Journal for ImmunoTherapy of Cancer

Ipilimumab and nivolumab combined with anthracycline-based chemotherapy in metastatic hormone receptor-positive breast cancer: a randomized phase 2b trial

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To cite: Andresen NK, Røssevold AH, Quaghebeur C, *et al*. Ipilimumab and nivolumab combined with anthracycline-based chemotherapy in metastatic hormone receptor-positive breast cancer: a randomized phase 2b trial. *Journal for ImmunoTherapy of Cancer* 2024;**12**:e007990. doi:10.1136/jitc-2023-007990

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2023-007990).

Accepted 27 December 2023

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ABSTRACT

Background Immune checkpoint inhibitors have shown minimal clinical activity in hormone receptor-positive metastatic breast cancer (HR$^+$mBC). Doxorubicin and low-dose cyclophosphamide are reported to induce immune responses and counter regulatory T cells (Tregs). Here, we report the efficacy and safety of combined programmed cell death protein-1/cytotoxic T-lymphocyte-associated protein 4 blockade concomitant with or after immunomodulatory chemotherapy for HR$^+$mBC.

Methods Patients with HR$^+$mBC starting first-/second-line chemotherapy (chemo) were randomized 2:3 to chemotherapy (pegylated liposomal doxorubicin 20 mg/m^2 every second week plus cyclophosphamide 50 mg by mouth/day in every other 2-week cycle) with or without concomitant ipilimumab (ipi; 1 mg/kg every sixth week) and nivolumab (nivo; 240 mg every second week). Patients in the chemo-only arm were offered cross-over to ipi/nivo without chemotherapy. Co-primary endpoints were safety in all patients starting therapy and progression-free survival (PFS) in the per-protocol (PP) population, defined as all patients evaluated for response and receiving at least two treatment cycles. Secondary endpoints included objective response rate, clinical benefit rate, Treg changes during therapy and assessment of programmed death-ligand 1 (PD-L1), mutational burden and immune gene signatures as biomarkers.

Results Eighty-two patients were randomized and received immune-chemo (N=49) or chemo-only (N=33), 16 patients continued to the ipi/nivo-only cross-over arm. Median follow-up was 41.4 months. Serious adverse events occurred in 63% in the immune-chemo arm, 39% in the chemo-only arm and 31% in the cross-over-arm. In the PP population (N=78) median PFS in the immune-chemo arm was 5.1 months, compared with 3.6 months in the chemo-only arm, with HR 0.94 (95% CI 0.59 to 1.51). Clinical benefit rates were 55% (26/47) and 48% (15/31) in the immune-chemo and chemo-only arms, respectively. In the cross-over-arm (ipi/nivo-only), objective responses were observed in 19% of patients (3/16) and clinical benefit in 25% (4/16). Treg levels in blood decreased after study chemotherapy. High-grade immune-related adverse events were associated with prolonged PFS. PD-L1 status and mutational burden were not associated with ipi/nivo benefit, whereas a numerical PFS advantage was observed for patients with a high Treg gene signature in tumor.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Therapies blocking the programmed cell death protein-1 (PD-1)-axis are approved for metastatic programmed death-ligand 1-positive triple-negative breast cancer (BC), whereas there is little knowledge on the activity of these drugs against hormone receptor-positive (HR$^+$) metastatic BC. Doxorubicin and cyclophosphamide reportedly have immunostimulatory properties, but clinical data on their potential synergy with immune checkpoint blockade are lacking.

WHAT THIS STUDY ADDS

⇒ This randomized open-label trial demonstrates that the concomitant addition of PD-1/cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockade to doxorubicin and cyclophosphamide increases the risk of high-grade adverse events without improving clinical activity compared with chemotherapy alone in metastatic HR$^+$ BC. However, a subgroup of patients obtained clinical benefit from ipilimumab and nivolumab administered after stopping chemotherapy.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings provide a rationale for further trials exploring dual PD-1/CTLA-4 blockade in HR$^+$ BC, but suggest that combination of these agents with chemotherapy should be sequential rather than concomitant.

Conclusion The addition of ipi/nivo to chemotherapy increased toxicity without improving efficacy. Ipi/nivo administered sequentially to chemotherapy was tolerable and induced clinical responses.
Trial registration number ClinicalTrials.gov Identifier: NCT03409198.

INTRODUCTION

Immune checkpoint blockade (ICB) shows efficacy against metastatic disease in many cancer forms,[1–4] but has not been extensively explored in hormone receptor-positive breast cancer (HR$^+$ BC), which represents about 75% of breast cancer cases.[5] In general, HR$^+$ BC is considered as immunologically cold, with most tumors having few infiltrating lymphocytes, low expression of programmed death-ligand 1 (PD-L1) and low mutational burden.[6–9] There is, however, some evidence of an ICB effect in HR$^+$ BC in the neoadjuvant setting.[10] In metastatic HR$^+$ BC, the response rates are low,[11–15] but there is a lack of data from studies combining ICB with chemotherapy. Data from a few single-arm cohorts have been reported,[16 17] but to our knowledge, only one randomized study. This was a phase II trial indicating no benefit from adding pembrolizumab to eribulin.[18] There is also a lack of ICB data from the early metastatic setting in HR$^+$ BC. The responses to ICB in metastatic triple-negative breast cancer (mTNBC) have been two to four times higher in first-line therapy, compared with later lines.[19]

Anthracycline-based chemotherapy is, along with taxanes, the most commonly used first-line chemotherapy against metastatic BC in Europe. Interestingly, anthracyclines and cyclophosphamide are shown to be potent inducers of immunogenic cell death.[20–22] Data also suggest that the survival benefit from anthracyclines in BC depends on the immune response.[20 23] Still, few studies have explored the potential synergy between anthracyclines and immunotherapy. In the TONIC trial, induction with doxorubicin gave the highest response rates to nivolumab in mTNBC.[24] Low-dose metronomic cyclophosphamide is reported to deplete regulatory T cells (Treg).[25] This has led to interest in the immunogenic effects of cyclophosphamide as an adjuvant in cancer vaccine trials, but with contradictory findings.[26 27]

Here, we report the results of the randomized phase IIb ICON trial investigating the potential of ICB in HR$^+$ mBC, using dual cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein-1 (PD-1) blockade in combination with selected chemotherapy, and applied in the early metastatic setting. In melanoma, the PD-L1-negative subpopulation has the greatest survival benefit from the addition of CTLA-4 blockade to PD-1 inhibition.[1] We hypothesized that ipilimumab (ipi) and nivolumab (nivo), combined with an immunostimulatory backbone of pegylated liposomal doxorubicin (PLD) and low-dose cyclophosphamide (cyclo) would be tolerable and induce clinical responses. PLD was selected instead of other anthracyclines to avoid steroids, minimize adverse cardiac effects and allow for continued treatment. To improve the safety and better control lymphopenia, PLD was administered every second week, instead of every fourth week. Ipilimumab was given in a reduced dosing schedule of 1 mg/kg every sixth week to improve tolerability.[28] Patients in the chemo-only arm were offered cross-over treatment with ipi/nivo after the end of PLD/cyclo-therapy. This cohort was planned as a substudy investigating the use of ipi/nivo after an immunostimulatory chemotherapeutic regimen, without concomitant chemotherapy.

