Personalized response-directed surgery and adjuvant therapy after neoadjuvant ipilimumab and nivolumab in high-risk stage III melanoma: the PRADO trial

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Neoadjuvant ipilimumab and nivolumab induces high pathologic response rates (pRRs) in clinical stage III nodal melanoma, and pathologic response is strongly associated with prolonged relapse-free survival (RFS). The PRADO extension cohort of the OpACIN-neo trial (NCT02977052) addressed the feasibility and effect on clinical outcome of using pathologic response after neoadjuvant ipilimumab and nivolumab as a criterion for further treatment personalization. In total, 99 patients with clinical stage IIIb–d nodal melanoma were included and treated with 6 weeks of neoadjuvant ipilimumab 1 mg kg^{-1} and nivolumab 3 mg kg^{-1}. In patients achieving major pathologic response (MPR, ≤10% viable tumor) in their index lymph node (ILN, the largest lymph node metastasis at baseline), therapeutic lymph node dissection (TLND) and adjuvant therapy were omitted. Patients with pathologic partial response (pPR; >10 to ≤50% viable tumor) underwent TLND only, whereas patients with pathologic non-response (pNR; >50% viable tumor) underwent TLND and adjuvant systemic therapy ± synchronous radiotherapy. Primary objectives were confirmation of pRR (ILN, at week 6) of the winner neoadjuvant combination scheme identified in OpACIN-neo; to investigate whether TLND can be safely omitted in patients achieving MPR; and to investigate whether RFS at 24 months can be improved for patients achieving pNR. ILN resection and ILN-response-tailored treatment were feasible. The pRR was 72%, including 61% MPR. Grade 3–4 toxicity within the first 12 weeks was observed in 22 (22%) patients. TLND was omitted in 59 of 60 patients with MPR, resulting in significantly lower surgical morbidity and better quality of life. The 24-month relapse-free survival and distant metastasis-free survival rates were 93% and 98% in patients with MPR, 64% and 64% in patients with pPR, and 71% and 76% in patients with pNR, respectively. These findings provide a strong rationale for randomized clinical trials testing response-directed treatment personalization after neoadjuvant ipilimumab and nivolumab.

Adjuvant immune checkpoint inhibition (CPI) and BRAF/MEK-targeted therapies after therapeutic lymph node dissection (TLND) have improved relapse-free survival (RFS) in patients with clinical stage III nodal melanoma. Despite these improvements, approximately 40–50% of patients have a relapse within 3–5 years after TLND[1–3]. Preclinical and early clinical trial data suggest that neoadjuvant CPI leads to superior anti-tumor immunity and survival benefit compared to adjuvant CPI[4,5]. Similarly to stage IV melanoma, the combination of anti-CLTA-4 and anti-PD-1 appears to be superior to anti-PD-1 monotherapy in the neoadjuvant setting[6,7]. Previous clinical trials (OpACIN (NCT02437279) and OpACIN-neo (NCT02977052)) testing neoadjuvant ipilimumab (anti-CTLA-4) plus nivolumab (anti-PD-1) in stage III melanoma demonstrated high pathologic response rates (pRRs; 74–78%) and a strong association between pathologic response and RFS, with 94–100% of responding patients remaining free of relapse at 2 years[5,7–9]. Similarly, long-term benefit was observed upon complete response to CPI in stage IV melanoma, even after cessation of CPI[10–12].

The association between response and survival; the observed ongoing responses after cessation of therapy in stage IV melanoma; and the substantial morbidity from TLND[13–16] that impairs

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health-related quality of life (HRQoL)[17,18] raised the question of whether TLND could be safely omitted in patients with major pathologic response (MPR, ≤10% viable tumor) after neoadjuvant CPI. Furthermore, we hypothesized that the addition of adjuvant systemic therapy ± adjuvant radiotherapy in patients with pathologic non-response (pNR) (>50% viable tumor) might reduce the relapse rate as compared to non-responding patients from previous neoadjuvant trials who did not receive adjuvant therapy[5,8].

In two previous studies, we demonstrated that the pathologic response in the index lymph node (ILN, the largest lymph node metastasis at baseline) was a reliable indicator of the response to neoadjuvant ipilimumab and nivolumab in the entire TLND specimen of stage III nodal melanoma[19,20].

This multicenter phase 2 PRADO expansion cohort (NCT02977052) of the OpACIN-neo trial investigated the role of assessing pathologic response in only the ILN to determine subsequent management, including TLND and adjuvant therapy. After baseline marker placement in the ILN, patients were treated with two cycles of ipilimumab 1 mg kg^{-1} plus nivolumab 3 mg kg^{-1} in week 0 and week 3, followed by ILN resection at week 6. Patients achieving MPR in the ILN did not undergo subsequent TLND or adjuvant treatment. Patients with pPR (>10–≤50% viable tumor) underwent TLND without adjuvant treatment, whereas patients with pNR (>50% viable tumor) underwent TLND and adjuvant nivolumab (*BRAF* wild-type tumors) or BRAF/MEK inhibitors (*BRAF*$^{V600E/K}$-mutated tumors) for 52 weeks ± local radiotherapy (Fig. 1a). Co-primary endpoints were pRR, 24-month RFS for patients achieving MPR and 24-month RFS for patients achieving pNR.

We report the first results from PRADO, including the efficacy and safety of neoadjuvant ipilimumab 1 mg kg^{-1} plus nivolumab 3 mg kg^{-1}; the feasibility of ILN resection and pathologic assessment; the effects of TLND and/or adjuvant therapy omission on morbidity and HRQoL; and the 24-month survival data after response-driven tailored treatment.

Results

In the PRADO trial, 99 patients with clinical stage III nodal melanoma and measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 were enrolled between November 2018 and January 2020 (Fig. 1b). Median age was 58 years; 65 (66%) patients were male; 45 (45%) patients had a *BRAF*^{V600} mutation; and 42 (42%) patients had more than one fluorodeoxyglucose-positive lymph node on positron emission tomography (PET)–computed tomography (CT) at baseline (Table 1). The ILN was marked pre-treatment using ultrasound guidance with a magnetic seed (53%), nitinol marker (34%), radioactive I-125 seed (9%) or hydrogel marker (4%) (Extended Data Fig. 1). At data cutoff (7 February 2022), the median follow-up from date of registration was 28.1 months (interquartile range (IQR), 25.0–33.8), with a minimum follow-up of 23.4 months for all patients alive.

Immunotherapy-related adverse events. In total, 89 (90%) patients received two scheduled treatment cycles, whereas ten (10%) patients received only one cycle due to immunotherapy-related adverse events (irAEs) (Fig. 1b). Grade 3–4 irAEs within the first 12 weeks were observed in 22 patients (22%; 95% confidence interval (CI): 14–32%) (Table 2). The most prevalent grade 3–4 irAEs were increased alanine transaminase (ALT) levels ($n=7$, 7%), increased aspartate aminotransferase (AST) levels ($n=6$, 6%) and diarrhea/colitis ($n=5$, 5%/$n=4$, 4%). No treatment-related deaths were observed. Grade 3–4 irAEs occurred in 30 (30%) patients up to the data cutoff, with increase of serum lipase levels being the most prevalent grade 3–4 toxicity ($n=9$, 9%) (Supplementary Table 1). Two patients with adjuvant therapy and six patients without adjuvant therapy developed their grade 3–4 irAE beyond week 12.

Radiologic response. At 6 weeks after the start of neoadjuvant CPI, pre-surgical CT showed a RECIST version 1.1 radiologic response in 45 (45%, 95% CI: 35–56%) patients and stable disease in 38 (38%) patients, resulting in a disease control rate of 84% (Fig. 2a); three patients (3%) were evaluated at later time points than as per protocol due to irAEs. Radiologic progression occurred in 13 (13%) patients, including seven (7%) patients with regional disease progression only who underwent the ILN resection according to protocol and six (6%) patients with distant metastases (of whom four had regional progressive disease and two had stable disease on CT).

Feasibility of ILN resection after neoadjuvant CPI. Of the 93 patients without distant metastases at week 6, 90 underwent a resection of the ILN; two proceeded direct to TLND (which was also delayed) due to grade 3–4 irAEs; and one did not undergo any surgery due to irAEs (Fig. 1b and Supplementary Table 2). Additionally, four of the six patients with distant metastases still underwent the ILN resection for regional control, resulting in a total of 94 of 99 patients (95%) undergoing the ILN resection (Fig. 1b). Grade 3–4 irAEs caused delays in the ILN resection in three patients (Supplementary Table 2).

Histopathologic assessment demonstrated that the marked ILN was successfully resected (that is, the marker was in the resection specimen) during the ILN resection in 90 of 94 (96%) patients at first attempt (Supplementary Table 2). In two patients, the ILN was resected during secondary surgery after it was noticed that the marked ILN was missing from the initial resected specimen. Additional lymph nodes other than the ILN (median 1, range 1–6) were resected in 38 (40%) patients, mainly due to localization in front of, or adjacent to, the ILN (Supplementary Table 2).

Pathologic response. Pathologic response was assessed based on the resection specimen of the ILN resection, except for the two patients who only underwent TLND and had no ILN resection due to irAEs (Fig. 1b). Response percentages were calculated over the total cohort of 99 patients. Pathologic responses were observed in 71 of 99 (72%; 95% CI: 62–80%) patients, including 48 (49%) with pathologic complete response (pCR) and 12 (12%) with near-pCR, resulting in an MPR rate of 61% (96% CI: 50–70%) (Fig. 2b). Partial responses were found in 11 (11%) patients. Thus, the radiologic response rate (45%) underestimated the pRR (72%), similarly to findings in previous trials[6,8,21] (Extended Data Fig. 2). Exploratory analyses showed that pathologic response was not associated with tumor burden at baseline or other demographics (Fig. 2c). In addition, no association was found between maximum-grade irAEs during the first 12 weeks and pathologic response (Supplementary Table 3). PD-L1 expression in baseline tumor biopsies was associated with pathologic response; the pRR was 56% in tumors with <1% PD-L1-expressing tumor cells, 92% in tumors with 1–50% PD-L1-expressing tumor cells and 100% in tumors with >50% PD-L1-expressing tumor cells ($P=0.004$) (Fig. 2c).

Response-directed tailored treatment. Based on the pathologic response assessment in the ILN, TLND was omitted in 59 of the 60 patients who achieved MPR at week 6. One patient underwent TLND despite having a near-pCR due to the presence of extranodal extension and viable tumor in the ILN surgical margins (Fig. 1b and Supplementary Table 2). Additionally, two patients with MPR had additional lymph nodes resected during follow-up surgery due to radiologically suspected residual disease on postoperative imaging (these additional lymph nodes showed pCR in both patients). Eight of the 11 patients with pPR underwent TLND (Fig. 1b and Supplementary Table 4); two patients refused TLND; and one patient had no TLND because of suspected distant metastases that later were diagnosed as pulmonary sarcoid-like reaction. All 19 patients who had pNR in the ILN and no distant metastases at

