Immune checkpoint inhibitors in mesothelioma

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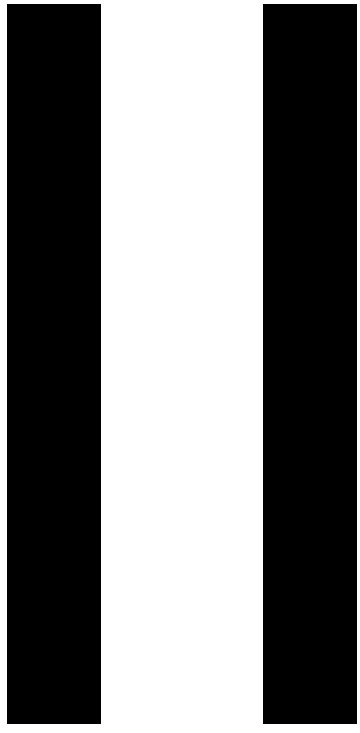

PART II

Clinical research

5

Chapter 5

Ipilimumab and nivolumab in the treatment of recurrent malignant pleural mesothelioma (INITIATE): results of a prospective, single-arm, phase 2 trial

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Abstract

Background

Single-drug checkpoint inhibition has shown activity in patients with recurrent malignant pleural mesothelioma. Here, we assessed the safety and efficacy of the combination of nivolumab, an anti-programmed death receptor 1 antibody, plus ipilimumab, an anti-cytotoxic-T-lymphocyte-associated antigen 4 antibody, in patients with previously treated and relapsed malignant pleural mesothelioma.

Methods

INITIATE was a prospective single-centre, single arm, phase 2 trial. Patients with malignant pleural mesothelioma who progressed after at least one line of platinum-containing chemotherapy were enrolled. Key eligibility criteria were measurable disease according to the modified Response Evaluation Criteria in Solid Tumours for mesotheliomas, Eastern Cooperative Oncology Group performance status 0 or 1, and adequate organ function. Patients received intravenous nivolumab (240 mg every 2 weeks) plus intravenous ipilimumab (1 mg/kg every 6 weeks up to four times). Treatment was continued for up to 2 years or until confirmed progression or unacceptable toxicity. The primary endpoint was disease control at 12 weeks. All patients who received at least one dose of therapy were included in safety analysis and all patients who received one dose of therapy and at least one radiological assessment were included in the primary analysis. This trial is registered at ClinicalTrials.gov, number NCT03048474.

Findings

Between Oct 5, 2016 and Aug 3, 2017, 38 patients were enrolled in the study, of which two patients were excluded because they were not eligible for biopsy. Of 36 eligible patients, one deteriorated before the start of the study so was not included in any analyses and one withdrew consent after one treatment cycle before radiological assessment so was included in the safety population only. 34 patients were evaluable for response assessment at 12 weeks. Of these, ten (29%) patients had a partial response and 13 (38%) patients had stable disease, thus disease control was achieved by 23 (68%, 95% CI: 50 - 83) of 34 patients. Treatment related adverse events were reported in 33 (94%) patients, the most common adverse events were infusion related reactions, skin disorders, and fatigue. Grade 3 treatment-related adverse events were reported in 12 (34%) of 35 patients.

Interpretation

In this single-centre phase 2 trial, the combination of nivolumab plus ipilimumab showed marked efficacy in patients with recurrent malignant pleural mesothelioma. The safety profile was consistent with known data on the combination regimen. Our results warrant further investigation of this combination in a phase 3 trial.

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Research in context

Evidence before this study

Few treatment options are available for patients with malignant pleural mesothelioma after one line of chemotherapy. We searched PubMed from January 1 2010 to June 1, 2018, with the following terms: "mesothelioma" AND "PD-1" OR "PD-L1" OR "CTLA-4" OR "checkpoint". We also searched clinical trial registers (ClinicalTrials.gov and WHO International Clinical Trials Registry Platform). This literature review indicated that there are several studies of monotherapy immune checkpoint inhibitors for malignant pleural mesothelioma and ongoing studies for combination checkpoint inhibitors. In both phase I and II studies monotherapy with a PD-1/PD-L1 antibody has meaningful efficacy and an acceptable safety profile, in contrast to monotherapy CTLA-4, which doesn't have clinical efficacy in a phase IIB study compared to placebo. Combination therapy with durvalumab and tremelimumab showed encouraging results. No phase III trials have been published.

Added value of this study

Results from the phase II INITIATE trial show that the combination of nivolumab plus ipilimumab has significant clinical efficacy for patients with malignant pleural mesothelioma after first line chemotherapy and a safety profile that is consistent with previously reported data.

Implications of all the available evidence

The clinical efficacy shown by our study suggests that combination checkpoint inhibition for malignant pleural mesothelioma should be tested in phase III studies in first and second line malignant pleural mesothelioma. The first-line phase III trial comparing nivolumab plus ipilimumab with platinum plus pemetrexed is ongoing (NCT02899299).

Introduction

Malignant pleural mesothelioma is an aggressive tumour originating from the mesothelial cells of the pleura. Asbestos exposure is the major risk factor for malignant pleural mesothelioma, with latency time from exposure to diagnosis varying from 20 to more than 50 years.1,2

The approved first-line treatment option for patients with malignant pleural mesothelioma who are not eligible for surgery is platinum-based chemotherapy with an antifolate.3,4 This treatment leads to a median overall survival of about 12-16 months, increasing to almost 19 months with the addition of the angiogenesis inhibitor bevacizumab.3,5

No approved second-line therapy exists yet. Responses with chemotherapy vary between 10% and 20% of patients and median overall survival ranges from 5.6 to 10.9 months.6-10

A few studies using a single-agent checkpoint inhibitor for second-line treatment of malignant pleural mesothelioma have been published. The programmed death receptor 1 (PD-1) checkpoint inhibitors pembrolizumab and nivolumab were used in the Keynote-028 and NivoMes trial respectively, with partial responses achieved by five (20%) of 25 patients and nine (26%) of 34 patients, disease control achieved by 18 (72%) of 25 patients and 16 (47%) of 34 patients, and survival at 12 months of 63% (95% CI 40-79) and 50% (36-70).11,12 In the Javelin phase 1b trial with programmed cell death ligand 1 (PD-L1) checkpoint inhibitor avelumab 5 (9.4%)of 53 patients had an overall response.13 But the randomized, double-blind, placebo-controlled phase 2 DETERMINE trial analysing second-line treatment with single-drug cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4) checkpoint inhibitor tremelimumab in 571 patients with mesothelioma did not show benefit.14

Preclinical data suggest a synergistic effect of CTLA-4 and PD-1 checkpoint inhibitors.15 The ongoing first-line CheckMate 743 phase 3 randomised controlled trial (NCT02899299) in patients with malignant pleural mesothelioma is comparing platinum-based chemotherapy plus pemetrexed with nivolumab plus ipilimumab. In the phase II single-arm NIBIT-MESO-1 trial, patients received a combination of tremelimumab (anti-CTLA-4 antibody) and durvalumab (anti-PD-L1 antibody) in the first or second line setting. 11 (28%) of 40 patients had an immune-related objective response and 25 (63%) achieved disease control. Median progression free survival (PFS) was 5.7 months and median overall survival (OS) 16.6 months.16 The MAPS2 randomised phase II trial by Scherpereel and colleagues assesed nivolumab with or without ipilimumab in patients with relapsed mesothelioma, and showed similar results for the combination treatment and monotherapy, 17 although a formal comparison was not done.