METHODS

Study design and participants

The ICON trial[29 30] was a randomized, open-label, phase IIb trial conducted at five hospitals in Norway and Belgium: Oslo University Hospital (trial sponsor), Stavanger University Hospital, Sørlandet Hospital, Institut Jules Bordet and CHU UCL Namur. The protocol was approved by the Norwegian Medicines Agency, the Belgian Federal Agency for Medicines and Health Products and the regional committees for medical research ethics. The protocol and statistical analysis plan are enclosed (online supplemental data files 1 and 2).

Eligible patients were required to have histologically confirmed metastatic estrogen receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer, measurable disease according to the Response Evaluation Criteria In Solid Tumors V.1.1 (RECIST V.1.1), Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 and maximum one previous line of chemotherapy in the metastatic setting. Previous endocrine and targeted therapies were allowed. A minimum of 12 months was required from anthracycline-containing or cyclophosphamide-containing (neo-) adjuvant therapy to disease recurrence. Patients with asymptomatic, treated brain metastases were eligible. The protocol at study initiation only allowed for patients with luminal B subtype (PAM50), and the randomization was stratified for PD-L1 status. These requirements were removed to simplify the screening process (protocol V.4.0 18 December 2018), after inclusion of 11 patients.

Randomization

Patients were randomly assigned 2:3 to receive chemotherapy alone (chemo-only) or the same chemotherapy in combination with immunotherapy (immune-chemo). Randomization was performed by the investigator using Viedoc (Viedoc Technologies AB, Uppsala, Sweden), based on listings with variable block size generated using Stata 14 (StataCorp, College Station, Texas, USA).

Study procedures

Study treatment was administered over 2-week cycles with PLD 20 mg/m^2 intravenously every second week and cyclophosphamide 50 mg per day in every other cycle (2 weeks on/2 weeks off). In the immune-chemo arm, chemotherapy was combined with ipilimumab 1 mg/kg intravenously every sixth week and nivolumab

240 mg intravenously every second week. Treatment was given until progression per RECIST V.1.1[31] or for a maximum of 24 months. Treatment beyond RECIST V.1.1 progression was allowed in patients with evidence of clinical benefit, absence of symptoms and signs indicating significant disease progression and without a decline in ECOG performance status attributed to disease progression. Patients treated beyond progression were followed using immune RECIST (iRECIST).[31] Patients stopping treatment in the chemo-only arm were offered cross-over to ipilimumab plus nivolumab without chemotherapy. To ease recruitment to the cross-over cohort, one treatment line outside of the trial was accepted before cross-over.

Dose reduction of PLD to 15 mg/m^2 was allowed and compulsory for grade 2 neutropenia or lymphopenia. Ipilimumab dosing interval was prolonged to 12 weeks if a grade ≥3 event related to ipilimumab occurred.

Tumor response was assessed according to RECIST V.1.1[32] as primary method and iRECIST[31] as secondary method. Tumor assessment was performed every 8 weeks the first 12 months and every 12 weeks thereafter. Patients stopping study therapy without disease progression continued tumor response assessments in follow-up for up to 12 months or until initiating other therapy.

Biomarker analyses

PD-L1 expression was assessed by immunohistochemistry (IHC) on prestudy formalin-fixed paraffin-embedded (FFPE) sections (77/82 patients) by the VENTANA SP142 assay (Roche Diagnostics, Rotkreuz, Switzerland) and scored on tumor-infiltrating immune cells, with a cut-off at ≥1%. Forty-five patients had more than one biopsy assessed and were categorized as PD-L1+ if any of the biopsies were positive.

Gene expression analysis was performed on bulk RNA isolated from prestudy FFPE sections (78/82 patients), using the nCounter BC360 assay (NanoString Technologies, Seattle, USA). Gene expression data were used to determine intrinsic molecular subtype, Tumor Inflammation Signature,[33] Treg signature and PD-L1 gene expression. In patients with more than one sample analyzed, the profile was based on the most recent sample.

Tumor-infiltrating lymphocytes (TILs) were assessed in H&E-stained slides of both pretreatment baseline biopsies (78 of 82 patients) and after 4 weeks of treatment. The abundance of lymphocytes within the borders of invasive tumor was scored from 0 to 3 and grouped as low (0–1) or high (2-3).

Tumor mutational burden (TMB) was assessed in study biopsies (67/82 patients) based on whole exome sequencing of tumor-normal pairs as previously described.[34] Briefly, data were analyzed by the nf-core/sarek pipeline[35] followed by TMB estimation on non-synonymous somatic variants.[36] For patients with more than one biopsy assessed, the highest TMB estimate was considered representative.

Flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood using LymphoPrep Cell Separation Media (Abbott Rapid Diagnostics AS, Oslo, Norway), frozen and stored in liquid nitrogen until assessed for T-cell populations by flow cytometry. PBMC were initially incubated with antibodies for surface markers CD3-BUV395, CD8-BUV563, CD4-BV510, CD25-BV605 (BioLegend, Nordic Biosite AS, Oslo, Norway) and Fixable Viability Dye eFluor780 (Thermo Fisher, Oslo, Norway) in fluorescence-activated cell sorting buffer (phosphate-buffered saline +2% fetal bovine serum+500 µM EDTA) containing Brilliant Violet Buffer (BD Bioscience). After fixation and permeabilization using eBioscience Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher), PBMC were incubated with an antibody to the intracellular transcription factor Foxp3-PE (Thermo Fisher). Samples were acquired using BD FACSymphony A5 flow cytometer (BD Biosciences, Franklin Lakes, New J, USA).

Study endpoints and statistical considerations

Primary endpoints were safety of the immune-chemo combination and a comparison of efficacy between the immune-chemo and chemo-only group, measured as progression-free survival (PFS). Safety was evaluated using Common Terminology Criteria for Adverse Events V.4.0 in the full analysis set (FAS), defined as all patients who started therapy with at least one study drug. The primary PFS analysis was performed in the per-protocol (PP) population, defined as all patients who were evaluated for response and received the equivalent of at least two treatment cycles. The PP population was introduced (protocol amendment May 2018) to counter the effect of patients leaving the trial early without enough time for an informative assessment. PFS was defined as the time from randomization to disease progression or death. Patients without disease progression or death were censored at the last tumor assessment date.