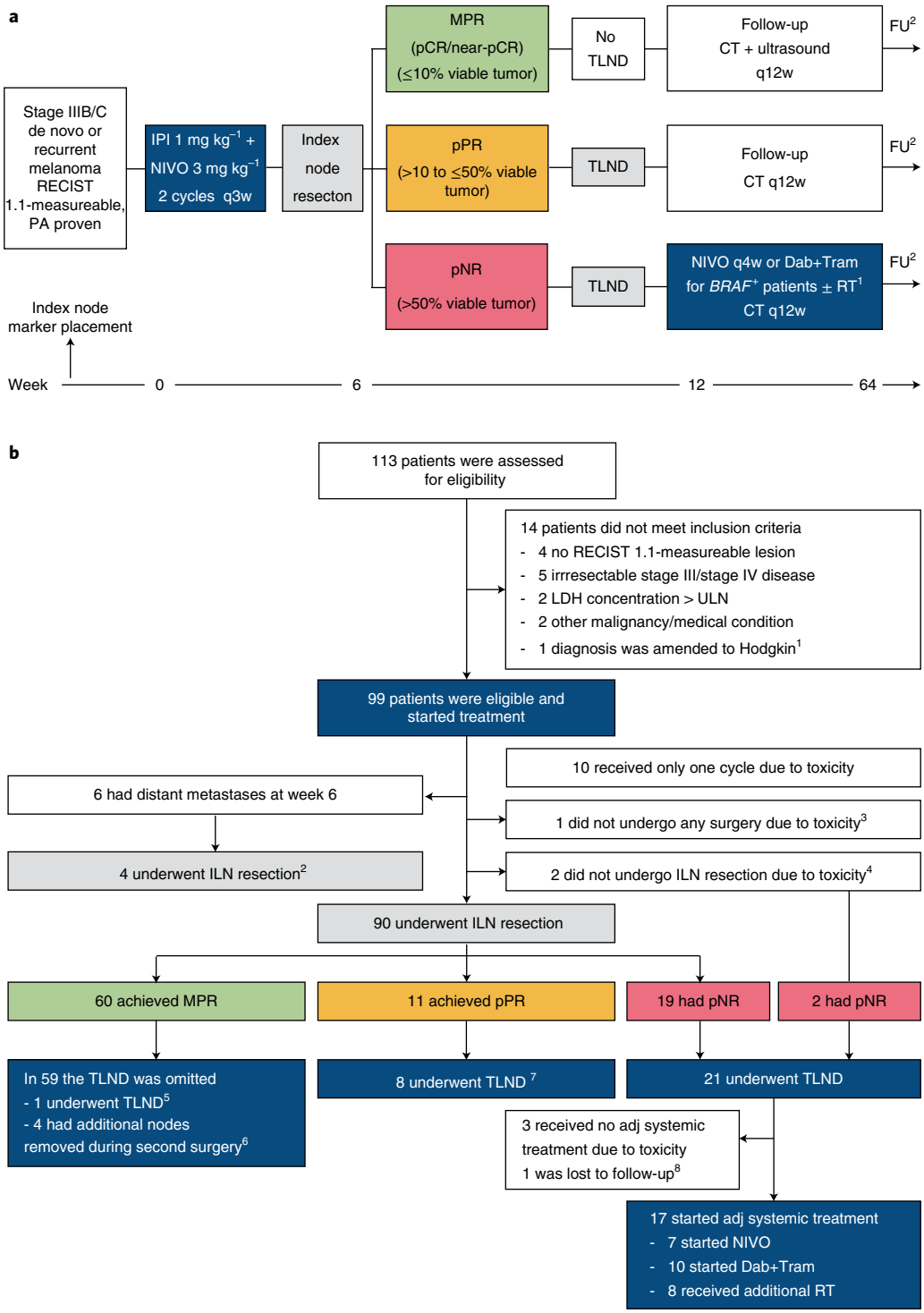


Fig. 1 | Study scheme and flowchart of PRADO. a, Study design of the PRADO trial. (1) Adjuvant radiotherapy according to patient and physician decisions and (2) according to institute standards. **b**, Flow chart of the PRADO trial. [1]For one patient, the diagnosis of melanoma was amended to Hodgkin lymphoma based on his pre-treatment tumor biopsy after inclusion into the trial. This patient went off study and was excluded from the data analyses. [2]Four patients with distant metastases underwent the ILN procedure for regional control ($n=3$) or because the distant metastases on CT were retrospectively identified after the ILN procedure ($n=1$). [3]One patient developed a myelitis transversa-like syndrome leading to constipation and colon perforation. This patient had a radiologic response. [4]Two patients had their ILN resected during TLND; their pathologic response assessment is based on the TLND. [5]One patient had a TLND despite achieving near-pCR due to viable tumor in the ILN margins, including extranodal extension. [6]Two patients had extra lymph nodes removed in a second surgery because of remaining suspect lymph nodes on postoperative CT scan. Second surgery showed no viable tumor. Two other patients had minor secondary surgery performed for removal of the marked ILN. [7]Two patients refused to undergo TLND after achieving pPR, and one patient did not have a TLND due to a pulmonary sarcoid-like reaction that was initially regarded as progressive disease. [8]All patients were *BRAF* wild-type; three patients did not receive adjuvant nivolumab due to an immunotherapy-related colitis, cholangitis and arthritis; and one patient was lost to follow-up. adj, adjuvant; Dab, dabrafenib; FU, follow-up; IPI, ipilimumab; NIVO, nivolumab; PA, pathology; RT, radiotherapy; Tram, trametinib; ULN, upper limit of normal.

Table 1 | Baseline characteristics of PRADO

Characteristic	Total cohort ($n=99$)	
Institute		
NKI	52	(53%)
MIA	34	(34%)
LUMC	5	(5%)
EMC	4	(4%)
UMCU	3	(3%)
UMCG	1	(1%)
Age, years (median, IQR)	58	(51.5–69.5)
Sex		
Men	65	(66%)
Women	34	(34%)
ECOG performance status score		
0	94	(95%)
1	5	(5%)
Primary tumor stage		
T1a/b	17	(17%)
T2a/b	26	(26%)
T3a/b	20	(20%)
T4a/b	21	(21%)
Tx	2	(2%)
Unknown primary	13	(13%)
Ulceration of primary tumor		
Yes	23	(23%)
No	58	(69%)
Unknown	18	(18%)
Location of affected lymph node		
Neck	24	(24%)
Axilla	39	(39%)
Groin	36	(36%)
Number of positive lymph nodes on PET-CT		
1	57	(58%)
>1–3	33	(33%)
>3	9	(9%)
Sum of diameter target lesions, mm (median, IQR)	25	(18–33)
Previous treatment		
Sentinel node procedure	24	(24%)
Lymph node dissection	2	(2%)
BRAF$^{V600E/K}$ mutation		
Yes	45	(45%)
No	43	(43%)
VE1 negative[a]	9	(9%)
Unknown	2	(2%)
LDH < ULN	97	(98%)

Data are median (IQR) or *n* (%). Percentages may not sum up to 100 because of rounding. [a]For these patients, the presence of *BRAF*^{V600E} mutation was assessed only by VE1 staining and negative (they achieved MPR, and no tumor material was left for formal testing). ECOG, Eastern Cooperative Oncology Group; ULN, upper limit of normal.

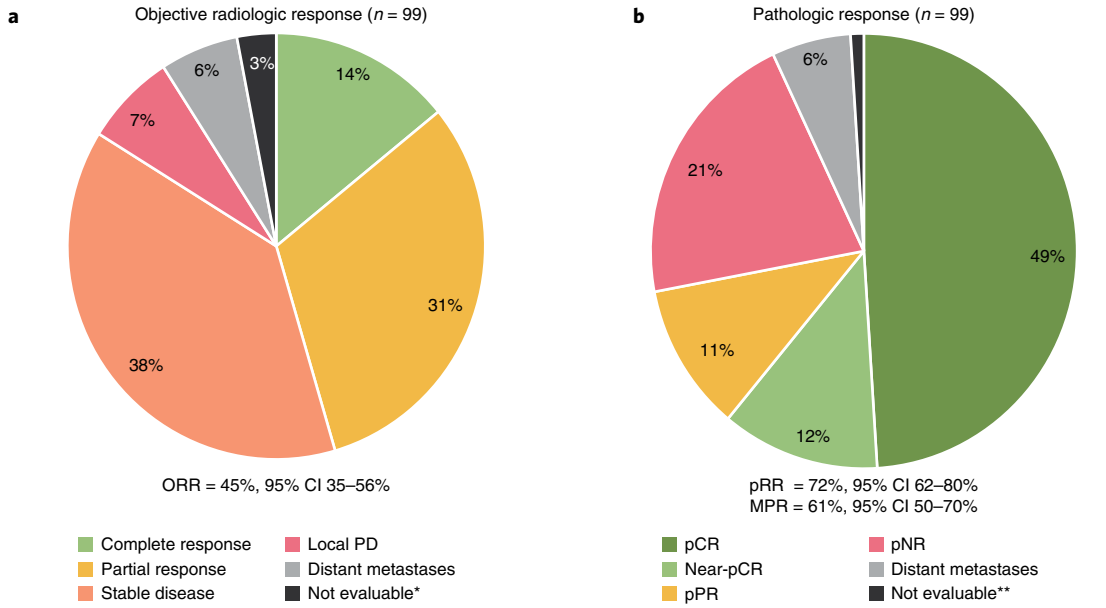
Table 2 | Immunotherapy-related adverse events within the first 12 weeks

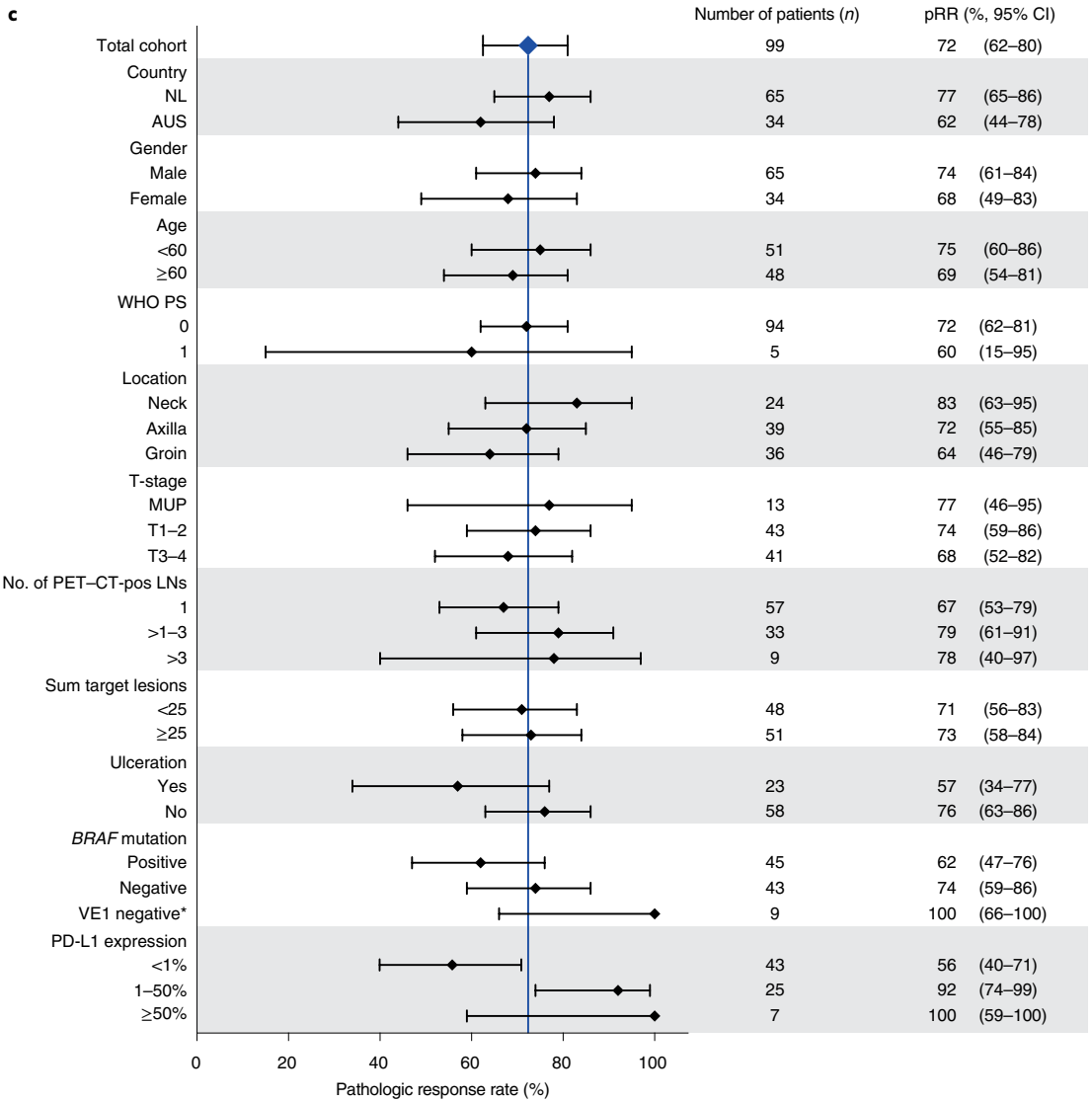
Immunotherapy-related adverse events	Total cohort ($n=99$)					
	Grade 1–2		Grade 3–4		Total	
Total number of patients with at least one adverse event[a]	74	(75%)	22	(22%)	96	(97%)
Fatigue	54	(55%)	0		54	(55%)
Rash	47	(47%)	3	(3%)	50	(51%)
Pruritus	28	(28%)	0		28	(28%)
Hyperthyroidism	23	(23%)	0		23	(23%)
ALT increased	15	(15%)	7	(7%)	22	(22%)
Diarrhea	17	(17%)	5	(5%)	22	(22%)
AST increased	13	(13%)	6	(6%)	19	(19%)
Nausea	18	(18%)	1	(1%)	19	(19%)
Dry mouth	17	(17%)	0		17	(17%)
Arthralgia	16	(16%)	0		16	(16%)
Hypothyroidism	16	(16%)	0		16	(16%)
Headache	12	(12%)	1	(1%)	13	(13%)
Myalgia	10	(10%)	0		10	(10%)
Infusion related reaction	8	(8%)	0		8	(8%)
Serum lipase increased	5	(5%)	3	(3%)	8	(8%)
Dry skin	7	(7%)	0		7	(7%)
Fever	7	(7%)	0		7	(7%)
Colitis	2	(2%)	4	(4%)	6	(6%)
Creatine kinase increased	5	(5%)	1	(1%)	6	(6%)
Dry eye	6	(6%)	0		6	(6%)
Dyspnea	5	(5%)	0		5	(5%)
Serum amylase increased	3	(3%)	1	(1%)	4	(4%)
GGT increased	1	(1%)	1	(1%)	2	(2%)
Myocarditis	0		2	(2%)	2	(2%)
Cholangitis	0		1	(1%)	1	(1%)
Functional decline[b]	0		1	(1%)	1	(1%)
Myelitis transversa-like syndrome	0		1	(1%)	1	(1%)

