Identification of the optimal combination dosing schedule of neoadjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma (OpACIN-neo): a multicentre, phase 2, randomised, controlled trial

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Summary

Background The outcome of patients with macroscopic stage III melanoma is poor. Neoadjuvant treatment with ipilimumab plus nivolumab at the standard dosing schedule induced pathological responses in a high proportion of patients in two small independent early-phase trials, and no patients with a pathological response have relapsed after a median follow up of 32 months. However, toxicity of the standard ipilimumab plus nivolumab dosing schedule was high, preventing its broader clinical use. The aim of the OpACIN-neo trial was to identify a dosing schedule of ipilimumab plus nivolumab that is less toxic but equally effective.

Methods OpACIN-neo is a multicentre, open-label, phase 2, randomised, controlled trial. Eligible patients were aged at least 18 years, had a WHO performance status of 0–1, had resectable stage III melanoma involving lymph nodes only, and measurable disease according to the Response Evaluation Criteria in Solid Tumors version 1.1. Patients were enrolled from three medical centres in Australia, Sweden, and the Netherlands, and were randomly assigned (1:1:1), stratified by site, to one of three neoadjuvant dosing schedules: group A, two cycles of ipilimumab 3 mg/kg plus nivolumab 1 mg/kg once every 3 weeks intravenously; group B, two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg once every 3 weeks intravenously; or group C, two cycles of ipilimumab 3 mg/kg once every 3 weeks directly followed by two cycles of nivolumab 3 mg/kg once every 2 weeks intravenously. The investigators, site staff, and patients were aware of the treatment assignment during the study participation. Pathologists were masked to treatment allocation and all other data. The primary endpoints were the proportion of patients with grade 3–4 immune-related toxicity within the first 12 weeks and the proportion of patients achieving a radiological objective response and pathological response at 6 weeks. Analyses were done in all patients who received at least one dose of study drug. This trial is registered with ClinicalTrials.gov, number NCT02977052, and is ongoing with an additional extension cohort and to complete survival analysis.

Findings Between Nov 24, 2016 and June 28, 2018, 105 patients were screened for eligibility, of whom 89 (85%) eligible patients were enrolled and randomly assigned to one of the three groups. Three patients were excluded after randomisation because they were found to be ineligible, and 86 received at least one dose of study drug; 30 patients in group A, 30 in group B, and 26 in group C (accrual to this group was closed early upon advice of the Data Safety Monitoring Board on June 4, 2018 because of severe adverse events). Within the first 12 weeks, grade 3–4 immune-related adverse events were observed in 12 (40%) of 30 patients in group A, six (20%) of 30 in group B, and 13 (50%) of 26 in group C. The difference in grade 3–4 toxicity between group B and A was –20% (95% CI –46 to 6; p=0·158) and between group C and group A was 10% (–20 to 40; p=0·591). The most common grade 3–4 adverse events were elevated liver enzymes in group A (six [20%]) and colitis in group C (five [19%]); in group B, none of the grade 3–4 adverse events were seen in more than one patient. One patient (in group A) died 9·5 months after the start of treatment due to the consequences of late-onset immune-related encephalitis, which was possibly treatment-related. 19 (63% [95% CI 44–80]) of 30 patients in group A, 17 (57% [37–75]) of 30 in group B, and nine (35% [17–56]) of 26 in group C achieved a radiological objective response, while pathological responses occurred in 24 (80% [61–92]) patients in group A, 23 (77% [58–90]) in group B, and 17 (65% [44–83]) in group C.

Interpretation OpACIN-neo identified a tolerable neoadjuvant dosing schedule (group B: two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg) that induces a pathological response in a high proportion of patients and might be suitable for broader clinical use. When more mature data confirm these early observations, this schedule should be tested in randomised phase 3 studies versus adjuvant therapies, which are the current standard-of-care systemic therapy for patients with stage III melanoma.

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Introduction

Patients with macroscopic resectable nodal stage III melanoma have a high risk for of relapse.[1] The standard treatment is lymph node dissection and, in high-risk cases, adjuvant radiotherapy improves local control but does not result in an overall survival benefit.[2] The outcomes of these patients are heterogeneous, with observed proportions of patients alive at 5 years of 93% for those with stage IIIA disease, 83% for those with stage IIIB, 69% for those with stage IIIC, and 32% for those with stage IIID.[1] Patients with lymph node metastasis are at an especially high risk of relapse after therapeutic lymph node dissection, with only 56–61% of patients remaining relapse free at 1 year and 30% at 5 years.[3,4]

Adjuvant high-dose ipilimumab (a human monoclonal antibody against CTLA-4 significantly improved 5-year relapse-free survival and overall survival of patients with stage III melanoma compared with placebo.[3] Adjuvant nivolumab and pembrolizumab (both PD-1-blocking antibodies), and dabrafenib plus trametinib (BRAF plus MEK inhibitor; *BRAF*^{V600} mutation-positive patients only) have been shown to improve median recurrence-free survival compared with ipilimumab alone or placebo.[4–7] However, a significant overall survival benefit has only been shown for adjuvant ipilimumab so far.[3]

In advanced melanoma, the combination of ipilimumab 3 mg/kg plus nivolumab 1 mg/kg every 3 weeks for four cycles (referred to here as the standard dosing schedule), followed by nivolumab 3 mg/kg consolidation, led to a higher proportion of patients achieving an objective response, and superior progression-free survival and overall survival compared with monotherapy with ipilimumab 3 mg/kg (significantly) or nivolumab 3 mg/kg (numerically).[8–10] However, these improved outcomes come at a cost of more toxicity, with grade 3–4 adverse events observed in 59% of patients treated with ipilimumab plus nivolumab, in 21% of patients treated with nivolumab, and in 28% of patients treated with ipilimumab.[9] Alternative combination dosing schedules that combine a lower dose of ipilimumab with nivolumab (Checkmate 511, NCT02714218)[11] or pembrolizumab (Keynote 029, NCT02089685)[12] reported a lower proportion of patients with grade 3–4 adverse events, but are yet to conclusively show equivalent efficacy in phase 3 randomised trials. Sequential application of ipilimumab directly followed by ipilimumab plus nivolumab using the standard dosing schedule, but at the cost of high toxicity (73–90% of patients had grade 3–4 adverse events). This toxicity raised the question as to whether alternative dosing schedules of ipilimumab plus nivolumab might show toxicity in a lower proportion of patients but with preserved efficacy.

Research in context

Evidence before this study

We searched PubMed with the terms "neoadjuvant therapy", "neoadjuvant treatment", "melanoma", "resectable", "stage III" for studies published in English from Jan 1, 1990, to Nov 1, 2018. Five single-arm neoadjuvant trials and four randomised trials testing neoadjuvant therapy were identified. The five single-arm trials tested neoadjuvant temozolomide, high-dose interferon alfa-2b, and three trials tested biochemotherapy (two trials tested chemotherapy plus interleukin[IL]-2 and interferon alfa-5; the other trial tested cchemotherapy plus IL-2 and interferon alpha-2a) showing that the proportion of patients achieving a response was between 16% for temozolomide and up to 50% for biochemotherapy. The biochemotherapy was not developed further due to substantial toxicity. In a small phase 1 pilot study, patients were randomly assigned to neoadjuvant or adjuvant therapy with hu14.18-IL-2 immunocytokine, showing a recurrence-free survival of 39% at 24 months. A randomised phase 2 study showed an improved event-free survival with the combination of 8 weeks neoadjuvant and 44 weeks adjuvant dabrafenib plus trametinib compared with standard of care (no adjuvant systemic therapy). Two small trials (NCT02519322 and OpACIN [NCT02437279]) showed pathological responses in a high proportion of patients (55–78%) and promising recurrence-free survival (median not reached after follow-up of 25–32 months) with neoadjuvant ipilimumab plus nivolumab using the standard dosing schedule, but at the cost of high toxicity (73–90% of patients had grade 3–4 adverse events). This toxicity raised the question as to whether alternative dosing schedules of ipilimumab plus nivolumab might show toxicity in a lower proportion of patients but with preserved efficacy.

