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

Nikolai Kragøe Andresen ,[1,2] Andreas Hagen Røssevold ,[1,2] Claire Quaghebeur,[3] Bjørnar Gilje,[4] Beate Boge,[5] Andrea Gombos,[6] Ragnhild Sørum Falk,[7] Randi R. Mathiesen,[8] Lars Julsrud,[9] Øystein Garred,[10] Hege G. Russnes,[2,11] Ragnhild Reehorst Lereim,[12] Sudhir Kumar Chauhan,[12] Ole Christian Lingjærde,[13,14] Claire Dunn,[12] Bjørn Naume,[2,8] Jon Amund Kyte [1,15]

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

For numbered affiliations see end of article.

Correspondence to
Professor Jon Amund Kyte; jonky@ous-hf.no

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

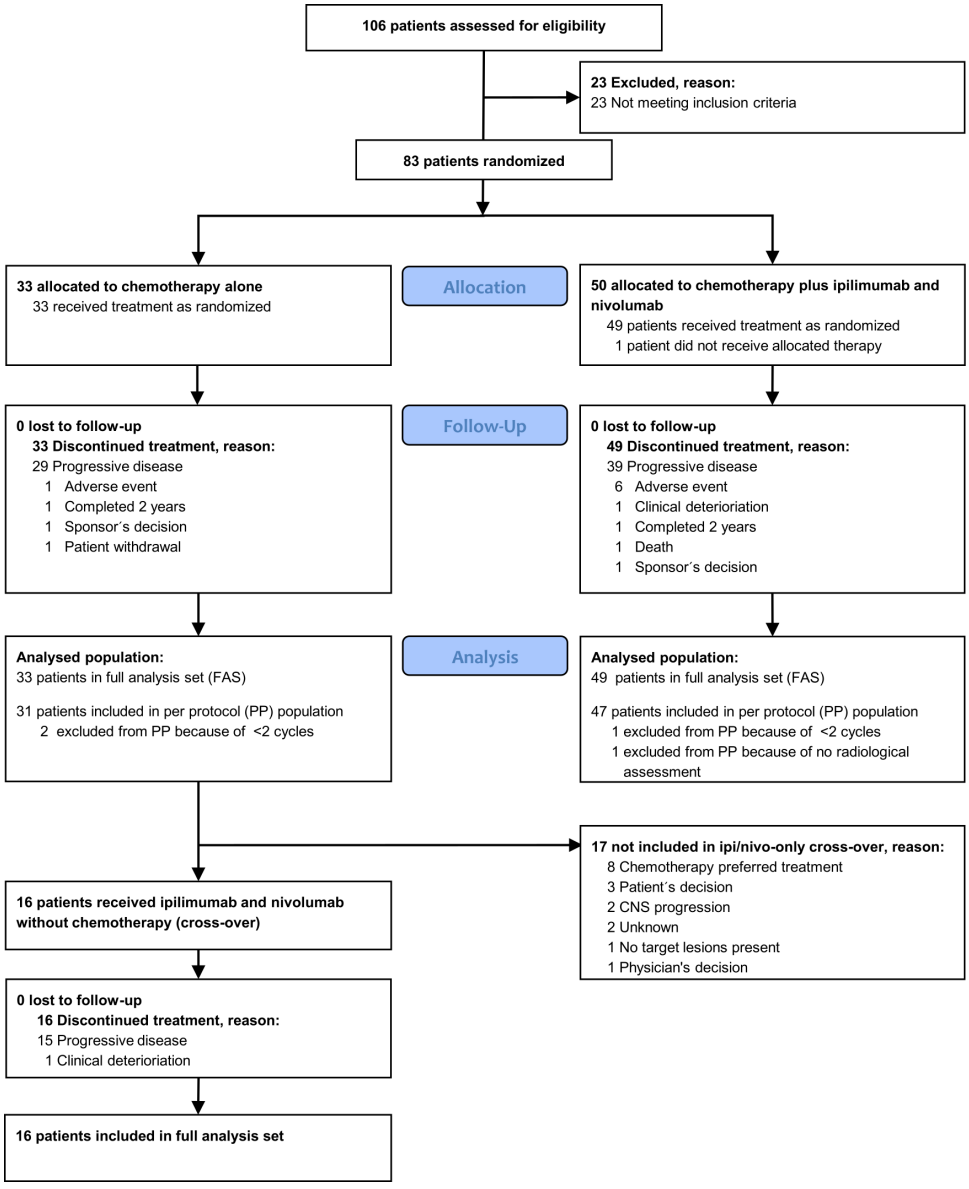


Figure 1 Consolidated Standards of Reporting Trials diagram. The FAS (full analysis set) is a modified intention-to-treat population including all patients starting allocated therapy. The PP (per-protocol) population includes all patients that received the equivalent of at least two treatment cycles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

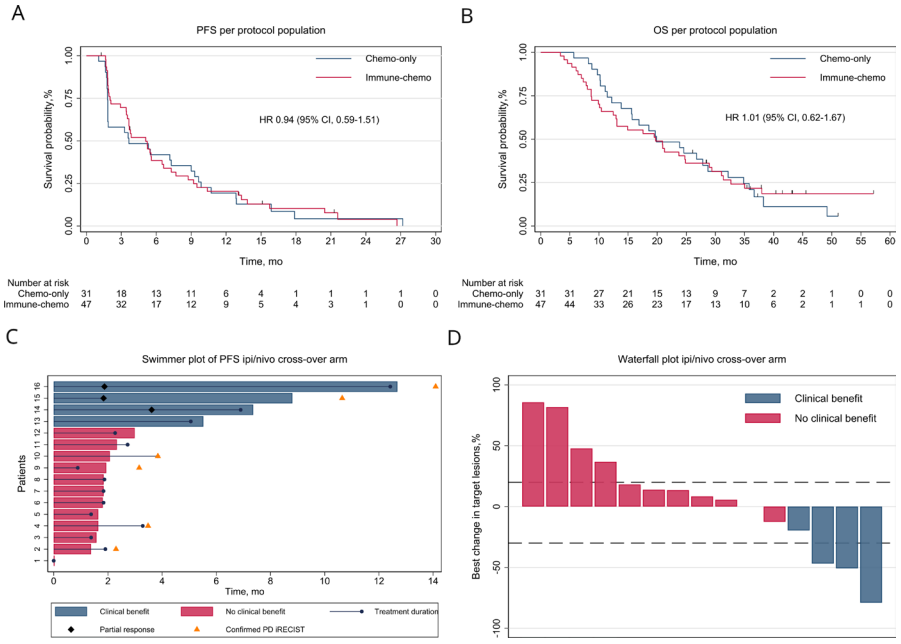


Figure 2 Clinical outcome. Kaplan-Meier plots of (A) PFS and (B) OS in the PP population. HRs are presented with a 95% CI. (C) Swimmer plot of the ipi/nivo-only cross-over arm. (D) Waterfall plot of best change in target lesions in ipi/nivo-only cross-over patients evaluated for response. Dashed lines 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

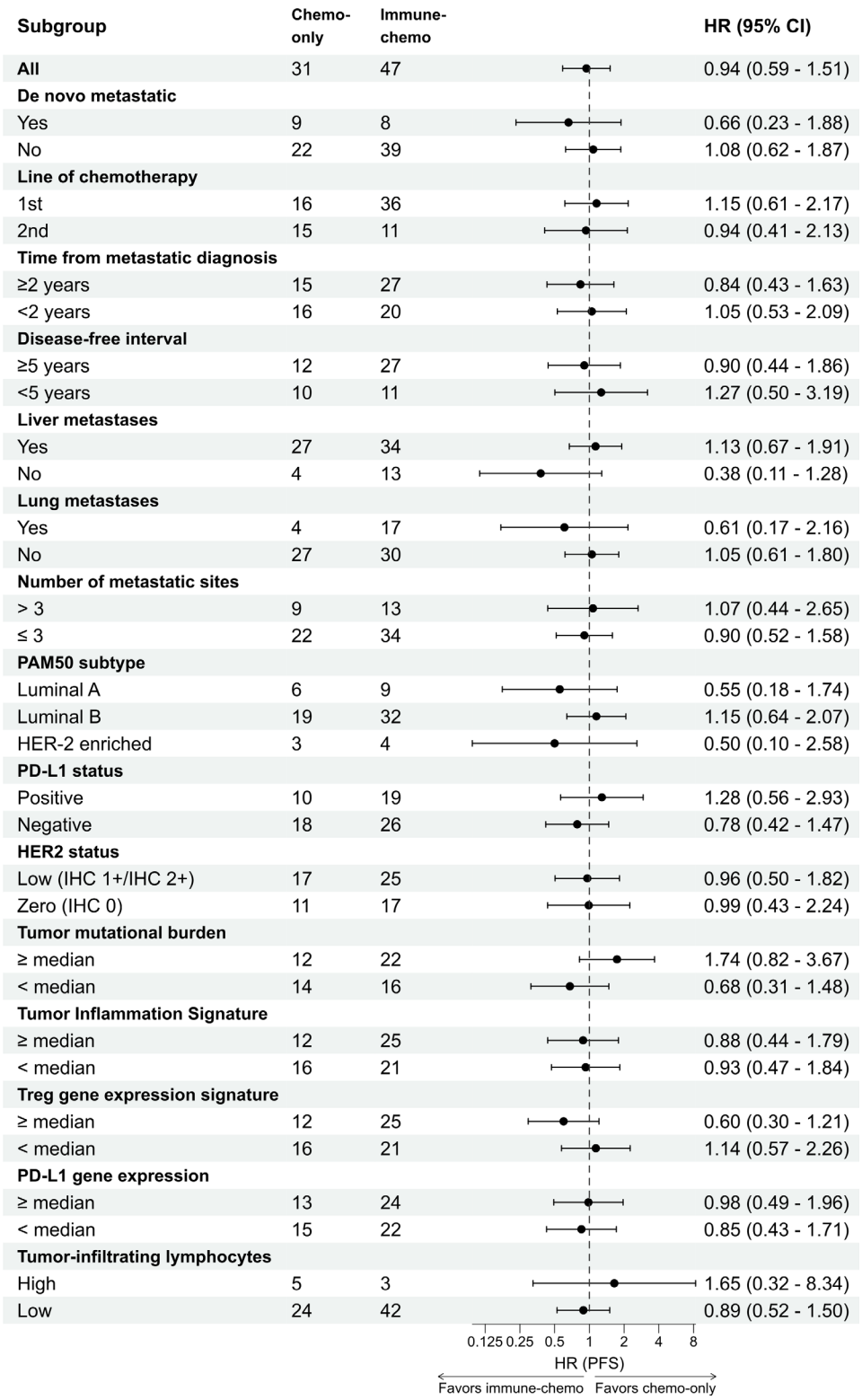


Figure 3 Progression-free survival in subgroups. Forest plot of PFS in subgroups of the PP population. PD-L1 expression was assessed by IHC in prestudy biopsies using the SP142 assay. PAM50 subtype, tumor inflammation signature, PD-L1 gene expression and Treg gene signature were obtained from bulk RNA 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.

