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ORIGINAL RESEARCH

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# Response and survival of metastatic melanoma patients treated with immune checkpoint inhibition for recurrent disease on adjuvant dendritic cell vaccination

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**ABSTRACT**

Vaccination with autologous dendritic cells (DC) loaded ex vivo with melanoma-associated antigens is currently being tested as an adjuvant treatment modality for resected locoregional metastatic (stage III) melanoma. Based on its mechanism of action, DC vaccination might potentiate the clinical efficacy of concurrent or sequential immune checkpoint inhibition (ICI). The purpose of this study was to determine the efficacy of ICI administered following recurrent disease during, or after, adjuvant DC vaccination. To this end, we retrospectively analyzed clinical responses of 51 melanoma patients with either irresectable stage III or stage IV disease treated with first- or second-line ICI following recurrence on adjuvant DC vaccination. Patients were analyzed according to the form of ICI administered: PD-1 inhibition monotherapy (nivolumab or pembrolizumab), ipilimumab monotherapy or combined treatment with ipilimumab and nivolumab. Treatment with first- or second-line PD-1 inhibition monotherapy after recurrence on adjuvant DC vaccination resulted in a response rate of 52%. In patients treated with ipilimumab monotherapy and ipilimumab-nivolumab response rates were 35% and 75%, respectively. In conclusion, ICI is effective in melanoma patients with recurrent disease on adjuvant DC vaccination.

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## Introduction

Melanoma is a highly malignant melanocyte-derived neoplasm. Surgical resection with curative intent is the primary treatment modality for local and locoregional disease. However, with advancing stage, surgical curation becomes increasing unlikely with 5-y melanoma-specific survival rates ranging from 93% (stage IIIA) to 32% (stage IIID) although the prognosis of melanoma patients having locoregional disease has likely improved since the advent of adjuvant systemic therapy.[1] In distant metastatic disease (stage IV melanoma), surgery has limited value and therapy mainly consists of systemic treatment with immune checkpoint inhibition (ICI) and targeted therapy.

ICI consists of monoclonal antibodies intended to enhance the cancer-eradicating capacity of the immune system by restraining the immune-inhibiting function of CTLA-4 (ipilimumab) and PD-1 (nivolumab and pembrolizumab). Stage IV melanoma patients can be treated with either antibody as monotherapy or with the combination of ipilimumab and nivolumab.[2–5] For resected stage III melanoma patients, all of the previous-mentioned agents are approved as monotherapy, with PD-1 inhibition outperforming ipilimumab.[6–8] Besides ICI, targeted therapy with combined BRAF inhibition and MEK inhibition (BRAF/MEKi) is approved for both the treatment of stage IV melanoma and the adjuvant treatment of stage III melanoma.[9–12]

Over the past years, we extensively studied dendritic cell (DC) vaccination in both stage III and stage IV melanoma patients.[13–24] DC vaccination involves the administration of autologous DC matured and loaded ex vivo with melanoma-associated antigens. DC vaccination aims to eradicate melanoma cells by activating melanoma-specific T-cells in vivo. In stage III patients, adjuvant DC vaccination protocols induced functional melanoma-specific T-cell responses in 71% of patients, compared to 23% in metastatic melanoma patients.[13,14] When retrospectively compared to matched historical controls, adjuvant DC vaccination improved overall survival (OS).[13] Although clinical response following DC vaccination has been observed in some stage IV patients, DC vaccination is considerably less effective in these patients compared to ICI and BRAF/MEKi.[15,25] Therefore, in melanoma, we focus on the adjuvant application of DC vaccination, with a phase III trial currently ongoing (NCT02993315).

The high rate of immune induction following adjuvant DC vaccination offers unique possibilities for its positioning

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within the systemic treatment landscape of melanoma. Based on its mechanism of action, DC vaccination might potentiate the clinical efficacy of concurrent or sequential ICI treatment. The potential synergy between ICI and DC vaccination can be explained using the cancer-immunity cycle proposed by Chen and Mellman.[26] This cycle illustrates the steps cytotoxic T-cells have to complete before cancer cells can successfully be eradicated. Failure to complete any of these processes results in the incomplete clearance of malignant cells. DC vaccination aims to improve the activation of naive T-cells, whilst ICI is intended to reduce T-cell inhibition. Therefore, both modalities may be complementary as they act on different steps of the cancer-immunity cycle.[27]

In this study, we explore the clinical outcome of patients treated with PD-1 inhibition monotherapy or ipilimumab-nivolumab following recurrence on adjuvant DC vaccination for completely resected stage III disease. In addition, we present updated data on ipilimumab monotherapy following recurrence on adjuvant DC vaccination.