Data are *n* (%). Immunotherapy-related adverse events that occurred in more than 5% of patients and all grade 3–4 events are displayed in the table. Within the first 12 weeks, no grade 5 adverse events were observed. [a]Some patients had more than one event. [b]Functional decline possibly caused by corticosteroid induced-myopathy. GGT, gamma-glutamyltransferase.

week 6 on radiologic imaging underwent TLND (Supplementary Table 4). Two additional patients who did not undergo the ILN procedure due to irAEs underwent TLND that showed pNR (Fig. 1b). Of these 21 patients with pNR, 17 were treated with adjuvant systemic therapy (seven patients received adjuvant nivolumab and ten were treated with adjuvant BRAF/MEK inhibition), whereas the four remaining patients did not receive adjuvant nivolumab due to irAEs ($n=3$, all *BRAF* wild-type) or were lost to follow-up ($n=1$). Eight patients received concurrent adjuvant radiotherapy (Fig. 1b).

TLND omission resulted in reduced morbidity and better HRQoL. For all patients who underwent TLND in the PRADO trial, the median time from the start of neoadjuvant CPI to TLND was 9.6 weeks (range, 8.1–22.1 weeks). TLND was delayed in five (16%) patients due to irAEs ($n=4$) or because there was no on-time theater slot available ($n=1$) (Supplementary Table 2). No unexpected surgical complications were observed. A significantly lower surgery-related adverse event rate according to Common


a
Objective radiologic response (n = 99)
14%
31%
38%
7%
6%
3%
ORR = 45%, 95% CI 35–56%
Complete response
Partial response
Stable disease
Local PD
Distant metastases
Not evaluable*
b
Pathologic response (n = 99)
49%
12%
11%
21%
6%
pRR = 72%, 95% CI 62–80%
MPR = 61%, 95% CI 50–70%
pCR
Near-pCR
pPR
pNR
Distant metastases
Not evaluable**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Number of patients (n)
pRR (%, 95% CI)
Total cohort 99 72 (62–80)
Country
NL 65 77 (65–86)
AUS 34 62 (44–78)
Gender
Male 65 74 (61–84)
Female 34 68 (49–83)
Age
<60 51 75 (60–86)
≥60 48 69 (54–81)
WHO PS
0 94 72 (62–81)
1 5 60 (15–95)
Location
Neck 24 83 (63–95)
Axilla 39 72 (55–85)
Groin 36 64 (46–79)
T-stage
MUP 13 77 (46–95)
T1–2 43 74 (59–86)
T3–4 41 68 (52–82)
No. of PET–CT-pos LNs
1 57 67 (53–79)
>1–3 33 79 (61–91)
>3 9 78 (40–97)
Sum target lesions
<25 48 71 (56–83)
≥25 51 73 (58–84)
Ulceration
Yes 23 57 (34–77)
No 58 76 (63–86)
BRAF mutation
Positive 45 62 (47–76)
Negative 43 74 (59–86)
VE1 negative* 9 100 (66–100)
PD-L1 expression
<1% 43 56 (40–71)
1–50% 25 92 (74–99)
≥50% 7 100 (59–100)
0
20
40
60
80
100
Pathologic response rate (%)

Fig. 2 | Radiologic and pathologic response. a, Objective radiologic response (ORR) of all patients in the PRADO trial ($n=99$) after 6 weeks of neoadjuvant ipilimumab plus nivolumab. *Three patients were not evaluable for radiologic response at week 6 due to irAEs. **b**, Pathologic response of the ILN of all patients in the PRADO trial ($n=99$) based on INMC criteria. **One patient did not have any surgery due to irAEs. **c**, Forest plot of data for all patients ($n=99$). pRRs with 95% CIs are displayed according to demographic, clinical and tumor characteristics. The 95% CIs were calculated using the Clopper–Pearson method. *All patients of whom only a VE1 staining was available achieved MPR, and no tumor material was left for formal testing. VE1, a monoclonal antibody against mutant $BRAF^{V600E}$ protein. AUS, Australia; NL, The Netherlands; LN, lymph node; MUP, melanoma of unknown primary; PD, progressive disease; WHO PS, World Health Organization performance status.

Terminology Criteria for Adverse Events (CTCAE) version 4 was observed in patients who only underwent ILN resection ($n=61$) as compared to patients who underwent both ILN resection and subsequent TLND ($n=31$) (46% versus 84%, $P<0.001$) (Fig. 3a and Extended Data Fig. 3a). Similarly, ILN-only patients had significantly lower Clavien–Dindo classification grades at week 12 than ILN+TLND patients ($P<0.001$) (Fig. 3b). Undergoing TLND was significantly associated with the presence of surgery-related adverse events, but the use of high-dose ($\geq 1\,mg\,kg^{-1}$) steroids within the first 12 weeks after the start of neoadjuvant CPI was not significantly associated with surgical morbidity (Supplementary Table 5).

Longitudinal HRQoL outcomes were compared between patients with MPR ($n=60$, of whom most underwent ILN resection only) (Extended Data Fig. 3b) and patients with non-MPR ($n=31$, most underwent ILN and TLND). Differences in scores were calculated while adjusting for age, gender, adjuvant treatment and relapse status (no/yes). Overall, patients with MPR scored significantly better on several HRQoL functioning domains than patients with non-MPR, including physical functioning, role functioning, global functioning, social functioning, the European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire-Core 30 (QLQ-C30) summary score and the melanoma (surgery) subscales. The biggest differences were detected at week 12, and all differences were clinically relevant (Fig. 3c, Extended Data Fig. 4 and Supplementary Table 6). Moreover, patients with MPR reported a lower symptom burden than patients with non-MPR with respect to fatigue and insomnia, with the biggest differences at week 12 (Fig. 3c, Supplementary Table 6 and Extended Data Fig. 4). After 2 years, significantly and small clinically relevant differences in scores were still present for physical functioning, fatigue and insomnia. Patients with non-MPR reported clinically important deterioration regarding physical and role functioning, fatigue and pain at week 12, nausea at week 36 and financial difficulties at week 48 and 60 (data not presented)[22]. Except for emotional functioning and insomnia, none of the HRQoL parameters was significantly different between both groups at week 6 (post-neoadjuvant CPI and pre-surgery) (Supplementary Table 7). To evaluate the effect of the patient's knowledge of his/her pathologic response on HRQoL outcomes, additional analyses were performed comparing the MPR to pPR and pNR subgroups (Extended Data Fig. 5). Statically significant and clinically relevant adjusted differences were observed in MPR versus pPR and MPR versus pNR patients. Because both the MPR and pPR patient groups were informed that they had a good prognosis (based on results from previous neoadjuvant trials[7]), the extent of surgery is likely to be an important contributing factor to the differences in HRQoL outcomes between patients with MPR and non-MPR.

Survival outcomes. After a median follow-up of 28.1 months (IQR, 25.0–33.8 months), median RFS (Fig. 4a), event-free survival (EFS) (Fig. 4c), distant metastasis-free survival (DMFS) (Fig. 4d) and overall survival (OS) (Fig. 4f) were not reached for the total cohort, with 24-month estimates being 85% (95% CI: 78–92%), 80% (95% CI: 72–88%), 89% (95% CI: 83–96%) and 95% (95% CI: 91 –99%), respectively.

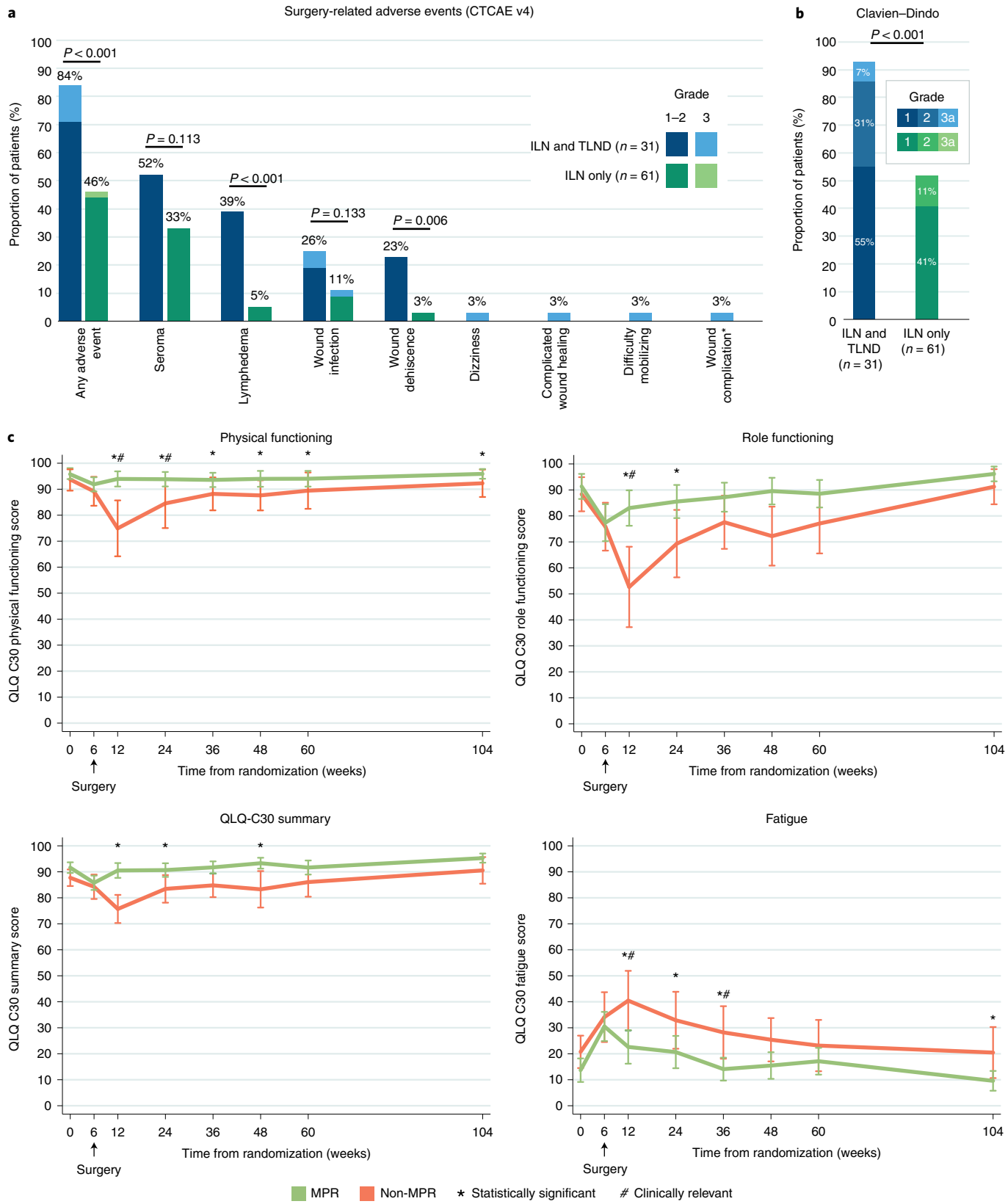
The estimated 24-month RFS rate for the patients achieving MPR was 93% (95% CI: 87–>99%) (Fig. 4b). Four of the 60 patients with MPR developed a regional recurrence (three pCR and one near-pCR in the ILN) (Supplementary Table 8). One of these patients developed later M1a disease, 23.5 months after ILN resection and 19.3 months after regional recurrence, resulting in a 24-month DMFS rate of 98% (95% CI: 94–>99%) for the MPR group (Fig. 4e). Notably, all four patients had two or more PET-positive lymph nodes at baseline and harbored a $BRAF^{V600E/K}$ mutation (versus 43% and 30% in MPR patients without relapse) (Supplementary Table 8). In total, 28 patients with MPR had two or more PET-positive lymph nodes at baseline, resulting in a recurrence rate of 4 of 28 (14%) in this group (Supplementary Table 9). Three of the four patients were treated by surgery followed by adjuvant therapy (nivolumab $n=2$, dabrafenib+trametinib $n=1$), and the fourth patient refused extended surgery and started BRAF/MEK inhibition with ongoing radiologic complete response. The subcutaneous lesions of the patient with M1a disease were surgically removed.