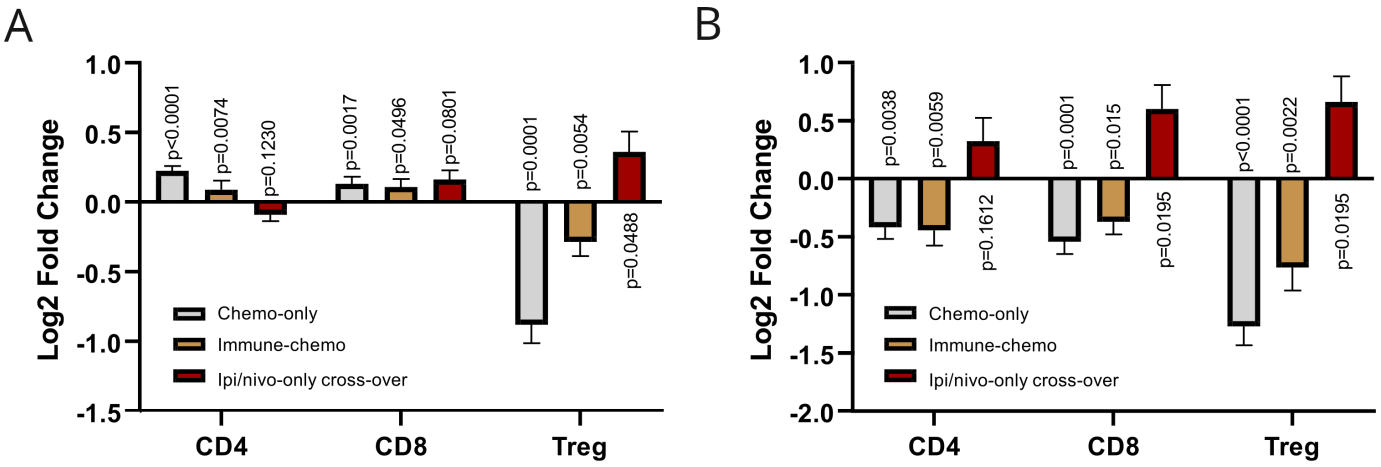


Figure 4 Impact of therapy on the phenotype and frequency of circulating T cells. PBMC from screening and week 8 were assessed for T-cell subsets by flow cytometry in 52 patients (chemo-only N=21, immune-chemo N=31, ipi/nivo cross-over N=11). Absolute cell counts were available from a subset of 41 patients (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.

Author affiliations
[1]Department of Clinical Cancer Research and Department of Cancer Immunology, Oslo University Hospital, Oslo, Norway
[2]Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[3]Department of Oncology, CHU UCL Namur - Site Sainte-Elisabeth, Namur, Belgium
[4]Department of Hematology and Oncology, Stavanger University Hospital, Stavanger, Norway
[5]Center for Cancer Treatment, Sørlandet Hospital Kristiansand, Kristiansand, Norway

[6]Department of Medical Oncology, Institut Jules Bordet, Bruxelles, Belgium
[7]Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway
[8]Department of Oncology, Oslo University Hospital, Oslo, Norway
[9]Department of Radiology and Nuclear medicine, Oslo University Hospital, Oslo, Norway
[10]Department of Pathology, Oslo University Hospital, Oslo, Norway
[11]Department of Pathology and Department of Cancer Genetics, Oslo University Hospital, Oslo, Norway
[12]Department of Cancer Immunology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway
[13]Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway
[14]Center for Bioinformatics, Department of Informatics, University of Oslo, Oslo, Norway
[15]Faculty of Health Sciences, Oslo Metropolitan University, Oslo, Norway

Twitter Andreas Hagen Røssevold @andreashr and Jon Amund Kyte @GroupKyte

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.

ORCID iDs
Nikolai Kragøe Andresen http://orcid.org/0000-0001-9386-1368
Andreas Hagen Røssevold http://orcid.org/0000-0002-7212-0111
Jon Amund Kyte http://orcid.org/0000-0002-2854-3694

REFERENCES

1 Larkin J, Chiarion-Sileni V, Gonzalez R, *et al*. Combined Nivolumab and Ipilimumab or monotherapy in untreated Melanoma. *N Engl J Med* 2015;373:23–34.
2 Hellmann MD, Paz-Ares L, Bernabe Caro R, *et al*. Nivolumab plus Ipilimumab in advanced non-small-cell lung cancer. *N Engl J Med* 2019;381:2020–31.
3 Schmid P, Adams S, Rugo HS, *et al*. Atezolizumab and NAB-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 2018;379:2108–21.
4 Cortes J, Cescon DW, Rugo HS, *et al*. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet* 2020;396:1817–28.
5 Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. *Nature* 2012;490:61–70.
6 Sobral-Leite M, Van de Vijver K, Michaut M, *et al*. Assessment of PD-L1 expression across breast cancer molecular subtypes, in relation to Mutation rate, Brca1-like status, tumor-infiltrating immune cells and survival. *Oncoimmunology* 2018;7:e1509820.
7 Denkert C, von Minckwitz G, Darb-Esfahani S, *et al*. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with Neoadjuvant therapy. *Lancet Oncol* 2018;19:40–50.
8 Luen S, Virassamy B, Savas P, *et al*. The Genomic landscape of breast cancer and its interaction with host immunity. *Breast* 2016;29:241–50.
9 Goldberg J, Pastorello RG, Vallius T, *et al*. The Immunology of hormone receptor positive breast cancer. *Front Immunol* 2021;12:674192.
10 Nanda R, Liu MC, Yau C, *et al*. Effect of Pembrolizumab plus Neoadjuvant chemotherapy on pathologic complete response in women with early-stage breast cancer: an analysis of the ongoing phase 2 Adaptively randomized I-Spy2 trial. *JAMA Oncol* 2020;6:676–84.
11 Rugo HS, Delord J-P, Im S-A, *et al*. Safety and antitumor activity of Pembrolizumab in patients with estrogen receptor-positive/human Epidermal growth factor receptor 2-negative advanced breast cancer. *Clin Cancer Res* 2018;24:2804–11.
12 Dirix LY, Takacs I, Jerusalem G, *et al*. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1B JAVELIN solid tumor study. *Breast Cancer Res Treat* 2018;167:671–86.
13 Santa-Maria CA, Kato T, Park J-H, *et al*. A pilot study of Durvalumab and Tremelimumab and Immunogenomic Dynamics in metastatic breast cancer. *Oncotarget* 2018;9:18985–96.

14 Bachelot T, Filleron T, Bieche I, *et al*. Durvalumab compared to maintenance chemotherapy in metastatic breast cancer: the randomized phase II Safir02-BREAST IMMUNO trial. *Nat Med* 2021;27:250–5.
15 Emens LA, Adams S, Cimino-Mathews A, *et al*. Society for Immunotherapy of cancer (SITC) clinical practice guideline on Immunotherapy for the treatment of breast cancer. *J Immunother Cancer* 2021;9:e002597.
16 Shah AN, Flaum L, Helenowski I, *et al*. Phase II study of Pembrolizumab and Capecitabine for triple negative and hormone receptor-positive, Her2-negative endocrine-refractory metastatic breast cancer. *J Immunother Cancer* 2020;8:e000173.
17 Pérez-García JM, Llombart-Cussac A, G Cortés M, *et al*. Pembrolizumab plus Eribulin in hormone-receptor-positive, Her2-negative, locally recurrent or metastatic breast cancer (KELLY): an open-label, Multicentre, single-arm, phase II trial. *Eur J Cancer* 2021;148:382–94.
18 Tolaney SM, Barroso-Sousa R, Keenan T, *et al*. Effect of Eribulin with or without Pembrolizumab on progression-free survival for patients with hormone receptor-positive, Erbb2-negative metastatic breast cancer: A randomized clinical trial. *JAMA Oncol* 2020;6:1598–605.
19 Adams S, Schmid P, Rugo HS, *et al*. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. *Ann Oncol* 2019;30:397–404.
20 Sistigu A, Yamazaki T, Vacchelli E, *et al*. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nat Med* 2014;20:1301–9.
21 Kroemer G, Senovilla L, Galluzzi L, *et al*. Natural and therapy-induced Immunosurveillance in breast cancer. *Nat Med* 2015;21:1128–38.
22 Bezu L, Gomes-de-Silva LC, Dewitte H, *et al*. n.d. Combinatorial strategies for the induction of Immunogenic cell death. *Front Immunol*;6.
23 Apetoh L, Ghiringhelli F, Tesniere A, *et al*. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* 2007;13:1050–9.
24 Voorwerk L, Slagter M, Horlings HM, *et al*. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. *Nat Med* 2019;25:920–8.
25 Ghiringhelli F, Menard C, Puig PE, *et al*. Metronomic cyclophosphamide regimen selectively depletes Cd4+Cd25+ regulatory T cells and restores T and NK Effector functions in end stage cancer patients. *Cancer Immunol Immunother* 2007;56:641–8.
26 Slingluff CL Jr, Petroni GR, Chianese-Bullock KA, *et al*. Randomized multicenter trial of the effects of Melanoma-associated helper peptides and cyclophosphamide on the Immunogenicity of a Multipeptide Melanoma vaccine. *J Clin Oncol* 2011;29:2924–32.
27 Emens LA, Asquith JM, Leatherman JM, *et al*. Timed sequential treatment with cyclophosphamide, doxorubicin, and an allogeneic granulocyte-macrophage colony-stimulating factor-Secreting breast tumor vaccine: a chemotherapy dose-ranging factorial study of safety and immune activation. *J Clin Oncol* 2009;27:5911–8.
28 Hellmann MD, Rizvi NA, Goldman JW, *et al*. Nivolumab plus Ipilimumab as first-line treatment for advanced non-small-cell lung cancer (Checkmate 012): results of an open-label, phase 1, Multicohort study. *Lancet Oncol* 2017;18:31–41.
29 Kyte JA, Andresen NK, Russnes HG, *et al*. ICON: a randomized phase IIb study evaluating Immunogenic chemotherapy combined with Ipilimumab and Nivolumab in patients with metastatic hormone receptor positive breast cancer. *J Transl Med* 2020;18:269.
30 Kyte JA, Andresen NK, Quaghebeur C, *et al*. ICON – a randomized phase IIb study evaluating chemotherapy combined with Ipilimumab and Nivolumab in metastatic hormone receptor-positive breast cancer. *Annals of Oncology* 2022;33:S636.
31 Seymour L, Bogaerts J, Perrone A, *et al*. iRECIST: guidelines for response criteria for use in trials testing Immunotherapeutics. *Lancet Oncol* 2017;18:e143–52.
32 Eisenhauer EA, Therasse P, Bogaerts J, *et al*. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228–47.
33 Ayers M, Lunceford J, Nebozhyn M, *et al*. IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest* 2017;127:2930–40.
34 Røssevold AH, Andresen NK, Bjerre CA, *et al*. Atezolizumab plus anthracycline-based chemotherapy in metastatic triple-negative breast cancer: the randomized, double-blind phase 2B ALICE trial. *Nat Med* 2022;28:2573–83.
35 Garcia M, Juhos S, Larsson M, *et al*. Sarek: A portable Workflow for whole-genome sequencing analysis of Germline and somatic variants. *F1000Res* 2020;9:63.
36 Merino DM, McShane LM, Fabrizio D, *et al*. Establishing guidelines to Harmonize tumor mutational burden (TMB): in Silico assessment of variation in TMB Quantification across diagnostic platforms: phase I of the friends of cancer research TMB harmonization project. *J Immunother Cancer* 2020;8:e000147.
37 Barroso-Sousa R, Li T, Reddy S, *et al*. Abstract Gs2-10: nimbus: A phase 2 trial of Nivolumab plus Ipilimumab for patients with Hypermutated Her2-negative metastatic breast cancer (MBC). *Cancer Res* 2022;82(4_Supplement):GS2–10
38 Alva AS, Mangat PK, Garrett-Mayer E, *et al*. Pembrolizumab in patients with metastatic breast cancer with high tumor mutational burden: results from the targeted agent and profiling utilization Registry (TAPUR) study. *J Clin Oncol* 2021;39:2443–51.
39 Yu J, Green MD, Li S, *et al*. Liver metastasis restrains Immunotherapy efficacy via macrophage-mediated T cell elimination. *Nat Med* 2021;27:152–64.
40 Goel S, DeCristo MJ, Watt AC, *et al*. Cdk4/6 inhibition triggers anti-tumour immunity. *Nature* 2017;548:471–5.
41 Rugo HS, Sohn J, Jerez Gilarranz Y, *et al*. KEYNOTE-B49: A phase 3, randomized, double-blind, placebo-controlled study of Pembrolizumab plus chemotherapy in patients with HR+/Her2- locally recurrent inoperable or metastatic breast cancer. *JCO* 2022;40(16_suppl):TPS1118.
42 Simpson TR, Li F, Montalvo-Ortiz W, *et al*. Fc-dependent depletion of tumor-infiltrating regulatory T cells Co-defines the efficacy of anti-CTLA-4 therapy against Melanoma. *J Exp Med* 2013;210:1695–710.
43 Arce Vargas F, Furness AJS, Litchfield K, *et al*. Fc Effector function contributes to the activity of human anti-CTLA-4 antibodies. *Cancer Cell* 2018;33:649–63.
44 Roussos Torres ET, Leatherman J, Rafie C, *et al*. 964Mo Entinostat, Nivolumab and Ipilimumab in advanced Her2-negative breast cancer (ETCTN-9844). *Annals of Oncology* 2021;32:S833.
45 Paz-Ares LG, Ciuleanu T-E, Pluzanski A, *et al*. Safety of first-line Nivolumab plus Ipilimumab in patients with metastatic NSCLC: A pooled analysis of Checkmate 227, Checkmate 568, and Checkmate 817. *J Thorac Oncol* 2023;18:79–92.
46 Reck M, Ciuleanu T-E, Cobo M, *et al*. First-line Nivolumab plus Ipilimumab with two cycles of chemotherapy versus chemotherapy alone (four cycles) in advanced non-small-cell lung cancer: Checkmate 9La 2-year update. *ESMO Open* 2021;6:100273.
47 Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016;16:626–38.
48 Unger JM, Vaidya R, Albain KS, *et al*. Sex differences in risk of severe adverse events in patients receiving Immunotherapy, targeted therapy, or chemotherapy in cancer clinical trials. *J Clin Oncol* 2022;40:1474–86.
49 Rugo HS, Kabos P, Beck JT, *et al*. Abemaciclib in combination with Pembrolizumab for HR+, Her2- metastatic breast cancer: phase 1B study. *NPJ Breast Cancer* 2022;8.
50 Masuda J, Sakai H, Tsurutani J, *et al*. Efficacy, safety, and biomarker analysis of Nivolumab in combination with Abemaciclib plus endocrine therapy in patients with HR-positive Her2-negative metastatic breast cancer: a phase II study. *J Immunother Cancer* 2023;11:e007126.