## Materials and methods

### Patients and treatment

We retrospectively analyzed patients treated with ICI (nivolumab, pembrolizumab or ipilimumab monotherapy, or ipilimumab-nivolumab) for recurrent disease after receiving DC vaccination for the adjuvant treatment of resected stage III cutaneous melanoma. All patients were treated with adjuvant DC vaccination between August 2004 and August 2018 in different study protocols (supplementary table 1). Briefly, vaccines consisted of autologous monocyte-derived or naturally circulating DC loaded with melanoma antigens. Patients were treated with three biweekly DC vaccinations (one cycle), with two additional cycles at six-month intervals in the absence of recurrent disease. Patients were evaluated every 3–6 months by medical history and physical examination. Imaging was performed at the discretion of the physician, except in the MIND-DC trial (NCT02993315) in which CT scanning was performed consistently during the follow-up visits. All DC vaccination studies were approved by the appropriate ethical review boards and written informed consent was obtained from all patients.

After disease recurrence on adjuvant DC vaccination, patients who received ICI as first- or second-line treatment for metastatic disease were evaluated for response, progression-free survival (PFS) and OS. Patients started ICI between October 2008 and December 2018. Later patients were excluded due to short follow-up at the time of analysis (March 2019). Patients were analyzed according to the type of ICI administered: PD-1 inhibition monotherapy, ipilimumab monotherapy or ipilimumab-nivolumab. Ipilimumab monotherapy was administered at a dose of 3 mg/kg for four cycles to all patients except one. This patient received ipilimumab monotherapy in a compassionate use program at a dose of 10 mg/kg for four cycles followed by 10 mg/kg every 12 weeks as maintenance therapy. Patients treated with PD-1 inhibition monotherapy received pembrolizumab 2 mg/kg every 3 weeks, nivolumab 3 mg/kg every 2 weeks or nivolumab 480 mg fixed dose every 4 weeks. All patients treated with ipilimumab-nivolumab received nivolumab at a dose of 1 mg/kg plus ipilimumab at a dose of 3 mg/kg every 3 weeks for four doses, followed by nivolumab at a dose of 3 mg/kg every 2 weeks. Patients were treated until scheduled therapy end, progressive disease (PD), unacceptable toxicity or a treatment pause in the setting of disease response.

### Immunological monitoring

In the DC vaccination trials, the immunological response was monitored after each DC vaccination cycle except in the MIND-DC trial in which immunological response was determined only following the first cycle. Immunological response was tested using delayed-type hypersensitivity (DTH) skin tests as described previously.[14] Briefly, patients received intradermal injections of DC loaded with melanoma antigens. After 48 h, 6 mm punch biopsies were taken from the injected skin. In these biopsies, skin-test infiltrating lymphocytes (SKIL) were analyzed for antigen-specific T-cells using multimeric-MHC complexes containing the relevant antigen epitopes. Furthermore, the presence of functional T-cells in the SKIL was assessed by measuring the interferon (IFN)-γ production upon stimulation with melanoma-associated antigen (supplementary figure 1). Patients with functional T-cells producing IFN-γ and/or having antigen-specific T-cells in at least one of the DTH skin tests were considered to have a melanoma-specific immunological response.

### Response evaluation

Patients underwent radiological evaluations during ICI using CT which were planned every 3 months with the possibility of extended intervals when patients experienced durable stable disease, partial (PR) or complete response (CR). Responses were assessed using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[28] Most patients (86%) were evaluated for the presence of cerebral metastases using MRI or CT prior to ICI start. The response rate is calculated as the portion of patients experiencing a PR or CR. The disease control rate is defined as the portion of patients experiencing stable disease, PR or CR.

### Statistical analysis

Survival data were calculated using the Kaplan–Meier method. OS is defined as the time from the initiation of ICI until death from any cause. PFS is the time from the first administration of ICI until PD. Median follow-up time was calculated with the Kaplan–Meier method, using the date of ICI start to the date of last follow-up and censoring for death.[29] Correlation between immunological outcome during DC vaccination and survival parameters on subsequent ICI

treatment was determined using a log-rank test. Correlation between immunological outcome during DC vaccination and clinical response was assessed using a Fisher's Exact test. SPSS software version 25 (SPSS Inc., Chicago, IL) and GraphPad version 5.03 (GraphPad Software Inc., San Diego, CA) were used for statistical analysis.