Added value of this study

OpACIN-neo identified a feasible combination dosing schedule: two cycles of neoadjuvant ipilimumab 1 mg/kg plus nivolumab 3 mg/kg, which induced a pathological response in a high proportion of patients, and at the time of data cutoff, no relapses have occurred in patients with a pathological response. This regimen seems to be suitable for broader clinical use and might be tested against the standard-of-care adjuvant therapies in a phase 3 trial.

Implications of all the available evidence

Neoadjuvant combination immune checkpoint inhibition is feasible and tolerable, and is effective in a large proportion of patients with macroscopic stage III nodal melanoma. If these data hold true with additional follow-up, this therapy might identify patients with long-term benefit after only 6 weeks of neoadjuvant treatment and thus could be used to personalise adjuvant therapy in individual patients according to their neoadjuvant response.

nivolumab or pembrolizumab also showed a lower proportion of patients with grade 3–4 adverse events, while efficacy seemed to be preserved, in our single-centre retrospective analysis.[13]

Neoadjuvant immunotherapy allows assessment of the clinical and pathological response for an individual patient in a short time period (weeks). Two early-phase trials testing neoadjuvant ipilimumab plus nivolumab therapy at the standard dosing schedule (NCT02519322 and OpACIN [NCT02437279]), recorded an unparalleled high proportion of patients achieving a pathological response (55% and 78%).[14,15] None of the seven of nine patients with a pathological response in the OpACIN trial[15] had relapsed after a median follow-up of 32 months, thus suggesting that pathological response might be a promising predictive marker of long-term outcome after neoadjuvant immunotherapy.[16] The OpACIN trial also indictated that neoadjuvant ipilimumab plus nivolumab may be superior to adjuvant therapy by expanding a broader range of tumour-resident T-cell clones.[15] However, toxicity from the standard dosing regimen (ipilimumab 3 mg/kg plus nivolumab 1 mg/kg every 3 weeks) was high, irrespective of whether the combination was administered in the neoadjuvant or adjuvant setting, with grade 3–4 toxicities observed in 73–90% of patients.[14,15,17] Single agent neoadjuvant PD-1 blockade (with three cycles of nivolumab[15] or one cycle of pembrolizumab[18]) has shown pathological responses in a lower proportion of patients (30–33%),[15,18] with some patients not being able to undergo surgery due to early progression.[15] Neoadjuvant dabrafenib plus trametinib induced pathological responses in a high proportion of patients and improved event-free survival compared with standard of care.[19] However, these responses did not always result in a long-term benefit.[20]

These data raised the question of whether neoadjuvant ipilimumab plus nivolumab can be dosed differently to reduce the toxicity while preserving efficacy. The observation in the OpACIN study that a high proportion of patients achieved a pathological response after only 6 weeks of treatment led us to reduce the neoadjuvant regimen to two cycles and to also test two alternative dosing schedules, one in combination with reduced ipilimumab dosing[11,12] and one with the drugs given sequentially,[13] both of which have previously shown in stage IV melanoma to be potentially less toxic than the standard dosing schedule.

As such, the objective of the current study was to compare three different neoadjuvant dosing schedules of ipilimumab plus nivolumab in macroscopic, nodal stage III, resectable melanoma in terms of the proportion of patients who developed grade 3–4 immune-related adverse events within the first 12 weeks after treatment initiation, the proportion of patients with a radiological response, and the proportion of patients with a pathological response after 6 weeks of neoadjuvant therapy.

See Online for appendix

Methods

Study design and participants

In the investigator-initiated, multicentre, open-label, phase 2, randomised, controlled OpACIN-neo trial, patients were enrolled at three medical centres, in Australia (Melanoma Institute Australia, Sydney, NSW), Sweden (Karolinska Institute, Stockholm), and the Netherlands (Netherlands Cancer Institute, Amsterdam; appendix p 2). The database lock for primary analysis took place on Sept 28, 2018 (12 weeks after the last patient received the first cycle of therapy). The study design is summarised in the appendix (p 6).

Eligible patients were 18 years or older with cytologically or histologically confirmed resectable stage III melanoma with one or more macroscopic lymph node metastases, measurable according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (>15 mm short axis).[21] A WHO performance status of 0 or 1, normal organ function and normal lactate dehydrogenase levels were required. Exclusion criteria included in-transit metastasis within the last 6 months, autoimmune disease, HIV infection, hepatitis B or C infection, previous radiotherapy, previous immunotherapy targeting CTLA-4, PD-1, or PD-L1, or immunosuppressive medication within 6 months before study inclusion. Full inclusion and exclusion criteria can be found in the full protocol in the appendix (pp 36–37).

The study was done in accordance with the protocol and Good Clinical Practice Guidelines as defined by the International Conference on Harmonization and the Declaration of Helsinki. The appropriate institutional review boards and ethics committees at each of the participating centres approved the protocol. The sponsor maintained the study database. A data safety monitoring board (DSMB) was installed and data of preplanned interim analyses were reported to the board. Furthermore, the sponsor reported annually to the medical ethics committee of the Netherlands Cancer Institute. All participating patients provided written informed consent before enrolment.

Randomisation and masking

Patients were randomly assigned (stratified according to centre) in a 1:1:1 ratio to receive neoadjuvant treatment with one of three dosing schedules with ipilimumab and nivolumab.

Patients were enrolled by the local investigators at participating sites. After verification of eligibility criteria, we used the ALEA randomisation software, which implements a minimisation technique described by Pocock and Simon,[22] to randomly assign patients and stratify them according to site. If patients no longer fulfilled the eligibility criteria after having signed informed consent but before initiation of treatment, they were withdrawn and were replaced by another patient. This was an open-label study; the investigators, site staff, and patients were aware of the treatment assignment

during the study participation. Pathologists were masked to the treatment allocation and all other data.

A planned interim analysis was done after 13 patients had been included in each group (see also statistical analysis). Based on the response and toxicity data that were available at this interim analysis, the data safety monitoring board (DSMB) advised to continue with all three groups of the trial.

Procedures

Patients in group A received two cycles of ipilimumab 3 mg/kg combined with nivolumab 1 mg/kg intravenously, every 3 weeks; patients in group B received two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg, intravenously, every 3 weeks; patients in group C received two cycles of ipilimumab 3 mg/kg every 3 weeks, intravenously, immediately followed by (starting on the same day as the last cycle of ipilimumab) two cycles of nivolumab 3 mg/kg, intravenously, every 2 weeks. Ipilimumab and nivolumab were manufactured and provided by Bristol-Myers Squibb (New York City, NY, USA). Lymph node dissection was scheduled for week 6 and performed by experienced melanoma surgeons. Patients were treated until unacceptable toxicity, withdrawal of consent, or completion of the total treatment schedule. Dose reductions and escalations were not permitted. Dose delays were only allowed in case they did not interfere with the timing of surgery. Standard discontinuation criteria in the event of immune-related toxicity are described in the study protocol (appendix pp 48–49). Permanent discontinuation of checkpoint inhibition due to adverse events did not preclude patients from having surgery. Adverse events and laboratory values were graded by the investigators throughout the study according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03, from the first study dose until 3 years after treatment initiation or start of other systemic therapy. Laboratory tests, haematology, and chemistry (including endocrine axis), were tested at baseline, week 3, week 6 (before surgery), week 9, and week 12 for all patients, and also at week 5 on the same day and before the last cycle of nivolumab for patients in group C (a full schedule of assessments is available in the appendix p 59).