Confidential

Oslo universitetssykehus

ICON CA209-9FN: A randomized phase IIb study evaluating immunogenic chemotherapy combined with ipilimumab and nivolumab in patients with luminal B breast cancer

SPONSOR:

Oslo University Hospital
The Norwegian Radium Hospital
Department of Oncology
Ullernchausseen 70
0379 Oslo
Norway

EudraCT number:	2017-000220-10
Protocol version number:	2.0
Protocol date:	12 Oct 2017

CONTACT DETAILS

Sponsor:	**Stein Kaasa, MD PhD, professor** Department of Oncology Oslo University Hospital Ph: +47 2293 4000
Principal Investigator:	**Jon Amund Kyte, MD PhD** Department of Oncology Oslo University Hospital Ph: +47 2293 5046/ +47 975 696 19
Co-Investigator:	**Bjørn Naume, MD PhD, professor** Department of Oncology Oslo University Hospital Ph: +47 2293 4000
Project manager	**Signe Øien Fretland** Department of Oncology Oslo University Hospital Ph: +47 2293 4000
Statistician:	**Ragnhild Sørum Falk, PhD** Oslo Center for Biostatistics and Epidemiology Oslo University Hospital Ph: +47 986 88 111

Confidential

SIGNATURE PAGE

Title: Randomized phase IIb study evaluating immunogenic chemotherapy combined with ipilimumab and nivolumab in patients with luminal B breast cancer

Protocol ID: ICON CA209-9FN

EudraCT no.: 2017-000220-10

I hereby declare that I will conduct the study in compliance with the Protocol, ICH GCP and the applicable regulatory requirements.

Name	Title	Role	Signature	Date
Stein Kaasa	Professor, MD PhD, Head of department	Sponsors representative		
Jon A. Kyte	MD PhD, senior consultant	Principal and coordinating Investigator		

TABLE OF CONTENTS

1.0 BACKGROUND & RATIONALE

Breast cancer is rarely curable after metastasis, and the therapeutic options are limited. Interestingly, the host immune response is strongly predictive for the effect of chemotherapy in subgroups of patients with breast cancer. In the present proposal, we aim at releasing the brake on the immune response by use of ipilimumab, which blocks CTLA-4 and may deplete regulatory T cells, combined with nivolumab (anti PD1). Importantly, it is possible that non-responders to nivolumab/ipilimumab (nivo/ipi) can be turned responders by use of immunogenic chemotherapy.

There is compelling evidence from animal studies, supported by data from humans, that some chemotherapeutic agents are immunogenic. Doxorubicin and cyclophosphamide have been shown to be particularly powerful inducers of immunogenic cell death. Both agents fulfil 5/5 criteria established for assessing the immunogenicity of different chemotherapeutic drugs (Table 1 in (1)). There is also strong evidence from humans, particularly in breast cancer, indicating that the clinical effect of doxorubicin and cyclophosphamide depends on the host immune response (2). Further, these agents have been shown to induce a Type I interferon immune response in breast cancer (3, 4). Taken together, there is a strong rationale for synergy between doxorubicin/cyclophosphamide and PD-1/CTLA-4 blockade (5).

In studies conducted at Oslo University Hospital and by a collaborator, we have observed that patients with hormone receptor positive breast cancer are highly diverse with regard to immune activation and up-regulation of inhibitory pathways, including PD1/PD-L1.

We will combine nivolumab and ipilimumab with established 1st choice chemotherapy in patients with metastatic luminal B breast cancer. We hypothesize that nivolumab/ipilimumab (nivo/ipi) may

i) potentiate the patient´s spontaneous anti-tumor immune response
ii) synergize with chemotherapeutic agents that induce immunological cell death

The prospect of clinical benefit from immunotherapy is probably best in patients that have not received multiple previous lines of chemotherapy, and we thus aim to bring the nivo/ipi combination into current early line regimes. Our chosen chemotherapeutic regime is a combination of antracyclin and cyclophosphamide, which is an acknowledged option. To facilitate rapid recruitment into the study, we suggest allowing for one previous line of chemotherapy, but with a requirement of good performance status (ECOG 0 or 1) and adequate organ function. Further, we suggest using the chemo drugs in a metronomic fashion (daily cyclophosphamide), rather than as high dose regimes administered every third week. We hypothesize that the metronomic regime will induce immunological cell death and counter T regulatory cells (6), while maintaining the leukocyte counts and the ability of the effector immune cells to respond. Indeed, a low-dose metronomic cyclophosphamide regime has been used in several cancer vaccine studies, in order to counter regulatory T cells and myeloid suppressor cells . Finally, we will use liposomal doxorubicin, which minimizes the adverse effects of antracyclins on the heart and allows for continued treatment beyond the otherwise mandatory antracyclin limits. This is of particular importance for immunotherapy, where the aim is to induce long term disease remission. It is important to identify a chemo regime that can be continued for an extended period of time, in combination with nivo/ipi.

2.0 OBJECTIVES AND HYPOTHESES

Objective	Hypothesis
Primary	
Assessment of toxicity of combined treatment with ipilimumab, nivolumab, pegylated liposomal doxorubicin and cyclophosphamide (ipi/nivo/chemo)	The ipi/nivo/chemo combination has acceptable safety and tolerability in metastatic Luminal B patients
Assessment of clinical response in ipi/nivo/chemo group compared to chemo only group: Progression-free survival (PFS); compare the PFS rates when 95% of patients in the control croup have PD	The ipi/nivo/chemo combination will produce improved PFS compared to chemo alone
Secondary	
Assessment of clinical response in ipi/nivo/chemo group compared to chemo only group: Objective tumor response rate (ORR), duration of response (DR), durable tumor response rate (DRR; >6 months), overall survival (OS)	The ipi/nivo/chemo combination will produce improved ORR, DR, DRR and OS compared to chemo alone
Assessment of toxicity of ipi/nivo (without chemotherapy) in cross-over arm	The ipi/nivo combination has acceptable safety and tolerability in metastatic Luminal B patients after cross over
Assessment of ORR, DR, DRR, PFS and OS in cross-over arm receiving ipi/nivo (without chemotherapy)	Patients from study arm A that have progressed on chemotherapy may respond to treatment with ipilimumab combined with nivolumab after cross over
Exploratory	
Assessment of immunological response	The ipi/nivo/chemo or ipi/nivo combination will induce T cell responses against antigens expressed in each patient´s tumor.
Identification of biomarkers for clinical response, toxicity and immune response	Genetical and immunological profiling of pre-treatment samples may identify biomarkers for predicting which patients will benefit from the ipi/nivo/chemo combination, or from ipi/nivo given without concomitant chemotherapy.
Characterization of tumor evolution and changes in immunological milieu induced by the combination therapy (ipi/nivo/chemo), as compared to chemo only, and by ipi/nivo without concomitant chemotherapy	Profiling of consecutive samples obtained before/during/after treatment may uncover the hallmarks of tumor/immunological evolution induced by the ipi/nivo/chemo combination therapy, as compared to chemo only, or by ipi/nivo given without concomitant chemotherapy

Assessment of patient reported outcomes, as measured by the Chalder Fatigue Questionnaire (FQ), an 11 point Numerical Rating Scale (NRS) for pain intensity and EORTC QLQ-C15-PAL	The ipi/nivo/chemo combination will give improved pain control and reduced fatigue compared to chemo alone

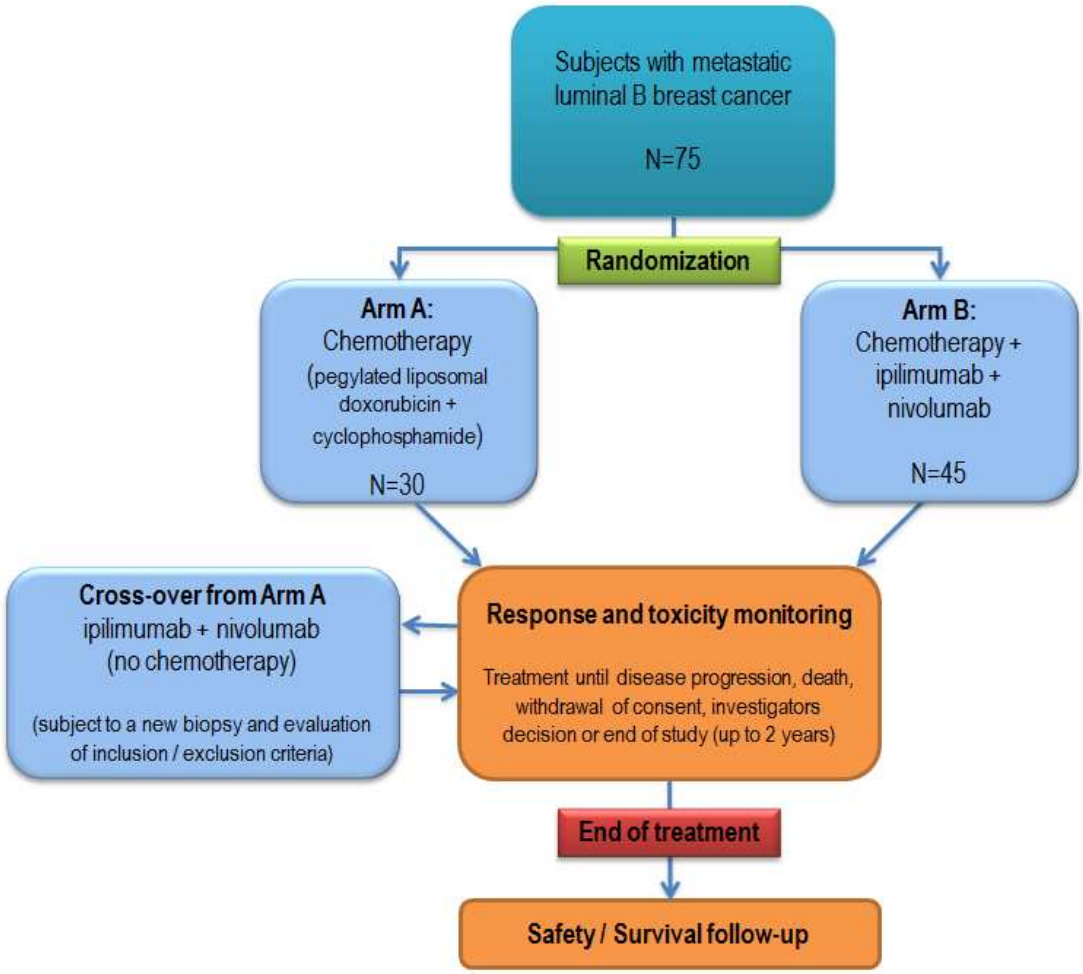
3.0 STUDY DESIGN

3.1 Description of study

This is a randomized phase IIb study evaluating the safety and efficacy of combining nivolumab and ipilimumab with immunogenic chemotherapy in subjects with metastatic luminal B breast cancer. The Investigational Medicinal Products (IMPs) are nivolumab, ipilimumab, pegylated liposomal doxorubicin and cyclophosphamide.