## Results

### Patient and treatment characteristics

A total of 51 patients received ICI as first- and/or second-line treatment for unresectable stage III or stage IV melanoma after recurrence on adjuvant DC vaccination. Median recurrence-free survival on adjuvant DC vaccination was 7.9 months. All patients received at least one DC vaccine with 47 patients completing at least one cycle of three DC vaccines. As introduced before, patients were analyzed in three separate treatment groups (PD-1 inhibition monotherapy, ipilimumab monotherapy and ipilimumab-nivolumab) (Figure 1). Baseline characteristics of the patients in each treatment group are shown in Table 1.

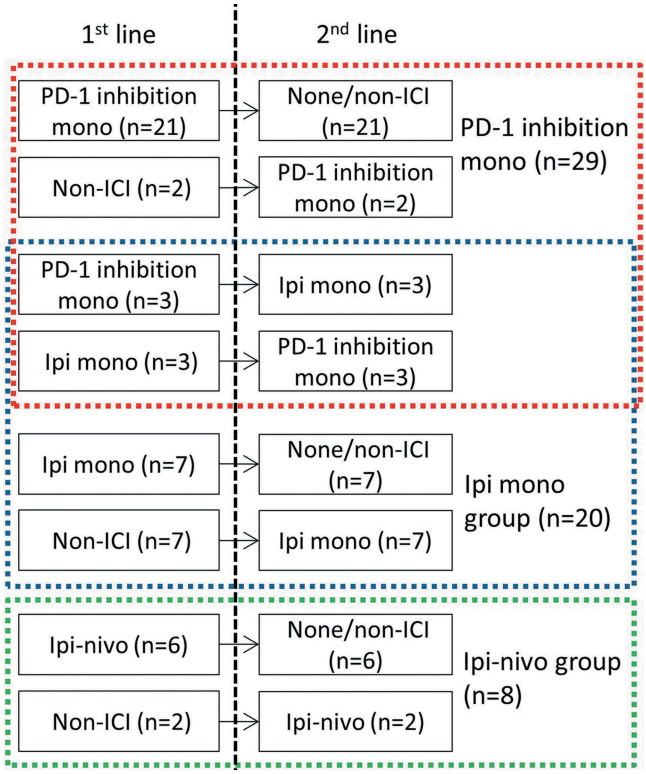


**Figure 1.** First- and second-line treatment in metastatic melanoma patients following recurrent disease on adjuvant dendritic cell vaccination. First- and second-line treatment is shown for the patients in the three different treatment groups. Three patients received first-line PD-1 inhibition monotherapy followed by second-line ipilimumab monotherapy, another three patients were treated with first-line ipilimumab monotherapy after which they received second-line PD-1 inhibition monotherapy. These six patients were analyzed in both the PD-1 inhibition monotherapy group (red) and the ipilimumab monotherapy group (blue). Therefore, the three treatment groups combined consisted of 57 analyzed patients.

Abbreviations: ICI, immune checkpoint inhibition; ipi, ipilimumab; ipi-nivo, combined treatment with ipilimumab and nivolumab; mono, monotherapy.

### Clinical efficacy of ICI following recurrence on adjuvant DC vaccination

Median follow-up time, from the first administration of ICI, was 10 months for patients treated with PD-1 inhibition monotherapy, 66 months for patients treated with ipilimumab monotherapy and 13 months for patients to whom ipilimumab-nivolumab was given.

Response rates following ICI are shown in Table 2. The response rate in patients treated with first- or second-line PD-1 inhibition monotherapy was 52%. In the ipilimumab-nivolumab group, the highest response rate (75%) was observed following first- or second-line treatment. In patients treated with first- or second-line ipilimumab monotherapy, the lowest response rate was seen, 35%.

Kaplan–Meier curves depicting PFS and OS of patients receiving first- or second-line ICI in different treatment groups are shown in Figure 2. There were no significant differences found in PFS and OS between first- and second-line PD-1 inhibition monotherapy or first- and second-line ipilimumab monotherapy (data not shown). First- and second-line ipilimumab-nivolumab were not analyzed separately as only two patients received second-line ipilimumab-nivolumab.