Patients had tumour assessments by CT before dosing at baseline, in week 6 before surgery, and in week 12. Response at week 6 was scored according to RECIST version 1.1 by the radiologist at the individual site, without central review. Patients who had progressive disease at week 6 that was limited to the affected lymph node region and judged as resectable by the multidisciplinary tumour board (MTB), could have surgery according to protocol. Patients who had progressive disease at week 6, including distant metastasis, or who had disease judged as unresectable by the MTB, could not have surgery according to protocol. However, if defined by the treating physician as being in their best interests, these patients could still receive surgery.

Starting at week 12, all patients were assessed for relapse by physical examination and laboratory testing every 3 months until development of distant metastasis, death, loss to follow-up, withdrawal of consent or for up to 3 years, and by radiological assessment with CT or PET–CT scans, with or without MRI of the brain, according to the institution's standard procedures (Netherlands Cancer Institute: physical examination every 3 months, CT every 3 months for patients with a pathological non-response (pNR), and every 6 months for patients with a pathological complete response (pCR), pathological near-complete response (near pCR), or pathological partial response (pPR); Melanoma Institute Australia: physical examination, CT plus MRI of the brain every 3 months, and yearly PET scan; Karolinska Institute: physical examinations every 3 months and CT every 6 months).

Pathological response was centrally reviewed by pathologists (BAvdW, CA, and RAS) experienced in judging response upon neoadjuvant checkpoint inhibition in stage III melanoma using the International Neoadjuvant Melanoma Consortium scoring system.[23]

Baseline tumour PD-L1 expression was assessed centrally (at the Netherlands Cancer Institute) with an automated laboratory-validated immunohistochemistry assay, with the use of the 22C3 antibody on a Ventana platform (BenchMark Ultra autostainer, Ventana Medical Systems, Tuscon, AZ, USA). RNA was isolated from freshly frozen pretreatment tumour biopsies in a central laboratory (Netherlands Cancer Institute) with the use of the AllPrep DNA/RNA kit (QIAgen, Hilden, Germany) for frozen material on the QIAcube according to the manufacturer's protocol. RNA expression analysis was done with the use of the nCounter PanCancer Immune Profiling panel (NanoString Technologies, Seattle, WA, USA), assessing the previously described IFN-γ signature.[24] Additional translational analyses, including DNA and RNA sequencing and immunohistochemistry analyses of pretreatment biopsies, RNA sequencing of peripheral blood mononuclear cells and cytokine analysis, are ongoing and will be included in a future manuscript describing this trial when the relapse-free survival data are more mature.

Outcomes

The primary objectives of OpACIN-neo were to compare safety and efficacy of three different neoadjuvant combination schemes of ipilimumab and nivolumab. The first primary endpoint of the study was the occurrence of grade 3–4 immune-related adverse events during the first 12 weeks after initiation of treatment. All adverse events that were deemed to be related to the neoadjuvant immunotherapy were defined as immune-related adverse events. Other primary endpoints were the

proportion of patients who achieved radiological objective responses, defined as the proportion of patients with a complete or partial response at week 6 according to RECIST version 1.1 (assessed by local radiologists),[21] and the proportion of patients with pathological responses (centrally reviewed, and defined as the proportion of patients with a pCR, near pCR, or pPR). pCR was defined as absence of viable tumour cells, near pCR was defined as more than 0% but 10% or less viable tumour cells, pPR as more than 10% but 50% or less viable tumour cells, pNR as more than 50% viable tumour cells according to the International Neoadjuvant Melanoma Consortium criteria.[23]

One of the secondary endpoints was recurrence-free survival. Data for the other secondary endpoints, including late adverse events and associations of baseline tumour markers (eg, mutational load and RNA tumour signatures) with outcome, other than PD-L1 expression and interferon-γ signature, will be reported separately.

Event-free survival (a post-hoc exploratory analysis) was defined as the time from randomisation until the date of first progression during neoadjuvant therapy (only in case of distant metastasis or local progression when unresectable), recurrence (local, regional, or distant metastasis), or death from any cause, whichever occurred first. Recurrence-free survival was defined as the time from surgery until the date of first recurrence (local, regional, or distant metastasis) or death from any cause.

Statistical analysis

We compared group B and group C with group A for the primary endpoint of the frequency of immunotherapy-related grade 3–4 adverse events within the first 12 weeks after the start of treatment. We followed up all patients for at least 12 weeks. Under the assumption that grade 3–4 toxicity would occur in 89% of patients treated in group A and in 50% of patients in groups B and C, we calculated that we would need 30 patients in each group to achieve 89% power at the two-sided α level 0·05 (based on Fisher's exact test). We based the 89% on the observed proportion of patients with grade 3–4 adverse events (89%) within the first 18 patients treated in the OpACIN trial.[25] We selected 50% as the highest proportion of patients with grade 3–4 adverse events in groups B and C that would be considered clinically acceptable to warrant further investigation of these dosing regimens in subsequent trials. Although the improvement in safety (a reduction in grade 3–5 adverse events) was the primary endpoint of the study (which was expected in group B and C compared with group A), our aim was that this improvement should not at the same time have a detrimental effect on efficacy (in terms of pathological response).

We applied a two-stage Simon's design to stop the trial early for futility in case of a low proportion of patients with a pathological response. We considered a proportion of 50% as futile and we tested against one-sided alternative of 75%. In the first stage, we required 13 patients in each group, after which we did an interim analysis. We closed a group if fewer than seven patients responded to treatment (based on the pathological response as assessed by local pathologists).

We planned to continue the study until 30 patients were included in each group. We required at least 21 of 30 patients in each group to achieve a pathological response in order claim that the proportion of patients with a pathological response was not futile. The interim and final results were discussed with the DSMB.

We did all analyses in all patients who received at least one dose of the study drug. We did not assess patients for pathological response if they did not have surgery and patients were not assessable for radiological response if they had not been radiologically assessed for response at week 6. We summarised the primary endpoints by the frequency and proportion per cohort with the two-sided 95% CIs, which we calculated using the Clopper-Pearson method. We calculated 95% CIs for difference in proportions of patients with grade 3–4 adverse events using an asymptotic method, and p values were calculated using Fisher's exact test. We estimated survival outcome curves (recurrence-free survival and event-free survival) for each group using the Kaplan-Meier method. Data for event-free survival and recurrence-free survival were censored at the last date of contact for patients with no evidence of disease.

We used the Kaplan-Meier method in an exploratory post-hoc analysis to compare recurrence-free survival in patients with and without a pathological response. We also did a descriptive exploratory analysis evaluating associations between baseline characteristics (including PD-L1 expression and IFN-γ signature gene expression) and pathological response and between maximum grade of toxic effect and pathological response. Analyses were done using R (version 3.3.1).