Randomized phase IIb trial in 75 patients comparing two arms:

- Arm A: Chemo only (pegylated liposomal doxorubicin + cyclophosphamide)
- Arm B: Chemo + ipilimumab + nivolumab
- Randomization 2:3 in favour of arm B
    - Randomization will be stratified according to tumor PD-L1 status (BMS-approved assay; no cancer treatment allowed between biopsy used for PD-L1 analysis and stratification)



Upon radiographic disease progression per RECIST v1.1, patients may continue to receive the study treatment until unacceptable toxicity or loss of clinical benefit, provided they meet all of the following criteria:

- Evidence of clinical benefit as assessed by the investigator
- Absence of symptoms and signs (including worsening of laboratory values) indicating clinically significant progression of disease
- No decline in ECOG performance status that can be attributed to disease progression

In patients who continue treatment beyond radiographic disease progression, tumor response will continue to be assessed using RECIST and iRECIST, according to the schedule outlined on the protocol.

3.2 Cross-over

Cross-over is allowed. The patients in arm A are offered nivo/ipi (without chemotherapy) after disease progression, if considered not in need of immediate chemotherapy. Patients with aggressive and widespread disease, and

acceptable tolerability for chemotherapy, should be recommended chemotherapy rather than cross-over to ipi/nivo therapy. The cross-over patients must have completed the treatment discontinuation visit before they can start treatment with nivo/ipi.

The inclusion / exclusion criteria must be met at the time of cross-over, except that an ECOG status of 2 is allowed. New tests for HIV/HCV/HBV, INR, aPTT and lipase are not required. There is no requirement of a wash-out period from PLD/syklofosfamid before start of ipi/nivo. A tumor biopsy after end of chemotherapy, and before start of nivo/ipi treatment, is mandatory.

From cross-over, the patients will re-enter the visit schedule from cycle 1 in the Study Flow Chart.

3.3 Patient recruitment plan

The plan is to recruit patients from all Norwegian health regions. The patient enrolment is expected to be completed within approximately 24 months and to reach the data-driven time point for PFS-analysis (95% PD in control group) approximately 3 years after the study opens. If the control group performs better than expected, the PFS-analysis will be performed 24 months after inclusion of the last patient.

3.4 Safety Monitoring Committee

A safety monitoring committee (SMC) will monitor safety on a periodic basis. Members of the SMC will be experienced clinicians at the Sponsor, independent from the study. The SMC will meet the first time when three patients have received three injections of nivolumab, but no later than 6 months after first patient in (FPI). Thereafter the SMC will meet approximately every 6 months to review safety and study conduct data. The safety data will include demographic data, adverse events and relevant laboratory data. No interim efficacy analysis is planned.

Following each data review, the SMC will provide recommendations to the PI as to whether the study should continue or be amended, or whether the study should be stopped on the basis of safety (i.e., evidence of harm). The Study Leadership will make a decision on the basis of the SMC recommendations.

Any outcomes of these safety reviews that affect study conduct will be communicated in a timely manner to the investigators.

4.0 INCLUSION AND EXCLUSION CRITERIA

4.1 Inclusion criteria

Patients must meet all of the following criteria to be eligible for study entry:

1. Metastatic hormone receptor positive breast cancer (primary or recurrent), defined as ER+ >1% in metastatic biopsy (archival material or study biopsy) and HER2 negative in the last biopsy evaluable for HER2. HER2-analysis is to be perfomed according to national criteria.
2. Luminal B breast cancer, defined by the PAM50 panel performed on primary tumor or metastasis biopsy
3. Adequate core or excisional study biopsy of a metastatic tumor lesion not previously irradiated. No anti-tumor

treatment is allowed between the time point for biopsy and study entry.

4. Measurable metastatic disease according to RECIST
5. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
6. Signed Informed Consent Form
7. Women or men aged ≥ 18 years
8. A minimum of 24 months since adjuvant/neoadjuvant chemotherapy with antracyclins
9. A maximum of one previous line with chemotherapy in the metastatic setting
10. Chemotherapy is considered as preferred treatment
11. Previous endocrine and targeted therapy is allowed
12. No use of systemic corticosteroids at study entry
13. Female subject of childbearing potential should have a negative urine or serum pregnancy within 7 days prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required
14. Female subjects of childbearing potential should agree to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of < 1% per year, during the treatment period and for at least 5 months after the last dose of study therapy.
15. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 7 months after the last dose of study therapy
16. Adequate organ function as defined in Table 1

Table 1 Adequate Organ Function Laboratory Values

System	**Laboratory Value**
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Lymphocyte count	≥0,800 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥40 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

4.2 EXCLUSION CRITERIA

The subject must be excluded from participating in the trial if the subject has/is:

1. Malignancies other than breast cancer within 5 years prior to randomization, with the exception of those with a negligible risk of metastasis or death and treated with expected curative outcome (such as adequately treated carcinoma in situ of the cervix or basal or squamous cell skin cancer)
2. Spinal cord compression not definitively treated with surgery and/or radiation, or previously diagnosed and treated spinal cord compression without evidence that disease has been clinically stable for > 8 weeks prior to randomization
3. Known CNS disease, except for treated asymptomatic CNS metastases, provided all of the following criteria are met:
    a. Measurable disease outside the CNS
    b. Asymptomatic for CNS disease > 4 months.
    c. Only supratentorial metastases allowed
    d. No evidence of progression after completion of CNS-directed therapy
    e. No ongoing requirement for dexamethasone as therapy for CNS disease
    f. No radiation of brain lesions within 4 months prior to randomization
    g. No leptomeningeal disease
4. Known BRCA mutation, unless the patient has already received carboplatin or for other reasons should not receive carboplatin treatment, according to own preferences or reccommendations by the treating physician.
5. Uncontrolled pleural effusion, pericardial effusion, or ascites. Patients with indwelling catheters (e.g., PleurX®) are allowed
6. Uncontrolled tumor-related pain. Patients requiring narcotic pain medication must be on a stable regimen at study entry. Symptomatic lesions (e.g., bone metastases or metastases causing nerve impingement) amenable to palliative radiotherapy should be treated prior to randomization. Asymptomatic metastatic lesions whose further growth would likely cause functional deficits or intractable pain (e.g., epidural metastasis that is not presently associated with spinal cord compression) should be considered for loco-regional therapy if appropriate prior to randomization
7. Ionized calcium > 1.2 x UNL. The use of bisphosphonates is allowed
8. Pregnant or breastfeeding
9. Evidence of significant uncontrolled concomitant disease that could affect compliance with the protocol or interpretation of results, including significant liver disease (such as cirrhosis, uncontrolled major seizure disorder, or superior vena cava syndrome)
10. Significant cardiovascular disease, such as New York Heart Association (NYHA) cardiac disease (Class II or greater), myocardial infarction within 3 months prior to randomization, unstable arrhythmias, or unstable angina Patients with a known left ventricular ejection fraction (LVEF) < 40% will be excluded. Patients with known coronary artery disease, congestive heart failure not meeting the above criteria, or LVEF < 50% must be on a stable medical regimen that is optimized in the opinion of the treating physician, in consultation with a cardiologist if appropriate
11. Severe infection within 21 days prior to randomization, requiring hospitalization
12. Received oral or IV antibiotics within 1 week prior to Cycle 1, Day 1. Patients receiving routine antibiotic prophylaxis (e.g., to prevent chronic obstructive pulmonary disease exacerbation or for dental extraction) are eligible

13. Major surgical procedure within 21 days prior to randomization or anticipation of the need for a major surgical procedure during the course of the study other than for diagnosis. Placement of central venous access catheter(s) is not considered a major surgical procedure and is therefore permitted
14. A history of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins
15. Known hypersensitivity to any of the components of the investigational products
16. A history of autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxin, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment. Patients with eczema, psoriasis, lichen simplex chronicus or vitiligo with dermatologic manifestations only (e.g., no psoriatic arthritis) are permitted provided that they meet all of the following conditions:
    a. Rash must cover less than 10% of body surface area.
    b. Disease is well controlled at baseline and only requiring low potency topical steroids
    c. No acute exacerbations of underlying condition within the last 12 months (not requiring PUVA [psoralen plus ultraviolet A radiation], methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, high potency or oral steroids)
17. Undergone allogeneic stem cell or solid organ transplantation
18. A history of idiopathic pulmonary fibrosis (including pneumonitis), drug-induced pneumonitis, organizing pneumonia (i.e., bronchiolitis obliterans, cryptogenic organizing pneumonia), or evidence of active pneumonitis on screening chest CT scan. History of radiation pneumonitis in the radiation field (fibrosis) is permitted
19. A positive test for HIV
20. Active hepatitis B (defined as having a positive hepatitis B surface antigen [HBsAg] test at screening) or hepatitis C. Patients with past hepatitis B virus (HBV) infection or resolved HBV infection (defined as having a negative HBsAg test and a positive antibody to hepatitis B core antigen [anti-HBc] antibody test) are eligible. Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction (PCR) is negative for HCV RNA
21. Active tuberculosis
22. Currently receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment
23. Received treatment with immune checkpoint modulators, including anti−CTLA-4, anti−PD-1, or anti−PD-L1 therapeutic antibodies
24. Received treatment with systemic immunostimulatory agents (including but not limited to interferons or IL-2) within 4 weeks or five half-lives of the drug (whichever is shorter) prior to randomization
25. Received treatment with systemic corticosteroids or other systemic immunosuppressive medications (including but not limited to prednisone, dexamethasone, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti−tumor necrosis factor [TNF] agents) within 2 weeks prior to randomization, or anticipated requirement for systemic immunosuppressive medications during the trial
    a. Patients who have received acute, low-dose, systemic immunosuppressant medications (e.g., a one-time dose of dexamethasone for nausea) may be enrolled in the study
    b. Patients with a history of allergic reaction to IV contrast requiring steroid pre-treatment should have baseline and subsequent tumor assessments performed using MRI
    c. The use of inhaled corticosteroids for chronic obstructive pulmonary disease, mineralocorticoids (e.g., fludrocortisone) for patients with orthostatic hypotension, and low-dose supplemental corticosteroids for adrenocortical insufficiency are allowed

26. Received anti-cancer therapy (medical agents or radiation) within 3 weeks prior to study Cycle 1, Day 1. However the following are allowed:
    a. Palliative radiotherapy for bone metastases > 2 weeks prior to Cycle 1, Day 1
27. A history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating Investigator
28. Known psychiatric or substance abuse disorders that would interfere with cooperation and the requirements of the trial
29. Received a live vaccine within 30 days of planned start of study therapy, or is expected to receive such a vaccine while on therapy
    a. *Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.*
30. Any reason why, in the opinion of the investigator, the patient should not participate

5.0 STUDY TREATMENT

5.1 Overview of Study Treatment

The following drugs are defined as IMPs:

Table 2: Study Treatment

Product Description / Class and Dosage Form	Potency	IMP/ Non-IMP	Blinded or Open Label	Packaging / Appearance	Storage Conditions (per label)
BMS-936558-01 (Nivolumab) Solution for Injection	100 mg (10 mg/mL) 40 mg (10 mg/mL)	IMP	Open-label	Clear to opalescent colorless to pale yellow liquid. May contain particles. 240 mg kit contains: 2 x 100 mg vials (10 mL/vial) and 1 x 40 mg vial (4 mL/vial) or carton containing 5 vials of 100 mg	Store at 2°-8°C. Protect from light and freezing.
Ipilimumab Solution for Injection	200 mg (5 mg/mL)	IMP	Open-label	Clear to slightly opalescent colorless to pale yellow liquid. May contain particles. Carton containing 4 vials of 200 mg 40 mL/vial	Store at 2°-8°C. Protect from light and freezing.
Pegylated liposomal doxorubicin Solution for Injection		IMP	Open-label	10 ml (20 mg) vials or 25ml (50 mg) vials	Store at 2°-8°C. Protect from freezing.