Of the 11 patients with pPR, four patients had recurrence, resulting in a 24-month RFS rate of 64% (95% CI: 41–99%) (Fig. 4b). Three patients developed distant recurrence, and the fourth patient developed regional followed by distant recurrence, yielding a 24-month DMFS rate of 64% (95% CI: 41–99%) (Fig. 4e). The patient and tumor characteristics of these patients are listed in Supplementary Table 10.

Fig. 3 | Effect of ILN procedure on surgical morbidity and HRQoL. a, Surgery-related adverse events of patients undergoing an ILN procedure only ($n=61$) versus those undergoing subsequent TLND ($n=31$) according CTCAE version 4.03. Only adverse events that occurred in three or more patients or were grade ≥3 are displayed in the figure. *P* values were calculated using Fisher's exact test. *The wound complication consisted of vacuum-assisted closure dressing of the wound and electrolyte monitoring. **b**, Clavien–Dindo classification at week 12 of patients undergoing ILN procedure only ($n=61$, green bar) versus patients undergoing subsequent TLND ($n=31$, blue bar). The *P* value was calculated using the linear-by-linear association test for ordinal data. **c**, Curves showing the unadjusted mean HRQoL scores of patients with MPR ($n=60$, green line) versus patients without MPR ($n=31$, orange line). Error bars indicate the 95% CI. The differences in mean HRQoL scores between patients with MPR and non-MPR (see also Supplementary Table 5) were adjusted for age, gender, adjuvant treatment and relapse status (no/yes). The adjusted score differences were interpreted in terms of statistical significance using a linear mixed-effects model with a two-tailed *P* value ($P<0.05$) and by clinical relevance according to the guideline of Cocks et al.[32]. Statistically significant adjusted differences were marked with *, and clinically relevant differences were marked with # (Supplementary Table 5). Results were considered clinically relevant if the adjusted difference in mean scores between the two groups was at least 'medium' and clinically irrelevant if differences in mean scores were 'trivial or small'. Questionnaire compliance rates in the MPR and non-MPR groups were 87% versus 97% at baseline, 98% versus 94% at week 6, 90% versus 81% at week 12, 88% versus 81% at week 24, 92% versus 84% at week 36, 85% versus 68% at week 48, 80% versus 77% at week 60 and 87% versus 61% at week 104 (year 2).

Six of 21 patients in the pNR group developed a melanoma recurrence ($n=5$) or died ($n=1$, recurrence status unknown) within the first 2 years after surgery, yielding a 24-month RFS rate of 71% (95% CI: 55–94%) (Fig. 4b). Relapses were regional ($n=1$), distant ($n=3$) or synchronous regional and distant ($n=1$), resulting in a 24-month DMFS rate of 76% (95% CI: 60–97%) (Fig. 4e).

At the data cutoff, recurrences were observed in two of seven patients treated with adjuvant nivolumab (24-month RFS rate 71%), in three of ten patients with adjuvant BRAF/MEK inhibition



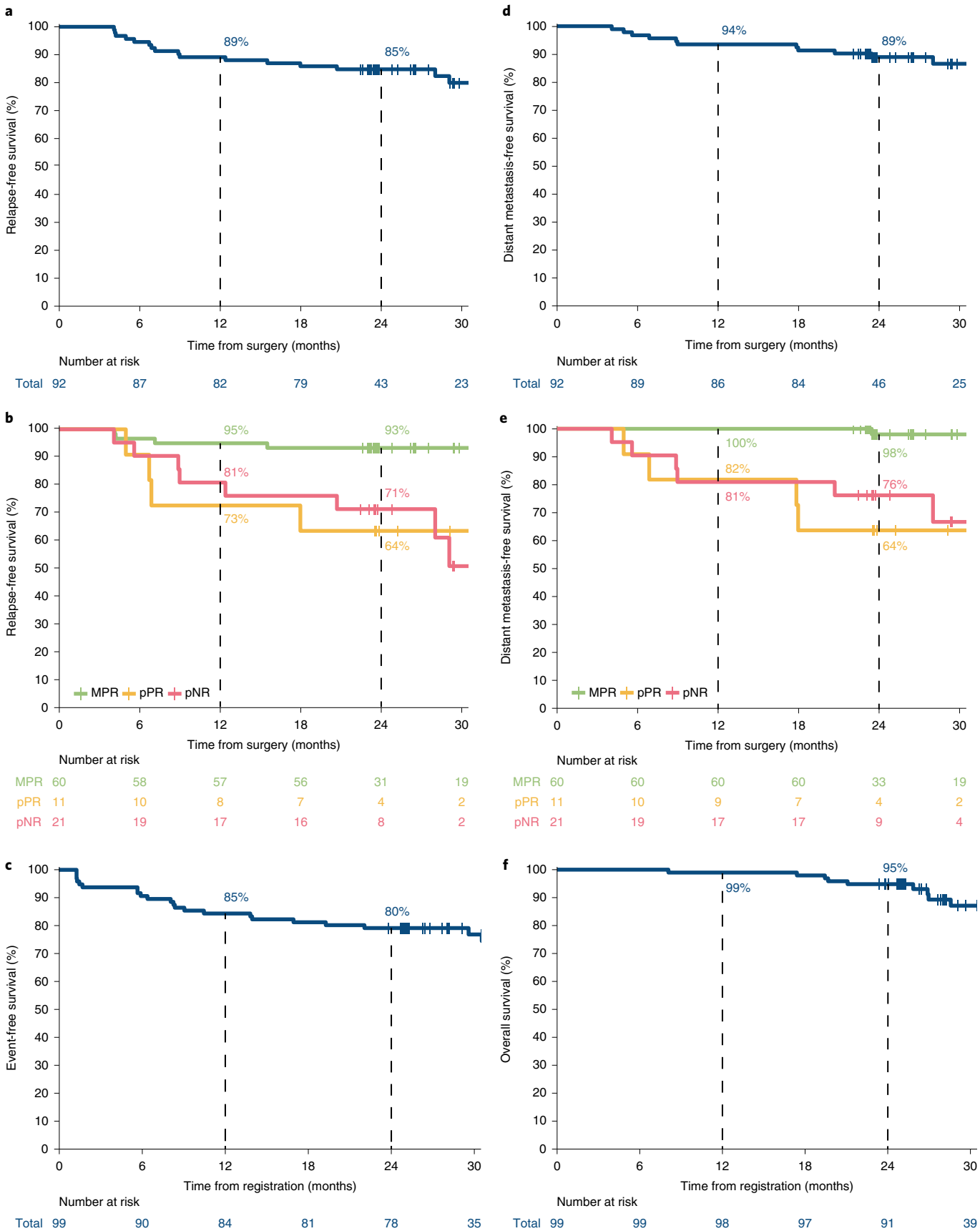


Fig. 4 | Survival outcomes. a, RFS for all patients who underwent surgery ($n=92$), six patients who progressed to stage IV disease before surgery and one patient who did not undergo surgery because of irAEs were excluded. **b**, RFS of the PRADO trial by pathologic response subgroup. Patients had MPR ($n=60$, green line), pPR ($n=11$, yellow line) or pNR ($n=21$, red line). **c**, EFS for the total population of the PRADO trial ($n=99$). **d**, DMFS for all patients who underwent surgery ($n=92$). **e**, DMFS of the PRADO trial by pathologic response subgroup. **f**, OS for the total population of the PRADO trial ($n=99$).

(24-month RFS rate 90%) and in two of three patients without systemic adjuvant therapy (24-month RFS rate 33%) (Extended Data Fig. 6). Of note, two patients with MPR in the ILN and one with pNR developed a new primary melanoma during follow-up. This was not counted as an event in the survival analyses.

Discussion

To our knowledge, PRADO is the first trial to demonstrate that the ILN procedure is feasible and enables response-directed tailored treatment. This approach enabled de-escalation of treatment (omission of TLND and adjuvant therapy) in most patients achieving MPR in their ILN (59 of 60), resulting in decreased morbidity and better HRQoL for these patients. Their 24-month RFS and DMFS rates were 93% and 98%, respectively, indicating that the response-driven tailored treatment did not impair their outcomes. In addition, our findings might be a first step in future efforts on reduction of health services use and costs for the treatment of stage III melanoma.

PRADO also confirmed the clinical outcomes observed in our prior neoadjuvant OpACIN-neo trial. The latter trial demonstrated that two cycles of ipilimumab 1 mg kg^{-1} plus nivolumab 3 mg kg^{-1} was the most favorable neoadjuvant treatment schedule, with pRR of 77% and 20% grade 3–4 irAEs within the first 12 weeks of treatment[8]. In the current PRADO trial, we observed pRR of 72% and 22% grade 3–4 irAEs within the first 12 weeks after neoadjuvant ipilimumab 1 mg kg^{-1} plus nivolumab 3 mg kg^{-1}, confirming the efficacy and safety of this treatment regimen for clinical stage III nodal melanoma.

Furthermore, we found that the implementation of the ILN resection in our neoadjuvant CPI treatment regimen was safe and feasible. The marked ILN was retrieved in 96% of cases, indicating that all four evaluated markers (magnetic Memaloc marker, nitinol UltraCor Twirl marker, radioactive I-125 seed and hydrogel marker) are suitable for identifying and removing the ILN. Broad experience with image-guided marker placement for locating axillary lymph nodes has already been gained with breast cancer surgery after neoadjuvant chemotherapy. Marker placement is regarded as safe and simple with high detection rates[23,24]. Individual institutional or surgeon preference, experience and availability of localization technique should direct the choice for the preferred marker. Delay or cancellation of the ILN procedure due to irAEs occurred in only a small subset of patients (6%). However, one needs to note that (high-dose) steroids were no contraindication for surgery in our participating institutes[25].

One of the co-primary endpoints of PRADO—the 24-month RFS in the MPR group—was not met based on the predefined measure of feasibility. The trial protocol stated that the null hypothesis could not be rejected in case of more than one recurrence in the MPR group, which was based on a 24-month RFS rate of 97% for responders (≤50% viable tumor) in OpACIN-neo[9]. Four patients with MPR had recurred at the data cutoff, resulting in a 24-month RFS rate of 93%. Nevertheless, only one patient developed distant metastasis (M1a disease 23.5 months after ILN resection). The other three patients developed only regional recurrences, enabling salvage TLND followed by adjuvant systemic therapy. None of these patients had additional recurrences at the data cutoff.

Similar results regarding distant metastasis were seen in OpACIN-neo, a trial in which all patients underwent TLND after neoadjuvant CPI. In this trial, 52 of 86 (60%) patients achieved MPR, and after a median follow-up of almost 4 years only one MPR patient had developed distant metastasis (M1d disease, 8.3 months after surgery)[9]. Based on previous large datasets that indicate that the vast majority of relapses occur within the first 12 months after surgery[2,3], our data on RFS and DMFS in the MPR group of PRADO can be considered relatively mature. Therefore, and in our view, immediate TLND might be safely omitted in patients achieving MPR in the ILN.

In contrast to earlier reports showing similar RFS in patients with pPR and MPR[7,9], pPR patients in PRADO had a worse outcome. Although not being able to exclude a sampling error due to the low patient number, this observation suggests that pPR patients should not be treated like MPR patients and might benefit from adjuvant therapy. This is supported by the RFS outcomes of patients with pNR who received additional adjuvant therapy in this trial. With only 29% of patients with pNR who had developed a melanoma recurrence at 2 years, their RFS was improved compared to the 65% of non-responding patients from OpACIN-neo who developed a recurrence[9]. Thus, PRADO suggested not only that treatment de-escalation is safe in patients with MPR but also that treatment escalation in non-responding patients might improve their outcome.

With more patients treated by the ILN approach after neoadjuvant CPI, one might define MPR patients with a higher chance for melanoma recurrence in the future. Notably, all four patients with MPR who recurred in PRADO had two or more PET-positive lymph nodes at baseline. We previously showed that the ILN response was highly concordant with the pathologic response of the entire tumor bed, supporting the current PRADO study design[20]. However, we also reported on two cases (out of 82 patients) showing a pathologic response in their ILN but also a non-response in a small non-index node that did not alter the pathologic response subgroup for the entire TLND tumor bed. This indicates the presence of less CPI-responsive tumor subclones in a minority of patients[20]. We speculate that such CPI-resistant tumor clones might have been the reason for the development of recurrences in these MPR patients, and that TLND in such patients improves their outcome. Currently, we are investigating genetic and transcriptomic differences between the ILN and recurrent node metastases to gain insights into potential mechanisms of resistance to neoadjuvant CPI.