This trial is registered with ClinicalTrials.gov, number NCT02977052.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Nov 24, 2016 and June 28, 2018, 105 patients were screened for eligibility, of whom 89 (85%) were enrolled and randomly assigned to one of the three different combination dosing schedules (30 patients to group A, 32 to group B, and 27 to group C; figure 1). Following the report of a serious adverse event of colitis in group C requiring colectomy, the DSMB requested an ad-hoc interim safety analysis (April, 2018, with an update in May, 2018), and subsequently advised the

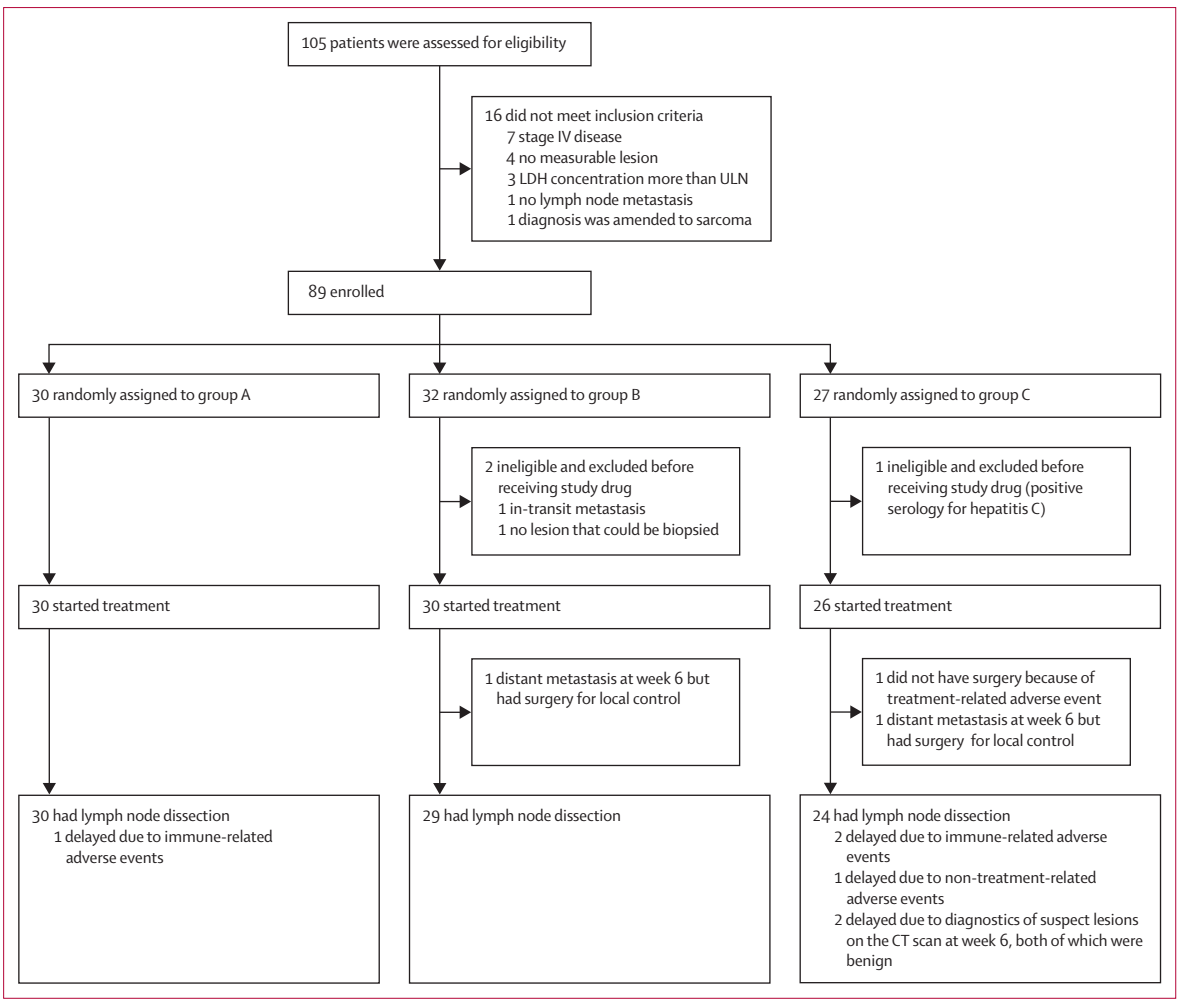

Figure 1: **Trial profile**
LDH=lactate dehydrogenase. ULN=upper limit of normal.

premature closure of group C (on June 4, 2018) because of a high incidence of severe adverse events, including five cases of grade 3 colitis and one case of grade 4 polyneuropathy (all requiring at least two lines of immune suppression and three of these patients required long-term hospital admission; for all serious events see appendix [p 3]). Therefore, a total of 86 patients, instead of the planned 90 patients, started treatment. Three patients (two assigned to group B and one assigned to group C) were excluded after randomisation but before receiving treatment because they were found to not meet the inclusion criteria by the central coordinating team (figure 1); thus, 30 patients in group A, 30 in group B, and 26 in group C actually started treatment and are included in this analysis.

Demographic and baseline characteristics (table 1), including primary tumour characteristics and lymph node metastasis burden, were similar between the groups, although group B had a higher proportion of women than the other two groups, and in group C a higher proportion of patients had a baseline PD-L1 expression of less than 1%. At data cutoff (Sept 28, 2018) median follow-up was 8·3 months (IQR 5·6–11·7) after randomisation, and all patients had completed the first 12 weeks after treatment initiation.

The two planned cycles of treatment were given to 26 (87%) of 30 patients in group A and 25 (83%) of 30 patients in group B. In group C, one (4%) patient received one single cycle of ipilimumab only, seven (27%) patients received two cycles of ipilimumab and one cycle of nivolumab, and 18 (69%) patients received all cycles of treatment. All treatment discontinuations were due to immune-related adverse events: in group A, four (13%) patients discontinued because of grade 3 elevated alanine aminotransferase (ALT; n=2), grade 3 rash (n=1), and grade 3 colitis (n=1); in group B, five (17%) discontinued due to grade 3 rash (n=1), grade 3 meningitis (n=1), grade 2 pneumonitis (n=1), grade 2 elevated ALT (n=1), and grade 2 arthralgia (n=1); and in group C, eight (31%) discontinued due to grade 3–4

	Group A (n=30)	Group B (n=30)	Group C (n=26)
Institute			
Netherlands Cancer Institute	15 (50%)	17 (57%)	14 (54%)
Melanoma Institute Australia	14 (47%)	12 (40%)	12 (46%)
Karolinska Institute	1 (3%)	1 (3%)	0
Age, years (IQR; range)	64 (41–71; 18–79)	54 (50–61; 31–74)	59 (42–62; 27–80)
Sex			
Male	19 (63%)	14 (47%)	16 (62%)
Female	11 (37%)	16 (53%)	10 (38%)
WHO performance status			
0	30 (100%)	29 (97%)	26 (100%)
1	0	1 (3%)	0
Primary tumour stage			
T1a	9 (30%)	8 (27%)	5 (19%)
T1b	3 (10%)	3 (10%)	1 (4%)
T2a	1 (3%)	2 (7%)	3 (12%)
T2b	0	2 (7%)	1 (4%)
T3a	2 (7%)	2 (7%)	3 (12%)
T3b	2 (7%	2 (7%)	3 (12%)
T4a	1 (3%)	0	1 (4%)
T4b	4 (13%)	2 (7%)	2 (8%)
Unknown primary	8 (27%)	9 (30%)	7 (27%)
Ulceration of primary tumour			
Yes	8 (27%)	7 (23%)	6 (23%)
No	14 (47%)	13 (43%)	12 (46%)
Unknown	8 (27%)	10 (33%)	8 (31%)
Sum of diameter target lesions, mm	23 (17–40)	25 (18–30)	24 (19–38)
Location of affected lymph node			
Neck	5 (17%)	3 (10%)	6 (23%)
Axilla	13 (43%)	17 (57%)	14 (54%)
Axilla and neck	3 (10%)	0	0
Groin	9 (30%)	9 (30%)	6 (23%)
Epitrochlear	0	1 (3%)	0
Pretreatment			
Sentinel node procedure	8 (27%)	8 (27%)	10 (38%)
Lymph node dissection	1 (3%)	3 (10%)	1 (4%)
Lactate dehydrogenase <ULN	30 (100%)	30 (100%)	25 (96%)
Absolute lymphocyte count >LLN	30 (100%)	29 (97%)	25 (96%)
C-reactive protein concentration <ULN	29 (97%)	26 (87%)	25 (96%)
PDL-1 expression on tumour cells			
<1%	13 (43%)	12 (40%)	16 (62%)
1–50%	9 (30%)	6 (20%)	4 (15%)
>50%	1 (3%)	4 (13%)	2 (8%)
Unknown	7 (23%)	8 (27%)	4 (15%)

Data are n (%) or median (IQR), unless otherwise specified. LLN= lower limit of normal. ULN=upper limit of normal.