Cyclophosphamide Tablets	50 mg	IMP	Open-label	Enpac (blister) 100 tablets	Ambient temperature (<25 °C)

Study arm A: Pegylated liposomal doxorubicin combined with cyclophosphamide
Study arm B: Nivolumab and ipilimumab, combined with pegylated liposomal doxorubicin and cyclophosphamide

5.2 Dosage

- Nivolumab 240mg administered intravenously every 2nd week until disease progression or for a maximum of 24 months
- Ipilimumab 1mg/kg administered intravenously every 6th week until disease progression or for a maximum of 24 months
    - If toxicity is unacceptable, apply a reduced dose level (dose minus 1) for ipilimumab of 1mg/kg every 12th week
- Chemotherapy will be administered as follows:
    - Pegylated liposomal doxorubicin 20mg/m^2 i.v. every 2nd week. An upper limit of 44mg per dose will be applied to patients with a body surface areae >2.2 m2.
    - Cyclophosphamide tablets 50 mg per day, daily as continuous treatment for every 2nd cycle (i.e. first 2 weeks of each 4 week period)
    - No upper limit for the administration time of pegylated liposomal doxorubicin /cyclophosphamide. Heart function will be monitored.

5.3 Duration of treatment

The patients will continue study treatment until disease progression per RECIST v1.1, death, withdrawal of consent, investigators decision or end of study (up to 2 yrs).

Upon radiographic disease progression per RECIST v1.1, patients may continue to receive the study treatment until unacceptable toxicity or loss of clinical benefit, provided they meet all of the following criteria:

- Evidence of clinical benefit as assessed by the investigator
- Absence of symptoms and signs (including worsening of laboratory values) indicating clinically significant progression of disease
- No decline in ECOG performance status that can be attributed to disease progression

If one of the IMPs are stopped/paused due to toxicity, the other IMPs may be continued.

5.4 Rationale for chemotherapy regime

The chemotherapy regime is regarded as appropriate therapy for this patient group, without nivo/ipi. The regime is expected to be well tolerated and applicable to most metastatic TNBC patients with ECOG 0-1, while also being sufficiently potent to suit those with an excellent performance status.

The chemotherapy regime and dosing schedule has been tailored to aid the effect of nivo/ipi, which depend on immune effector cells for its activity. First, the chosen chemotherapeutic agents (antracyclins and cyclophosphamide) are known to induce immunogenic cell ceath. Second, we apply the outlined dosage regime, rather than a high dose regime administered every third/fourth week, in order to maintain the leukocyte counts and

the ability of the effector immune cells to respond. Antracyclins are routinely administered at intervals ranging from one to four weeks in metastatic breast cancer patients. Tregs and MDSCs represent important mediators of tumor tolerance and may oppose the effect of nivo/ipi. The metronomic cyclophosphamide dosage chosen in the present study has been widely used to counter Tregs and MDSCs and is also considered safe, as it has been combined with multiple other chemotherapeutic agents without causing important toxicity (7). We include 14-day intervals without cyclophosphamide to allow for unsuppressed T cell proliferation and activity, which may be important for the nivo/ipi effect.

We will use pegylated liposomal doxorubicin to minimize the adverse effects of anthracyclines on the heart and allow for continued treatment beyond the otherwise mandatory anthracycline limits. The possibility of long term treatment is important in order to appropriately test checkpoint inhibitors, as these drugs are known to induce durable responses in other patient groups. Pegylated liposomal doxorubicin is also administered without any need for corticosteroids, which is desirable for immunotherapy.

The standard pegylated liposomal doxorubicin dose for breast cancer is 40-50mg/m^2 every 4th week. In Norway, the most widely used dose is 40 mg/m2. The dose chosen in this study is expected to be well tolerated, as the 40mg/m^2 is divided into two doses of 20mg/m^2 given every 2nd week. Some studies in breast cancer have used pegylated liposomal doxorubicin at 15-30 mg/m^2 every 2nd week , or in combination with cyclophosphamide (500 mg/m^2), and 5-fluorouracil (500 mg/m^2) every 3rd week (7-10). A dose of 20mg/m^2 has been well tolerated in combination with cyclophosphamide (50mg/day) even in fragile, older patients (11) and is also tolerated by HIV positive patients with Kaposi sarcoma. In our study, a detailed dose reduction plan will be outlined in case of adverse effects.

5.5 Rationale for nivolumab and ipilimumab dosage

Combined therapy with nivolumab and ipilimuab has been shown to be effective in metastatic melanoma (12), for which the combination is approved by FDA, EMA and the Norwegian Medical Agency (SLV). There is evidence suggesting that the nivo/ipi combination is more effective than either agent alone, in particular for patients with PD-L1 negative tumors, as is the case for the majority of breast cancer patients. The dosage approved for melanoma (ipi 3mg/kg Q3 and nivo 1mg/kg Q3) has, however, produced increased toxicity compared to ipi or nivo monotherapy. The ipi/nivo combination was evaluated in patients with stage IIB-IV non-squamous cell lung cancer (NSCLC) in the CA209012 study. Early combination cohorts evaluated two dosing schedules: nivolumab 1 mg/kg + ipilimumab 3 mg/kg and nivolumab 3 mg/kg + ipilimumab 1 mg/kg, both regimens were given every 3 weeks x 4 doses, followed by maintenance nivolumab 3 mg/kg q 2 weeks. Compared to melanoma, these regimens resulted in more toxicity in the NSCLC population, with 39% of patients discontinuing treatment due to treatment-related adverse events. Thus, additional combination cohorts were iniated, using lower doses of both nivolumab and ipilimumab, or the approved dose of nivolumab with less frequent dosing of ipilimumab. These new regimens were better tolerated, and the safety profile was comparable to what has been observed in the nivolumab monotherapy cohort. The clinical response rates were higher in cohorts evaluating the approved dose of nivolumab 3 mg/kg, compared to lower nivolumab doses. Those with PD-L1 expression ≥ 1% had response rates of 48%, when nivo (3mg/kg) was combined with ipilimumab at 1mg/kg every 6th or 12th week (cohort Q and P). Other data suggest that a lower dose of ipilimumab than 1mg/kg reduces the effect of this drug. As of 18-Feb-2016, 38 subjects have been treated in cohort P and 39 subjects have been treated in cohort Q. As of the database lock date, 21% of subjects remain on therapy in cohorts P+Q. The primary reason for treatment discontinuation was disease progression in 18 subjects (47.4%) in cohort P, and 20 subjects (51.3%) in cohort Q.

The toxicity data for nivolumab suggests that this drug can be safelty administered as monotherapy up to 10mg/kg, and that there are only minor differences in clinical activity of nivolumab in range from 3-10 mg/kg. These data

suggest that a flat dose of 240mg should be applicable. Taking the data together, BMS has decided to move forward with a dosage of ipilimuab 1mg/kg and nivolumab at flat dose of 240mg in several new clinical trials. We have chosen this dosage in the present ICON trial, for combination with chemotherpy.

5.6 Risk benefit assessment

Immunotherapy with checkpoint inhibitors have produced durable clinical responses across several cancer forms (13, 14). The PD1 blockers have showed particularly strong efficacy and are generally well tolerated. In breast cancer, the data are so far limited. However, phase I/II studies with PD1 blockers as monotherapy have shown clinical responses in subsets of breast cancer patients, and limited adverse effects(15). Further, as outlined above, there is strong evidence indicating that the immune response is important for the clinical outcome in breast cancer and predictive for the the effect of antracyclins and cyclophosphamide(2-4). There is a strong rationale for synergy between ipilimumab and nivolumab, as these agents modulate different phases in the immune response. Ipilimumab blocks CTLA-4 and is considered to act primarily in the initiation of immunity and through depletion of regulatory T cells. Nivolumab blocks PD-1 and thereby enhances the effector phase of the immune reaction, by enabling T cells to kill tumor cells and engage effectively with other PD-L1 expressing targets.

Based on the biological characteristics of Luminal B breast cancer, we consider that there is a strong rationale for combining immunogenic chemotherapy with CTLA4- and PD-1 blockade in this patient population (see above). Further, metastatic Luminal B breast cancer carries an unfavourable prognosis. Of note, only patients where chemotherapy is considered the treatment-of-choice will be considered for this trial. These patients generally have a poor prognosis, with disease progression on endocrine/targeted therapy and/or an aggressive disease pattern.

The safety data for ipilimumab and nivolumab are briefly described herein, and more extensively in the respective Investigator´s Brochures. There is an unknown potential for toxicity when ipi/nivo is combined with chemotherapy. In view of this, we have selected a relatively low dose of ipilimumab in the present trial. As described above, the available data from ipi/nivo combination studies suggest that the toxicity is closely related to the ipi dose. It should be noted, that there is no reason to expect that the autoimmune side effects of ipi/nivo will in general be enhanced by chemotherapy. Most patients with autoimmune diseases experience no increase in symptoms from their autoimmune disease during chemotherapy. Rather, some patients observe an improvement in their autoimmune condition, possibly related to an attenuated immune system. In breast cancer, data from studies combining the PD-L1 blocker atezolizumab with chemotherapy has not shown any increase in treatment discontinuation due to side effects, as compared with atezolizumab given without chemotherapy.

In summary, the study treatment combining nivo/ipi with antracyclins and cyclophosphamide offers potential clinical benefit for the selected patient group. The risk of important side effects is limited, and the patients will be carefully monitored for side effects. We thus consider that the potential benefit outweighs the risks associated with the study treatment.

5.7 Administration of nivolumab and ipilimumab

Nivolumab will be administered as a 30 minute infusion and ipilimumab as a separate 30 minute infusion. When study nivolumab and ipilimumab are to be administered on the same day, nivolumab is to be administered first and separate infusion bags and filters should be used for each infusion. Nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the ipilimumab infusion. The second infusion will always be the ipilimumab study drug and will start after the infusion line has been flushed, filters changed and subject has been observed to ensure no infusion reaction has occurred. The time between infusions must be a

minimum of 30 minutes. Nivolumab and ipilimumab may be diluted in 0.9% Sodium Chloride Solution or 5% Dextrose solution.

Dosing calculation for ipilimumab should be based on the body weight. If the subject's weight on the day of dosing differs by > 10% from the weight used to calculate the prior dose, the dose must be recalculated. Doses can be rounded per institutional standards'. There will be no dose escalations or reductions of nivolumab and ipilimumab allowed, apart from what is stated above for dose level minus 1.

Subjects may be dosed with nivolumab no less than 12 days from the previous dose.

5.8 Administration of pegylated liposomal doxorubicin

Pegylated liposomal doxorubicin will be labelled by the study hospital pharmacies as IMP. The drug is to be administrated after the administration of nivolumab and ipilimumab and according to the standard procedure at the study hospitals and the European gudelines (ema.europa.eu). The use of prophylactic antiemetics including netupitant/aprepitant, ondansetron/palonosetron and metoclopramide is allowed and encouraged. Pyridoxine is allowed. Corticosteroids should be avoided if possible.

5.9 Administration and compliance registration for cyclophosphamide

Cyclophosphamide will be labelled by the study hospital pharmacies as IMP and administrated by the patient. The compliance will be monitored by the study personell every 8th week, by counting of the number of tablets in each labelled box and registering of this number in the eCRF.