Patients with mesothelioma usually have moderate expression of PD-L1, with 20–40% of tumours expressing PD-L1 in more than 1% of cells. PD-L1 expression is more common in the non-epithelioid histological subtype than in the epithelioid subtype. In cohorts of patients who have not been treated with checkpoint inhibitors, patients with PD-L1-positive tumours have a substantially worse prognosis (median survival 4.8 months) than those with negative tumours (16.3 months), independent of histology (epithelioid or non-epithelioid subtypes). The heterogeneity of tumour biopsy procedures in malignant pleural mesothelioma, and non-uniformity of staining procedures, including differences in cutoff levels, makes comparison between studies difficult.18-21

In line with our previous study on nivolumab monotherapy, we here report the efficacy and safety data of our INITIATE trial assessing the combination of ipilimumab and nivolumab in the treatment of malignant pleural mesothelioma, including results of PD-L1 expression.

Methods

Study design and participants

INITIATE is a prospective single-centre, single arm, phase 2 trial for patients with unresectable malignant pleural mesothelioma who have disease progression or recurrence after at least one line of platinum-containing systemic therapy. The study was approved by the institutional review board and in accordance to the Declaration of Helsinki. All patients provided written informed consent before enrollment.

Patients were aged at least 18 years, had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1 and could have any subtype of histologically confirmed recurrent malignant pleural mesothelioma. Additional inclusion criteria were measurable disease on CT scan according to the modified Response Evaluation Criteria in Solid Tumours (mRECIST), 22 life expectancy greater than 12 weeks and adequate hematologic and organ function within the 14 days prior to first study treatment.23

Exclusion criteria were previous treatment with any checkpoint inhibitor or current treatment with systemic immunosuppressive medication (use of systemic prednisolone at maximum dosage of 10mg/day or equivalent was allowed), previous malignancy (except adequately treated basal cell, squamous cell skin cancer, superficial or in-situ cancer of the bladder or other cancer for which the patient had been disease-free for at least five years), brain metastases and patients with only peritoneal malignant mesothelioma.

Other exclusion criteria were a history of active autoimmune disease, idiopathic pulmonary fibrosis, severe infections in the 4 weeks before start of study treatment, active

tuberculosis, significant cardiovascular disease, myocardial infarction in the 6 months before enrolment, unstable angina, or unstable arrhythmias, pulmonary or hepatic disease constituting a high risk for investigational treatment as per investigator's judgement, and unresolved (drug-induced) pneumonitis, organizing pneumonia, or active pneumonitis on CT scan. Relevant gastrointestinal disease, prior allogeneic bone marrow or solid organ transplantation or a history of HIV were also exclusion criteria, as well as any major surgical procedures within the 28 days before starting study treatment.

Patients with uncontrolled pleural or peritoneal effusion requiring recurrent (once monthly or more frequently) drainage procedures, and patients with uncontrolled tumour-related pain were excluded. Pain medication had to be on a stable regimen at study entry and lesions amenable to palliative radiotherapy had to be treated prior to enrollment.

Procedures

After giving informed consent, histological tumour biopsies and peripheral blood were collected before treatment administration. Thoracoscopy was the preferred method, but ultrasound or CT-guided transthoracic needle core biopsies (6 x 16 Gauge) were allowed. After six weeks of treatment, a second tumour biopsy was obtained for research purpose. PD-L1 expression on formalin-fixed, paraffin-embedded tissue samples was assessed with immunohistochemistry using the 22C3 pharmDx antibody (Agilent, Santa Clara, CA, USA).

Patients received the PD-1 checkpoint inhibitor nivolumab in combination with CTLA-4 checkpoint inhibitor ipilimumab (Bristol-Myer Squibb, New York, NY, USA). Nivolumab was administered intravenously over at least 30 min at a fixed dose of 240 mg, every 2 weeks. Ipilimumab was administered intravenously in 30 min at a dose of 1 mg/kg, after nivolumab infusion, every 6 weeks for up to four doses, on the basis of results of melanoma trials. Patients received nivolumab therapy for a maximum of 2 years, or until disease progression or unacceptable toxicity. Treatment delay criteria include any grade ≥ 2 non-skin, drug-related adverse event as assessed with Common Terminology Criteria for Adverse Events (CTCAE version 4.03) with a few exceptions as specified in full protocol. Re-treatment could be given when all toxicities had resolved to grade 1, or according to the protocol.

Tumour imaging via CT scan was done in the 28 days before start of therapy and for response assessments every 6 weeks of treatment until disease progression was observed. Evaluation of CT scans was done by one independent reviewer using mRECIST criteria for mesothelioma.23 Laboratory tests were performed every 2 weeks and included a standard hematology and chemistry panel. Thyroid and adrenal function tests were performed every 6 weeks.

After treatment completion patients had follow-up visits every 6 weeks for the first 48 weeks, then every 12 weeks, until progression or death. All patients with progressive disease had follow-up visits every 3 months to assess survival.

Outcomes

The primary outcome of the study was the proportion of patients who achieved disease control at 12 weeks after start of nivolumab plus ipilimumab. Disease control was defined as either complete response, partial response or stable disease according to the modified RECIST criteria for mesothelioma.

Secondary outcomes were safety, objective response (complete or partial response) at 6 months, disease control at 6 months, progression-free survival (time from first treatment to progression or death) and overall survival (time from first treatment to death of any cause) and immunological changes of mesothelioma before and after 6 weeks of treatment. Immunological results will be presented elsewhere.

Statistical analysis

To test the hypothesis that combination treatment of nivolumab plus ipilimumab will improve disease control from 20% to 50%,6 an optimal two-stage design was used with the type I error rate (α) being 0.02 and the power (1−β) being 90%.24 The null hypothesis that 20% of patients will receive a true response, was tested against a one-sided alternative. The planned sample size was 33 patients, with an interim analysis after 12 patients. The study would be stopped for futility if at the time of interim analysis 3 or less out of 12 patients showed disease control at 12 weeks. Treatment with nivolumab plus ipilimumab is deemed successful if the study is not stopped at interim analysis and at least 12 out of 33 patients show disease control at 12 weeks.