Secondary efficacy endpoints were overall survival (OS), objective tumor response rate (ORR), duration of response (DOR), durable response rate (>6 months) (DRR), and clinical benefit rate (CBR, response or stable disease until radiological assessment at week 24±10 days). All efficacy endpoints were analyzed in the PP, FAS, and the PD-L1-positive population by both RECIST V.1.1 and iRECIST. Biomarker assessments (tumor mutational burden, immune gene expression, intrinsic subtype) and patient-reported outcomes (not reported here) were also secondary endpoints.

One patient, randomized to chemo-only, was withdrawn after one cycle due to a need for urgent radiotherapy. At a later time point, she was re-screened and randomized to immune-chemo, where she fulfilled the PP population criteria. She was therefore in the FAS population for both arms, but in the PP population only for the immune-chemo arm. A sensitivity analysis for the primary endpoint (PFS) indicated that the exclusion

of this patient from both arms would have had a negligible effect (online supplemental figure S1A). She was censored for survival in the chemo-only arm at the date of the second randomization.

The sample size calculation was based on a two-sided alpha level of 10% and a power of 80% to detect an absolute reduction of 15% in the proportion of patients with progression or death in the immune-chemo versus the chemo-only arm at 20 months. Based on these calculations, the study planned to randomize 75 patients. Comparisons between treatment arms are presented as HRs with 95% CIs using the Cox proportional hazards model. For categorical data, proportions with 95% CI calculated using the Wilson score method are presented. Median follow-up time was calculated using the reverse Kaplan-Meier method. Wilcoxon paired signed-rank test was used for statistical comparison of flow cytometry data. All p values given are two-tailed. Statistical analyses were performed using Stata V.17 (StataCorp, College Station, Texas, USA) and R V.4.1.2. PBMC data were analyzed with FlowJo V.10.8.1 (BD Biosciences, Ashland, Oregon, USA) and GraphPad Prism software V.9.

RESULTS

Patient characteristics and treatment exposure

From February 2018 to November 2020, the study completed enrolment with a total of 83 patients randomized, of which 82 started allocated therapy in the immune-chemo (N=49) or chemo-only (N=33) arms

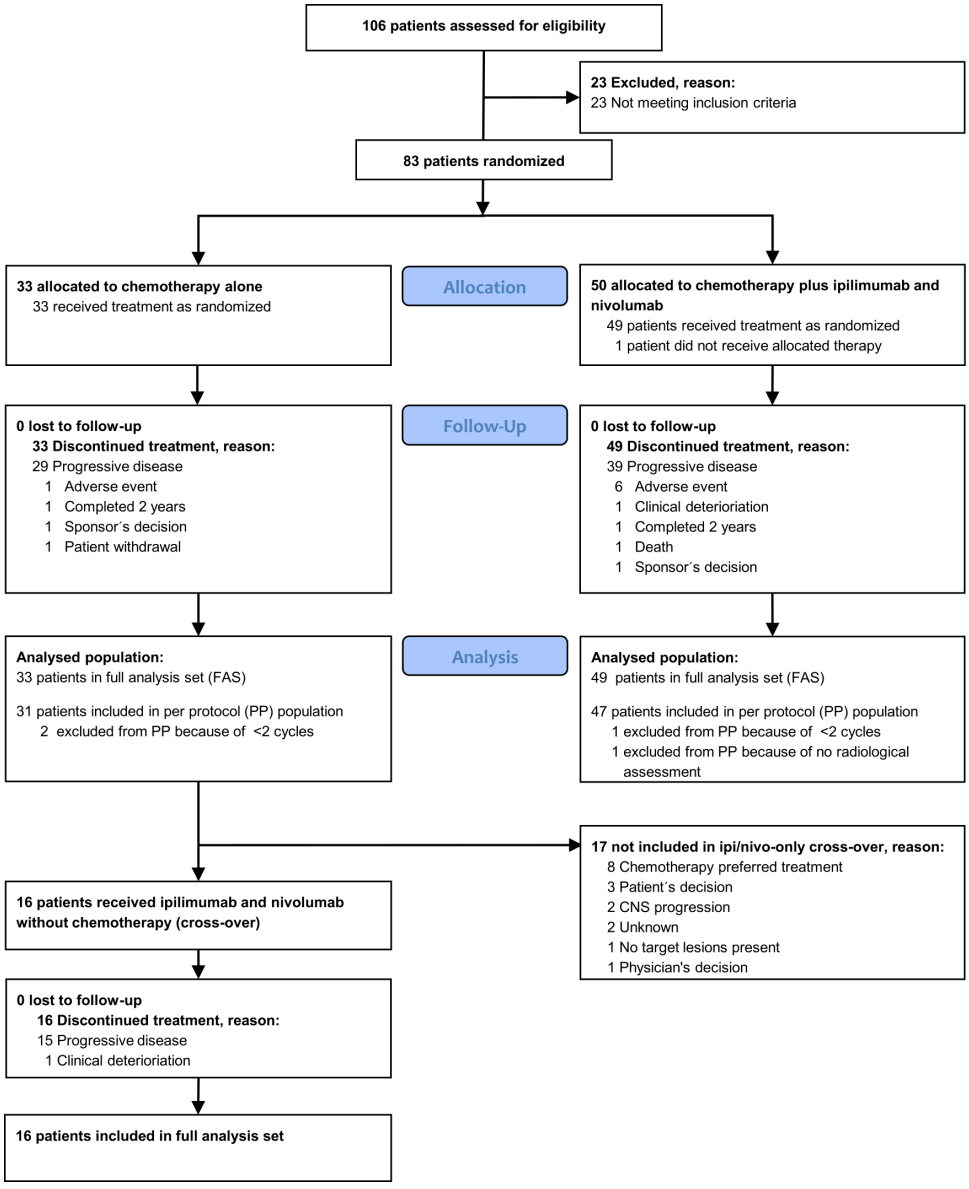

Figure 1 Consolidated Standards of Reporting Trials diagram. The FAS (full analysis set) is a modified intention-to-treat population including all patients starting allocated therapy. The PP (per-protocol) population includes all patients that received the equivalent of at least two treatment cycles and were evaluated for tumor response.