5.10 Storage of Study Drug

The pharmacy should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as determined by BMS. If concerns regarding the quality or appearance of the study drug arise, the study drug should not be dispensed and BMS should be contacted immediately. Study drug not supplied by BMS will be stored in accordance with the package insert.
Investigational product documentation (whether supplied by BMS or not) must be maintained that includes all processes required to ensure drug is accurately administered. This includes documentation of drug storage, administration and, as applicable, storage temperatures, reconstitution, and use of required processes (eg, required diluents, administration sets). Infusion-related supplies (eg, IV bags, in-line filters, 0.9% sodium chloride injection, 5% dextrose injection) will be purchased locally.

Please refer to the current version of the Investigator Brochure (IB) / SPC and/or pharmacy reference sheets for complete storage, handling, dispensing, and infusion information.

5.11 Destruction or Return of Investigational Product

For this study, IMP (those supplied by BMS, a vendor or sourced by the investigator) such as partially used study drug containers, vials and syringes may be destroyed on site.
Any unused study drugs can only be destroyed after being inspected and reconciled by the responsible Study Monitor unless study drug containers must be immediately destroyed as required for safety, or to meet local regulations (eg, cytotoxics or biologics).

On-site destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. The procedures must be filed with the site's SOPs.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The method of disposal, ie, incinerator, licensed sanitary landfill, or licensed waste disposal vendor must be documented.
- Accountability and disposal records are complete, up-to-date, and available for the Monitor to review throughout the clinical trial period.

6.0 OUTCOME MEASURES

6.1 Safety Outcome Measures

- Incidence, nature, and severity of adverse events graded according to NCI CTCAE v4.0
- Changes in vital signs, physical findings, and clinical laboratory results

6.2 Efficacy Outcome Measures

The primary efficacy outcome measure is to be assessed in the ITT population as follows:

- PFS, defined as the time from randomization to the time of radiographic progression (as assessed by RECIST v1.1) or death from any cause during the study

The secondary efficacy outcome measures will be assessed in the **ITT population and in the PD-L1-positive subpopulation** as follows:

- Overall survival (OS), defined as the time from the date of randomization to the date of death from any cause
- Objective tumor response rate (ORR), defined as the proportion of patients with an objective tumor response (either partial response [PR] or complete response [CR] using RECIST v1.1)
- Durable response rate (DRR), defined as the proportion of patients with an objective tumor response lasting at least 6 months, according to RECIST v1.1
- Duration of objective response (DOR) among patients with an objective response, according to RECIST v1.1
- PFS in the PD-L1-positive subpopulation assessed by RECIST v1.1
- PFS, ORR, DRR and DOR assessed by iRECIST
- Investigator-assessed PFS using RECIST v1.1, with no censoring at missing scans
- Investigator-assessed PFS using iRECIST, with no censoring at missing scans
- PFS, ORR, DRR and DOR for patients after cross-over, assessed by RECIST v1.1
- PFS, ORR, DRR and DOR for patients after cross-over, assessed by iRECIST

6.3 Exploratory Outcome Measures

- Assessment of immunological response
- Identification of biomarkers for clinical response, toxicity and immune response

- Characterization of tumor evolution and changes in immunological milieu induced by the immune/chemo combination therapy, as compared to chemo only, and by ipi/nivo (without chemo) in the cross-over arm
- Development in FQ score. The analyses will include time to deterioration (TTD) in the FQ score, defined by a minimally clinically important difference (MCID) of ≥ 3 points. The maximum total FQ score is 33 points. For mean score, a separate analysis will be perfomed for subjects with a baseline FQ score ≥ 21 points
- Development in NRS pain intensity score. The analyses will include TTD in the pain intensity score, defined by a minimally clinically important difference (MCID) of ≥ 2 points (scale 0-10). For mean score, a separate analysis will be performed for subjects with a baseline score for ≥ 4 points.
- Mean changes and TTD in item 15 of the EORTC QLQ-C15-PAL, defined by a MCID ≥ 20 points at patient individual level. A change of ≥10 points is considered to be of clinical importance at group level. The development of other scales and items of QLQ-C15-PAL will also be recorded.

7.0 TRIAL FLOW CHART

7.1 Study Flow Chart

Trial Period:	**Screening Phase**		**End of Treatment**					**Post-Treatment**		
Treatment Cycle (C)/Title: (Each cycle is 2 weeks)	Informed consent (Visit 1)	Main Study Screening (Visit 2)	To be repeated C1	 C2	 C3	 C4	Treatment discontinuation	Safety Follow-up	Patients not progressed during treatment Follow Up Visits	Patients progressed during treatment Follow Up Visit
Scheduling Window (Days)[a]:		-21 to -1	± 3	± 3	± 3	± 3	At time of discon	30 days[q] post discon ± 7 days	Every 12 weeks ± 7 days post discon for 12 months, or until disease progression	16 weeks ± 10 days post discon
Administrative Procedures										
Informed Consent[b]	x									
Inclusion/Exclusion Criteria		x								
Demographics and Medical History		x								
Prior and Concomitant Medication Review[c]		x	x	x	x	x	x	x		
Trial Treatment Administration			x	x	x	x				
Post-study anticancer therapy status									x	x
Clinical Procedures/Assessments										
Review Adverse Events[d,e]		x	x	x	x	x	x	x[e]	x[e]	x[e]
Full Physical Examination		x	x[f]					x		
Directed Physical Examination				x	x	x	x		x	x
Vital Signs and Weight		x	x	x	x	x	x	x	x	x
ECOG Performance Status		x	x	x	X	x	x	x	x	x
Electrocardiogram (ECG)		x	x[f]							
LVEF assesment		x[f]								
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory										
Pregnancy Test – Urine or Serum -HCG[g]		x	x	x	x	x	x	x		

Trial Period:	**Screening Phase**		**End of Treatment**					**Post-Treatment**		
Treatment Cycle (C)/Title: (Each cycle is 2 weeks)	Informed consent (Visit 1)	Main Study Screening (Visit 2)	To be repeated C1	 C2	 C3	 C4	Treatment discontinuation	Safety Follow-up	Patients not progressed during treatment Follow Up Visits	Patients progressed during treatment Follow Up Visit
Scheduling Window (Days)[a]:		-21 to -1	± 3	± 3	± 3	± 3	At time of discon	30 days[q] post discon ± 7 days	Every 12 weeks ± 7 days post discon for 12 months, or until disease progression	16 weeks ± 10 days post discon
INR and aPTT[h]		x								
CBC with Differential[i]		x	x	x	x	x	x	x	x	x
Comprehensive Serum Chemistry Panel[i]		x	x	x	x	x	x	x	x	x
Urine analysis[i]		x	x[f]				x	x		
FT4 and TSH, anti TPO[i]		x	x[f]				x	x	x	x
MUC-1, CA 125, CEA, amylase		x	x[f]		x		x		x	x
HIV/HCV/HBV-tests, lipase		x								
Efficacy Measurements										
Tumor Imaging[i, l]		x	x[f]				x		x	
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood										
Tumor Biopsy[m]		x			x		x			
PBMC collection[n]			x	x			x		x[k]	x
Plasma and serum sample[o]			x	x	x		x		x[k]	x
Urine sample		x	x[f]				x			
CTC collection (only if sufficient resources)			x		x		x			
Patient Reported Outcomes										
FQ, NRS, EORTC QLQ-C15-PAL[p]			x				x		x	x

a. General, assessments/procedures are to be performed on Day 1 and prior to the first dose of treatment for each cycle unless otherwise specified. Treatment cycles are 2 weeks; however the treatment cycle interval may be increased due to toxicity according to the dose modification guidelines provided in Section 10.1.2

b. Written consent must be obtained prior to performing any protocol specified procedure. Results of a test performed prior to the subject signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame in the protocol. Subject number will be assigned when the study informed consent is signed.

c. Prior medications – Record all medications taken within 30 days of screening visit. Concomitant medications – Enter new medications started during the trial through the Safety Follow-up visit. Record all medications taken for AEs.

d. AEs and laboratory safety measurements will be graded per NCI CTCAE version 4.0. All AEs, whether gradable by CTCAE or not, will also be evaluated for seriousness.

e. After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. After initiation of study drug, all adverse events will be reported until 30 days after the last dose of study treatment or until initiation of another anti-cancer therapy, whichever occurs first. After this period, investigators should report any serious adverse events and adverse events of special interest that are believed to be related to prior treatment with study drug. The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all serious adverse events considered to be related to study drug or trial-related procedures until a final outcome can be reported.

f. The full clinical examination is to be replaced by a directed clinical examination at C1 day 1. FT4, TSH, anti-TPO and urin analyses are not to be performed at C1 day 1, but at screening and at day 1 of C5 and every 4th cycle thereafter. MUC-1, CA 125, CEA and amylase analyses are not to be performed at C1 day 1, but at screening and at day 1 of C3 and every 2nd cycle thereafter.Tumor assessment is not to be performed at C1 day 1, but at screening, and as indicated in footnote i and l. For cross-over patients the tumor assessment at the discontinuation visit will serve as screening assessment. Urine sampling for the biobank is only to be performed at screening, day 1 of C5 and at time of progression. ECG is to be performed at screening (not C1), C5 day 1 and every 4th cycle thereafter. Left ventricular ejection fraction (LVEF) is to be measured at screening, cyclus 9 and every 8th week thereafter, if administration of pegylated liposomal doxorubicin is continued. LVEF may be measured by Multi Gated Acquisition Scan (MUGA) or echocardiography.

g. Women of childbearing potential must have a negative serum pregnancy test result ≤3 days prior to the first dose of nivolumab. A serum or urine pregnancy test (investigator's discretion) must be performed ≤3 days prior to Day 1 of every cycle during the treatment phase. A serum pregnancy test must be performed at the End of Treatment visit.

h. Coagulation factors (PT/INR and aPTT) should be tested as part of the screening procedures for all subjects. Any subject receiving anticoagulant therapy should have coagulation factors monitored closely throughout the trial.

i. Tumor assessments performed as standard of care prior to obtaining informed consent and within 21 days of Cycle 1, Day 1 may be used rather than repeating tests. All measurable and evaluable lesions should be assessed and documented at the screening visit. Radiologic imaging performed during the screening period should consist of 1) CT of the chest/abdomen/pelvis, alternatively MRI 2) bone scan (MRI, PET scan or scintigraphy), and 3) any other imaging studies (CT neck, plain films, etc.) as clinically indicated by the treating physician. The same radiographic procedures and technique must be used throughout the study for each patient (e.g., if the patient had CT chest/abdomen/pelvis performed during screening, then she/he should subsequently undergo CT performed using the same radiologic protocol throughout the remainder of the study). Results must be reviewed by the investigator before dosing at the next cycle. Tumor assessments will be performed at baseline, every 8 weeks from C1 day1 (± 1 week) for the first 12 months following randomization, and every 12 weeks (± 10 days) thereafter, with additional scans as clinically indicated. If iUPD is detected, a new radiological scan should be performed after 4-8 weeks, in accordance with iRECIST. Tumor response will be evaluated using both iRECIST criteria and RECIST v1.1. In the absence of disease progression per iRECIST, tumor assessments should continue regardless of whether patients discontinue study treatment, unless they withdraw consent or the study is terminated by the Sponsor, whichever occurs first.

j. Unresolved abnormal labs that are drug related AEs should be followed until resolution. Labs do not need to be repeated after the end of treatment if labs are within normal range.

k. For patients not progressed during treatment, plasma/serum and PBMC collection to be performed at first FU visit only (12 weeks after discontinuation). The Sponsor may ask for additional blood /PBMCs in selected cases.

l. In subjects who discontinue study therapy without confirmed disease progression, a radiologic evaluation should be performed at the time of treatment discontinuation (i.e., date of discontinuation ± 4 weeks). If a previous scan was obtained within 4 weeks prior to the date of discontinuation, then a scan at treatment discontinuation is not mandatory. Radiological assessments performed as standard of care can replace the tumor scans at follow-up visits, if performed ± 4 weeks of the scheduled time point.

m. Fresh frozen and FFPE tumor biopsies before start of treatment (mandatory), 4 weeks from C1 day 1 (± 5 days), 6 months from C1 day 1 (± 10 days) and at time of treatment discontinuation (fine needle aspiration is not sufficient). The prestudy biopsy may be obtained any time after signed informed consent. Archival tumor tissue

can be used instead of pretreatment biopsy, but must be obtained within three months of Cycle 1, Day 1. No anti-tumor treatment is allowed between the time point for biopsy and study entry. If the archival biopsy does not include fresh frozen tumor, a new pre-treatment biopsy for preservation as fresh-frozen material is mandatory.

n. PBMC; at day 1 of C2 (or screening) and day 1 of C2, C5, C9, C13, C25 and at time of progression. 100 ml ACD blood to be drawn at C1day1/screening. 70 ml ACD blood to be drawn at all later time points. Samples for PBMC collection should always be taken before infusion.

o. Plasma and serum to be collected at day 1 and day 2 of C1 and C5, and at the day of nivolumab injection (day 1) of C2, C3, C4, C6, C9, C13, C25 and at time of progression.

p. PRO forms to be completed at day 1 (+/- 7 days) of C1, C5, C9, C13, C25, C39 and at time of progression. The forms should be completed prior to the evaluation visit with the study doctor.

q. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy.