Anticipating possible drop-out cases, we included 36 patients, which yielded 34 evaluable patients. To account for this change from the planned population size, the adjusted p-value was calculated as the conditional probability (under the null hypothesis of a DCR of 20%) of finding at least the obtained number of patients with disease control in 34 patients, conditional on at least four patients having disease control among the first 12 patients. This adjusted p-value was then compared against the pre-specified type I error rate of 2%.

All patients who received at least one dose of immunotherapy and at least one radiologic evaluation were considered evaluable. All patients who received at least one dose of immunotherapy and had at least one follow-up visit were included in the safety analysis.

Time-to-event endpoints (ie, progression-free survival and overall survival) were estimated with the Kaplan-Meier method. Treatment outcomes (partial response, stable disease

or progressive disease at 12 weeks, described as an ordinal variable) were compared between PD-L1 positive and negative patients using the linear-by-linear association test. Clinical benefit (partial response or stable disease for > 6 months) was compared between PD-L1 positive and PD-L1 negative patients using the Fisher's exact test. PD-L1 expression in tumour cells was scored as the percentage of all tumour cells that expressed PD-L1. PD-L1 expression in tumour-infiltrating immune cells was categorised into four groups according to the percentage of immune cells (ie, non-tumour cells) that were PD-L1 positive as follows: less than 1% was scored as 0, at least 1% to less than 5% was scored as 1, at least 5% to less than 10% was scored as 2, and at least 10% was scored as 3. All analyses were done in R statistical software (version 3.4.0). The trial was registered with ClinicalTrials.gov, number NCT03048474.

Role of funding source

The funder had a role in study design, but not data collection, analysis, or interpretation or writing of the report. All authors had full access to the raw data. All authors confirmed the accuracy and completeness of the data and made the decision to submit the manuscript.

Results

Patients and treatment

Between Oct 5, 2016 and Aug 3, 2017, 38 patients with progression of malignant pleural mesothelioma after at least one line of chemotherapy gave informed consent. Of these, 36 patients were eligible for inclusion (figure 1). One patient deteriorated quickly and could not begin immunotherapy at the planned start of treatment so was excluded from analyses.

Most patients in the cohort were men (27 [77%] of 35) and most had the epithelioid subtype (30 [86%]); the median age was 65 years (IQR 62–71; range 37–79 years; table 1). All patients had received at least one line of chemotherapy containing a platinum doublet with pemetrexed, 22 cisplatin and 13 carboplatin. Other previous therapies included gemcitabine (five [14%] of 35 patients), vinorelbine (one [3%]), pemetrexed monotherapy (three [9%]), and bevacizumab (one [3%]). Some patients were previously treated in a clinical trial with either anetumab ravtansine (a mesothelin-targeting antibody–drug conjugate), nintedanib, tazemetostat (competitive inhibitor of histone methyl transferase EZH2), or dendritic cell therapy (each in one [3%] patient).

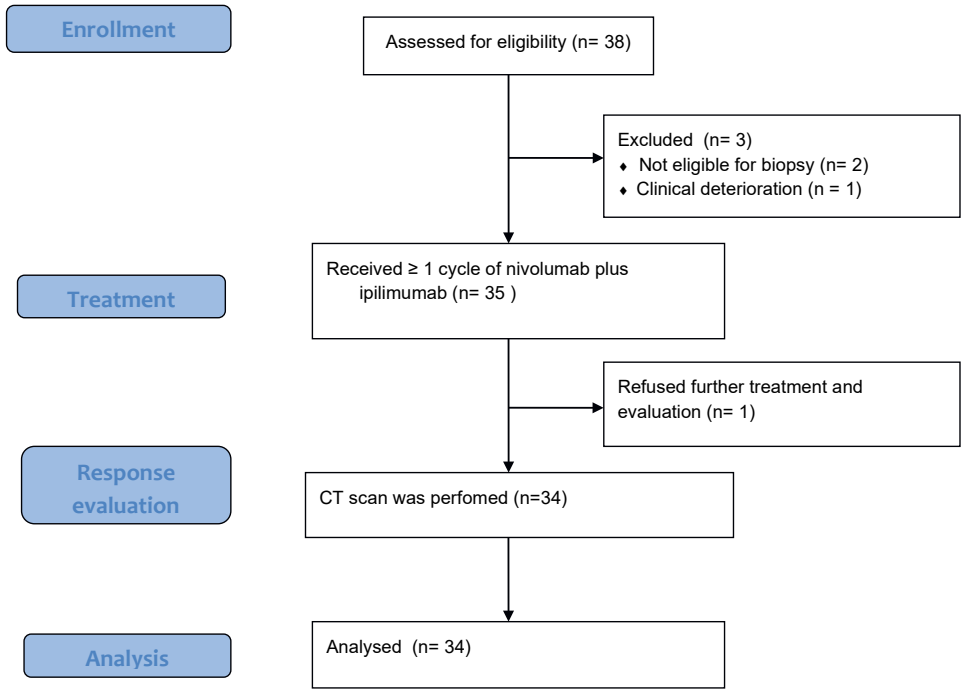

Figure 1 patient flow chart

A large variation existed in time between diagnosis and time of enrollment in study, ranging from 2·2 months until 95·4 months (almost 8 years), with a median of 12 months (IQR 8.8 – 22.7). The median time between the last systemic treatment to enrollment in the study was 6.4 months (range 1 – 61, IQR 3.2 – 20.1).

Of the 36 patients eligible patients one deteriorated quickly and could not begin immunotherapy at the planned start of treatment. Another refused any further treatment or control visits after only 1 cycle of immunotherapy and was not included in the analysis. A total of 34 patients received at least one dose of immunotherapy and a radiologic evaluation and thus were evaluable for response assessment. A total of 35 patients received immunotherapy and had at least one follow-up visit. The first patient started treatment on November 9, 2016 and the last patient on August 28, 2017.

Table 1. Baseline characteristics (n = 35).

Median age (years)	65
range	37-79
Sex	
men	27 (77%)
women	8 (23%)
Histology	
Epithelioid	30 (85%)
Sarcomatoid	3 (9%)
Mixed	2 (6%)
ECOG performance status at registration	
0	10 (29%)
1	25 (71%)
Ethnicity	
Caucasian	34 (97%)
Negroid	1 (3%)
Prior lines of therapy	
1	29 (83%)
2	4 (11%)
3	1 (3%)
4	1 (3%)
Disease stage	
I - III	21 (60%)
IV	14 (40%)
Smoking status	
Never	12 (34%)
Former	17 (49%)
Current	6 (17%)
PD-L1 expression on tumour cells	
Negative (<1%)	19 (54%)
Positive (≥ 1%)	15 (43%)
Not scored	1 (3%)

At time of data cut-off (June 1, 2018) patients who started treatment had a median of 12 doses of nivolumab (range 1–37, IQR 8.3 – 21.8 doses) and a median of 4 doses of ipilimumab (range 1–4, IQR 3-4 doses) administered. In 13 patients (37%) (in 30 cycles) nivolumab was postponed, mainly due to toxicities and/or corticosteroid use for toxicities, but also due to flu (2 patients – 6%) and family circumstances (2 patients – 6%). In five patients (14%) (6 cycles) ipilimumab was delayed because of toxicity.