TLND omission significantly reduced surgical morbidity and was associated with better HRQoL. These data are in line with work on surgical morbidity from randomized trials and single-arm studies comparing morbidity and cancer control between completion lymph node dissection and observation after a positive sentinel lymph node biopsy in patients with melanoma[14–16,26–28]. The fact that adjuvant therapy and relapse status were included in the mixed-effects model, and differences in HRQoL outcomes were observed in patients with MPR and pPR, indicate that the differences in HRQoL outcomes are likely to be attributed to the different extent of surgery. Additional factors contributing to the lower physical functioning status and higher fatigue scores after 2 years in non-MPR patients might be the two sequential surgeries and anesthesia within a short time period (±3 weeks)[17,18,29].

This trial is limited by the small sample sizes per pathologic subgroup, especially for patients with pPR and pNR, impeding definitive conclusions on survival outcomes after response-tailored treatment. Moreover, PRADO did not randomize TLND versus the ILN approach or response-tailored adjuvant therapy versus adjuvant therapy, allowing for only indirect comparisons to historical cohorts from previous neoadjuvant and adjuvant studies. The non-randomized study design also did not allow for a strict comparison of HRQoL between patients with and without TLND.

The randomized phase 3 NADINA trial (NCT04949113) currently investigates standard TLND followed by adjuvant nivolumab versus neoadjuvant ipilimumab plus nivolumab followed by TLND and adjuvant nivolumab or BRAF/MEK inhibition (in non-MPR patients only) in clinical stage III melanoma. NADINA includes, unlike most previous neoadjuvant immunotherapy trials, patients with in-transit metastases. Two small case series have shown that the pathologic response after neoadjuvant CPI in in-transit metastases or locally advanced primary tumors is concordant with the response in the lymph node metastases[30,31]. Another randomized phase 2 trial (SWOG S1801, NCT03698019) in clinical stage III–IV melanoma

compares neoadjuvant plus adjuvant pembrolizumab versus adjuvant pembrolizumab. Although these trials will define the benefit of neoadjuvant systemic CPI in melanoma versus adjuvant anti-PD-1, a large randomized trial analyzing the ILN approach versus TLND is pending.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-022-01851-x.

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Methods

Study design and participants. The PRADO trial included patients who were 18 years of age or older with histologically confirmed resectable stage III nodal melanoma and at least one node measurable according to RECIST version 1.1 (≥15 mm short axis). Normal lactate dehydrogenase (LDH) levels and a World Health Organization performance status score of 0 or 1 were required. Major exclusion criteria were prior treatment with CPI targeting CTLA-4/PD-1/PD-L1, BRAF±MEK inhibition or radiotherapy, a history of in-transit metastases within 6 months before inclusion and a history of autoimmune diseases. Full inclusion and exclusion criteria can be found in the appendix (protocol pages 41–43). Patients were enrolled in Australia at the Melanoma Institute Australia (MIA, Sydney) and in the Netherlands at the Netherlands Cancer Institute (NKI, Amsterdam), Leiden University Medical Center (LUMC), Erasmus Medical Center (EMC, Rotterdam), University Medical Center Utrecht (UMCU) and University Medical Center Groningen (UMCG). The medical ethics review committee of the Netherlands Cancer Institute and ethical committees at Melanoma Institute Australia approved the trial. The trial was conducted in accordance with the protocol and Good Clinical Practice guidelines as defined by the International Conference on Harmonization and the Declaration of Helsinki. All participating patients provided written informed consent before enrollment.

Patients were treated with two cycles ipilimumab 1 mg kg^{-1} plus nivolumab 3 mg kg^{-1} after placement of a marker in the ILN (largest melanoma-containing lymph node at baseline). Removal of the marker lymph node (ILN procedure) was planned after 6 weeks. Patients who achieved pCR or near-pCR (both together termed MPR) in their ILN did not undergo TLND nor received any adjuvant treatment. Patients with pPR underwent TLND without adjuvant treatment, and patients with pNR underwent TLND plus adjuvant systemic treatment (nivolumab or dabrafenib+trametinib) for 52 weeks with or without local radiotherapy (Fig. 2a). Enrollment continued until a minimum of 50 patients had achieved MPR in their ILN.

Randomization and blinding. In the PRADO trial, there was no randomization and no blinding.

Treatment and assessments. Before initiation of neoadjuvant treatment, the ILN was marked using ultrasound guidance. Different markers were used depending on the participating sites' preference, including a magnetic Memaloc marker, nitinol UltraCor Twirl marker, hydrogel marker and radioactive I-125 seed. The ILN procedure was scheduled after 6 weeks from the start of CPI, during which only the marked ILN was planned to be resected. Additional radiologically suspected or biopsy-proven lymph nodes other than the ILN were allowed to remain in situ and were planned to be resected (only in case of pPR or pNR in the ILN) during TLND, which was planned after 9 weeks (range, 7–12 weeks) from the start of CPI. Treatment of irAEs of the neoadjuvant CPI with steroids was no contraindication to proceeding to surgery.

Patients were treated until the end of the treatment schedule, unacceptable toxicity or withdrawal of consent. Discontinuation criteria due to irAEs are described in the appendix (trial protocol pages 57–58). Permanent discontinuation of CPI due to irAEs did not preclude patients from undergoing the ILN procedure or TLND. All treatment-related adverse events and laboratory values were recorded and graded by the investigators according to the CTCAE version 4.03. Surgery-related morbidity was also graded according to the Clavien–Dindo classification. Laboratory assessments were performed at baseline and at week 3, week 6, week 9 and week 12. Radiologic tumor assessments by CT were done at baseline, in week 6 before the ILN procedure and in week 12. Radiologic responses were assessed using RECIST version 1.1 guidelines by the radiologists at the participating centers without central review. Patients who progressed to stage IV disease went off study according to the protocol and were treated according to standard of care.

The pathologic responses were centrally revised by experienced pathologists (B.A.v.d.W., A.J.C. and R.A.S. at MIA or NKI) according to International Neoadjuvant Melanoma Consortium (INMC) guidelines[33]. Pathologic responses of the ILN were categorized as being pCR (0% viable tumor), near-pCR (1–≤10% viable tumor), pPR (>10–≤50% viable tumor) or pNR (>50% viable tumor). Subsequent response-tailored treatment was based on the pathologic response of the ILN, except for two patients who only underwent TLND and no ILN resection due to irAEs. The pathologic responses of non-index nodes that were resected during the ILN procedure and TLND are shown in Supplementary Table 4. Starting at week 12, patients without pathologic response received adjuvant treatment with nivolumab or BRAF/MEK inhibition for 52 weeks ± local radiotherapy in parallel. All patients were assessed for recurrence of disease by radiologic assessment with CT or PET–CT, physical examination and laboratory testing for every 12 weeks until development of distant metastases, death, lost to follow-up or withdrawal of consent for up to 2 years after surgery and in years 3, 4 and 5 according to institute standards. The data cutoff for collection of survival and toxicity data was 7 February 2022.

Baseline tumor PD-L1 expression analysis was performed centrally (NKI) on formalin-fixed, paraffin-embedded tumor sections with an automated laboratory-validated immunohistochemistry assay, using the 22C3 antibody on a Ventana platform. PD-L1 expression was determined by the tumor proportion score (the percentage of tumor cells with complete or partial membranous staining at any intensity).

HRQoL. HRQoL scores were assessed by use of the EORTC QLQ-C30 and melanoma (surgery)-specific questions of the Functional Assessment of Cancer Therapy-Melanoma (FACT-M). The HRQoL assessments took place before treatment (at baseline), at week 6 (post-neoadjuvant CPI, pre-surgery) and at weeks 12, 24, 36, 48, 60 and 104 (year 2). The data cutoff for collection of HRQoL data was 1 February 2022. Missing items from the EORTC QLQ-C30 were imputed according to EORTC guidelines. More information on the questionnaires can be found in the Supplementary Materials (page 16).

Endpoints. The primary objective of the PRADO trial was to confirm the pRR of the most favorable treatment arm of OpACIN-neo (arm B: two cycles ipilimumab 1 mg kg^{-1} plus nivolumab 3 mg kg^{-1}). Co-primary objectives were to investigate whether TLND could be safely omitted in patients achieving MPR in the ILN and whether RFS of patients with pNR could be prolonged by adding adjuvant treatment. Primary endpoints were pRR and 24-month RFS in patients achieving MPR and pNR.

Secondary endpoints were grade 3–4 irAE rate during the first 12 weeks after CPI initiation, radiologic response rate, DMFS, EFS, OS, ongoing long-term irAEs, comparison of surgical morbidity between marked ILN resection and TLND, HRQoL and biomarker analyses. For definitions of pathologic response and survival endpoints, see Supplementary Table 11.

Statistical analyses. *Sample size and power.* When designing the trial, we planned to enroll 100–110 patients with the goal to include at least 50 patients with MPR. This goal was earlier achieved, so that eventually 99 patients with melanoma were accrued. The first objective of the trial was to confirm the pRR (pCR, near-CR and pPR) of the most favorable treatment schedule from OpACIN-neo (arm B). A pRR of 55% was considered unacceptable, and we expected 70% of patients to respond to treatment. An exact test for one proportion has 85% power to test this hypothesis at the two-sided alpha level of 0.05. At least 65 responders were required, which implies the actual significance level of 0.043.

Co-primary objectives of the PRADO cohort were to assess (1) whether it is safe to omit TLND in patients achieving MPR (pCR or near-pCR) and (2) improvement of the RFS rate at 24 months for patients with pNR by adding adjuvant treatment. Our assumption was that no recurrences within 24 months in patients achieving MPR would occur, and RFS at 24 months of 90% or less would be considered unsafe. Power calculation for this objective was performed via simulations accessing the lower bound of the one-sided 95% CI, applied to Kaplan–Meier estimate of RFS at 24 months, using beta product confidence procedure (BPCP)[34]. For at least 50 patients who were expected to achieve MPR and assuming 24-month RFS under the alternative hypothesis of 98%, there was 75.5% power, and under the alternative hypothesis of 99%, there was 91.5% power. With 60 MPR patients having two or more years of follow-up, the lower boundary of the one-sided 95% BPCP CI would exceed 90% if no more than one recurrence occurred. In total, 21 patients were included in the pNR group. The BPCP method has 81% power to reject RFS at 24 months of 20% at one-sided alpha of 0.05 in case of improvement of the RFS at 24 months to 45%. With 21 pNR patients having two or more years of follow-up, the lower boundary of the one-sided 95% BPCP CI would exceed the 20% if no more than 13 recurrences occurred. No interim analyses were planned for PRADO. However, if one relapse in the MPR patient cohort was observed before the end of patient inclusion in the trial, the Data and Safety Monitoring Board and Bristol Myers Squibb would be immediately informed and the further procedure of the trial discussed. If, at any moment, there were two relapses, the trial would be amended by reintroducing TLND in this cohort.

Response, toxicity and survival. For the PRADO trial, analyses on pathologic response, radiologic response and irAEs were performed in all patients with melanoma who received at least one dose of the study drug. For pathologic response, patients were not evaluable if they did not undergo any surgery due to irAEs, and patients with stage IV disease at week 6 were allocated to the 'distant metastases' subgroup independent of undergoing the ILN procedure or not. Patients were not assessable for radiologic response if they had not been radiologically evaluated for response at week 6. The pathologic and radiologic responses as well as adverse events were summarized as proportions of the total cohort with the two-sided 95% CI calculated using the Clopper–Pearson method. For analyses on surgical-related toxicity, patients who underwent only the ILN resection ($n=61$) were compared to patients who underwent the ILN resection followed by TLND and those who proceeded immediately to TLND ($n=29$ and $n=2$, respectively). Patients who underwent no surgery ($n=3$) or a small secondary surgery for removal of some additional lymph nodes ($n=4$) were excluded from the analysis. CIs for difference in proportions of patients with surgical toxicity were calculated using the asymptotic method, and the provided P values come from Fisher's exact test. The P value for differences in the Clavien–Dindo classification was calculated by linear-by-linear association test for ordinal data. Odds ratios and P values for the association between TLND and steroid use on the presence of surgical morbidity were calculated using a

multivariate logistic regression model. Survival outcome curves (RFS, EFS, DMFS and OS) for the total cohort and pathologic response subgroups were estimated using Kaplan–Meier methodology. CIs were calculated using the Greenwood formula and log transformation. Analyses were performed using R (version 3.5.1) and SPSS Statistics (version 27). This trial is registered with ClinicalTrials.gov (NCT02977052) and is ongoing for survival analysis.