8.0 TRIAL PROCEDURES

8.1 Trial Procedures

The Trial Flow Chart, Section 8.1, summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the Investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

8.1.1 **Administrative Procedures**

8.1.1.1 **Informed Consent**

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial and prior to any trial-related procedures.

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

8.1.1.2 **Inclusion/Exclusion Criteria**

All inclusion and exclusion criteria will be reviewed by the Investigator or qualified designee to ensure that the subject qualifies for the trial.

8.1.1.3 **Medical History**

A medical history will be obtained by the Investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

8.1.1.4 **Prior and Concomitant Medications Review**

8.1.1.4.1 **Prior Medications**

The Investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject the last 30 days before screening visit. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

8.1.1.4.2 **Concomitant Medications**

The Investigator or qualified designee will record medication, if any, taken by the subject during the trial.

8.1.1.4.3 **Disease Details**

The Investigator or qualified designee will obtain prior and current details regarding disease status.

8.1.1.4.4 **Prior Treatment Details**

The Investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

8.1.1.4.5 **Subsequent Anti-Cancer Therapy Status**

The Investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy.

8.1.1.5 **Assignment of Subject Number**

All consented subjects will be given a unique subject number that will be used to identify the subject for all procedures that occur during the screening period, and for all subjects eligible for treatment, during the treatment and follow-up period.
Each subject will be assigned only one subject number. Subject numbers must not be re-used for different subjects. Any subject who is screened multiple times will retain the original subject number assigned at the initial screening visit.

8.1.1.6 **Randomization**

Randomization will be performed using the eCRF. The subject will be allocated to a randomization number.

8.1.1.7 **Electronic Case Report Forms (eCRFs)**

The designated investigator staff will enter the data required by the protocol into the eCase report forms (eCRF). The Investigator is responsible for assuring that data entered into the eCRF is complete, accurate, and that entry is performed in a timely manner.

The signature of the investigator will attest the accuracy of the data on each eCRF. If any assessments are omitted, the reason for such omissions will be noted on the eCRFs. Corrections, with the reason for the corrections will also be recorded.

8.1.2 **Clinical Procedures/Assessments**

8.1.2.1 **Full Physical Exam**

A complete physical examination should include an evaluation of the throat, heart, lungs, abdomen, skin, lymph node regions, musculoskeletal and neurological systems. Any abnormality identified at baseline should be recorded on the General Medical History. Height and weight should be measured and recorded in the CRF.
At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events in the CRF.

The Investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening, and every 8th week during study treatment as well as at the 30 days safety follow-up visit.

8.1.2.2 **Directed Physical Exam**

For cycles that do not require a full physical exam per the Trial Flow Chart, the Investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

8.1.2.3 **Vital Signs**

The Investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart. Vital signs should include temperature, pulseoxygen saturation, weight and blood pressure. Height and respiratory rate will be measured at screening only.

8.1.2.4 **Eastern Cooperative Oncology Group (ECOG) Performance Scale**

The Investigator or qualified designee will assess ECOG status at screening, prior to the administration of each dose of trial treatment, at discontinuation of trial treatment and during follow-up as specified in the Trial Flow Chart.

8.1.2.5 **Cardiac assessment**

The left ventricular ejection fraction (LVEF) should be measured at screening, cyclus 9 and every 8th week thereafter, if therapy with pegylated liposomal doxorubicin is continued. LVEF may be measured by MUGA of eccocardiography. ECG is to be done at screening and repeated every 4th cyclus. In addition, LVEF-determination, ECG and other cardiac assessments should be performed when clinically indicated. More frequent LVEF monitoring may be warranted if the cumulative dose of doxorubicin exceeds 450 mg/m2.

8.1.2.6 **Laboratory tests**

Laboratory tests for hematology, chemistry, urin analysis and other relevant tests are specified in Table 3.

Results must be reviewed by the Investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

Table 3

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	
Platelet count	Alanine aminotransferase (ALT)	Protein	INR
WBC (total and differential)	Aspartate aminotransferase (AST)		aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam if abnormal results are noted	
Absolute Neutrophil Count	Total protein		Free tyroxine (T4)
Absolute Lymphocyte Count	CRP	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Creatinine		TPO
	Uric Acid		
	Ionized/free Calcium		
	Chloride		Amylase
	Glucose		Lipase
	Phosphorus		HBV sAg and HCV Ab or HCV RNA
	Potassium		HIV-test
	Sodium		
	Magnesium		
	Total Bilirubin		MUC-1 CA125 CEA
	Direct Bilirubin *(If total bilirubin is elevated)*		Blood for correlative studies
† Perform on women of childbearing potential only.			

8.1.2.7 **Tumor Imaging and Assessment of Disease**

The tumor response will be assessed according to RECIST v1.1 as primary method, and by iRECIST as secondary method. After baseline tumor assessments, evaluation of tumor response will be performed every 8 weeks for the first 12 months following randomization (± 7 days) and every 12 weeks thereafter (± 10 days), with additional scans performed as clinically indicated. The same radiographic procedures used to assess measurable disease sites at screening should be used throughout the study (e.g., the same contrast protocol for CT scans and/or MRI). All known sites of disease must be documented at screening and re-assessed at each subsequent tumor evaluation.

If the radiological scan shows unconfirmed disease progression (iUPD) according to iRECIST, and the patient does not start a new cancer therapy, a new scan should be performed between 4 and 8 weeks after the previous scan. If disease progression per iRECIST is not confirmed, the radiological evaluation should continue as originally planned in the study, unless more frequent scanning is indicated.

If a new scan cannot be obtained for a patient with iUPD, the date for PD according to RECIST v1.1 should be used in the recording of PD according to iRECIST in this study.

8.1.2.8 **Tumor Tissue Collection and Correlative Studies Blood/Urine Sampling**

The time points for correlative studies tumor/blood/urine collection are listed in the study flow chart.

Tumor biopsies are to be collected pre (mandatory), during and post therapy (time of progression). If possible, three biopsies from the same lesion are to be obtained at each time point and preserved as follows:

- Formalin fixed paraffin embedded (FFPE)
- Fresh frozen tumor biopsies
- Fresh tumor cells to be frozen as cell suspensions for functional assays and CyTOF

The biopsies are to be collected from the same lesion throughout the study, if possible.

Blood samples are to be collected pre-, during and post-therapy:

- Peripheral blood mononuclear cells
- Plasma/serum
- Circulating tumor cells (only if sufficient resources available)

Urine samples collected pre-, during and post-therapy

The samples will be processed and stored according to existing protocols at OUS.

8.1.2.9 **PD-L1 analysis**

The pre-study biopsies will be analysed by IHC for PD-L1 expression, using a protocol recommended by BMS.

8.1.3 **Other Procedures**

8.1.3.1 **Withdrawal/Discontinuation**

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 10.4.

8.1.4 **Post-Treatment Visits**

8.1.4.1 **Safety Follow-Up Visit**

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to

the Safety Follow-Up Visit should be recorded. After this period, investigators should report any serious adverse events and adverse events of special interest that are believed to be related to prior treatment with study drug. The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all serious adverse events considered to be related to study drug or trial-related procedures until a final outcome can be reported.

8.1.4.2 **Follow-up Visits**

All subjects who discontinue trial treatment will move into the Follow-Up Phase. The patients that progressed during study treatment will be assessed at one Follow-up visit 16 weeks (± 10 days) after discontinuation. Patients who discontinue without progression will be assessed every 12 weeks (± 7 days) for the next 12 months. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, or end of the study. For details, see Study Flow Chart section 8.1.

8.1.4.3 **Survival Follow-up**

Survival data will be collected for 5 years after inclusion of the last patient. Information related to survival status will be collected from the Norwegian Population Registry. Cause of death will be collected from the patient´s local hospital or GP.

9.0 SAFETY ASSESSMENT

9.1 Safety plan

Administration of study treatment will be performed in a setting with emergency medical facilities and staff who are trained to monitor for and respond to medical emergencies. All adverse events and serious adverse events will be recorded during the trial and for up to 30 days after the last dose of study drug or until the initiation of another anti-cancer therapy, whichever occurs first. After this period, investigators should report serious adverse events and adverse events of special interest that are believed to be related to prior treatment with study drug. The potential safety issues anticipated in this trial, as well as measures intended to avoid or minimize such toxicities, are outlined in the following sections.

9.1.1 **Eligibility Criteria**

Eligibility criteria were selected to guard the safety of patients in this trial. Patients with a prior diagnosis of autoimmune disease, patients with evidence of acute infections, and patients who have received a live-attenuated viral vaccine within 4 weeks of randomization are excluded from the study.

9.1.2 **Monitoring**

Safety will be evaluated in this study through the monitoring of all serious and non-serious adverse events defined and graded according to NCI CTCAE v4.0. Patients will be assessed for safety (including laboratory values). Laboratory values must be reviewed prior to each administration of IMP.

General safety assessments will include serial interval histories, physical examinations, and specific laboratory studies, including serum chemistries and blood counts.

During the study, patients will be closely monitored for the development of any adverse events, including signs or symptoms of autoimmune conditions and infection.

All serious adverse events and protocol-defined events of special interest will be reported in an expedited fashion.

Patients will be followed for AE for 30 days, and for SAE and AESI for 100 days, following their last dose of study drug.

Patients who have an ongoing study drug related adverse event upon study completion or at discontinuation from the study will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, the patient is lost to follow-up, the patient withdraws consent, or until it has been determined that study treatment or participation is not the cause of the adverse event.

9.1.3 **Management of adverse events**

For recommendations on the management of adverse events related to nivolumab or ipiliumumab, please see the last updated versions of the Investigator´s Brochures for nivolumab and ipilimumab.

9.2 Dose Modification

9.2.1 **General Notes Regarding Dose Modification**

Reasons for dose modifications or delays, the supportive measures taken, and the outcomes will be documented in the patient's chart and recorded on the eCRF. The severity of adverse events will be graded according to the NCI CTCAE v4.0 grading system.

- Dose reduction of nivolumab or ipilimumab is not permitted, apart from changing the dosing intervals for ipilimumab to every 12th week.

- For any concomitant conditions already apparent at baseline, the dose modifications will apply according to the corresponding shift in toxicity grade, if the investigator feels it is appropriate. For example, if a patient has Grade 1 asthenia at baseline that increases to Grade 2 during treatment, this will be considered a shift of one grade and treated as Grade 1 toxicity for dose-modification purposes.

- When several toxicities with different grades of severity occur at the same time, the dose modifications should be according to the highest grade observed.

- If, in the opinion of the investigator, a toxicity is considered to be due to one/two/three component(s) of the treatment (i.e. nivo, ipi, cyclophosphamide or pegylated liposomal doxorubicin) and the dose of that/those component(s) is/are delayed or modified in accordance with the guidelines below, the other component(s) may be administered if there is no contraindication.