Table 1: **Baseline characteristics**

colitis (n=4), grade 3 radiculitis (n=1), grade 3 rash (n=1), grade 2 colitis (n=1), and grade 2 fever (n=1).

Grade 3–4 immune-related adverse events within the first 12 weeks were reported in 12 (40%) patients in group A, in six (20%) patients in group B, and in 13 (50%) patients in group C (table 2), indicating that the group B regimen was the best tolerated. The difference in the proportion of patients with grade 3–4 immune-related adverse events between groups B and A was –20% (–46 to 6; p=0·158) and 10% (–20 to 40; p=0·591) between groups C and A. The most common grade 3–4 immune-related adverse events were increased ALT concentrations in group A (six [20%] of 30 patients), and colitis in group C (five [19%] of 26 patients). In group B, none of the observed grade 3–4 immune-related adverse events were seen in more than one (5%) patient (table 2). Immune-related serious adverse events occurred in five (17%) patients in group A, two (7%) in group B, and 13 (50%) in group C, of which the most common was colitis (five [19%]; appendix p 3). Any immune-related toxicity (of any grade) within the first 12 weeks was observed in 29 (97%) patients in group A, 29 (97%) in group B, and 26 (100%) in group C (table 2). Data for all immune-related adverse events that occurred up to data cutoff are provided in the appendix (p 4). Only two patients (one in group B and one in group C) developed a grade 3 or worse immune-related adverse event for the first time beyond 12 weeks after treatment initiation.

The frequency of surgery-related adverse events was similar across the three groups, and no cases were attributed to the neoadjuvant therapy (table 2). Surgery was delayed in one patient in group A and two patients in group C due to immune-related adverse events and was delayed in three additional patients in group C (one due to non-immune related adverse event and two due to need for diagnostics of suspect lesions on the CT scan at week 6, both of which turned out to be benign; figure 1; appendix p 5). One patient treated in group A died 9·5 months after the start of treatment, without disease recurrence, due to complications after a late-onset of immune-related encephalitis (camouflaged by an initially presumed stroke, hypophysitis associated hypothyroidism, and bacterial cholecystitis complicated by septic shock 8 weeks after surgery; at that timepoint the patient received low-dose steroids [10 mg prednisone once daily; steroids were initiated 11 weeks earlier with a maximum daily dose of methylprednisolone 1 g that was used for only a few days]).

Radiological objective responses were recorded in 45 (52%) of 86 patients overall: 19 (63% [95% CI 44–80]) of 30 patients in group A, 17 (57% [37–75]) of 30 in group B (notably, one patient in group B could not be assessed because the target lesion was not depicted on the CT performed at week 6), and nine (35% [17–56]) of 26 patients in group C (table 3). Progressive disease was observed in nine (10%) of 86 patients overall (two in group A, two in group B, and five in group C); seven of these patients had local progression only and had lymph

node dissection according to protocol, while two patients developed distant metastasis but nevertheless had palliative (partial) lymph node dissection for local tumour control (table 3).

Pathological response could be assessed for all patients in group A and B, and for 25 (96%) of 26 patients in group C. One patient in group C achieved a complete response on CT, but did not have surgery due to severe polyradiculitis and therefore pathological response assessment could not be done (this patient had not relapsed after 16 months follow-up after start of therapy). Pathological responses were observed in 64 (74%) of 86 treated patients, with a pCR in 37 (43%), a near-pCR in 15 (17%), a pPR in 12 (14%), and a pNR in 21 (24%) of 86 patients overall. A pathological response was observed in 24 (80% [95% CI 61–92]) patients in group A (14 [47%]

	Group A (n=30)			Group B (n=30)			Group C (n=26)		
	Grade 1–2	Grade 3	Grade 4*	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Immune-related adverse events									
Total number of patients with at least one immune-related adverse event†	17 (57%)	11 (37%)	1 (3%)	23 (77%)	5 (17%)	1 (3%)	13 (50%)	11 (42%)	2 (8%)
Fatigue	19 (63%)	0	0	17 (57%)	0	0	14 (54%)	0	0
Rash	16 (53%)	2 (7%)	0	10 (33%)	1 (3%)	0	15 (58%)	3 (12%)	0
Pruritus	12 (40%)	0	0	10 (33%)	0	0	9 (35%)	0	0
Elevated ALT concentration	6 (20%)	6 (20%)	0	5 (17%)	0	1 (3%)	7 (27%)	2 (8%)	0
Elevated AST concentration	7 (23%)	5 (17%)	0	5 (17%)	0	1 (3%)	7 (27%)	1 (4%)	0
Hyperthyroidism	12 (40%)	0	0	2 (7%)	0	0	8 (31%)	1 (4%)	0
Diarrhoea	6 (20%)	1 (3%)	0	3 (10%)	1 (3%)	0	8 (31%)	3 (12%)	0
Headache	7 (23%)	1 (3%)	0	5 (17%)	0	0	4 (15%)	0	0
Fever	4 (13%)	0	0	3 (10%)	1 (3%)	0	7 (27%)	0	0
Dry mouth	6 (20%)	0	0	3 (10%)	0	0	3 (12%)	0	0
Colitis	0	2 (7%)	0	1 (3%)	0	0	2 (8%)	4 (15%)	1 (4%)
Hypothyroidism	5 (17%)	0	0	2 (7%)	0	0	3 (12%)	0	0
Nausea	4 (13%)	0	0	1 (3%)	0	0	3 (12%)	1 (4%)	0
Arthralgia	2 (7%)	0	0	3 (10%)	0	0	3 (12%)	0	0
Dry eye	2 (7%)	0	0	3 (10%)	0	0	2 (8%)	0	0
Flu-like symptoms	1 (3%)	0	0	4 (13%)	0	0	2 (8%)	0	0
GGT increased	2 (7%)	2 (7%)	0	1 (3%)	1 (3%)	1 (3%)	0	0	0
Infusion related reaction	0	0	0	5 (17%)	0	0	2 (8%)	0	0
Abdominal pain	3 (10%)	0	0	0	0	0	3 (12%)	0	0
Anaemia	2 (7%)	0	0	1 (3%)	0	0	3 (12%)	0	0
Malaise	3 (10%)	0	0	3 (10%)	0	0	0	0	0
Serum amylase increased	2 (7%)	0	1 (3%)	1 (3%)	1 (3%)	0	1 (4%)	0	0
Anorexia	1 (3%)	0	0	2 (7%)	0	0	2 (8%)	0	0
Dysgeusia	1 (3%)	0	0	1 (3%)	0	0	3 (12%)	0	0
Lipase increased	1 (3%)	1 (3%)	0	2 (7%)	0	0	1 (4%)	0	0
Sarcoid-like reaction	0	0	0	3 (10%)	0	0	2 (8%)	0	0
Adrenal insufficiency	1 (3%)	0	0	0	0	0	1 (4%)	1 (4%)	0
Hyponatremia	0	0	0	1 (3%)	1 (3%)	0	0	1 (4%)	0
Radiculitis	0	0	0	1 (3%)	0	0	0	1 (4%)	0
Stomach pain	0	0	0	1 (3%)	0	0	0	1 (4%)	0
Diabetic ketoacidosis	0	0	0	0	0	0	0	1 (4%)	0
Blood bilirubin increased	0	1 (3%)	0	0	0	0	0	0	0
Hypotension	0	1 (3%)	0	0	0	0	0	0	0
Meningitis	0	0	0	0	1 (3%)	0	0	0	0
Peripheral motor neuropathy	0	0	0	0	0	0	0	0	1 (4%)