CNS, central nervous system

(FAS population; figure 1). Sixteen patients stopping treatment in the chemo-only arm due to disease progression or toxicity received cross-over treatment with ipi/nivo without chemotherapy. The safety follow-up was completed in May 2022. Baseline patient characteristics are summarized in table 1. The two main arms were mostly well balanced, but the proportions with ECOG 0, de novo metastatic disease or previous chemotherapy in the metastatic setting were higher in the chemo-only arm. Median duration of treatment was similar between the arms (immune-chemo 4.5 months; chemo-only 4.6 months). The mean dose intensity for PLD, defined as percentage of full dose per protocol, was lower in the immune-chemo arm (68% vs 81%).

Safety

Table 2 gives a summary of adverse events (AEs) regardless of relation to study drugs in the FAS population (N=82). A list of all AEs occurring in more than one patient is available in online supplemental table S1. Serious AEs occurred in 63% of patients in the immune-chemo arm versus 39% in the chemo-only arm. Six patients (12%) in the immune-chemo arm and one patient (3%) receiving chemo-only discontinued all study drugs because of AEs. Immune-related adverse events (irAE) were observed in 65% of patients in the immune-chemo arm, most commonly thyroid events (45%), adrenocortical insufficiency/hypophysitis (10%) and pneumonitis (8%). Grade ≥3 irAE occurred in 31% of patients in the immune-chemo arm. Two grade 5 events were recorded, both in the immune-chemo arm. None of these events were considered related to study therapy. One event was considered related to disease progression. The other event was a *pneumocystis jirovecii* lung infection that emerged after treatment with corticosteroids for colitis. The patient had not received trial therapy for >2 months preceding the start of the *pneumocystis jirovecii* infection. Among the 16 cross-over patients receiving ipi/nivo-only, serious AEs were observed in 5 patients (31%) and grade ≥3 irAE in 3 patients (19%) (table 2). Eleven patients (22%) in the immune-chemo arm and four patients (25%) in the ipi/nivo-only arm discontinued ipi/nivo because of treatment-related AEs. An exploratory analysis indicated that patients with irAE had a shorter interval from stopping endocrine treatment, while the time from stopping therapy with CDK4/6 inhibitors (CDK4/6i) was not related to irAE (**data not shown**).

Efficacy

At data cut-off on 20 January 2023, the median follow-up time was 41.4 months (IQR 37.1–45.4). The primary endpoint analysis (PP population; N=78) indicated no difference in PFS between the study arms (HR 0.94, 95% CI 0.59 to 1.51) (figure 2A). Median PFS was 5.1 months (95% CI 3.4 to 6.5) in the immune-chemo arm and 3.6 months (95% CI 1.8 to 9.0) in the chemo-only arm. The proportion of patients without progression or death at 20 months, the time point used for sample size calculations, was 9.1% (95% CI 3.6 to 21.2) versus 3.3% (0.6–16.7) in the immune-chemo and chemo-only arms.

Figure 3 shows PFS for the subgroups of the PP population. The largest numerical difference was observed for patients without liver metastases (HR 0.38; 95% CI 0.11 to 1.28) or with a high Treg gene signature (HR 0.60, 95% CI 0.30 to 1.21). Neither PD-L1 status by IHC, PD-L1 gene expression, nor the Tumor Inflammation Signature[33] were associated with a PFS benefit. The median TMB was 1.4 mut/Mb (IQR 1.1–2.8). No PFS benefit was observed in patients with TMB ≥median, and the only patient with TMB >10 mut/Mb had progressive disease as best response (immune-chemo arm).

In the analyses of secondary endpoints, RECIST V.1.1 and iRECIST gave identical results, with no cases of pseudoprogression. PFS in the FAS population is presented in online supplemental figure S1B. ORR, CBR, DRR, and DOR were similar between the arms (online supplemental table S2). The development of responses over time in each patient is shown in online supplemental figure S2A,B. Median OS was also similar between the arms, both in the PP and FAS populations (figure 2B; online supplemental file S1C). All patients still alive at data cut-off either belonged to the immune-chemo arm or had received ipi/nivo after cross-over.

As exploratory analyses, we investigated if high-grade irAE or recent treatment with a CDK4/6i were associated with PFS benefit. To avoid a bias related to more time for development of irAE among subjects with a long PFS, a landmark analysis was performed for irAE occurring the first 4 months (online supplemental figure S3A). The results indicated prolonged PFS for the group that developed high-grade irAE (HR 0.34; 95% CI 0.13 to 0.93). Recent CDK4/6i exposure was not associated with a PFS benefit for the immune-chemo arm (online supplemental figure S3B).

Fourteen out of the 16 cross-over patients did not receive any treatment between end of study chemotherapy and start of ipi/nivo, whereas two patients received other treatment (paclitaxel) in between. The median time from the end of the last chemotherapy cycle to the start of ipi/nivo was 2.1 weeks (IQR 1.3–7.0). Median PFS was 1.9 months (IQR 1.6–5.5) (figure 2C) and the CBR was 25% (95% CI 10.2 to 49.5). Five patients had a measurable reduction in target lesions (figure 2D), none of whom received other treatment between the study chemotherapy and ipi/nivo. Three of these patients had a confirmed partial response, with response durations of 3.7, 7.0, and 10.8 months (figure 2C; online supplemental figure S2C). Paired biopsies before and 4 weeks into ipi/nivo therapy were available for TIL assessment from four out of five patients with target lesion reduction. An increase in TIL score was recorded in all four cases. By contrast, none of the five patients with paired biopsies and no target lesion reduction had an increase in TIL score. None of the three objective responders had PD-L1-positive disease assessed by IHC, or a high TMB. An overview of candidate biomarkers in patients with/without clinical benefit is presented in online supplemental table S3. Exploratory