One patient (3%) decided to stop due to toxicity (malaise grade 2), after receiving all four doses of ipiliumumab. The patient who withdrew consent stopped treatment after one cycle of immunotherapy. Ten (29%) patients were still on treatment at the time of data cutoff. All others with data available (23 patients [66%]) had to stop immunotherapy because of radiological progression.

Efficacy

For the primary endpoint at 12 weeks, 23 (68%; 95% CI 50–83) of 34 patients had achieved disease control (ten [29% had a partial response and 13 [38%] had stable disease; table 2, figure 2). 11 (32%) patients had progressive disease and none had a complete response at 12 weeks. Disease control in 23 (68%) patients was enough to refute the null hypothesis of 20% disease control at the one-sided preplanned 98% confidence level (98% one-sided CI 49–100, accounting for the planned interim analysis after 12 patients). In fact, these numbers exceeded our expectations. The results reject our own alternative hypothesis of 50% disease control with 95% confidence (95% one-sided CI 52–100).

Table 2. Clinical activity.

Radiological response at twelve weeks	
Complete response	0
Partial response	10 (29%)
Stable disease	13 (38%)
Progressive disease	11 (32%)
Disease control rate	23 (68%, 50 – 83) *
Objective response	13 (38%, 22 – 56)
Ongoing response **	11 (32%, 17 – 51)
Median follow up time (months)	14.3 (12.7 – 15.7)
Median duration of response (months) ***	14·3 (6·4 - NR)
Median progression-free survival (months)	6·2 (4·1- NR)
Progression-free survival at 6 months	50% (36-70)
Median overall survival (months)	NR (12·7 - NR)
1 year overall survival	64% (50 – 83)

Data are n (%), n (%; 95% CI), median (95% CI), or % (95% CI). NR=not reached

* confidence interval calculated accounting for the planned interim analysis after 12 patients.

**patients with partial response or stable disease for more than 6 months, on study drugs or at end of treatment.

*** time from start of response to progression

At data cutoff, three more patients had achieved a partial response, two after 18 weeks and one after 24 weeks of treatment, resulting in a total of 13 patients (38%) with a partial response as their best response. The median time to response was 2.6 months (95% CI 2.4–not reached). The median duration of response (time from start of response to progression) was 14.3 months (95% CI 6·4-not reached).

At six months 13 (38%) of 34 patients had a partial response, four (12%) patients had stable disease and 17 (50%) patients had progressive disease; thus, disease control at six months was achieved by 17 (50%) patients (95% CI 32% – 68%;appendix). Objective response at six months was 38% (95% CI 22 - 56).

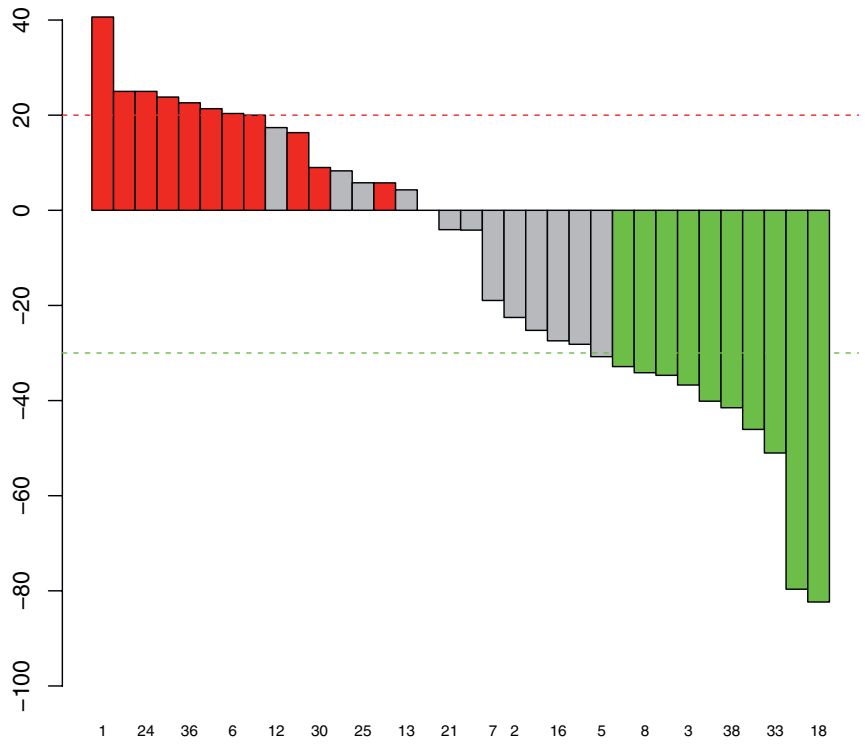

Figure 2: Percentage change in tumour size, baseline to week 12.

Change in sum of target lesions measured according to modified Response Evaluation Criteria in Solid Tumours by independent reviewer at 12 weeks as percentage change from baseline. Horizontal dotted line at 30% decrease shows cutoff for partial response and dotted line at 20% increase shows cutoff for progressive disease. Some patients have progressive disease based on non-target lesions. Orange shows progressive disease; blue shows stable disease; and green shows partial response.

At data cutoff, 10 (29%) patients were still receiving immunotherapy in this study, six of them for more than a year. Median progression free survival was at least 6.2 months (95% CI: 4.1 months – not reached; table 2, figure 3). The proportion of patients achieving progression free survival at six months was 50% (95% CI: 36-70; table 2, figure 3).