(Table 2 continues on next page)

	Group A (n=30)			Group B (n=30)			Group C (n=26)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
(Continued from previous page)									
Surgery-related adverse events									
Total number of patients with at least one surgery-related adverse event*	19 (63%)	4 (13%)	0	15 (50%)	4 (13%)	0	14 (44%)	4 (5%)	0
Seroma	13 (43%)	0	0	12 (40%)	0	0	7 (27%)	1 (4%)	0
Wound infection	9 (30%)	4 (13%)	0	6 (20%)	2 (7%)	0	7 (27%)	3 (12%)	0
Pain	4 (13%)	0	0	3 (10%)	0	0	3 (12%)	0	0
Lymphoedema	3 (10%)	0	0	4 (13%)	0	0	2 (8%)	0	0
Wound dehiscence	2 (7%)	0	0	2 (7%)	0	0	3 (12%)	0	0
Limb oedema	1 (3%)	0	0	3 (10%)	0	0	1 (4%)	0	0
Fever	2 (7%)	0	0	1 (3%)	0	0	2 (8%)	0	0
Chyle leakage	0	0	0	0	1 (3%)	0	0	0	0
Intraoperative haemorrhage	0	0	0	0	1 (3%)	0	0	0	0
Postoperative haemorrhage	0	0	0	0	0	0	0	1 (4%)	0

Data are n (%). Treatment-related adverse events that occurred in more than 5% of patients in the total cohort and all grade 3–4 adverse events are shown. ALT=alanine aminotransferase. AST=aspartate aminotransferase. GGT=gamma-glutamyltransferase. *Some patients had more than one event. Within the first 12 weeks no treatment-related deaths were observed. †One patient in group A died due to complications from late-onset immune-related encephalitis 9·5 months after treatment initiation.

Table 2: **Treatment-related adverse events during the first 12 weeks**

	Group A (n=30)	Group B (n=30)	Group C (n=26)
Radiological response			
Complete repsonse	2 (7%)	3 (10%)	1 (4%)
Partial response	17 (57%)	14 (47%)	8 (31%)
Stable disease	9 (30%)	10 (33%)	12 (46%)
Progressive disease	2 (7%)	2 (7%)	5 (19%)
Local progressive disease	2 (7%)	1 (3%)	4 (16%)
Distant metastasis	0	1 (3%)	1 (4%)
Not evaluable	0	1 (3%)*	0
Patients who achieved an objective response	19 (63% [44–80])	17 (57% [37–75])	9 (35% [17–56])
Pathological response			
Pathological complete response	14 (47%)	17 (57%)	6 (23%)
Near pathological complete response	7 (23%)	2 (7%)	6 (23%)
Pathological partial response	3 (10%)	4 (13%)	5 (19%)
Pathological non-response	6 (20%)	7 (23%)†	8 (38%)
Not evaluable	0	0	1 (4%)‡
Patients who achieved a pathological responses	24 (80% [61–92])	23 (77% [58–90])	17 (65% [44–83])

Data are n (%) or n (% [95% CI]). *For one patient the target lesion was not pictured on the CT images and could not be evaluated for response. †One patient had only palliative resection of largest lymph node. ‡Surgery was not done because of toxicity (severe polyradiculitis with peripheral motor neuropathy); this patient had a radiological complete response.

Table 3: **Response to treatment**

with a pCR), 23 (77% [58–90]) in group B (17 [57%] with a pCR), and 17 (65% [44–83]) in group C (six [23%] with a pCR; table 3). These results suggest that radiological response underestimated pathological response because the proportion of patients achieving objective responses, according to RECIST version 1.1, was 52% and the proportion of patients achieving pathological responses was 74% across all three groups (figure 2A). A post-hoc exploratory analysis showed that pathological response did not vary by demographic characteristics or tumour burden at baseline (appendix p 7). We also observed no significant association between the maximum grade of immune-related adverse events and proportion of patients who achieved pathological responses within any of the groups (appendix p 8).

Median event-free survival (post-hoc analysis; figure 2B) and recurrence-free survival (figure 2C) were not reached in any of the groups. Although these data are not mature, event-free and recurrence-free survival seem to be similar across the three groups (figure 2B, 2C). In group A two patients have relapsed and one patient has died, in group B one patient developed distant metastasis before surgery and three patients have relapsed, and in group C one patient developed distant metastasis before surgery and two patients have relapsed. None of the 64 patients who achieved a pathological response (pCR, near pCR, or pPR) have relapsed so far, whereas nine (43%) of 21 patients with pNR have relapsed (post-hoc analysis; n=7; figure 2D) or progressed before surgery (n=2). Two patients had a local relapse and were treated by surgery, seven patients had distant metastasis and were treated with systemic therapy. Two patients died during follow-up. One patient who achieved a pCR after treatment in group A died from complications from

late-onset immune-related encephalitis without melanoma relapse (figure 2C). One patient with a pNR after treatment in group A and who harboured a *NRAS* mutation died of melanoma, this patient did not receive any systemic therapy because she was ineligible for inclusion in an early-phase targeted therapy trial due to fast clinical deterioration.

Baseline biopsy samples were analysed for the presence of an IFN-γ signature with the use of NanoString panCancer Immune Panel analysis. In an exploratory analysis, two (6%) of 32 patients clustering into the IFN-γ signature high subgroup have relapsed, while four (19%) of 21 patients clustering into IFN-γ signature intermediate subgroup, and three (25%) of 12 patients clustering into IFN-γ signature low subgroup have relapsed. Five (42%) of 12 patients clustering into the IFN-γ low subgroup had no pathological response compared with five (24%) of 21 patients in the intermediate subgroup and seven (22%) of 32 patients in the high subgroup (appendix p 9). PD-L1 expression on tumour cells was not associated with pathologic response (appendix p 7). In our previous OpACIN trial the IFN-γ signature analysed by RNA sequencing attributed the relapses more precisely. Therefore, we reanalysed these samples by the NanoString gene-expression analysis on available patient samples from the OpACIN trial (n=18). The comparison of these two techniques revealed high concordance in assignment of tumour samples with a high IFN-γ signature. Discordance was observed in two (11%) of 18 cases, both discordant samples had a low or intermediate IFN-γ signature (appendix p 10).

