nivolumab monotherapy.[20]

In 2021, 43% of patients had CR as BOR.[3] Now we report an ORR of 80%, with 50% of the patients obtaining CR. In the phase III trial CheckMate 067, CRs were registered in 19% of patients receiving nivolumab monotherapy and in 22% of the patients treated with ipilimumab and nivolumab.[2] In RELATIVITY-047, the CR rate was 14.2% in the nivolumab group and 16.3% in the nivolumab plus anti-LAG3-antibody relatlimab group.[20] Thus, we report remarkably higher CR rates than could be expected for patients treated with dual checkpoint inhibitor therapy.

The mPFS of 25.5 months (95% CI 8.8 to 39) remained twice as long as registered for patients treated with ipilimumab plus nivolumab (11.5 months)[2 21] and nivolumab plus relatlimab (10.2 months).[20] The registered adverse events have not changed notably since data was published in 2021.[3]

For cohort A patients, the mDoR was 27 months (95% CI 14.2 to NR) whereas the mDOR in Checkmate-067 was NR; neither in patients receiving nivolumab monotherapy nor nivolumab-plus-ipilimumab. In the ipilimumab monotherapy group the mDoR was 14 months.[2] The reason for these differences in response durability is not known. However, it could be speculated that some cohort A patients that would otherwise have progressed on anti-PD1 monotherapy, by adding the vaccine might have achieved temporary responses on dual vaccination trial treatment.

Explorative subgroup analyses should be taken cautiously as a result of the low number of patients. However, the analyses indicated that patients with unfavorable baseline characteristics such as elevated LDH levels, stage M1c, or PD-L1$^-$ tumors also have impressive response rates with an ORR of 61.5% for PD-L1$^-$ patients, 79% for patients with elevated LDH, and 88% for patients with M1c. Patients with M1a+b had a slightly lower ORR of 69%. It could be speculated that the differences were influenced by the slightly smaller size of the M1a+b subgroup compared with the M1c group or the fact that two patients in the M1a+b group had PD on ipilimumab prior to trial inclusion (online supplemental table 1A,B). The patients with M1a+b could also, by chance, have had other unregistered poor prognostic factors.

The same speculations could be applied to the differences in mPFS in the subgroups. The mPFS for patients with elevated LDH and M1c were longer compared with those with non-elevated LDH and stage M1a+b. Patients with elevated LDH levels (n=11) were almost half the size of group with normal LDH (n=19). Also, only one patient with elevated LDH levels had received ipilimumab therapy prior to trial inclusion compared with two patients in the group with normal LDH (online supplemental table