HRQoL. HRQoL outcomes were evaluated using mixed-effects linear regressions for longitudinal data with patient-specific intercepts and an autoregressive covariance matrix structure. All models were adjusted for MPR status (no-MPR/MPR), age (measured in years), gender (female/male), adjuvant treatment (no/yes, measured as a time-dependent variable), relapse status (no/yes, measured as a time-dependent variable) and time (baseline and weeks 6, 12, 24, 36, 48, 60 and 104). Additionally, for comparison of outcomes between MPR and no-MPR at specific time points, interaction terms between MPR status and time were added to the models. Coefficients of all covariates were considered as fixed effects. Results were interpreted in three ways: (1) statistically significant difference was defined with a two-sided P value ≤ 0.05; (2) medium to large differences were defined as clinically relevant according to the guideline of Cocks et al.[32]; and (3) domain-specific thresholds were used to identify functional impairments and symptoms that limit patients' daily life[22]. All analyses were conducted using STATA version 15.1 software (StataCorp).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

To minimize the risk of patient re-identification, de-identified individual patient-level clinical data are available under restricted access. Upon a scientifically sound request, data access can be obtained via the NKI's scientific repository at repository@nki.nl, which will contact the corresponding author (C.U.B.). Data requests will be reviewed by the institutional review board of the Netherlands Cancer Institute (NKI) and will require the requesting researcher to sign a data access agreement with the NKI.

References

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Author contributions

C.U.B. designed the trial and wrote the trial protocol. E.A.R. provided additional input and wrote the amendment for the PRADO extension cohort during the 20th Workshop on 'Methods in Clinical Cancer Research' (Zeist, Netherlands). G.V.L. and A.v.A. reviewed the protocol. A.H.B. wrote the HRQoL part of the protocol. I.L.M.R., A.M.M., J.M.V., R.P.M.S., T.P., E.A.R., E.K., A.A.M.v.d.V., K.P.M.S., G.A.P.H., W.M.C.K., W.J.v.H., J.A.v.d.H., D.J.G., M.W.W., A.J.W., C.L.Z., J.M.L., K.F.S., S.C., J.S., A.S., A.v.A., G.V.L. and C.U.B. recruited and treated patients and collected data. A.J.C., R.V.R., R.A.S. and B.A.v.d.W. reviewed and scored the pathology of all cases. N.M.J.v.d.H., I.L.M.R., M.G., L.V.v.d.P.-F., A.H.B. and C.U.B. collected and interpreted data on HRQoL. K. Sikorska and I.L.M.R. performed statistical analysis of the clinical data. A.H.B. and K.J. performed the HRQoL statistical analyses. A.T.A. and L.G.G.-O. contributed to central and local data management. R.Z. and M.G. were clinical project managers of the trial. I.L.M.R., A.H.B. and C.U.B. wrote the first draft of the manuscript. All authors interpreted the data, reviewed the manuscript and approved the final version.

Competing interests

No author has received financial support for the work on this manuscript, and no medical writer was involved at any stage of the preparation of this manuscript. A.M.M. has served on advisory boards for Bristol Myers Squibb (BMS), Merck Sharp & Dohme (MSD), Novartis, Roche, Pierre Fabre and QBiotics. R.P.M.S. has received honoraria for advisory board participation from MSD, Novartis and Qbiotics and speaking honoraria from BMS and Novartis. E.K. received honoraria for consultancy/advisory relationships (all paid to the institute) from BMS, Novartis, Merck and Pierre Fabre and received research grants not related to this paper from BMS. A.A.M.v.d.V. received compensation for advisory roles and honoraria (all paid to the institute) from BMS, MSD, Merck, Roche, Eisai, Pfizer, Sanofi, Novartis, Pierre Fabre and Ipsen. K.P.M.S. received compensation for advisory roles and honoraria (all paid to the institute) from BMS, MSD, Roche, Novartis, Pierre Fabre and Abbvie and received research funding from Novartis, TigaTx and BMS. G.A.P.H. received compensation for consulting and advisory roles (all paid to the institute) from Amgen, Roche, MSD, BMS, Pfizer, Novartis and Pierre Fabre and received research grants (paid to the institute) from BMS and Seerave. W.J.v.H. received compensation for advisory roles (all paid to the institute) from BMS, Amgen and Sanofi. D.J.G. received compensation for advisory roles (all paid to the institute) from Amgen and Novartis. M.W.W. received compensation for advisory roles (all paid to the institute) from Novartis. A.J.S. has served on an advisory board for QBiotics and received fees for professional services from Eli Lily Australia. J.B.A.G.H. received compensation (all paid to the institute) for advisory roles from AIMM, Amgen, BioNTech, BMS, GlaxoSmithKline, Ipsen, MSD, Merck Serono, Molecular Partners, Neogene Therapeutics, Novartis, Pfizer, Roche/Genentech, Sanofi, Seattle Genetics, Third Rock Ventures and Vaximm; stock option ownership of Neogene Therapeutics; and institutional research funding from Amgen, BioNTech, BMS, MSD and Novartis. B.A.v.d.W. has served on the advisory board for BMS. A.v.A. had served on advisory boards and received consultancy honoraria (all paid to the institute) for Amgen, BMS, Novartis, MSD, Merck-Pfizer, Pierre Fabre, Sanofi, Sirius Medical and 4SC and received research grants (all paid to the institute) from Amgen and Merck-Pfizer. R.A.S. has received fees for professional services from F. Hoffmann-La Roche, Evaxion, Provectus Biopharmaceuticals Australia, Qbiotics, Novartis, MSD, NeraCare, Amgen, BMS, Myriad Genetics and GlaxoSmithKline. A.H.B. has received a research grant from BMS. G.V.L. is consultant advisor for Aduro, Amgen, Array Biopharma, Boehringer Ingelheim, BMS, Evaxion, Hexal AG (Sandoz Company), Highlight Therapeutics, MSD, Novartis, Oncosec, Pierre Fabre, Provectus, QBiotics and Regeneron Pharmaceuticals. C.U.B. reports receiving compensation for advisory roles from BMS, MSD, Roche, Novartis, GlaxoSmithKline, AstraZeneca, Pfizer, Eli Lilly, GenMab, Pierre Fabre and Third Rock Ventures and receiving research funding from BMS, MSD, Novartis, 4SC and NanoString. Furthermore, C.U.B. reports to be co-founder of Immagene BV. All compensations and funding for C.U.B. were paid to the institute, except for Third Rock Ventures and Immagene. The other authors declare no conflicts of interest.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-022-01851-x.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-022-01851-x.

Correspondence and requests for materials should be addressed to Christian U. Blank.

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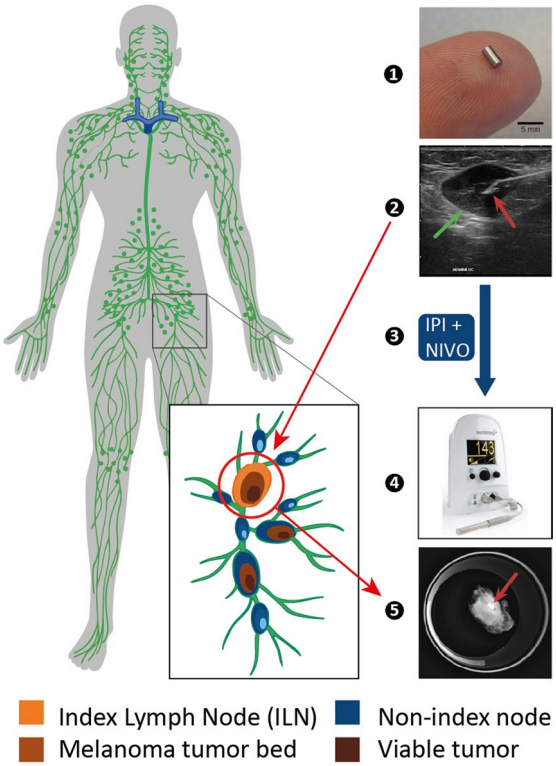
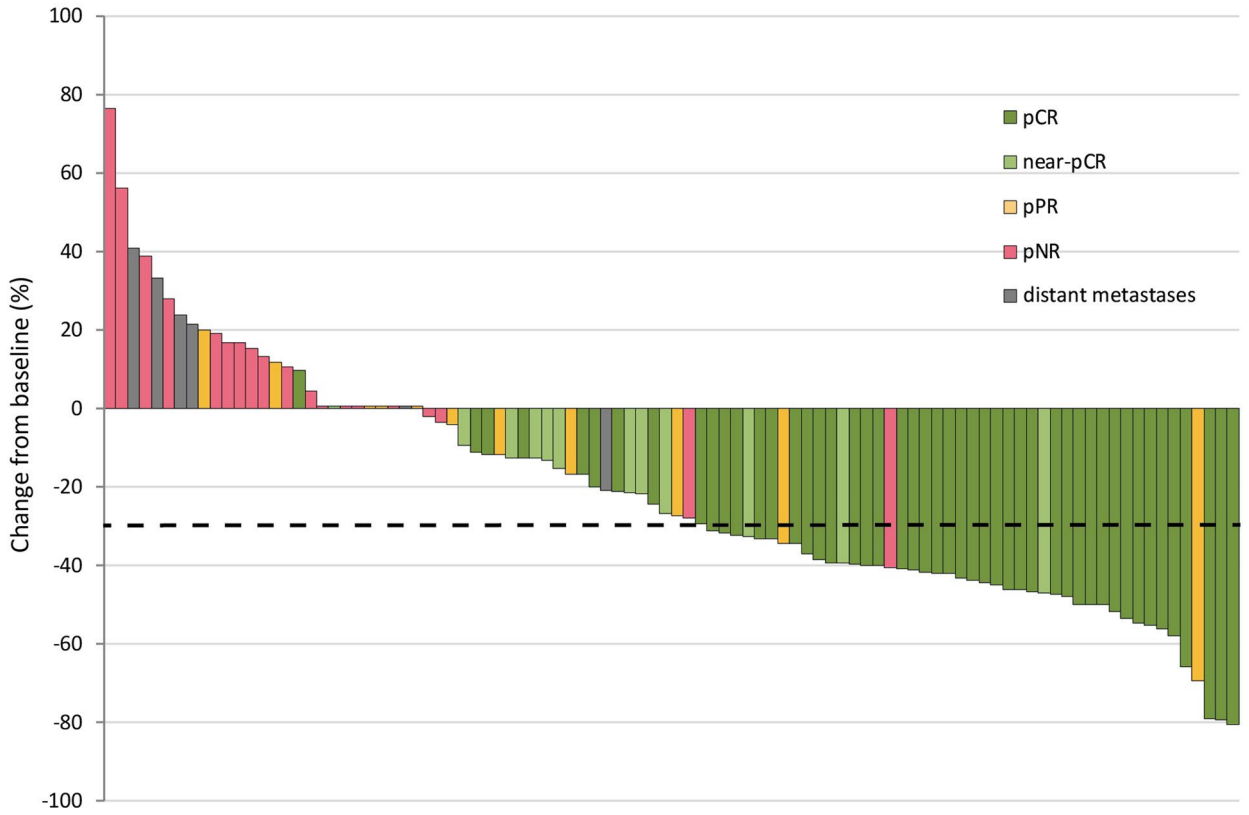
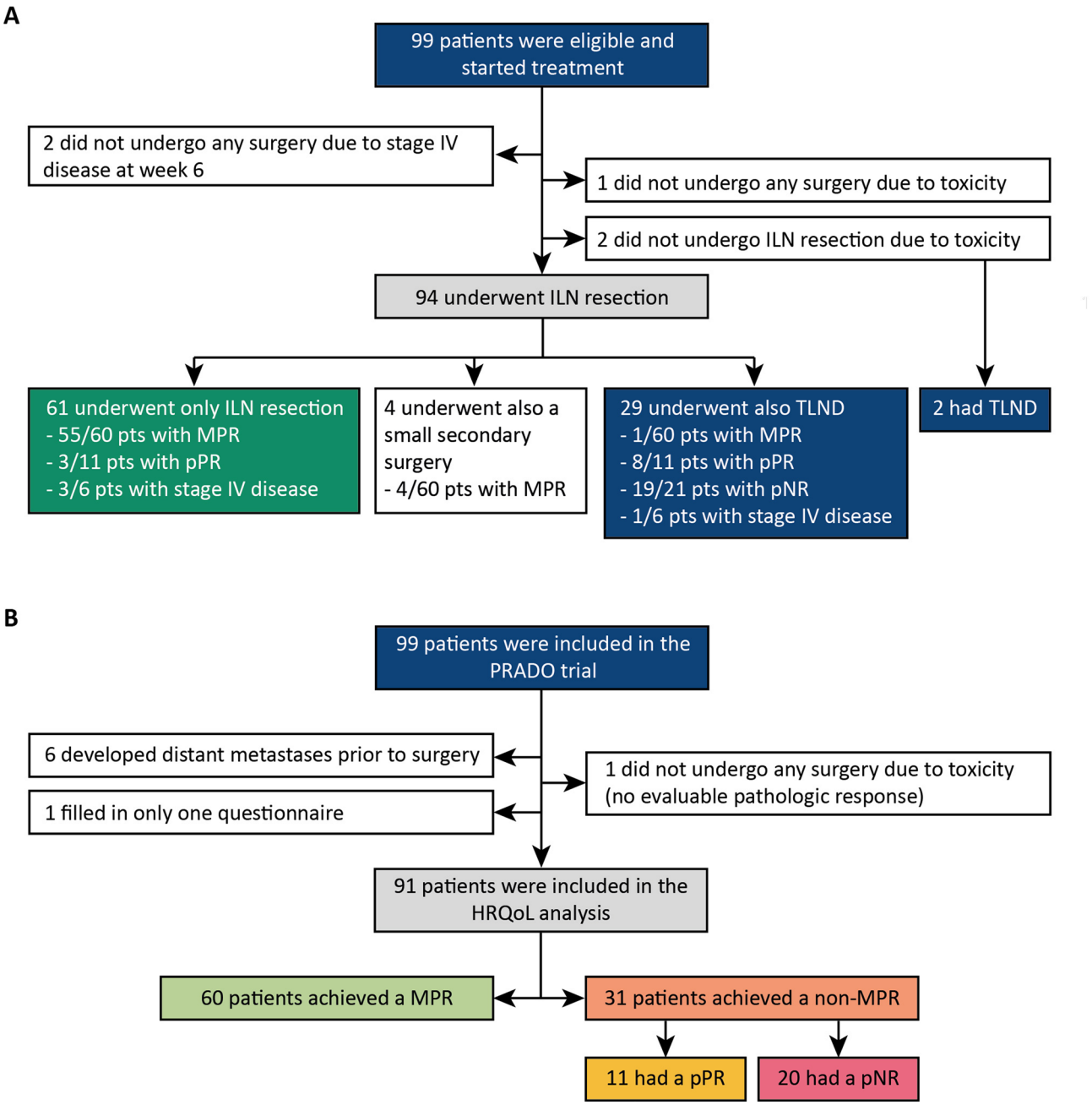