Discussion

The results of the OpACIN-neo study show that the treatment regimen in group B—two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg—seems to be the best tolerated dosing schedule of neoadjuvant ipilimumab and nivolumab, while still inducing a pathological response in the same high proportion of patients with high-risk, nodal, resectable stage III melanoma as standard dosing. Thus, OpACIN-neo met its primary objective by identifying a combination schedule that is

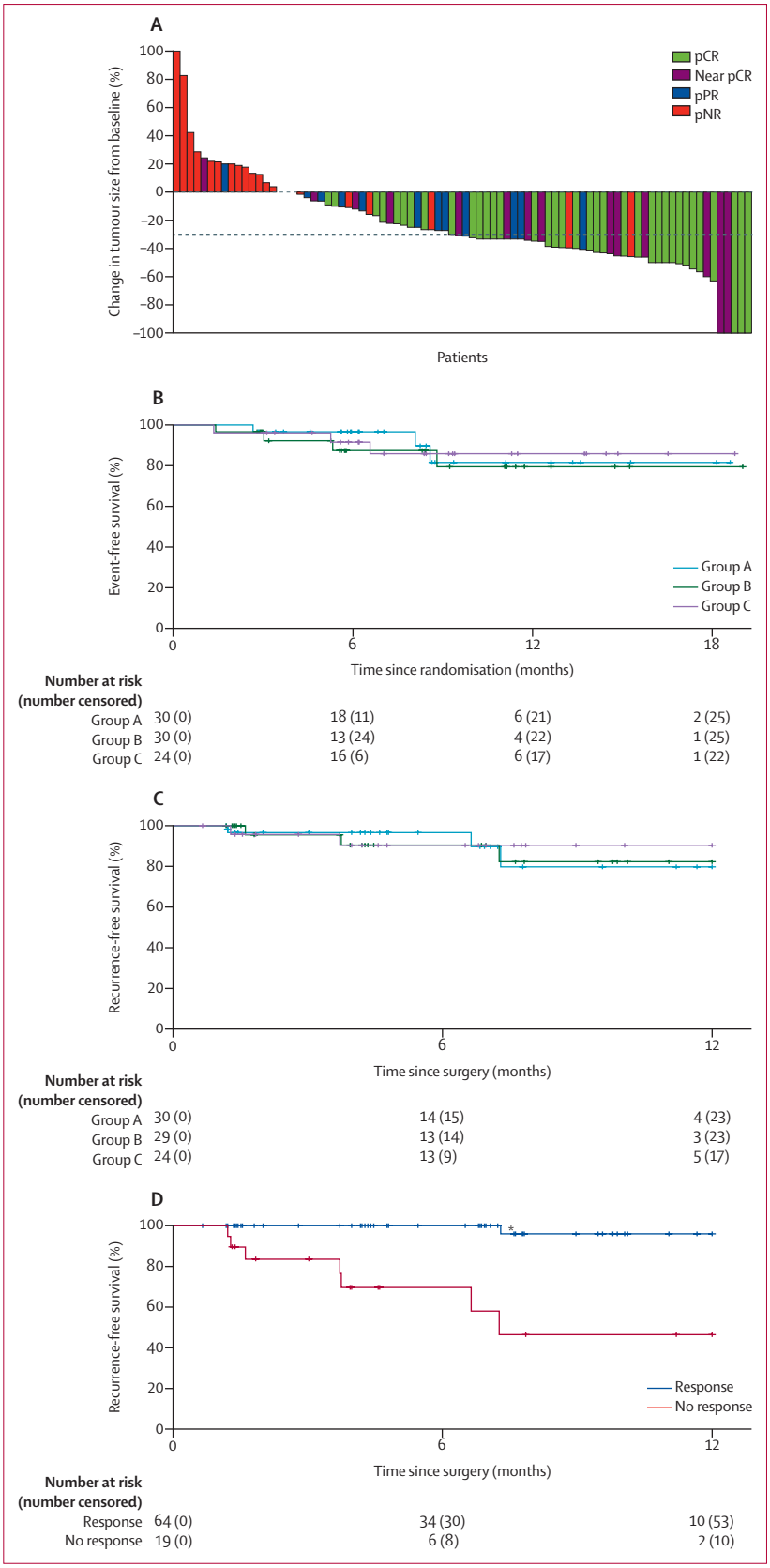

Figure 2: **Antitumour activity, event-free survival, and recurrence-free survival**

(A) Best percentage change from baseline to week 6, calculated by subtracting the sum of the longest diameters of the target lesions in patients who had a CT scan at baseline and week 6. We truncated changes from baseline of more than 100% at 100%. The dashed line represents a reduction in tumour size of more than 30% according to RECIST version 1.1. One patient did not have surgery due to toxicity; two patients already had distant metastasis before surgery (at week 6) and are therefore not included in the relapse-free survival analysis. (B) Event-free survival. (C) Recurrence-free survival. (D) Recurrence-free survival according to pathological response. *One patient who achieved a pCR after treatment in group A died due to complications from late-onset immune-related encephalitis without melanoma relapse. Near pCR=near pathological complete response. pCR=pathological complete response. pNR=pathological non-response. pPR=pathological partial response.

substantially less toxic than the previously observed proportions of 73–90% grade 3–4 adverse events upon neoadjuvant application of the standard ipilimumab plus nivolumab dosing schedule.[14,15] Furthermore, with a median follow-up of more than 8 months, none of the patients with a pathological response (n=64) have relapsed across all groups. To the best of our knowledge, OpACIN-neo is the largest trial so far to assess neoadjuvant CTLA-4 plus PD-1 blockade (ipilimumab plus nivolumab) in patients with resectable, stage III melanoma.

Despite being immature for recurrence-free survival analysis, this trial is already mature for toxicity analysis. Toxicity within the frist 12 weeks was chosen as primary endpoint because in 18 (90%) of 20 patients in our previous trial (OpACIN) the highest grade adverse events were observed within the first 12 weeks.[14] Indeed, so far, in OpACIN-neo only two patients developed for the first time grade 3 or worse imune-related adverse events beyond 12 weeks after treatment initiation. The proportion of patients in groups A and B with grade 3–4 immune-related adverse events did not differ significantly because the proportion of patients with grade 3–4 adverse events in group A was lower than anticipated. This outcome might be due to sampling errors in the previous trials,[14,15] possibly overestimating the occurrence of grade 3–4 immune-related adverse events. Moreover, in the present study, patients in group A received only a maximum of two cycles of combination therapy, instead of a maximum of three to four cycles in the previous studies.[14,15] The lower proportion of patients with grade 3–4 immune-related adverse events within group B compared with the other two groups indicates that the dose level of ipilimumab seems to be the dose-limiting factor in stage III melanoma treatment (since the dose of ipilimumab was only 1 mg/kg in group B *vs* 3 mg/kg in groups A and C). In line with this observation, ipilimumab 10 mg/kg as adjuvant therapy induced grade 3–4 adverse events in a higher proportion of patients with stage III melanoma compared with stage IV disease (54% *vs* 37%),[3,26] whereas for nivolumab the proportions of patients who developed grade 3–4 adverse events were similar for stage III and stage IV disease (14% *vs* 16%).[5,8] Within group C, we observed a higher proportion of patients with grade 3–4 immune-related adverse events than expected—even higher than for the standard dosing regimen (group A). We do not have a clear explanation for this observation, and translational analyses to gain more insights into this issue are underway. The reduction in the number of cycles and reduction of the dose of ipilimumab identified a combination scheme (group B) with an acceptable toxicity profile, without impairing the proportion of patients achieving a pathological response and thus is likely to be suitable for a broader application.

OpACIN-neo confirms in a larger patient cohort the high proportion of patients with radiological and pathological responses seen in previous, small, early-phase trials testing neoadjuvant ipilimumab plus nivolumab.[14,15] In line with our previous OpACIN trial, the radiological response again underestimated the pathological response. Additionally, although follow-up is still short, this trial showed once more that patients achieving a pathological response after neoadjuvant checkpoint inhibition have a low risk of relapse, consistent with findings in previous early-phase studies.[14,15,18] This observation is in contrast to immune checkpoint inhibition in late-stage melanoma, in which a substantial proportion of patients develop disease progression after initial response, including a small subset of those who had a complete response, albeit after longer follow-up.[10,27] A possible (thus far unproven) conceptual interpretation of these data is that expression of T-cell checkpoint molecules is the dominant parameter within the cancer immunogram interfering with tumour control in patients with stage III melanoma,[28] whereas other parameters, such as human leukocyte antigen loss or systemic immune suppression, become of increasing importance for patients with stage IV disease.