Table 1 Patient demographics and clinical characteristics

Baseline characteristics	Chemotherapy only (N=33)	Chemotherapy plus ipilimumab and nivolumab (N=49)	P value	Ipilimumab and nivolumab only cross-over (N=16)
Median age, years	55 (37–74)	53 (36–75)	1.00	56 (39–73)
Gender			1.00	
Female	33 (100)	48 (98)		16 (100)
ECOG performance status			0.16	
0	18 (55)	19 (39)		11 (69)
1	15 (45)	30 (61)		5 (31)
De novo metastatic disease	9 (27)	9 (18)	0.34	4 (25)
Sites of metastases				
Bone metastases	28 (85)	45 (92)	0.47	14 (88)
Liver metastases	28 (85)	36 (73)	0.22	15 (94)
Lung metastases	6 (18)	18 (37)	0.07	3 (19)
>3 sites of metastases	9 (27)	14 (29)	0.90	4 (25)
Previous (neo-) adjuvant chemotherapy	20 (61)	35 (71)	0.31	10 (63)
Previous lines of metastatic chemotherapy			0.08	
0[a]	18 (55)	36 (73)		
1	15 (45)	13 (27)		
Type of first-line metastatic chemotherapy			0.15	
Paclitaxel	10 (30)	4 (8)		
Capecitabine	3 (9)	7 (14)		
Taxane-based combinations	2 (6)	2 (4)		
Previous CDK4/6 inhibitor	30 (91)	44 (90)	1.00	15 (94)
Previous lines of metastatic endocrine therapy			1.00	
0	2 (6)	2 (4)		1 (6)
1–2	21 (64)	31 (63)		10 (63)
≥3	10 (30)	16 (33)		5 (31)
PD-L1 expression (IHC, SP142 clone)			0.53	
Positive	10 (30)	19 (39)		5 (31)
Negative	20 (61)	28 (57)		11 (69)
Missing	3 (9)	2 (4)		0
HER2 status			0.75	
HER2 zero (IHC 0)	13 (39)	17 (35)		6 (38)
HER2 low (IHC 1+/IHC 2+)	17 (52)	26 (53)		10 (63)
Missing	3 (9)	6 (12)		0
PAM50 subtype			1.0	
Luminal A	6 (18)	9 (18)		3 (19)
Luminal B	21 (64)	34 (69)		11 (69)
HER2 enriched	3 (9)	4 (8)		1 (6)
Basal	0	1 (2)		0
Missing	3 (9)	1 (2)		1 (6)

Data are presented as median (range) for continuous measures and N (%) for categorical measures. PD-L1 expression (SP142 assay) and PAM50 subtype (nCounter BC360) were assessed in prestudy biopsies. HER2 status was based on available pathology reports from prestudy biopsies (primary tumors N=31, metastases N=42). HER2 zero was defined as IHC 0 and HER2 low defined as either IHC 1+ or IHC 2+ with a negative in situ hybridization assay. Two-sided p values were calculated using the Wilcoxon rank-sum test for continuous measures and Fisher's exact or χ^2 test for categorical data. [a]Patients who had started/received first-line chemotherapy with anthracyclines and not progressed (four in chemo-only, eight in immune-chemo), were classified as continuation of first-line treatment.
CDK4/6, cyclin-dependent kinase 4 and 6; ECOG, Eastern Cooperative Oncology Group; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1.

Table 2 Summary of adverse events

	Chemotherapy only (N=33)		Chemotherapy plus ipilimumab and Nivolumab (N=49)		Ipilimumab and nivolumab only cross-over (N=16)	
	All grades N (%)	Grade ≥3 N (%)	All grades N (%)	Grade ≥3 N (%)	All grades N (%)	Grade ≥3 N (%)
Any AE	33 (100)	16 (48)	49 (100)	41 (84)	15 (94)	4 (25)
Any TRAE	32 (97)	13 (39)	48 (98)	36 (73)	14 (88)	3 (19)
Any SAE	13 (39)	4 (12)	31 (63)	26 (53)	5 (31)	3 (19)
Immune-related adverse events (irAE)						
Any irAE	1 (3)	0	32 (65)	15 (31)	8 (50)	3 (19)
Thyroid events	1 (3)	0	22 (45)	1 (2)	2 (13)	0
Adrenocortical insufficiency/hypophysitis	0	0	5 (10)	5 (10)	2 (13)	0
Pneumonitis	0	0	4 (8)	1 (2)	1 (6)	0
Hepatitis	0	0	3 (6)	3 (6)	2 (13)	1 (6)
Colitis/diarrhoea	0	0	3 (6)	2 (4)	2 (13)	1 (6)
Type 1 diabetes mellitus	0	0	2 (4)	2 (4)	0	0
Pancreatitis/lipase increased	0	0	2 (4)	2 (4)	0	0
Rash	0	0	1 (2)	1 (2)	1 (6)	1 (6)
Nephritis	0	0	1 (2)	0	0	0
Most common adverse events						
Fatigue	16 (48)	1 (3)	27 (55)	4 (8)	6 (38)	0
Lymphocyte count decreased	15 (45)	7 (21)	32 (65)	18 (37)	0	0
Rash	13 (39)	3 (9)	27 (55)	8 (16)	5 (31)	1 (6)
Nausea	16 (48)	0	26 (53)	2 (4)	2 (13)	0
Constipation	18 (55)	1 (3)	16 (33)	0	0	0
Stomatitis	12 (36)	1 (3)	20 (41)	1 (2)	2 (13)	0
PPE	10 (30)	0	16 (33)	2 (4)	0	0
Neutrophil count decreased	10 (30)	5 (15)	11 (22)	3 (6)	1 (6)	0
Musculoskeletal pain	2 (6)	0	11 (22)	0	5 (31)	0
Fever	2 (6)	0	8 (16)	2 (4)	4 (25)	0
Pruritus	2 (6)	0	3 (6)	0	5 (31)	0

Adverse events in the FAS population are graded according to NCI CTCAE V.4.0 and presented as N (%) by treatment arm. Individual adverse events are listed regardless of relation to study therapy. Repeated adverse events in the same subject are counted only once. The table includes all immune-related adverse events, defined as 'adverse events of special interest' according to the protocol, and all adverse events occurring in ≥25% of patients in any treatment group.
AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events; FAS, full analysis set; NCI, National Cancer Institute; PPE, palmar-plantar erythrodysesthesia syndrome; SAE, serious AE; TRAE, treatment-related adverse event.

analysis of overall survival by clinical benefit is shown in online supplemental figure S1D. Among the three patients with objective response, one survived for 33 months after cross-over, and the other two were alive at data-cut off (23+ months, 30+months).

Changes in circulating T cells during therapy

We investigated if the applied therapy led to changes in the composition of circulating T cells. To this aim, paired PBMC samples (pretreatment and week 8) from 52 patients were analyzed by flow cytometry. The lymphocyte populations were identified as shown in online supplemental figure S4. The percentage of Tregs was reduced in both chemotherapy-containing arms ($p<0.05$), consistent with the hypothesized effect of metronomic cyclophosphamide (figure 4A). By contrast, patients in the ipi/nivo-only cohort had a relative increase in Tregs. The absolute counts decreased for all T-cell subsets in both the chemo-only and immune-chemo arm, but increased in patients receiving ipi/nivo-only (figure 4B).