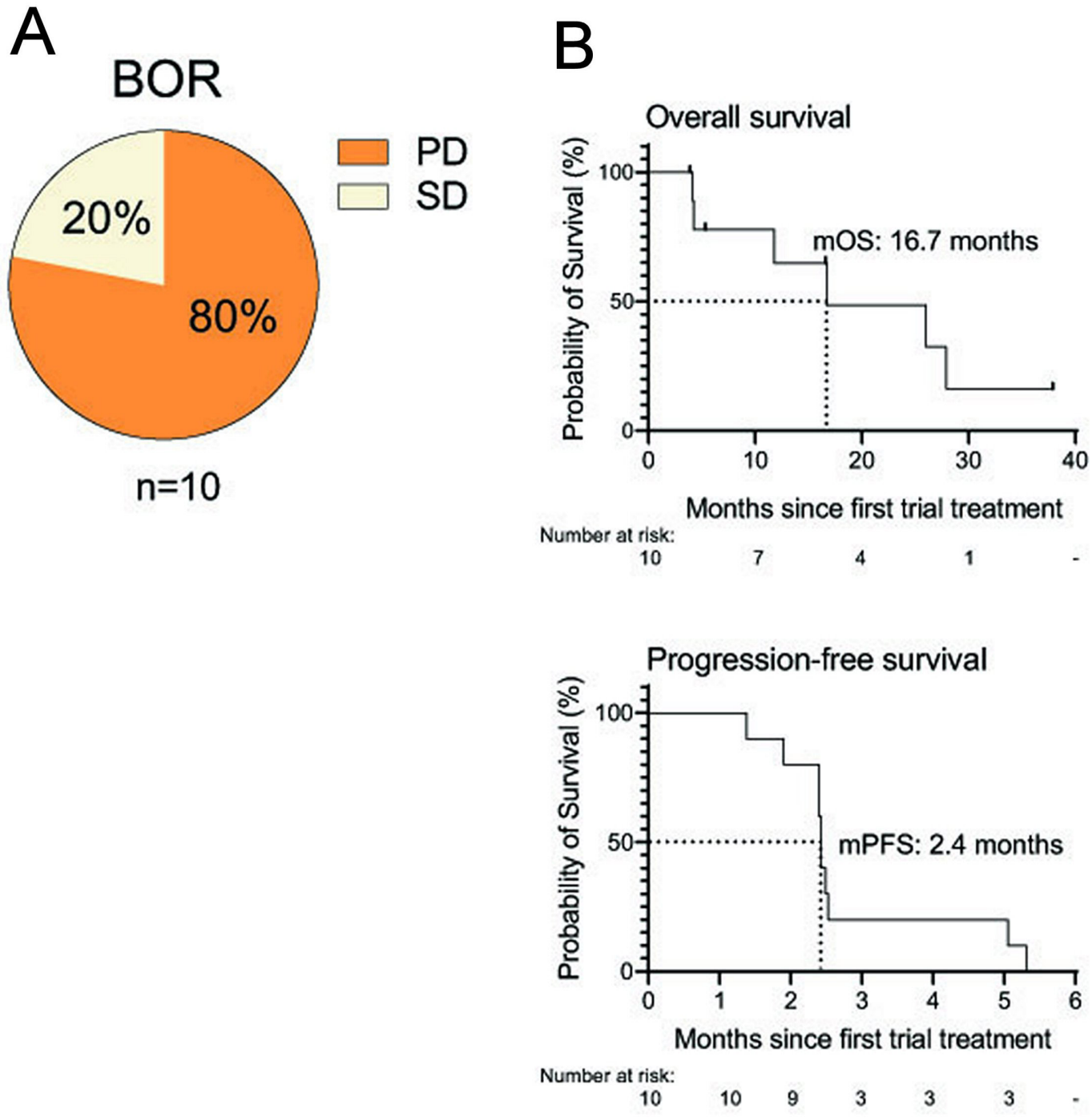

Figure 4 (A) Pie chart showing the BOR of all evaluable patients in cohort B (n=9). (B) Kaplan-Meier curves of mOS and mPFS of patients in cohort B (n=10). BOR, best overall response; mOS, median overall survival; mPFS, median progression-free survival; ORR, overall response rate; PD, progressive disease; SD, stable disease.

1A,B). The patients with elevated LDH and M1c could also, by chance, have had other unregistered favorable prognostic factors.

The mPFS for patients with PD-L1$^-$ tumors was notably lower than that of patients with PD-L1$^+$ tumors (7.1 months (95% CI 2.0 to 25.6) and 30.9 months (95% CI 16.8 to NR), respectively). Still, a mPFS of 7.1 months for PD-L1$^-$ patients was twice the reported for the same patient group in CheckMate 067 (2.8 months)[2] and RELATIVITY-047 (2.9 months)[20] indicating an improved efficacy for the full patient population. Based on the registered PFS from RELATIVITY-047, the European Medicines Agency approved combined nivolumab and relatlimab treatment only for patients with PD-L1$^-$ tumors (<1%), whereas the US Food and Drug Administration approved the combination regardless of the PD-L1 status.[22 23]

We amended the trial to allow for a second patient cohort (cohort B) in search of an efficacy signal of the combination treatment in patients with PD during anti-PD-1 therapy. All 10 patients included in cohort B were

evaluable at the data cut-off. Two patients had SD for 5 months, and eight had PD as BOR. Despite the small patient cohort and the diversity of treatment prior to inclusion, a meaningful signal of efficacy was not demonstrated in this patient cohort. Apart from progressing on anti-PD-1, the patients in cohort B did not have more disadvantageous baseline characteristics than those in cohort A (table 1). Notably, all patients in cohort B had BRAF wild-type tumors. This selection was probably due to the obvious possibility of offering treatment with BRAF and MEK inhibitors for patients with BRAF-mutated tumors.