PFS rates after 1 and 2 y were 53% and 34% for patients treated with PD-1 inhibition monotherapy, respectively. After 1 y, 37% of the patients treated with ipilimumab monotherapy were free of progression. The 2- and 5-y PFS rates following ipilimumab monotherapy were 37% and 31%, respectively.

Following PD-1 inhibition monotherapy, 93% of patients were alive after 1 y, after 2 y this was 66%. One, 2- and 5-y OS rates for ipilimumab monotherapy were 73%, 50% and 39%, respectively. For patients treated with ipilimumab-nivolumab, 1-y PFS and OS rates were 50% and 66%, respectively, but follow-up in this treatment group is limited.

### Immunological response on DC vaccination and the subsequent clinical efficacy of ICI

No correlation between the presence of a melanoma-specific immunological response after DC vaccination and PFS or OS after ICI treatment was found in any of the treatment groups (data not shown). Neither was a melanoma-specific immunological response during DC vaccination more prevalent in ICI-responding patients compared to patients not responding to ICI (supplementary figures 1 and 2).

## Discussion

ICI following recurrence on adjuvant DC vaccination led to clinical benefit in a considerable portion of metastatic melanoma patients. Clinical response was observed in 52% of the patients treated with first- or second-line PD-1 inhibition monotherapy. In the ipilimumab monotherapy and the ipilimumab-nivolumab groups, 35% and 75% of patients responded to treatment, respectively.

Of the patients treated with PD-1 inhibition monotherapy, the majority had no cerebral metastases and a normal lactate dehydrogenase (LDH), both positive predictors for response and survival.[30,31] This patient selection resulted from

**Table 1.** Patient baseline characteristics at the start of immune checkpoint inhibition.

| | PD-1 inhibition monotherapy after DC vaccination (n = 29) | Ipilimumab monotherapy after DC vaccination (n = 20) | Ipilimumab-nivolumab after DC vaccination (n = 8) |
|---|---|---|---|
| Age | | | |
| Mean (range) | 55 (37–74) | 53 (24–69) | 60 (43–78) |
| Sex | | | |
| Male | 17 (59%) | 17 (85%) | 8 (100%) |
| Female | 12 (41%) | 3 (15%) | 0 |
| Number of completed cycles of DC vaccination | | | |
| 0 (1 or 2 vaccines) | 2 (7%) | 0 | 2 (25%) |
| 1 | 14 (48%) | 3 (15%) | 4 (50%) |
| 2 | 6 (21%) | 6 (30%) | 1 (13%) |
| 3 | 7 (24%) | 11 (55%) | 1 (13%) |
| Stage (AJCC 7th ed.) at start of ICI | | | |
| Unresectable stage III | 5 (17%) | 0 | 0 |
| M1a | 5 (17%) | 3 (15%) | 1 (13%) |
| M1b | 8 (28%) | 5 (25%) | 0 |
| M1c | 11 (38%) | 12 (60%) | 7 (88%) |
| BRAF mutation | | | |
| V600 mutation | 16 (55%) | 10 (50%) | 6 (75%) |
| No V600 mutation | 13 (45%) | 5 (25%) | 2 (25%) |
| Unknown | 0 | 5 (25%) | 0 |
| Lactate dehydrogenase | | | |
| ≤ULN | 26 (90%) | 16 (80%) | 3 (38%) |
| >ULN | 3 (10%) | 4 (20%) | 5 (63%) |
| Cerebral metastases | | | |
| Yes | 0 | 4 (20%) | 2 (25%) |
| No | 24 (83%) | 14 (70%) | 6 (75%) |
| Unknown | 5 (17%) | 2 (10%) | 0 |
| Local treatment for cerebral metastases[a] | | | |
| No treatment | N/A | 0 | 1 (50%) |
| Surgery | N/A | 1 (25%) | 0 |
| Radiotherapy | N/A | 3 (75%) | 1 (50%) |
| Line of treatment | | | |
| First | 24 (83%) | 10 (50%) | 6 (75%) |
| Second | 5 (17%) | 10 (50%) | 2 (25%) |
| Prior systemic treatment | | | |
| None | 24 (83%) | 10 (50%) | 6 (75%) |
| Dacarbazine | 0 | 3 (15%) | 0 |
| PD-1 inhibition monotherapy | N/A | 3 (15%) | 0 |
| BRAF/MEKi | 2 (7%) | 0 | 2 (25%) |
| BRAFi monotherapy | 0 | 4 (20%) | 0 |
| Ipilimumab monotherapy | 3 (10%) | N/A | 0 |

[a]percentage of patients having cerebral metastases.