In terms of efficacy outcomes, this study was limited to reporting proportions of radiological and pathological responses and patients who have relapsed within each of the groups but was not powered to compare efficacy between the groups. Despite this, efficacy seems numerically similar between groups A and B.

The OpACIN trials, in addition to the trials of our colleagues,[15,18] form the basis for a possible new framework in the treatment of high-risk, measurable, stage III melanoma—namely, the administration of a short course of (combined) checkpoint inhibition before potentially curative surgery. By contrast with adjuvant therapy, neoadjuvant checkpoint inhibition has several advantages. First, the presence of the full tumour mass at the start of immunotherapy allows the induction of a broader and stronger T-cell response.[14] Second, the response to therapy can be established in each individual patient. This response provides information about individual prognosis (allowing tailored follow-up) and guidance for possible additional adjuvant therapies (such as dabrafenib and trametinib for patients not responding to neoadjuvant therapy and harbouring a $BRAF^{V600}$ mutation). Third, extensive surgery might conceivably be omitted in patients who achieve a pCR or near pCR. This hypothesis is being tested in the PRADO extension cohort (NCT02977052) in which an extra 100 patients will be given the most favourable dose schedule (the group B schedule: two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg). After neoadjuvant therapy, all these patients will first undergo resection of one marked lymph node only. The patients with a pCR or near pCR within this lymph node will be spared lymph node dissection and subsequent adjuvant systemic therapy. This approach might reduce morbidity, improve quality of life, and be more cost effective. Finally, and from a more translational research perspective, by providing high-quality datasets from

homogenous patient populations, trials such as OpACIN-neo form a useful platform to investigate primary resistance to checkpoint inhibitor combinations or other novel treatments, which is an unmet need for patients with stage III and IV melanoma. By defining baseline biomarkers that are associated with response and recurrence-free survival upon neoadjuvant checkpoint inhibition, such studies could facilitate the development of truly personalised immunotherapy in stage III and IV disease.

A first explorative biomarker analysis performed on pretreatment tumour biopsy samples showed that IFN-γ signature (based on gene expression analysis with NanoString nCounter) was associated with relapse status, and therefore might potentially be a good biomarker for the outcome upon neoadjuvant ipilimumab plus nivolumab. These data are in line with data from the OpACIN trial[14] that used RNA sequencing to assess IFN-γ signatures, and showed that none of the patients with a high or intermediate IFN-γ signature had relapsed. However, in the present study, the discriminatory power of the IFN-γ signature was not absolute and the association with pathological response was less strong than the association with relapse status. A possible explanation might be the use of different techniques for determining the IFN-γ signatures. Comparison of RNA sequencing and NanoString gene-expression revealed concordance in assignment of tumour samples as high IFN-γ signatures, but discordance was observed in assignment of tumour samples as low or intermediate IFN-γ signatures. Nevertheless, if the association with relapse status holds true after additional follow-up, novel, alternative, neoadjuvant combination regimens should thus be tested preferentially in patients with a low IFN-γ signature. Additional biomarker research in this trial is ongoing, with the focus on finding a marker that is even more discriminating and predictive for pathological response.

In conclusion, OpACIN-neo identified a short course (only 6 weeks), well tolerated, neoadjuvant combination scheme of two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg (group B) that induces a pathological response in a high proportion of patients, including a high proportion of pCRs. At data cutoff, none of the patients with a pathological response had relapsed. If more mature data from this trial remain in line with these early results, neoadjuvant ipilimumab plus nivolumab versus standard adjuvant therapy should be tested in phase 3 randomised trials.

Contributors

CUB designed the study and wrote the study protocol. ACJvA and TNS provided additional input to the study design. EAR, AMM, ACJvA, JVvT, ADG, StM, AMK, KSh, SC, HE, JS, JBAGH, OEN, WMCK, CLZ, RPMS, WJvH, AJS, JH, GVL, and CUB recruited and treated patients and collected data. CA, CB, BAvdW, and RAS reviewed and scored the pathology of all cases, including grading pathological responses. OK performed, under the supervision of DSP, the bioinformatics analysis. KSi and HvT did the statistical analysis. MG recruited patients and collected data. AB coordinated and contributed to translational laboratory logistics and immunohistochemistry and molecular laboratory work. ATA and LGG-O contributed to central and local data management. LJB coordinated NanoString gene expression analysis. LMP and MG were clinical project managers of the trial. SH scored radiological response. EAR and CUB wrote the first draft of the manuscript. TNS and GVL co-wrote the manuscript. All authors interpreted the data, reviewed the manuscript, and approved the final version.

Declaration of interests

EAR received travel support from Merck Sharpe & Dohme (MSD) and NanoString. AMM reports personal fees as a consultant advisor for Bristol Myers Squibb (BMS), MSD, Novartis, Roche, and Pierre-Fabre. ACJvA reports personal fees as a consultant advisor for Amgen, BMS, Novartis, MSD Merck, Merck–Pfizer, and 4SC, and received grant support from Amgen, BMS, and Novartis all paid to the institution (Netherlands Cancer Institute). RAS reports personal fees as a consultant advisor for MSD, Neracare, Myriad, and Novartis. OK reports grant support from BMS. JVvT has served as a consultant adviser for Pfizer and Novartis, for which the institution (Netherlands Cancer Institute) received funding. ADG received travel support from Merck KgA and Sun Pharma and has served as a consultant advisor for BMS, Pfizer, Merck KgA, Regeneron, and Sun Pharma. JBAGH has served as a consultant advisor for BMS, MSD, Pfizer, AstraZeneca–MedImmune, Roche–Genentech, Ipsen, Bayer, Immunocore, Novartis, Seattle Genetics, Neon Therapeutics, Celsius Therapeutics, Gadet, and GlaxoSmithKline (GSK), for which the institution (Netherlands Cancer Institute) received funding, and has received grant support from BMS, MSD, Novartis, and Neon Therapeutics. DSP reports grant support from BMS. JH reports personal fees as a consultant advisor for BMS, MSD, and Novartis. TNS has served as a consultant advisor for Adaptive Biotechnologies, AIMM Therapeutics, Allogene Therapeutics, Amgen, Merus, Neon Therapeutics, and Scenic Biotech; is recipient of grant and research support from MSD, BMS, and Merck KGaA; is a stockholder in AIMM Therapeutics, Allogene Therapeutics, Merus, Neogene Therapeutics, and Neon Therapeutics; and is a venture partner at Third Rock Ventures. GVL reports personal fees as a consultant advisor to Aduro, Amgen, BMS, Mass Array, MSD, Novartis, Oncosec, Pierre-Fabre, and Roche. CUB reports personal fees as a consultant advisor for BMS, MSD, Roche, Novartis, Lilly, Pfizer, GSK, GenMab, and Pierre Fabre for which the institution (Netherlands Cancer Institute) received funding, and has received research grants from BMS, Novartis, and NanoString all paid to the institution (Netherlands Cancer Institute). All other authors declare no competing interests.

Data sharing

De-identified data will be available on request after all primary and secondary endpoints have been analysed and published, and after signing of a data transfer agreement with the Netherlands Cancer Institute. Requests for data sharing can be made to the corresponding author, including a research proposal that must be approved by the principal investigators of all participating centres.

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