In CheckMate 066 and 067, a selected cohort of patients was treated with nivolumab beyond PD. A retrospective analysis showed that 24 (28%) of 85 patients had PR on continued nivolumab therapy after initial progression, and the mPFS was 7.6 months.[24] The responses may well, in part, be explained by patients with pseudoprogression and not PD when selected for nivolumab therapy beyond PD. Consequently, these patients could potentially have responded without further nivolumab treatment. It is unlikely that the patients in cohort B with SD could have benefited from pseudoprogression to prior therapy. With the expanding knowledge of pseudoprogression, we know that this phenomenon mainly occurs within the first months of checkpoint inhibitor treatment, and follow-up scans are performed to confirm the treatment response if pseudoprogression is suspected.[25 26]

Hence, the remarkable clinical effects seen as a first-line treatment in patients with metastatic melanoma in cohort A were not apparent in the anti-PD1 refractory patient cohort B. The trial data indicate that the IDO/PD-L1 vaccine should be added upfront to the anti-PD1 treatment to boost the efficacy. In the patients with PD on anti-PD1 treatment, adding the vaccine could not reverse resistance, which challenge the observed up-front combination activity. To this end, the phase III randomized trial with IDO inhibitor epacadostat plus pembrolizumab versus placebo plus pembrolizumab failed to confirm the promising survival rates obtained in a non-randomized phase II trials (NCT02752074).[27] On the other hand, the lack of responses in anti-PD1 refractory patients could, however, be explained by the clinical sequence of the vaccine and the anti-PD1 treatment. It was recently described that anti-PD-1 therapy, followed by later concomitant treatment with anti-PD-1 therapy and vaccines, did not induce significant therapeutic benefits in animal tumor models. When the combination treatment of anti-PD-1 therapy and vaccines was given without anti-PD-1 pretreatment, a synergistic antitumor effect was induced, and tumor growth was reduced. The difference could be explained by a population of anti-PD-1 induced $PD\text{-}1^{+}CD38^{+}CD8^{+}$ T cells that interfere with antitumor immunity and prevent therapeutic responses.[28]

The trial was limited by the non-randomized setup and low patient number. Furthermore, comparing patients from different clinical trials is difficult due to different trial setups such as inclusion criteria and evaluation methods. In cohort A, the subgroup analyses were explorative, based on few patients, and only three prognostic factors while data on other prognostic factors such as tumor burden was not available. Based on data from this clinical trial, a larger randomized phase III trial testing pembrolizumab+/-IDO/PD-L1 vaccination in first line is now ongoing (NCT05155254). Results from this trial will decide the future role of the vaccine as an up-front anti-PD1 combination agent.

CONCLUSION

This long-term follow-up reveals that 30 anti-PD-1 therapy naïve patients with metastatic melanoma treated with IDO and PD-L1 vaccines in combination with nivolumab obtained impressive response rates that translated into a strong OS and PFS. Half of the patients achieved CR as BOR, the ORR was 80%, and the median follow-up was 45.3 months (IQR 34.8–59.2). The benefit seemed to be across well-known unfavorable baseline characteristics.

Patients in cohort B with PD during anti-PD-1 therapy did not obtain meaningful clinical efficacy. Notably, the immune-related adverse events were comparable to those reported for anti-PD-1 monotherapy in both patient cohorts. The data support further development of the dual vaccine in combination with anti-PD-1 therapy. An ongoing phase III trial is now examining the IDO/PD-L1 vaccine (IO102-IO103) in combination with the anti-PD-1 treatment pembrolizumab in patients with advanced melanoma (NCT05155254).

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Contributors CLL and JWK recruited and treated the patients in the trial. CLL wrote the manuscript. IMS, EE, MHA and JWK critically revised the manuscript. All the authors made contributions to the article, and all authors approved the versions for submission. IMS was the guarantor.

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Competing interests MHA has patent applications allocated to the biotech company IO Biotech ApS concerning IDO and PD-L1 peptide therapy. MHA is a shareholder, founder, and advisor for the company IO Biotech. EE is employed at IO Biotech. IMS has either lectured for or had relationships regarding advisory board with Novo Nordisk, MSD, Novartis, Pierre Fabre Sanofi Aventis, BMS, IO Biotech, and TILT Biotherapeutics. IMS received research grants from BMS, IO Biotech, Adaptimmune, Lytix biopharma, and TILT Biotherapeutics. IMS is the shareholder and cofounder of the biotech company IO Biotech ApS. CLL and JWK do not have conflicts of interest.

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ORCID iDs
Cathrine Lund Lorentzen http://orcid.org/0000-0003-2131-2188
Mads Hald Andersen http://orcid.org/0000-0002-2914-9605

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