Abbreviations: AJCC, American Joint Committee on Cancer; BRAF/MEKi, BRAF/MEK inhibition; BRAFi, BRAF inhibition; DC, dendritic cell; ICI, immune checkpoint inhibition; N/A, not applicable; ULN, upper limit of normal.

treatment guidelines in the institutions were patients received ICI. According to these guidelines, patients lacking an elevated LDH, cerebral metastases, high tumor load and rapid disease progression should preferably be treated with PD-1 inhibition monotherapy instead of ipilimumab-nivolumab. When taking the favorable characteristics in account, the response rate of 52% of the PD-1 inhibition monotherapy cohort is similar to the 51% response rate reported in comparable patients (i.e. patients with a normal LDH and no active cerebral metastases) following nivolumab monotherapy.[31]

The observed response rate of 35% in patients treated with ipilimumab monotherapy is higher than the response rates of 11–19% reported of ipilimumab monotherapy in melanoma patients without prior DC vaccination.[32–34] However, the comparison between our cohort and the published data is complicated by differences in patient characteristics. In the published trials, the presence of active cerebral metastases was an exclusion criterion (with 5–11% of patients having treated cerebral metastases). In our cohort, 20% of patients had cerebral metastases of which 75% were treated. The portion of patients having an elevated LDH was slightly lower in our cohort (20%), compared to 33–39% in published trials. Lastly, in our cohort, a portion of patients received prior PD-1 inhibition monotherapy (15%) or BRAF inhibition (20%). In the landmark studies, a minority (0–20%) of patients received prior targeted therapy with no patients receiving PD-1 inhibition monotherapy before ipilimumab monotherapy. As responses to ipilimumab monotherapy after progressive disease on PD-1 inhibition monotherapy are reported to be similar to first-line ipilimumab monotherapy, we regard the influence of prior PD-1 inhibition monotherapy on response rates to be limited.[35]

All patients in our ipilimumab-nivolumab cohort had an elevated LDH, cerebral metastases and/or rapid disease progression before the start of ICI. Despite these unfavorable characteristics, our ipilimumab-nivolumab cohort showed a response rate of 75% which is higher than the 58% response rate described in literature.[34] However, our results may be biased as our small cohort is prone to sampling errors, complicating extrapolation to larger numbers of patients.

**Table 2.** Clinical efficacy of immune checkpoint inhibition following dendritic cell vaccination.

| | PD-1 inhibition monotherapy after DC vaccination (n = 29) | Ipilimumab monotherapy after DC vaccination (n = 20) | Ipilimumab- nivolumab after DC vaccination (n = 8) |
|---|---|---|---|
| Response rate | 15 (52%) | 7 (35%) | 6 (75%) |
| Disease control rate | 21 (72%) | 10 (50%) | 6 (75%) |
| Best response on ICI | | | |
| Complete response | 7 (24%) | 4 (20%) | 2 (25%) |
| Partial response | 8 (28%) | 3 (15%) | 4 (50%) |
| Stable disease | 6 (21%) | 3 (15%) | 0 |
| Progressive disease | 8 (28%) | 10 (50%) | 2 (25%) |
| Median progression-free survival (months) | 13.1 | 3.9 | 5.6 |
| Median overall survival (months) | 32.5 | 30.0 | NR |
| Systemic treatment after progressive disease on ICI[a] | | | |
| No treatment for progressive disease | 7 (41%) | 4 (29%) | 2 (50%) |
| Dacarbazine | 0 | 1 (7%) | 0 |
| BRAF/MEKi | 5 (29%) | 1 (7%) | 2 (50%) |
| BRAFi monotherapy | 0 | 3 (21%) | 0 |
| (Re-introduction) Ipilimumab monotherapy | 4 (24%) | 0 | 0 |
| (Re-introduction) PD-1 inhibition monotherapy | 2 (12%) | 7 (50%) | 0 |
| Ipilimumab-nivolumab | 1 (6%) | 1 (7%) | 0 |
| Treatment in a clinical trial | 0 | 3 (21%) | 0 |

[a]percentage of the number of patients with progressive disease, patients may have been treated with multiple agents after progressive disease on immune checkpoint inhibition.