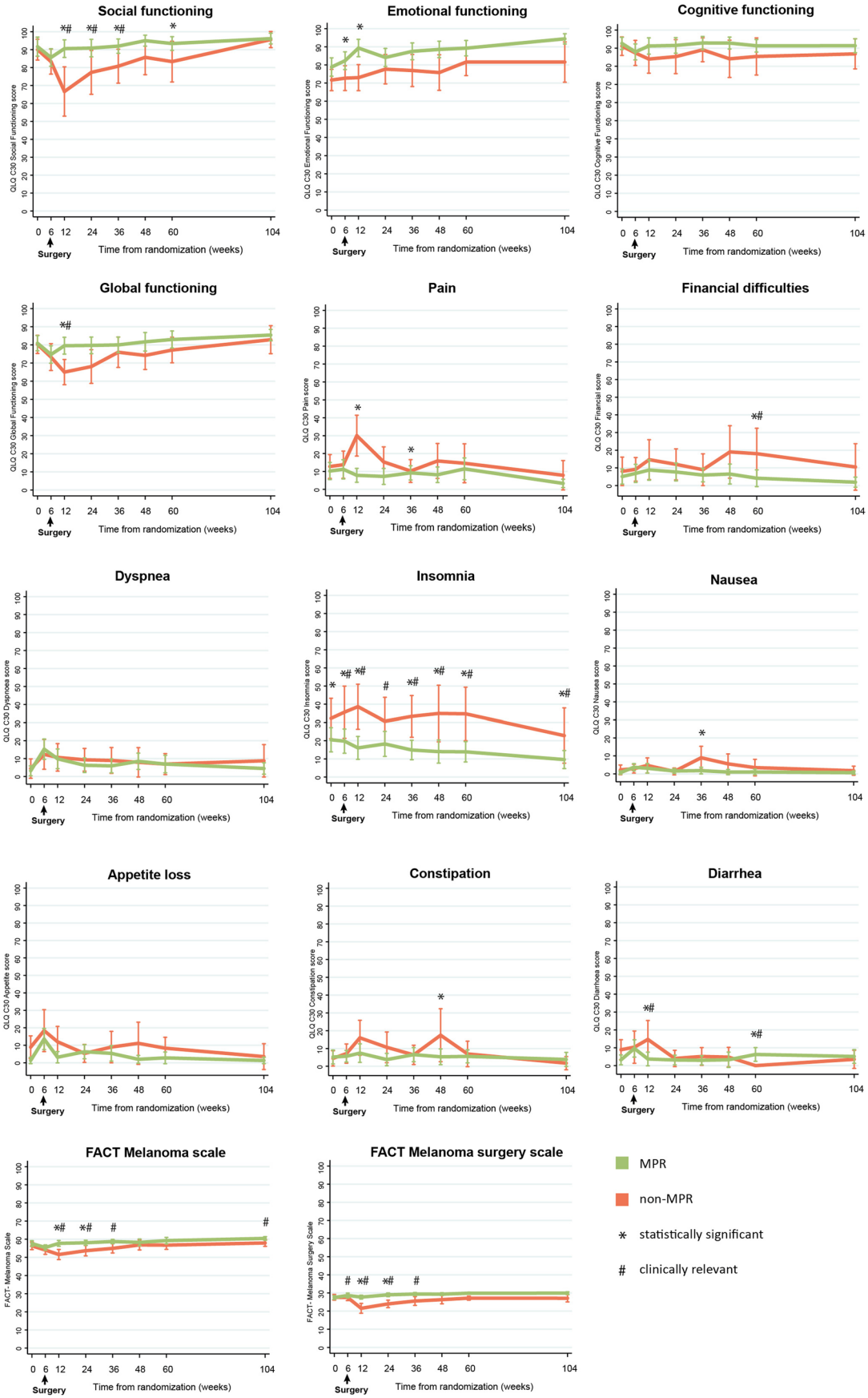
Extended Data Fig. 1 | Marker placement in the ILN. Schematic overview of magnetic seed placement in the ILN and retrieval of the ILN during the ILN procedure. (1) Magnetic seed, (2) Ultrasound image of positioning of the needle tip (red arrow) in the ILN (green arrow) before implantation of the magnetic seed, (3) Two cycles of ipilimumab plus nivolumab are given after the magnetic seed is implanted, (4) Magnetic detector (*Endomag Sentimag®*) used during surgery for seed detection, (5) Postoperative specimen X-ray with magnetic seed (red arrow) in situ. This image has been adapted from *Schermers B, Br J Surg, 2019*[19].



Extended Data Fig. 2 | Objective radiologic response underestimates pathologic response. Waterfall plot of the radiologic change in target lesions (in %) between baseline and week 6 of all PRADO patients with evaluable CT-scan ($n = 96$). Colours indicate the responses as pCR (dark green), near-pCR (light green), pPR (yellow), pNR (red) and distant metastases (grey). The dotted line indicates the cutoff for RECIST version 1.1 radiologic response.

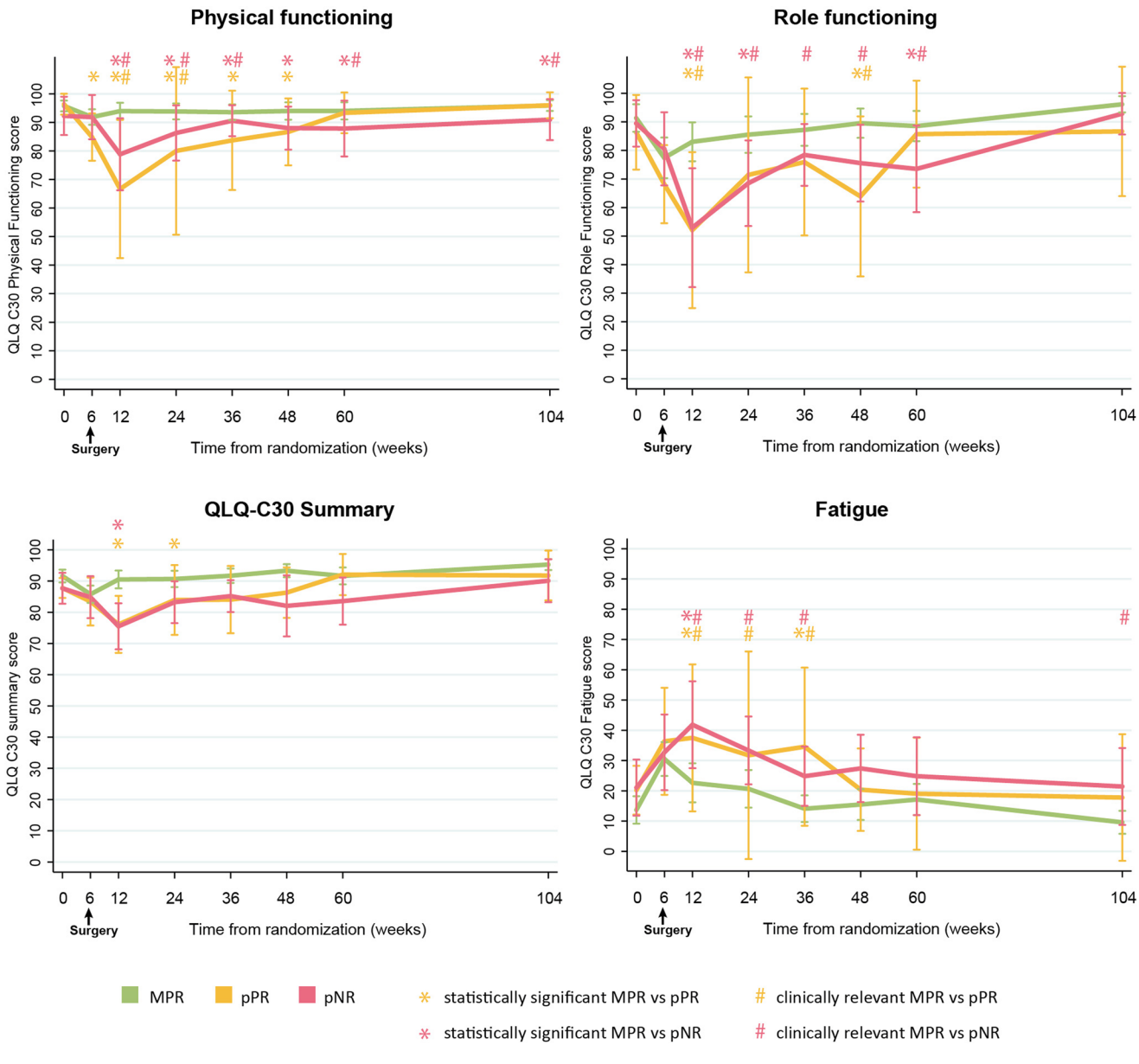


Extended Data Fig. 3 | Flowchart for patient inclusion for surgical morbidity and HRQoL analyses. a, Flow chart of patient inclusion for surgical morbidity analyses. For information regarding the execution of the ILN resection and TLND, see also Supplementary Table 2. **b**, HRQoL analyses of the PRADO trial.