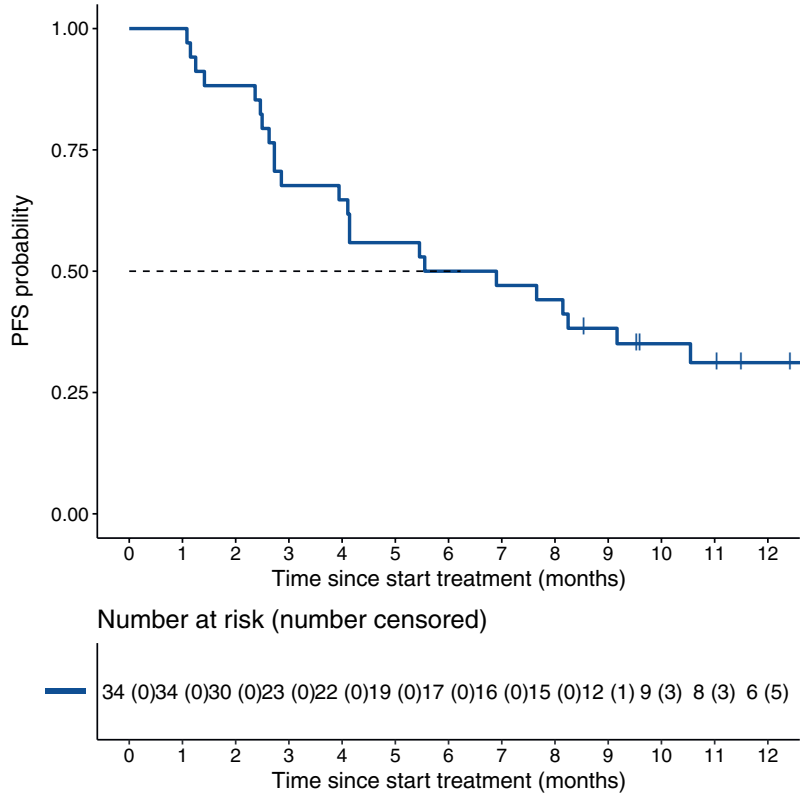

Figure 3: Kaplan-Meier curve of progression-free survival

Median follow-up (since first treatment) was 14.3 months (95% CI 12.7 – 15.7). Median overall survival was not yet attained, since only 13 patients (38%) had died, but with 95% confidence, the median overall survival will be greater than 12.7 months. Overall survival at six months was 85% (95% CI: 74 – 98) and overall survival at twelve months was 64% (95% CI: 50 – 83; table 2).

The small number of tumours with non-epithelioid histology did not allow a meaningful comparison between histological subtypes.

Safety

33 patients (94%) reported any treatment-related adverse event (table 3). The most frequent were infusion related reactions and skin disorders (each in 17 [49%] of 35 patients), including pruritus (11 [31%]) and dry skin (eight [23%]). Other treatment-related adverse events were fatigue (nine [26%] patients), anorexia (seven [20%]), diarrhoea (seven [20%]), nausea (six [17%]), and increased aspartate transaminase (five

[14%]). All other adverse events occurred in four patients or fewer. In the 33 patients, 134 treatment-related adverse events occurred. 12 patients (34%) had one or more grade 3 events related to treatment, including diarrhoea (three patients [9%]), increased alanine aminotransferase, anorexia, increased aspartate transaminase, and pleural effusion (all in two patients [6%]). Only one grade 4 event occurred, an increase in γ-glutamyltransferase, which decreased after a delay of one cycle of nivolumab. No grade 5 adverse events were reported. One patient discontinued treatment because of several toxicities, in particular malaise, but also mucositis, dysgeusia, pruritus, fatigue, hypothyroidism, and arthralgia.

Notably, many patients had infusion-related reactions (49%), grade 1 or 2, starting at first or second nivolumab dose. In those patients, the infusion was interrupted and symptomatic treatment was given (acetaminophen or antihistaminic drug, or both), with a prompt response. At all following immunotherapy cycles, prophylactic treatment (acetaminophen with or without antihistamine drug) was given and the infusion rate of nivolumab was slowed down, preventing further reactions. No patients required a prolonged admission or had to stop treatment because of infusion-related reactions. It was not possible to attribute adverse events to either nivolumab or ipilimumab, with the exception of infusion-related reactions, which seemed to be caused by nivolumab, based on time of onset of reaction. Six treatment-related serious adverse events (all grade 3) occurred in five patients, including pleural effusion (two patients), dyspnoea (in one patient; the same patient as one of the pleural effusion events), asthma cardiale, diarrhoea, and adrenal insufficiency (each in one patient).

Concomitant systemic corticosteroids for treatment of immune-related adverse events were administered in eight (23%) of 35 patients; for adrenal insufficiency (two patients [6%]), arthralgia (two [6%]), colitis (two [6%]), decrease of renal function (two [6%]), and pneumonitis (one [3%]). All these patients were re-treated with immunotherapy, but only when toxicity had decreased to a lower grade and patients were off steroids or on low-dose steroids. Some patients had more than one treatment related toxicity for which they needed steroids at different timepoints. In one patient, treatment stopped because of progressive disease while on systemic corticosteroids for treatment-related toxicity for the second time. Incidence of treatment-related toxicities was compared between those who achieved a partial response and those who had stable disease or progressive disease, but the occurrence of any of these adverse events did not differ between the two groups.

Table 3. Treatment-related adverse events (n=35)

	All grades (1-5)	Grade 1	Grade 2	Grade 3	Grade 4
Adrenal insufficiency	3 (9%)	0	2 (6%)	1 (3%)	0
Alanine aminotranferase (ALT) increase	3 (9%)	1 (3%)	0	2 (6%)	0
Anorexia	7 (20%)	4 (11%)	1 (1%)	2 (6%)	0
Arthralgia	4 (11%)	1 (3%)	3 (9%)	0	0
Aspartate transaminase (AST) increase	5 (14%)	2 (6%)	1 (1%)	2 (6%)	0
Asthma cardiale	1 (3%)	0	0	1 (3%)	0
Diarrhea	7 (20%)	3 (9%)	1 (1%)	3 (9%)	0
Dyspnea	4 (11%)	2 (6%)	1 (3%)	1 (3%)	0
Fatigue	9 (26%)	5 (14%)	4 (11%)	0	0
Gamma-glutamyltransferase (GGT) increase	1 (3%)	0	0	0	1 (3%)
Infusion related reaction	17 (49%)	2 (6%)	15 (43%)	0	0
Malaise *	3 (9%)	0	3 (9%)	0	0
Mucositis oral	1 (3%)	0	0	1 (3%)	0
Myalgia	4 (11%)	2 (6%)	2 (6%)	0	0
Nausea	6 (17%)	0	6 (17%)	0	0
Pleural effusion	2 (6%)	2 (6%)	0	2 (6%)	0
Pleural infection	1 (3%)	0	0	1 (3%)	0
Skin disorder	17 (49%)	10 (29%)	6 (17%)	1 (3%)	0
Pruritus	11 (31%)	10 (29%)	1 (3%)	0	0
Dry skin	8 (23%)	5 (14%)	3 (9%)	0	0
Rash	10 (29%)	5 (14%)	4 (11%)	1 (3%)	0

Data are n (%). For grades 1–2 events, only those that occurred in 10% or more patients are reported. All grade 3 and 4 events are reported. No grade 5 events occurred. *Resulted in treatment discontinuation for one patient.

We did a post-hoc analysis of clinical benefit (partial response or stable disease for more than 6 months) and treatment outcome (partial response, stable disease, or progressive disease at 12 weeks), according to PD-L1 expression status. Pretreatment biopsies of all 34 evaluable patients were scored for PD-L1 expression (22C3 antibody). 15 (44%) samples had PD-L1 expression on at least 1% of tumour cells (table 4), of which 12 (80%) were epithelioid, one (7%) was mixed, and two (13%) were sarcomatoid. Both patients with sarcomatoid subtype had a PD-L1 expression of 50%. Five (15%) patients had PD-L1 expression of at least 50%. Responses at 12 weeks for the 15 PD-L1-positive patients (ie, PD-L1 expression of ≥1%) were partial response in seven (47%), stable disease in six (40%), and progressive disease in two (13%), which were significantly better than responses for the 19 PD-L1-negative patients, which were partial response in three (16%), stable disease in seven (37%), and progressive disease in nine (47%; p=0·018, linear-by-linear association test). PD-L1 positivity (vs negativity) was significantly associated with clinical benefit (ie, partial response or stable disease for >6 months; p=0.037, Fisher's exact test). 11 (73%) of

the 15 PD-L1-positive patients had clinical benefit, whereas only six (32%) of 19 PD-L1-negative patients had clinical benefit (table 4).