Abbreviations: BRAF/MEKi, BRAF/MEK inhibition; BRAFi, BRAF inhibitor; DC, dendritic cell; ICI, immune checkpoint inhibition; NR, not reached.

The present study has some limitations. First, data obtained from literature may represent a slightly different patient population with regard to prognosis, impeding a fair comparison with our cohort. Second, OS in this study may have been confounded as a portion of patients received other treatment lines besides first- or second-line ICI. However, response rates are influenced little by prior treatment lines and not at all by subsequent treatment lines.

No correlation between the presence of a melanoma-specific immunological response after DC vaccination and clinical response or survival on subsequent ICI treatment was found. This is unsupportive of the concept that DC vaccination activates the immune system resulting in improved clinical outcome on subsequent ICI. The absence of such a correlation may have several reasons. First, all patients analyzed in this study were refractory to DC vaccination. Therefore, although melanoma-specific T cells could be detected after DC vaccination, the T cells might not have been susceptible for stimulation with ICI. Second, the response to the chosen target (melanoma-associated antigens) might be too weak to translate into clinical effect (possibly in contrast to neo-antigens). Third, it may be that our method of immunological monitoring does not capture the complete spectrum of immune induction following DC vaccination. Finally, our immunomonitoring method only conveys a snapshot of the T-cell status at the moment of testing, and may therefore not be representative of the T-cell status at the time of ICI.

Still, sequential DC vaccination potentially has synergy with ICI. Recent work by Linette et al. strengthens this idea as it implicates immunological ignorance of clonal neoantigens as the basis for ineffective T-cell immunity and suggested to employ DC vaccination as an adjunct to ICI.[36] Our group has previously demonstrated that treatment with ipilimumab following recurrence on DC vaccination might result in improved clinical efficacy of ipilimumab.[37] Furthermore, concurrent administration of DC vaccination and ipilimumab has been tested in two clinical studies, showing the suggestion of synergy with little added toxicity.[38,39] Studies investigating whether DC vaccination potentiates PD-1 inhibition in the treatment of metastatic melanoma are currently ongoing.

This study shows that sequential ICI treatment following recurrence on adjuvant DC vaccination remains at least as effective as ICI treatment without prior adjuvant DC vaccination. This is important as it is currently unclear how to treat patients when recurrence occurs during, or shortly after, adjuvant treatment with either BRAF/MEKi or ICI. Unless a long treatment-free interval is present, re-introducing the same drug to treat recurrent disease arising during adjuvant therapy will most likely not be beneficial. Although vaccination is currently not an approved agent for the adjuvant treatment of stage III melanoma, it may prove to be effective in the currently ongoing phase III trial (NCT02993315). This possibly creates the opportunity to treat patients with DC vaccination in the adjuvant setting and leave ICI as a treatment option in case of recurrence.

In conclusion, ICI remains a viable treatment option for melanoma patients in case of recurrence on adjuvant DC vaccination. This adds to the notion that DC vaccination as an adjunct to ICI (either sequentially or concurrently) may have a role within the future treatment landscape of melanoma. Evidently, the therapeutic efficacy of adjuvant DC vaccination has to be proven, a phase III trial to that end is currently ongoing.