DISCUSSION

The ICON trial is to our knowledge the first randomized study in any form of mBC employing dual PD-1/CTLA-4 blockade, and the first to combine it with chemotherapy. There was a clear rationale for exploring the selected combination, based on the efficacy of PD-1/CTLA-4 blockade in PD-L1-negative melanoma and lung

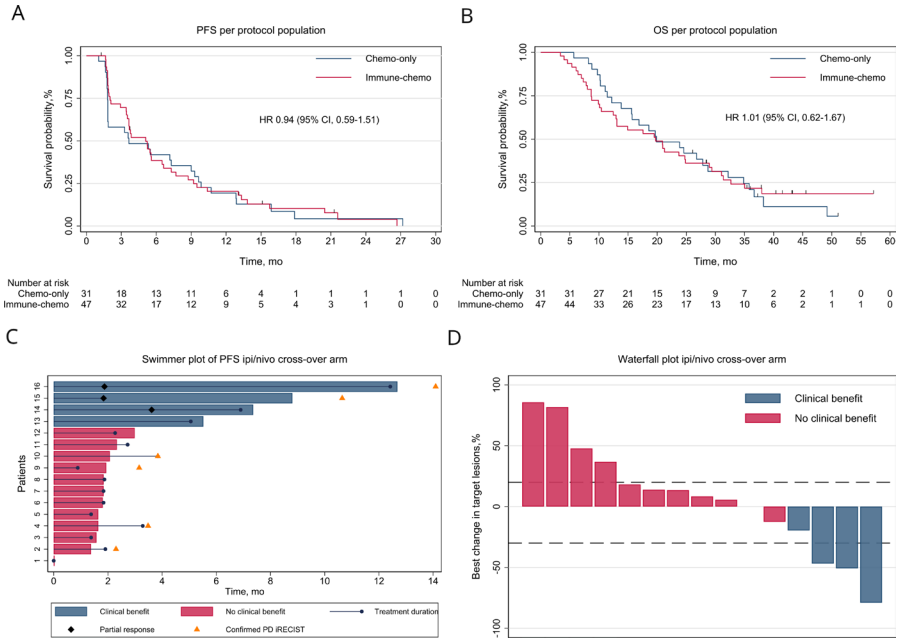

Figure 2 Clinical outcome. Kaplan-Meier plots of (A) PFS and (B) OS in the PP population. HRs are presented with a 95% CI. (C) Swimmer plot of the ipi/nivo-only cross-over arm. (D) Waterfall plot of best change in target lesions in ipi/nivo-only cross-over patients evaluated for response. Dashed lines represent 20% increase and 30% reduction in target lesions. ipi, ipilimumab; iRECIST, immune Response Evaluation Criteria In Solid Tumors; mo, months; nivo, nivolumab; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PP, per-protocol.

cancer,[1 2] and the perceived immunogenic properties of anthracyclines and effects of low-dose cyclophosphamide on Tregs. We did not observe any PFS advantage from the concomitant addition of ipi/nivo to chemotherapy, and considerable toxicity. In patients receiving cross-over treatment with ipi/nivo after stopping chemotherapy, we still observed clinical benefit in 25% of patients.

The number of patients in the ipi/nivo-only cross-over arm was limited. It is still interesting that their response rates were not inferior to biomarker-enriched ICB trials in HR$^+$ mBC,[11 37 38] which only enrolled patients with a high mutational burden or PD-L1$^+$ disease. Furthermore, despite a modest duration of response to ipi/nivo-only, long-term survival was observed in the responders. We detected an increased number of circulating Tregs after ipi/nivo therapy. This may be a compensatory consequence of immune activation. The apparent association between TIL increase and target lesion reduction suggests that on-treatment biomarkers should be further explored. It is interesting that we observed responses from ipi/nivo-only, without any signal of benefit from the concomitant addition of ipi/nivo to chemotherapy. This duality may be incidental, but could reflect that the scheduling of chemotherapy before ipi/nivo was beneficial. All patients with clinical benefit in the ipi/nivo-only arm started ipi/nivo directly after PLD/cyclo. The hypothesized immunomodulatory actions of PLD/cyclo, including the observed reduction in Tregs, may have created a fertile ground for ipi/nivo-activity. In mTNBC, the SAFIR02-BREAST IMMUNO and TONIC trials have indicated a benefit of PD-L1/PD-1 blockade after induction chemotherapy.[14 24] An immunostimulatory effect of PLD/cyclo would be in line with our recently reported ALICE study in mTNBC, employing the same chemotherapy backbone.[34] The ALICE data indicated a benefit from the addition of atezolizumab for both PD-L1-positive and PD-L1-negative mTNBC, whereas studies with other chemotherapy backbones have not suggested ICB benefit for PD-L1-negative disease.[3 4] Contrary to ICON, there was no substantial difference in the dose reductions of PLD/cyclo between the arms in the ALICE study.

The observed association in ICON between high-grade irAE and prolonged PFS in the immune-chemo arm is intriguing. It is conceivable that a moderate effect of ipi/nivo in the randomized comparison was nullified by the more frequent dose reduction of chemotherapy in the immune-chemo arm. Liver metastases are described to be more resistant to ICB.[39] In our study, patients without liver metastases had a numerically improved PFS in the immune-chemo arm, but the number of patients without liver lesions was small. CDK4/6 inhibitors are reported to have pro-inflammatory effects,[40] but no association between recent CDK4/6i exposure and benefit from the immune-chemo combination was observed.

The immune microenvironment in HR$^+$ mBC differs from TNBC.[9] This may imply a need for other biomarkers and therapeutic targets. In the ICON study, we observed no advantage for the immune-chemo arm in patients with a baseline high PD-L1 expression, TIL score or Tumor Inflammation Signature. With regard to PD-L1, our finding is in line with trials combining eribulin with pembrolizumab in HR$^+$ mBC.[17 18] The role of PD-L1 expression in this population will be clarified by the ongoing phase III KEYNOTE-B49 trial assessing