PD-L1 expression on immune cells (scored 0–3) was significantly associated with response, with higher expression corresponding to better response (p=0.001, linear-by-linear association test). Most notably, of the 11 patients who progressed at 12 weeks, ten (91%) had PD-L1 expression of less than 1% on immune cells (score 0). Seven (21%) of the total 34 patients had PD-L1 expression of at least 5% (score ≥2), and all had clinical benefit. For the ten patients with both PD-L1 expression on tumour cells and immune cells, nine (90%) had clinical benefit. The hazard ratio of tumour cell PD-L1 expression versus no expression was 0.39 (95% CI 0.17–0.94) for progression-free survival (figure 4A) and 0.16 (0.04–0.73) for overall survival (figure 4B), indicating both clinical and statistical significance. The hazard ratio of immune cell PD-L1 expression versus no expression was significant (0.18; 95% CI 0.04–0.78) for progression-free survival (figure 4C) but non-significant (0.30; 0.08–1.1) for overall survival (figure 4D).

Table 4. clinical benefit by PD-L1 expression

	Tumour cell PD-L1 expression, as a percentage of all tumour cells			Tumour-infiltrating immune cell PD-L1 expression, as a percentage of all non-tumour cells		
	Negative	Positive ≥1%	≥50%	Negative IC 0	Positive IC ≥ 1	IC ≥ 2
pre-treatment biopsy (n=34)						
clinical benefit	6	11	4	5	12	7
no clinical benefit	13	4	1	14	3	0
total	19	15	5	19	15	7
on-treatment biopsy (n=32)						
	Negative	Positive ≥1%	≥50%	Negative IC 0	Positive IC ≥ 1	IC ≥ 2
clinical benefit	3	8	2	1	15	8
no clinical benefit	7	6	0	4	11	6
Total *	12	14	2	5	26	14

Clinical benefit was partial response or long-term stable disease (≥6 months). *Six patients did not have a tumour at the time of on-treatment biopsy, so PD-L1 expression in tumour cells could not be measured; in one patient, tumour-infiltrating immune cell PD-L1 expression could not be scored. PD-L1=programmed cell death ligand 1.

After 6 weeks of treatment, we obtained biopsy samples from 32 patients; in one (3%) patient, no accessible tumour remained and one (3%) patient was not fit for a thoracoscopy. Six on-treatment biopsy samples showed no tumour cells; in five of them a

dense infiltration of immune cells was seen. Of the 19 patients that were PD-L1 negative at baseline, eight (42%) were positive during treatment, of which four (21%) had clinical benefit and the other four (21%) did not. Conversely, of the 15 patients that were PD-L1 positive at baseline, three (20%) were negative during treatment (appendix). When assessing PD-L1 expression on tumour cells in on-treatment samples, an association with response was noted (p=0.053, linear-by-linear association test), but the association was less strong than in the pretreatment samples. The on-treatment samples also showed that PD-L1-positive patients had a better response at 12 weeks (29% partial response, 50% stable disease, and 21% progressive disease) than PD-L1-negative patients (8% partial response, 33% stable disease, and 58% progressive disease). No association was noted when analysing on-treatment PD-L1 expression on immune cells.

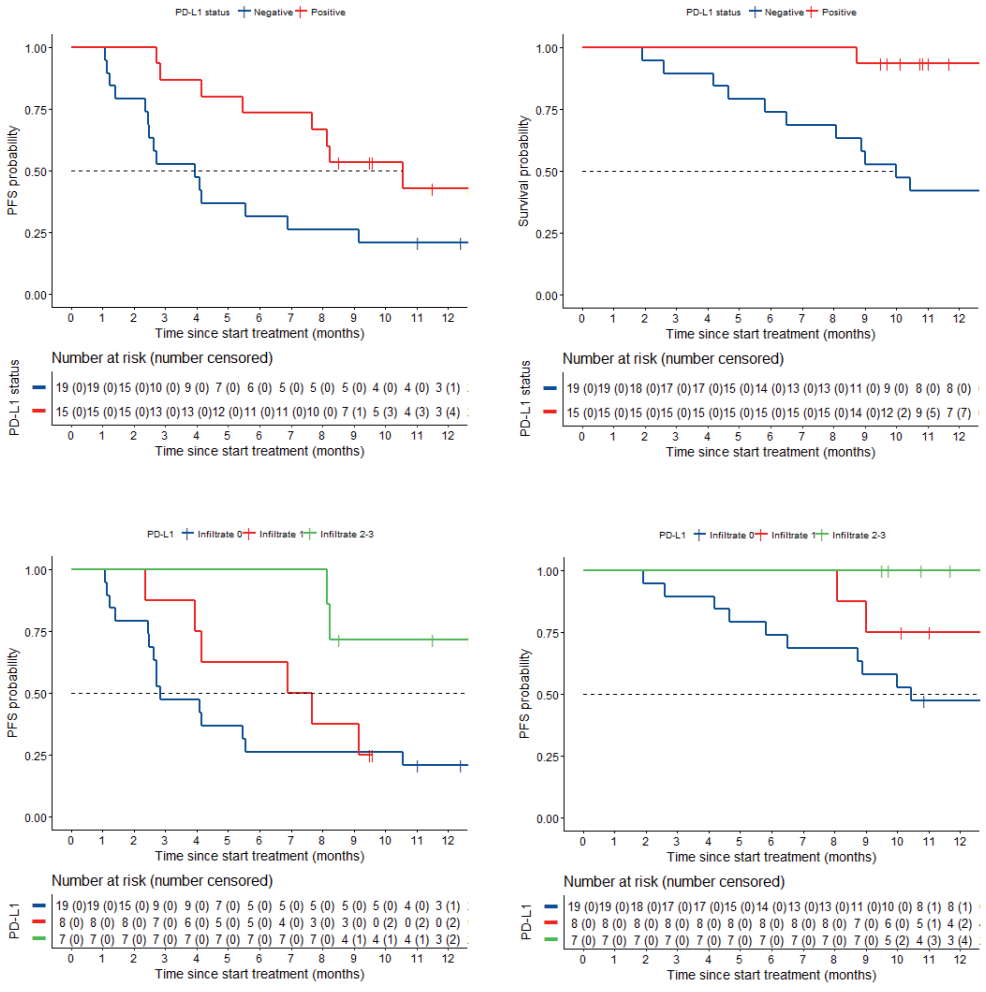

Figure 4: Kaplan-Meier survival curves in patient subgroups

Progression-free survival (A) and overall survival (B) by PD-L1 tumour cell expression level at baseline and progression-free survival (C) and overall survival (D) by PD-L1 expression in immune infiltrate. PD-L1=programmed cell death ligand 1.