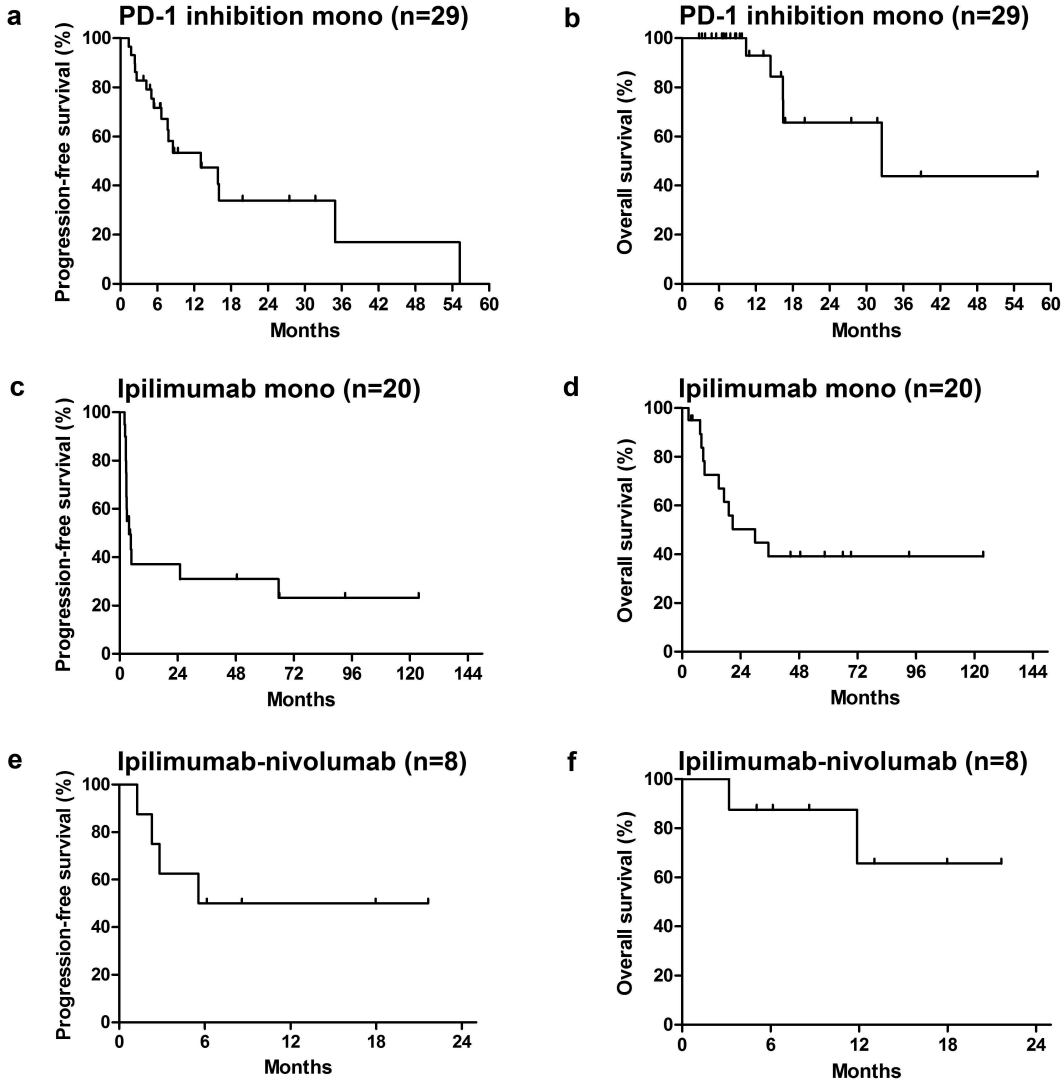


**Figure 2.** Progression-free and overall survival of patients treated with immune checkpoint inhibition following recurrence on adjuvant dendritic cell vaccination. Kaplan–Meier curves showing the progression-free and overall survival following PD-1 inhibition monotherapy (panels a, b); ipilimumab monotherapy (panels c, d) and ipilimumab-nivolumab (panels e, f) after recurrence on adjuvant DC vaccination. Survival data of first- and second-line therapy combined are shown in these panels.

Abbreviation: mono, monotherapy.

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## Disclosure of potential conflicts of interest

AAMvdV received consultancy fees for participation in advisory boards of BMS, Ipsen, MSD, Novartis, Pfizer, Pierre Fabre, Roche and Sanofi. JBAGH has provided consultation, attended advisory boards, and/or provided lectures for AIMM, Amgen, AZ, Bayer, BMS, Celsius Therapeutics, Gadeta, GSK, Immunocore, Ipsen, Merck Serono, MSD, Neogene therapeutics, Neon Therapeutics, Novartis, Pfizer, Roche/Genentech, Sanofi, Seattle Genetics and Vaximm, JBAGH received grant support from BMS, MSD, Neon Therapeutics and Novartis. JWBdG received consultancy fees for participation in advisory boards of BMS, MSD, Novartis, Pierre Fabre and Servier. MJBS received consultation fees for participation in advisory boards of Bristol-Myers Squibb, MSD and Pierre Fabre. RHK received personal grants and consultancy fees for participation in advisory boards of Astra Zeneca, BMS, MSD, Novartis and Pierre Fabre. RHTK participated in educational sessions of BMS and MSD. RHTK received grants from Roche. WRG received speakers' fees from ESMO and MSD. WRG received consultancy fees for participation in advisory boards of BMS, IMS Health, IQVIA, Janssen-Cilag, MSD and Sanofi. WRG received research grants from Astellas, Bayer, Janssen-Cilag and Sanofi.

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