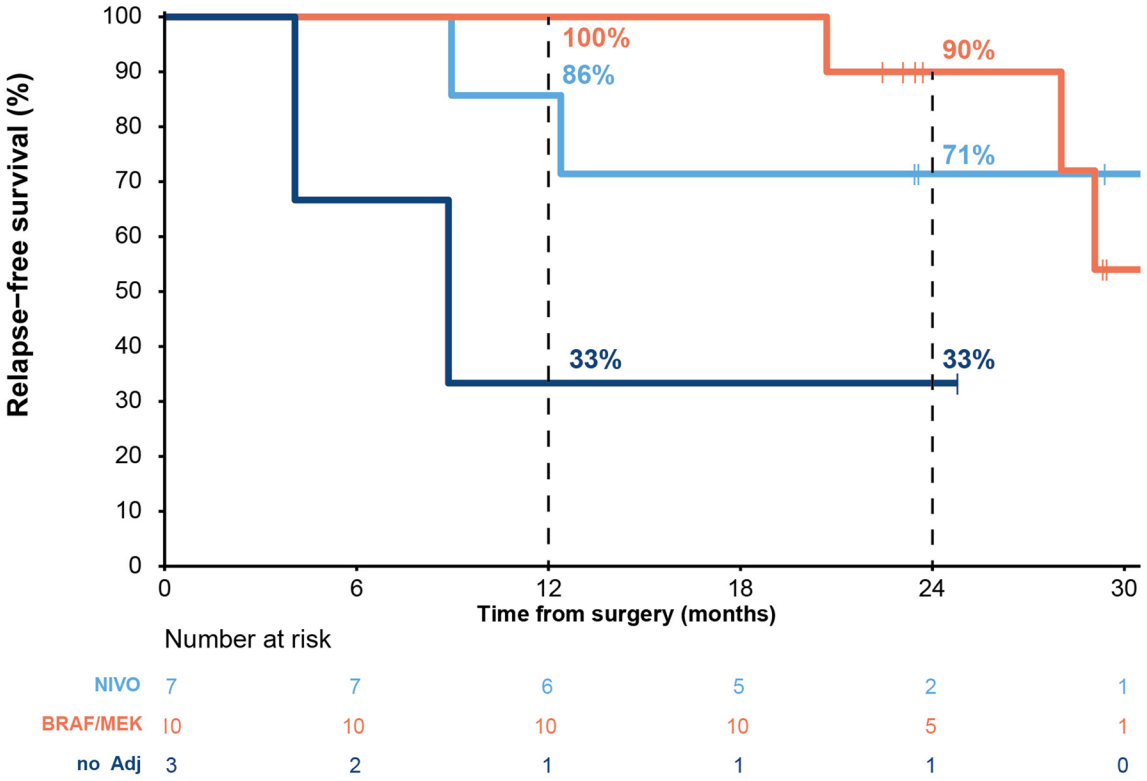


Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Effect of pathological response and treatment on HRQoL outcomes. Curves showing the unadjusted mean HRQoL scores of patients with MPR ($n = 60$, green line) versus patients without MPR ($n = 31$, orange line). Error bars indicate the 95% CI. The differences in mean HRQoL scores between patients with MPR and non-MPR (see also Supplementary Table 5) were adjusted for age, gender, adjuvant treatment and relapse status (no/yes). The adjusted score differences were interpreted in terms of statistical significance using a linear mixed effect model with a two tailed *P* value ($P < 0.05$), and by clinical relevance according to the guideline of Cocks et al[32]. Statistically significant adjusted differences were marked with * and clinically relevant differences were marked with # **(**Supplementary Table 5**)**. Results were considered clinically relevant if the adjusted difference in mean scores between the two groups was at least 'medium' and clinically irrelevant if differences in mean scores were 'trivial or small'. Questionnaire compliance rates in the MPR and non-MPR group were 87% vs 97% at baseline, 98% vs 94% at week 6, 90% vs 81% at week 12, 88% vs 81% at week 24, 92% vs 84% at week 36, 85% vs 68% at week 48, 80% vs 77% at week 60 and 87% vs 61% at week 104 (year 2).



Extended Data Fig. 5 | HRQoL comparison between patients with MPR, pPR and pNR. Curves showing the unadjusted HRQoL scores between patients with MPR ($n=60$, green line), pPR ($n=11$, yellow line) and pNR ($n=20$, red line). Error bars indicate the 95% CI. The differences in mean HRQoL scores between patients with MPR versus pPR and MPR versus pNR were adjusted for age, gender, adjuvant treatment and relapse status (no/yes). The adjusted score differences were interpreted in terms of statistical significance using a linear mixed effect model with a two tailed P value ($P<0.05$), and by clinical relevance according to the guideline of Cocks et al[32]. Statistically significant adjusted differences were marked with * and clinically relevant differences were marked with #. Results were considered clinically relevant if the adjusted difference in mean scores between the two groups was at least 'medium' and clinically irrelevant if differences in mean scores were 'trivial or small'.



Extended Data Fig. 6 | RFS by adjuvant treatment. RFS of patients with pNR from the PRADO trial by adjuvant therapy. Patients were treated with adjuvant nivolumab ($n=7$, light blue line), adjuvant BRAF/MEK inhibition ($n=10$, orange line) or no adjuvant therapy ($n=3$, dark blue line). The patient who was lost to follow-up was excluded.

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☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	The clinical data including was collected and processed in electronic case report forms (eCRF) using Tenalea (version 18.1) at the Netherlands Cancer Institute, Melanoma Institute Australia, Leiden University Medical Centre, Erasmus Medical Center, University Medical Centre Utrecht and University Medical Centre Groningen. Quality of Life data was collected via questionnaires and also entered in the eCRF.
Data analysis	Statistical analyses of the PRADO study were performed using R (version 3.5.1). The Health-related quality of life analyses were performed using STATA (version 15.1, StataCorp, College Station, Texas, USA).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

To minimize the risk of patient re-identification, de-identified individual patient-level clinical data are available under restricted access. Upon scientifically sound request, data access can be obtained via the NKI's scientific repository at repository@nki.nl, who will contact corresponding author CUB. Data requests will be reviewed by the institutional review board (IRB) of the Netherlands Cancer Institute (NKI), and will require the requesting researcher to sign a data access agreement with the NKI.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	During designing of the trial we planned to enroll 100-110 patients. Eventually 99 melanoma patients were accrued. The first goal of the study was to confirm the pathologic response rate (pCR, near-CR, pPR), of the most favorable treatment schedule from Opacin-neo (arm B). A pathologic response rate of 55% was considered unacceptable, while we expected 70% of patients to respond to treatment. An exact test for one proportion has 85% power to test this hypothesis at the two-sided alpha level of 0.05. At least 65 responders were required, which implies the actual significance level of 0.043. Co-primary objectives of the PRADO cohort were to assess 1) whether it is safe to omit TLND in patients achieving a MPR (pCR or near-pCR) and 2) improvement of the RFS rate at 24 months for patients with pNR by adding adjuvant treatment. We expected no recurrences within 24 months in patients achieving MPR, and RFS at 24 months of 90% or less would be considered unsafe. Power calculation for this objective was performed via simulations accessing the lower bound of the one-sided 95% confidence interval, applied to Kaplan-Meier estimate of RFS at 24 months, using beta product confidence procedure (BPCP)29. With at least 46 patients achieving MPR and followed for 24 months, this method has 80-90% power to test H0: 90% versus HA: >90%. In total, 21 patients were included in the pNR group. The BPCP method has 81% power to reject RFS at 24 months of 20% at one-sided alpha of 0.05 in case of improvement of the RFS at 24 months to 45%.
Data exclusions	100 patients were registered in the study and 1 patient was excluded after registration because the diagnosis of melanoma was amended to Hodgekin based on the baseline biopsy of the lymph node. Inclusion and exclusion criteria were prespecified in the study protocol (protocol p.41-43). For the comparison of surgical morbidity between patients who underwent only the ILN procedure and patients who also subsequently underwent TLND, patients who underwent no surgery (n=3) or who underwent a small secondary surgery for removal of some additional lymph nodes (n=4) were excluded from the analysis. For the comparison of health-related quality of life between patients with MPR and no-MPR, patients were excluded if they had distant metastases prior to surgery (n=6, patients went off study after six weeks), if they did not undergo any surgery (n=1), or if they filled in only one questionnaire (n=1).
Replication	Replication of the clinical results is not applicable as this manuscript reports results of a phase II clinical trial. Pathological responses were centrally revised by experienced pathologists from the Netherlands Cancer Institute and Melanoma Institute Australia. Replication of tumor biopsy analyses is limited due to precious tumor material; a standard automated lab-validated immunohistochemistry assay was followed for PD-L1 staining.
Randomization	PRADO used a single arm trial design with personalized response-directed surgery and adjuvant therapy following neoadjuvant checkpoint inhibition, thus randomization is not relevant to the study.
Blinding	The trial was nog randomized and all patients were allocated to receive the same neoadjuvant treatment. Blinding was therefore not performed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	Resectable stage III melanoma patients with one or more measurable lymph node metastases (according to RECIST v1.1) that can be biopsied, no history of in-transit metastases within the last 6 months, naïve for CTLA-4/PD-1/PD-L1 immunotherapy, and more than 18 years old. Of all the patients included the median age was 58 years and 66% was male, 95% had an ECOG performance status of 0, 2% had an elevated LDH level at baseline, and 45% had a BRAF V600 mutation.
Recruitment	All patients with clinically detected stage III nodal melanoma who were treated in one of the participating centers and were deemed eligible for the study were invited to participate. Patients were recruited by either surgical oncologists or medical oncologist from the melanoma cancer clinics in the Netherlands Cancer Institute, Melanoma Institute Australia, Leiden University Medical Centre, Erasmus Medical Center, University Medical Centre Utrecht and University Medical Centre Groningen. Patients were generally referred to the participating study centers by outside hospitals. No specific bias in recruitment was identified.
Ethics oversight	Medical ethics review committee of the Netherlands Cancer Institute and ethical committees at Melanoma Institute Australia approved the trial. The trial was conducted in accordance with the ICH Harmonized Tripartite Guideline for Good Clinical Practice and the principles of the Declaration of Helsinki. All patients provided written informed consent.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	This trial is registered with ClinicalTrials.gov (NCT02977052)
Study protocol	The full trial protocol can be found in the supplementary appendix.
Data collection	Patients were enrolled between November 2018 and January 2020 in the Netherlands Cancer Institute, Melanoma Institute Australia, Leiden University Medical Centre, Erasmus Medical Center, University Medical Centre Utrecht and University Medical Centre Groningen. The data cut-off in the current manuscript was Februari 7th, 2022 (and for the health-related quality of life analyses this was Februari 1st, 2022). Clinical data was collected through an eCRF by the clinical trial department of the Netherlands Cancer Institute. Clinical data was analyzed by the department of biostatistics at the Netherlands Cancer Institute.
Outcomes	The primary objective of the PRADO trial was to confirm the pathologic response rate of the most favorable treatment arm of OpACIN-neo (Arm B: 2 cycles ipilimumab 1mg/kg plus nivolumab 3mg/kg). Co-primary objectives were to investigate whether a TLND could be safely omitted in patients achieving a pCR/near-pCR in the ILN, and whether RFS of patients with a pNR could be prolonged by adding adjuvant treatment. Primary endpoints were pathologic response rate and 2-year RFS of patients with MPR and pNR. When designing the trial, we planned to enroll 100-110 patients with the goal to include at least 50 patients with MPR. This goal was earlier achieved, so that eventually 99 melanoma patients were accrued. A pathological response rate of 55% was considered unacceptable, while we expected 70% of patients to respond to treatment. An exact test for one proportion has 85% power to test this hypothesis at the two-sided alpha level of 0.05. At least 65 responders were required, which implies the actual significance level of 0.043. Our assumption was that no recurrences within 24 months in patients achieving MPR would occur, and RFS at 24 months of 90% or less would be considered unsafe. Power calculation for this objective was performed via simulations accessing the lower bound of the one-sided 95% confidence interval, applied to Kaplan-Meier estimate of RFS at 24 months, using beta product confidence procedure (BPCP). For at least 50 patients that were expected to achieve MPR and assuming 24-months RFS under the alternative hypothesis of 98%, there was 75.5% power, and under the alternative hypothesis of 99% there was 91.5% power. With 60 MPR patients having ≥2 years follow-up, the lower boundary of the one-sided 95% BPCP confidence interval would exceed 90% if no more than 1 recurrence occurred. In total, 21 patients were included in the pNR group. The BPCP method has 81% power to reject RFS at 24 months of 20% at one-sided alpha of 0.05 in case of improvement of the RFS at 24 months to 45%. With 21 pNR patients having ≥2 years follow-up, the lower boundary of the one-sided 95% BPCP confidence interval would exceed the 20% if no more than 13 recurrences occurred. Secondary objectives were confirmation of the toxicity rate of OpACIN-neo arm B (defined as the grade 3-4 immunotherapy-related adverse event rate during the first 12 weeks), radiologic response rate, distant metastases free survival, event-free survival, overall survival, ongoing toxicity at 3 years, comparison of surgical morbidity between patients undergoing only the ILN procedure and ILN procedure plus subsequent TLND, health-related quality of life, and biomarker analyses.