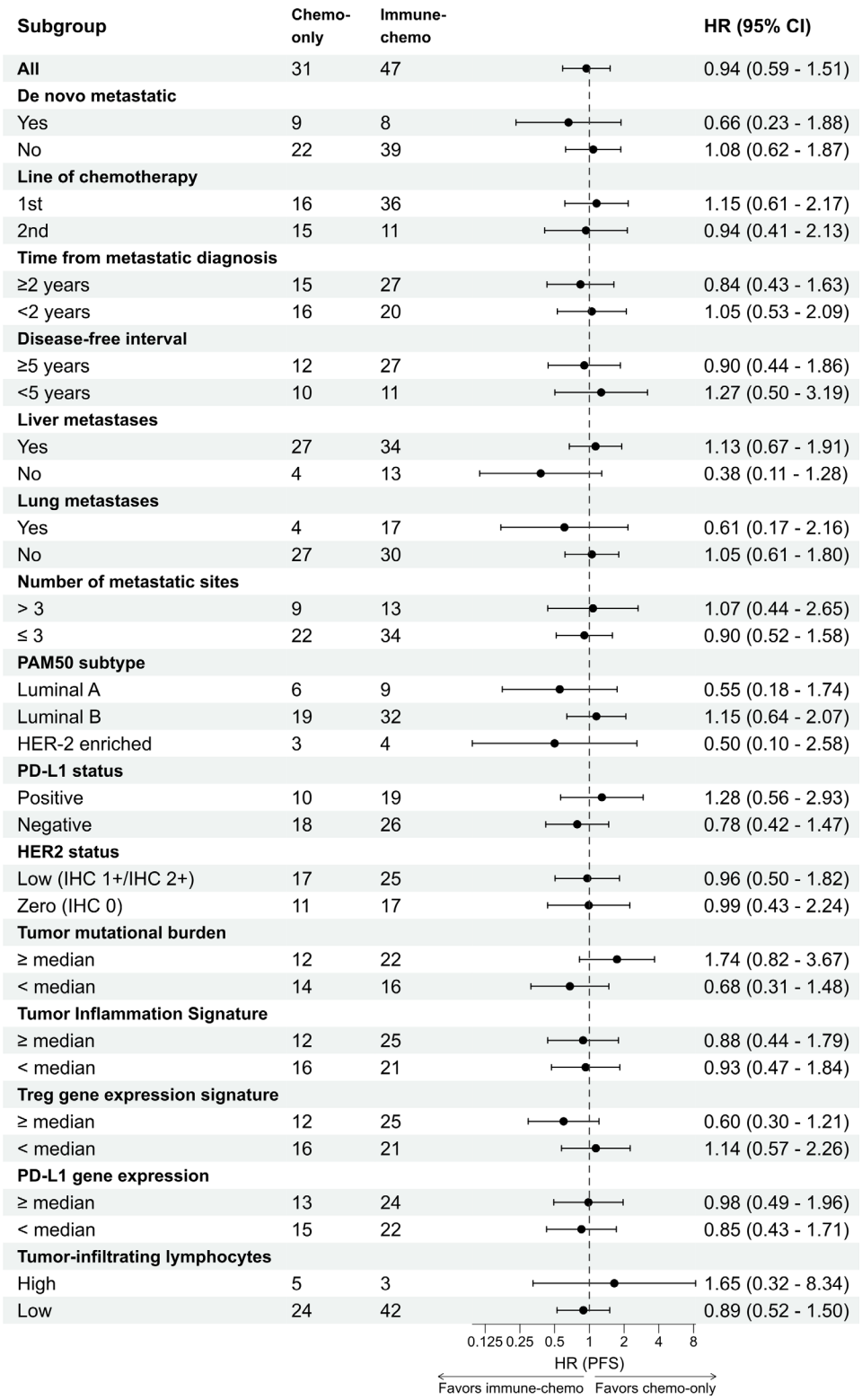

Figure 3 Progression-free survival in subgroups. Forest plot of PFS in subgroups of the PP population. PD-L1 expression was assessed by IHC in prestudy biopsies using the SP142 assay. PAM50 subtype, tumor inflammation signature, PD-L1 gene expression and Treg gene signature were obtained from bulk RNA isolation from prestudy biopsies (nCounter BC360 assay). Tumor-infiltrating lymphocytes (TILs) were scored from 0 to 3 on H&E stained slides and categorized as low (0–1) or high (2-3) infiltration. TILs were assessed in pretreatment screening biopsies (N=55) or if not sufficient material the most recent prestudy biopsy available (N=19). HER2 status was based on pathology reports from prestudy biopsies (primary tumors N=31, metastases N=42). HER2 zero defined as IHC 0 and HER2 low defined as either IHC 1+ or IHC 2+ with a negative in situ hybridization assay. HRs are presented with 95% CIs. HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ipi, ipilimumab; nivo, nivolumab; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PP, per-protocol; Treg, regulatory T cell.

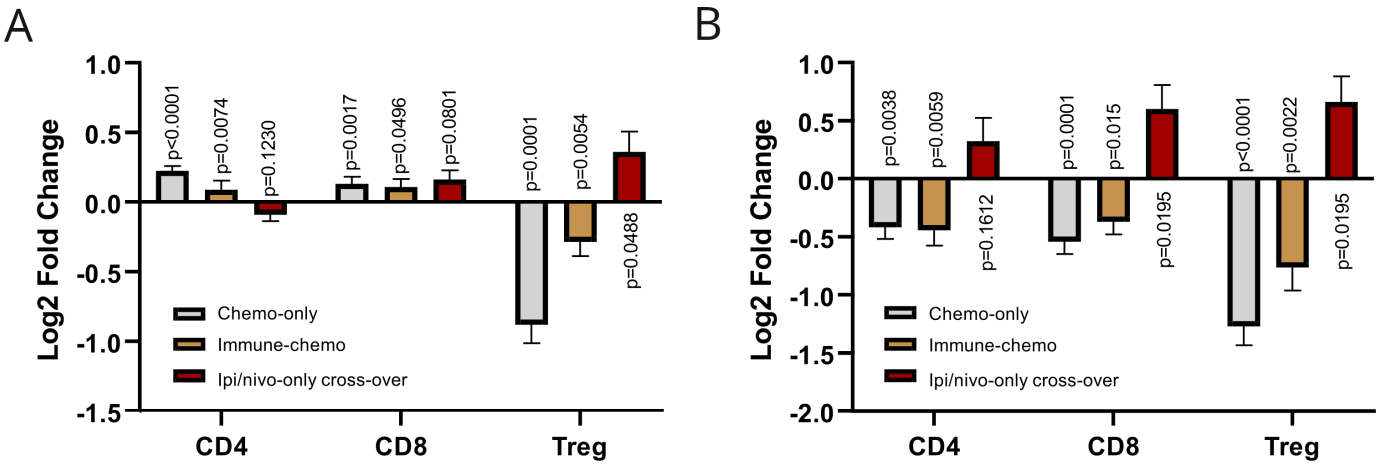

Figure 4 Impact of therapy on the phenotype and frequency of circulating T cells. PBMC from screening and week 8 were assessed for T-cell subsets by flow cytometry in 52 patients (chemo-only N=21, immune-chemo N=31, ipi/nivo cross-over N=11). Absolute cell counts were available from a subset of 41 patients (chemo only N=17, immune-chemo N=24, ipi/nivo cross-over N=10). Fold changes from screening to week 8 were calculated and log2 transformed. Data are presented as mean±SEM. CD4+ and CD8+ T-cell subsets are shown as a percentage of total lymphocytes and regulatory T cells are shown as a percentage of CD4+ T cells. (A) Percentage of T-cell subsets. (B) Absolute cell counts. P values were calculated using the Wilcoxon matched-pairs signed-rank test. Treg, regulatory T cell; ipi, ipilimumab; nivo, nivolumab; PBMC, peripheral blood mononuclear cell.

pembrolizumab in combination with chemotherapy in PD-L1-positive HR$^+$ mBC.[41] In our study, a numerical PFS benefit for the immune-chemo arm was observed for patients with a high Treg gene signature in tumor. This finding is of particular interest as preclinical studies have suggested that ipilimumab may deplete Tregs.[42 43] Even in the cross-over arm, the clinical benefit from ipi/nivo was not associated with PD-L1 IHC positivity, PD-L1 gene expression, the Tumor Inflammation Signature, or a high TMB, which are biomarkers for response to PD-1 blockade. Taken together, our observations support the role of ipilimumab in the clinical responders. Previous data from CTLA-4 blockade in patients with HR$^+$ BC are limited[13 37 44] and more studies would be valuable.