Discussion

Our study shows that the combination of nivolumab and ipilimumab has marked clinical activity in previously treated relapsed patients with malignant pleural mesothelioma. The regimen was well tolerated and toxicity was reversible and considered manageable when adhering to protocol guidelines.

Four (31%) of the 13 patients who achieved a partial response did so by the 6-week assessment six (46%) did so by the 12-week assessment, and three (23%) did so after 12 weeks. The median time to response was 2.6 months, which is similar to time to response with nivolumab in another study of patients with malignant pleural mesothelioma (12).

The objective response of 36% is much better than the response reported for second line chemotherapy (10-20%) (6,7,10) or monotherapy with a checkpoint inhibitor (10-20%) for malignant pleural mesothelioma (11-14). However, these studies are difficult to compare because of a potential selection bias, related to the heterogeneity between studies with respect to included patients, inclusion criteria and treatment history.

Regarding the CTLA-4 checkpoint inhibitor tremelimumab, single center phase II studies seemed promising (25,26), but the multicenter randomized phase IIB study was negative, compared to placebo (14). Whether this result was due to selection bias or variations in tumour or patient biology is unclear. No positive phase III studies have been published for checkpoint inhibitors in mesothelioma yet. Whether our results will translate to a survival benefit for patients with mesothelioma needs to be investigated in a phase III trial. Results for the first line multicenter phase III study comparing nivolumab plus ipilimumab with platinum plus pemetrexed (Checkmate 743) are awaited.

The same combination of checkpoint inhibitors nivolumab plus ipilimumab was also analysed in the MAPS-2 trial (17). Our study and the MAPS2 trial showed similar proportions of patients achieving 12-week disease control. Our median progression-free survival of 6.2 months (95% CI 4.1–not reached) is similar to the MAPS2 median progression-free survival of 5.6 months (3.1–8.3) for the combination treatment, as is our overall survival at 12 months of 64% (50–83%) to their result of 58% (46–70%). Another combination of checkpoint inhibitors (anti-PD-L1 durvalumab plus anti-CTLA-4 tremelimumab) as first-line and second-line treatment was tested in the NIBIT-MESO-1 clinical trial and similar efficacy and toxicity results were obtained (16).

Metaxas and colleagues (27) did a real-world analysis of varying regimens of pembrolizumab in patients with malignant pleural mesothelioma. The general observation was that in the unselected population, including patients with a performance status of 2, treatment with a checkpoint inhibitor was feasible. However, as described in a comment by De Gooijer and Baas (28), there were many limitations of the analysis, including the absence of a control group and the large proportion of patients with a high performance status.

Although all patients but one experienced any treatment-related adverse event, only 12 (34%) patients had a grade 3 or 4 adverse event. Most treatment-related AEs were reversible and considered manageable when adhering to protocol guidelines. Only one patient

discontinued treatment due to toxicities. Of all 577 planned cycles of immunotherapy, 32 cycles (6%) were not given due to treatment-related AEs.

The combination of CTLA-4 and PD-1 inhibitors increased toxicity in our study compared to with other monotherapy trials, we mainly attributed the high numbers of toxicities to the CTLA-4 inhibitor (11,12,14,16). Many patients had grade 1 or 2 toxicities, and these did not delay treatment and were considered manageable with standard protocols.

The reason for the many infusion-related reactions (IRR) to nivolumab (in 49% of all patients) is not clear. For nivolumab monotherapy in malignant pleural mesothelioma (Nivomes trial) two (6%) IRR were described (12), although conditions were similar to our study (240mg infused over 30 min). In the Keynote-028 trial assessing pembrolizumab monotherapy only one (4%) patient had an infusion-related reaction (11). In Checkmate-057, which assessed nivolumab monotherapy (3 mg/kg infused over 60 min) for patients with non-small-cell lung carcinoma, 3% of patients had infusion related reactions.29 In other studies, with combination treatment of nivolumab plus ipilimumab in melanoma (1mg/kg nivolumab over 60 min and 3 mg/kg ipilimumab over 90 min) grade 1 or 2 infusion related reactions occurred in 3% of patients (30). The discrepancies with our study might be related to the combination therapy plus the differences in infusion rate (30 min in our study), even though safety studies for shorter infusion rates of combined nivolumab and ipilimumab and other monoclonal antibodies showed acceptable safety (30,31). We also observed a variety of skin-related toxicity (50%), including pruritus, dry skin, and rash. This toxicities responded well to symptomatic local treatment.

Limitations of this study include the small sample size and single-arm setting. Despite recruiting almost all patients that were referred to our hospital, a limited selection of participants were enrolled. The median time from diagnosis to start of study in our trial was 12 months and greater than 4 years in two patients, whereas the mean overall survival for mesothelioma is only 12–16 months (3,5). Because few patients with mesothelioma have a performance status of 0–1 after one or more lines of therapy, our cohort does not resemble the general population of patients with malignant pleural mesothelioma who have relapsed after treatment; our patients progressed more slowly or were more sensitive to treatment.

In a few clinical trials of checkpoint inhibitors, PD-L1 expression was measured with variable response results. One of the inclusion criteria for the Keynote-028 study was PD-L1 expression in more than 1% of tumor cells, assessed by the 22C3 antibody. Whether a higher expression resulted in a better or longer response was not reported (11). In the Javelin trial with avelumab for malignant pleural mesothelioma, 43 patients were evaluable for PD-L1 expression, with a cutoff for positivity of more than 5% of tumour

cells. Objective response was achieved by three (19%) of 16 PD-L1-positive patients and two (7%) of 27 PD-L1-negative patients (13). In the Nivomes trial (12) assessing nivolumab in patients with malignant pleural mesothelioma, PD-L1 expression of more than 1% (assessed with 28-8 antibody) was measured in 27% of patients, with no clear association with clinical benefit. Baseline tumour PD-L1 expression (SP-263 assay) in the NIBIT-MESO-1 trial16 did not correlate with response or survival. In the MAPS-2 trial, PD-L1 expression of at least 1% significantly correlated with objective response, and high PD-L1 expression (≥25%) was correlated with both objective response and disease control. In our study PD-L1 expression on tumour cells was significantly correlated with response. But like in other studies, not all patients with PD-L1 expression achieved a response and some who were PD-L1 negative did respond. We noted a change in PD-L1 expression between pre- and on-treatment biopsies (appendix), this might be due to the (known) heterogeneity of malignant pleural mesothelioma (32), or the effect of therapy (33).