There was a clear difference in high-grade and serious AEs between the arms. The irAEs mainly represented endocrine events, most commonly hypothyroidism. In the immune-chemo arm, 45% developed hypothyroidism, compared with 13% in the ipi/nivo-only cross-over arm. The frequency of hypothyroidism was 13.6% in a pooled analysis of three lung cancer trials with equivalent ipi/nivo dosing, and 16% in a lung cancer study combining chemotherapy with ipi/nivo.[45 46] The reason for the high frequency of endocrine irAE in the ICON immune-chemo arm is not clear. It could be related to a Treg-depleting effect of the chemotherapy or to the study population. Autoimmune diseases are more frequent in women,[47] as are AEs from cancer immunotherapy,[48] and previous radiotherapy may predispose for thyroid disorders. However, other mBC studies with PD-1/PD-L1 blockade plus chemotherapy have reported a frequency of hypothyroidism of 13–16%.[3 4 18] Most ICON patients had recently stopped endocrine therapy (ET), and the interval from stopping ET to randomization was shorter in those developing irAE. Data from trials combining PD-1 inhibitors with CDK4/6 inhibitors and ET have shown high rates of irAEs.[49 50] Estrogen contributes to the differences in immune responses between men and women,[47] and immunogenic effects of altered estrogen signaling could be a contributing factor to irAEs in these trials and in ICON.

There are several limitations to this study. First, the trial was not powered to detect a small difference in efficacy between the two arms. Second, imbalances between the arms represent a limitation in smaller randomized trials. In ICON, the immune-chemo group had a higher proportion without previous chemotherapy in the metastatic setting, but also an inferior ECOG status and a lower proportion with de novo metastatic disease. Third, several subgroups of interest are too small for an informative assessment.

This study indicates that the concomitant administration of ipi/nivo with PLD and low-dose cyclophosphamide causes a high risk of immune-related toxicity without improving therapeutic efficacy in HR$^+$, HER2-negative mBC. Ipi/nivo administered after PLD/low-dose cyclophosphamide was tolerable and induced responses in a clinically meaningful proportion of patients. Further trials combining CTLA-4 and PD-1 inhibitors in HR$^+$ mBC without concomitant chemotherapy should be considered, including trials employing pre-conditioning with immunomodulatory chemotherapy.

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Acknowledgements The authors thank the patients, their families, the user representative and the personnel at the study hospitals for their contributions and excellent patient care. We would also like to acknowledge the organizers of the ESMO Congress 2022 for letting us present the first analysis of data from the main treatment cohorts of the ICON trial[30]. Special thanks to Ester-Johanne Sande, Ahmad Awada, Elin Borgen, Cecilie Bendigtsen Schirmer, Ingrid Bakka, Jon Lømo, Tonje Lien, Tormod Guren, Signe Fretland, Marius Stensland, Christian Kersten, Mai Nguyen, Thea Jahr, Gaute Hagen, Gjertrud Skorstad, and Veronica E Feilum. We are also grateful to the team at Bristol Myers Squibb Norway, NanoString Technologies, the Oslo University Hospital Bioinformatics and Genomics Core Facilities, and the contributing units at Oslo University Hospital Department of Pathology, headed by Mette Førsund, Inger Johanne Ryen, and Anne Renolen.

Contributors JAK was the coordinating investigator of the study and was responsible for study conception and design, and acquisition of funding and approvals. He also contributed as an investigator and medical monitor, and to patient recruitment, data collection, and data interpretation. The manuscript was written by JAK and NKA, with contributions from all authors. NKA and AHR were investigators at Oslo University Hospital and medical monitors for the other sites, and contributed to patient recruitment and data acquisition, curation, and analysis. AG, CQ, BG and BB were principal investigators at their respective study sites. RSF and OCL were study statisticians. BN contributed to the study conception and design, and to patient recruitment and data interpretation. LJ performed radiological assessments. RRM was an investigator and medical monitor. HGR and ØG were study pathologists. CD, SKC and RRL performed translational laboratory analyses. JAK and NKA are responsible for the overall content as guarantors. All authors approved the final version of this manuscript.

Funding Bristol Myers Squibb supported the study (CA209-9FN) with free drug (ipilimumab and nivolumab) and a funding contribution. The BC360 panel and kit were provided by NanoString Technologies. The study was also supported by grants from Norwegian Health Region South-East (grants 2017100 and 2018090 to JAK; grant 2019014 to RRM) and the Norwegian Cancer Society/Norwegian Breast Cancer Society (grant 214972 to JAK).

Competing interests JAK has in the last 5 years received research support from Bristol Myers Squibb, F. Hoffmann-La Roche, NanoString, and NEC OncoImmunity and has previously received advisory board/lecture honoraria from pharmaceutical companies, including Bristol Myers Squibb. CQ has received honoraria for advisory board from AstraZeneca. BG has received honoraria for advisory boards from Eli Lilly, Gilead, Daiichi Sankyo, Roche, and Pierre Fabre. LJ has received lecture honoraria from Pfizer, Novartis, and AstraZeneca. AG has received travel grants or honoraria for advisory boards from Lilly, Daiichi Sankyo, Seagen, Pfizer, and AstraZeneca. HGR has received research support from Illumina and NanoString. OCL has over the last 2 years received honoraria for work as statistical advisor for Novartis. All other authors declare no competing interests.

Patient consent for publication Not applicable.

Ethics approval Ethics committee approval in Norway: Regional Committee for Medical Research Ethics south-east D, Norway reference# 2017/1283. Ethics committee approval in Belgium: Commissie Medische Ethiek, Roeselare, Belgium reference# 19021. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Any request for raw or analyzed data will be reviewed by the study team, and a response can be expected within 14 days. Requests should be made to the corresponding author (jonky@ous-hf.no). The data generated in this study is subject to patient confidentiality, and the transfer of data or materials will require approval from the Regional Committee for Medical and Health Research Ethics South-East Norway.

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