We noted a significant association of immune cell PD-L1 expression with outcome, in line with research in other types of cancer (34). These immune cells might be of different subtypes, which could be the reason for the better outcome. This will be focus of our ongoing translational research.

PD-L1 expression on both tumour cells and immune cells at baseline might serve as a prognostic biomarker for the effect of checkpoint inhibitors in patients with malignant pleural mesothelioma. But both are insufficient for prediction of response. Patient characteristics and other biomarkers need to be studied prospectively to establish which subgroup of patients will benefit from checkpoint inhibitors.

In conclusion, in this single-centre phase II study, the combination of nivolumab and ipilimumab has marked clinical efficacy in patients with malignant pleural mesothelioma. The safety profile is consistent with previously reported data of combination checkpoint inhibitors. Our results add to the growing evidence that immunotherapy is a promising treatment, warranting further research in a phase 3 trial.

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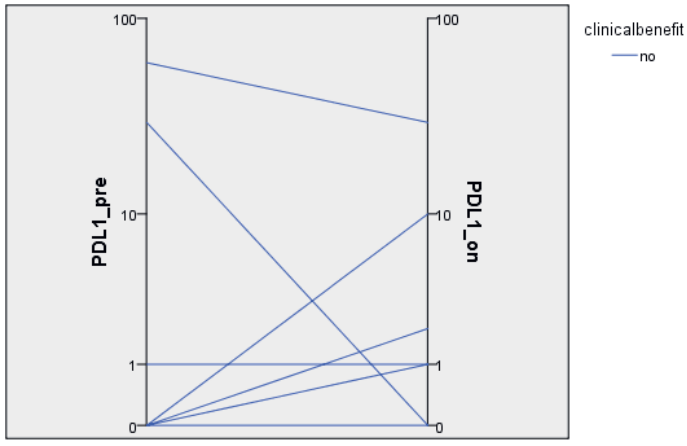
Supplementary data

Supplementary figure 1: change in PD-L1 expression during treatment.

A:



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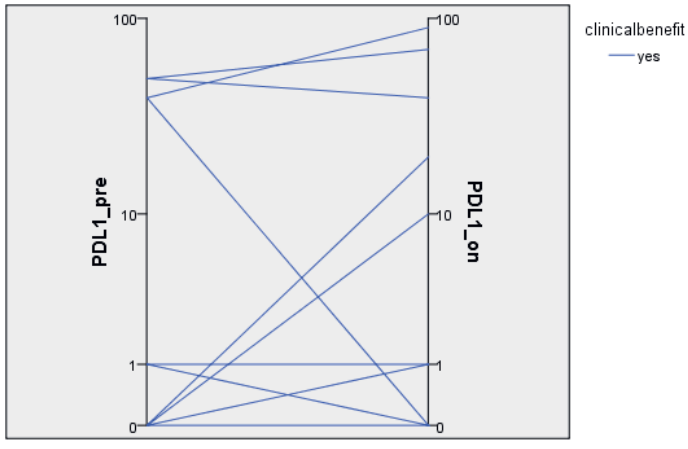
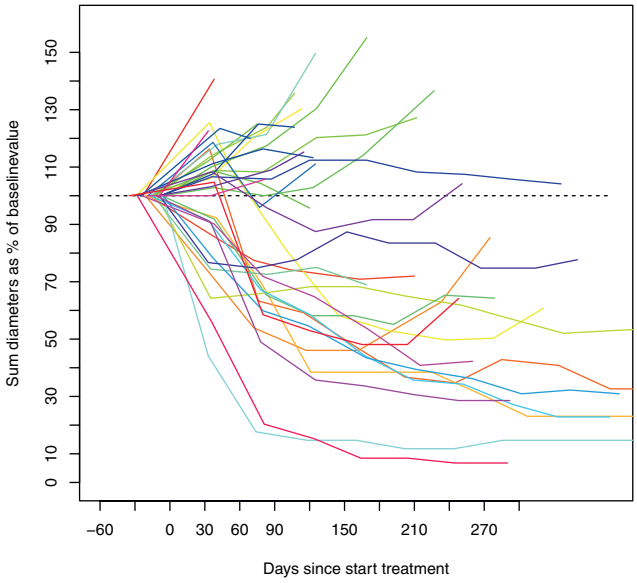


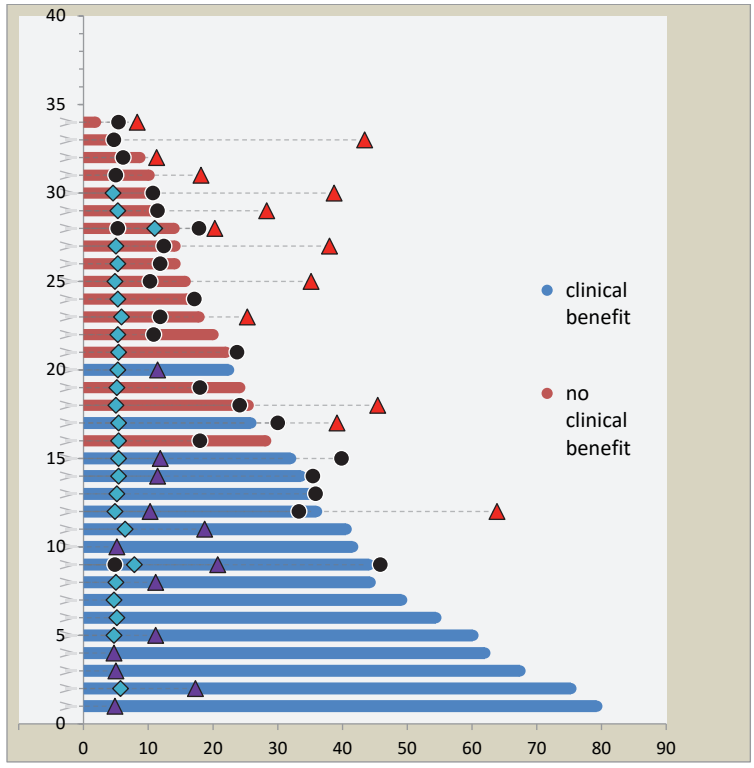
Change in PD-L1 expression on tumor cells during treatment in patients without clinical benefit (A) and with clinical benefit (B). Left y-axis is PD-L1 expression in pre-treatment biopsies and right y-axis is PD-L1 expression in on-treatment biopsies. Both as a percentage of all tumor cells on a logarithmic scale.
In A: nine patients do not have change in expression from 0.

Supplementary figure 2. plot representing the change in sum of target lesions from baseline over time in days (%).



Percentage change in sum of target lesions from baseline over time in days. Positive change indicates tumour growth and negative change indicates tumour reduction. N = 34

Figure 3. Swimmer plot: treatment exposure and response duration in weeks.



The length of each bar corresponds with treatment duration in weeks. Response symbols represent the time when first reported (and not best response). We defined clinical benefit as partial response or stable disease for more than 6 months.