- When treatment is temporarily interrupted because of toxicity, the treatment cycles will be restarted such that the nivolumab and chemotherapy infusions remain synchronized.

- If it is anticipated that chemotherapy will be delayed by ≥ 10 days, then nivo/ipi should be given without the chemotherapy if there is no contraindication.

The treating physician may use discretion in modifying or accelerating the dose modification guidelines described below depending on the severity of toxicity and an assessment of the risk versus benefit for the patient, with the goal of maximizing patient compliance and access to supportive care.

9.2.2 **Nivolumab/Ipilimumab Dose Modification**

There will be no dose reduction for nivolumab in this study.

If a patient experiences a grade 3 or 4 adverse event considered probably related to ipilimumab, the ipilimumab dosing interval should be extended to every 12 week. The ipilimumab dose should be kept at 1mg/kg.

Patients may temporarily suspend study treatment with Nivolumab/Ipilimumab if they experience an adverse event that requires a dose to be held.

- If nivolumab is held because of adverse events for > 42 days (6 weeks) beyond the last dose, then the patient will be discontinued from nivolumab treatment. If, in the judgment of the investigator, the patient is likely to derive clinical benefit from resuming nivolumab after a hold > 42 days, study drug may be restarted with the approval of the Sponsor. If a patient must be tapered off steroids used to treat adverse events, nivolumab may be held for > 42 days until steroids are discontinued or reduced to prednisone dose (or dose equivalent) ≤ 10 mg/day. The acceptable length of interruption will depend on agreement between the investigator and the Sponsor.
- If ipilimumab is held because of adverse events for >126 days (18 weeks) beyond the last dose, then the patient will be discontinued from ipilimumab treatment . If, in the judgment of the investigator, the patient is likely to derive clinical benefit from resuming ipilimumab after a hold > 126 days, study drug may be restarted with the approval of the Sponsor. If a patient must be tapered off steroids used to treat adverse events, ipilimumab may be held for > 126 days until steroids are discontinued or reduced to prednisone dose (or dose equivalent) ≤ 10 mg/day. The acceptable length of interruption will depend on agreement between the investigator and the Sponsor.
- Dose interruptions for reason(s) other than adverse events, such as surgical procedures, may be allowed with Sponsor approval. The acceptable length of interruption will depend on agreement between the investigator and the Sponsor.
- Pateints who discontinue treatment with either nivolumab or ipilimumab due to AE may continue treament with the other IMPs.

9.2.3 **Dose-modification of chemotherapy**

Pegylated liposomal doxorubicin will be administered in accordance with standard procedures and established practice at the study hospitals, including criteria for hematological counts. Cardiac function will be monitored if clinically indicated and according to routine practice. Dose reduction and delay of treatment is allowed when performed in accordance with standard practice and in line with the guidelines given in the pegylated liposomal doxorubicin Product Information, as listed at ema.europa.eu. This includes guidelines on the management of stomatitis, palmar–plantar erythrodysesthesia and haematological toxicity.

The following adjustments to the standard guidelines apply:

- A grade 3 or 4 lymphopenia is to be handled as the corresponding grade of neutropenia. The dose of pegylated liposomal doxorubicin is withheld until the neutropenia resolves to grade ≤ 1 and the lymphopenia to grade ≤ 2,

Pegylated liposomal doxorubicin may be reduced in dose by up to 25%, i.e. to 15mg/m^2 or paused with up to two weeks when considered necessary by the investigator. If the investigator considers that a more extended pause is necessary, the investigator should consult a member of the Study Leadership. Dose reductions below 15mg/m2 are not allowed.

Metronomic cyclophosphamide, as used in the study, is expected to be well tolerated. If considered necessary by the investigator, the drug may be omitted for up to two weeks. If the investigator considers that a more extended pause is necessary, the investigator should consult a member of the Study Leadership.

9.3 Safety parameters and definitions

Safety assessments will consist of monitoring and recording of adverse events (including serious adverse events and non-serious adverse events of special interest), performing protocol-specified safety laboratory assessments, measuring protocol-specified vital signs, and conducting other protocol-specified tests that are deemed critical to the safety evaluation of the study.

9.3.1 **Adverse Events**

According to the ICH guideline for Good Clinical Practice, an adverse event is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. An adverse event can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition), except events that are clearly consistent with the expected pattern of progression of the underlying disease
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)

9.3.2 **Serious Adverse Events (Immediately Reportable to the Sponsor)**

A serious adverse event is any adverse event that meets any of the following criteria:

- Fatal (i.e., the adverse event actually causes or leads to death)
- Life threatening (i.e., the adverse event, in the view of the investigator, places the patient at immediate risk of death) This does not include any adverse event that had it occurred in a more severe form or was allowed to continue might have caused death
- Requires or prolongs inpatient hospitalization

- Results in persistent or significant disability/incapacity (i.e., the adverse event results in substantial disruption of the patient's ability to conduct normal life functions)
- Congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug
- Significant medical event in the investigator's judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (rated as mild, moderate, or severe or according to NCI CTCAE criteria); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each adverse event recorded on the CRF.
Serious adverse events are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event).

9.3.3 **Adverse Events of Special Interest (Immediately Reportable to the Sponsor)**

*A*dverse events of special interest are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event). Adverse events of special interest for this study include the following conditions which may be suggestive of an autoimmune disorder:

- Immune related Pneumonitis
- Immune related Colitis
- Immune related adrenal insufficiency
- Immune related Hepatitis
- Immune related Hypothyroidism
- Immune related Hyperthyroidism
- Immune related Pancreatitis
- Immune related Diabetes Mellitus
- Immune related Nephritis
- Treatment related Rash
- Immune related Hypophysitis

9.4 Immediate reporting requirements from investigator to sponsor

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- Serious adverse events
- Non-serious adverse events of special interest
- Pregnancies

9.4.1 Reporting Requirements for Serious Adverse Events and Non-Serious Adverse Events of Special Interest

9.4.1.1 Events that Occur Prior to Study Drug Initiation

After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. The Serious Adverse Event should be reported to Sponsor immediately (i.e., no more than 24 hours after learning of the event).

9.4.1.2 Events that Occur After Study Drug Initiation

After initiation of study drug, serious adverse events and non-serious adverse events of special interest will be reported until 100 days after the last dose of ipilimumab/nivolumab or until initiation of another anti-cancer therapy, whichever occurs first. Investigators should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) and forward these to the Sponsor.

9.5 Reporting Requirements for Pregnancies

If, following initiation of the study drug, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of study exposure, including during at least 5 half lives after product administration, the investigator must immediately notify the Sponsor of this event and complete and forward a Pregnancy Form to Sponsor within 24 hours of awareness of the event and in accordance with SAE reporting procedures.

In this case, the study drug will be permanently discontinued in an appropriate manner.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Form.

Any pregnancy that occurs in a female partner of a male study participant should be reported to Sponsor. Information on this pregnancy will be collected on the Pregnancy Form.

9.6 Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

9.7 Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs.

Potential drug induced liver injury is defined as:
1. AT (ALT or AST) elevation > 3 times upper limit of normal (ULN), AND
2. Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase), AND

3. No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic

9.8 Follow-up of patients after adverse events

9.8.1 Investigator Follow-Up

During the study period, resolution of adverse events (with dates) should be documented on the Adverse Event CRF and in the patient's medical record to facilitate source data verification. If, after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded on the Adverse Event CRF. Every effort should be made to follow all serious adverse events considered related to study drug or trial-related procedures until a final outcome can be reported. All pregnancies reported during the study should be followed until pregnancy outcome.

9.8.2 Sponsor Follow-Up

For serious adverse events, non-serious adverse events of special interest, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

9.9 Post-study adverse events

At the treatment discontinuation visit, the investigator should instruct each patient to report to the investigator any subsequent adverse events that the patient's personal physician believes could be related to prior study drug treatment or study procedures.

Investigators should notify the Sponsor of any serious adverse events and adverse events of special interest that are believed to be related to prior drug treatment or study procedures that occur at any time after a patient has discontinued study participation. The Sponsor should also be notified if the investigator becomes aware of the development of cancer or a congenital anomaly/birth defect in a subsequently conceived offspring of a patient who participated in this study.

9.10 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to Competent Authorities and Investigators in accordance with applicable national laws and regulations.

9.10.1 Suspected Unexpected Serious Adverse Reactions (SUSARs)

Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to the Competent Authority according to national regulation. The following timelines should be followed:

The sponsor will ensure that all relevant information about SUSARs that are fatal or life-threatening is recorded and reported as soon as possible to the Competent Authority concerned in any case no later than seven (7) days after knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional eight (8) days.

All other SUSARs will be reported to the Competent Authority concerned as soon as possible but within a maximum of fifteen (15) days of first knowledge by the sponsor.

SUSARs will be reported using the CIOMS form since Oslo University Hospital (sponsor) is not connected to EudraVigilance.

9.10.2 **Serious Adverse Events and Non-serious adverse event of special interest**

9.10.2.1 **Reporting from site to sponsor**

SAEs, whether related or not related to study drug, must be reported to Sponsor's representative (via eCRF or other approved form) within 24 hours after lerning of the event.

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to Sponsor's representative using the same procedure used for transmitting the initial SAE report.

9.10.2.2 **Reporting from sponsor to BMS**

All Serious Adverse Events (SAEs) that occur after the subject's written consent and within 100 days of discontinuation of ipilimumab/nivolumab, must be reported to BMS Worldwide Safety. The SAE should be reported to BMS within 24 hours after lerning of the event using an SAE reporting method approved by BMS, whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy). The BMS protocol ID number must be included on whatever form (CIOMS, etc) is submitted by the Sponsor/Investigator.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: +1 609-818-3804

10.0 COLLATERAL RESEARCH

An extensive research program will be conducted. The patient informed consent form will allow for performing biomarker analyses, immunological studies, gene profiling and studies of tumor evolution/heterogeneity during treatment, as well as comparison with data/material from other studies.

10.1 Biobanking:

Please see the study flow chart for information on the time points for collection of biopsies, peripheral blood and urine.

- Tumor biopsies collected pre, during and post therapy (time of progression). If sufficient tissue is avaliable, three biopsies will be obtained at each timepoint, and prioritized in the following order:

  - FFPE tissue
  - Snap-frozen tumor biopsies
  - Fresh tumor cells/ tumor infiltrating lymphocytes frozen as cell suspension for CYTOF/flow cytometry, analysis of T cell specificity with multimers and functional immune assays
- Blood samples collected pre-, during and post-therapy:
  - Peripheral blood mononuclear cells, processed with gradient centrifugation and frozen on liquid nitrogen
  - Plasma/serum, separated and frozen.
  - Circulating tumor cells (only if sufficient resources available)
- Urine samples collected pre-, during and post-therapy

10.2 Plans for translational research addressing exploratory endpoints

The list below does not represent a mandatory list of assays that are to be performed, but provides an overview of the current plans. The prioritation of assays will be subject to review during the trial. Additional assays may be performed, reflecting ongoing developments in the field. Some of these assays will only be performed on selected patients, due to cost and work load.

- Gene profiling with selected panels (e.g. PAM50, customized immune-gene and tumor gene panels)
- Pathology/immunohistochemistry (incl. PD-L1 and markers for leucocyte subpopulations)
- DNA sequencing of tumor and normal PBMCs (mutations, mutation load, HLA types/loss, SNPs)(16)
- RNA transcriptome sequencing of tumor from selected patients (gene expression profiles, neoantigens)
- Serum biomarkers/soluble biopsies, including
  - circulating DNA
  - circulating micro RNA(17)
  - Bioplex cytokine- and MMP panels
- Characterization of cell suspensions from tumor and peripheral blood:
  - CYTOF (investigating subpopulations/heterogeneity/evolution, both within tumor cells, immune cells and other stromal cells; relate CYTOF data to gene profiling and mRNA expression)
  - Flow cytometry, including the use of panels for regulatory T cells and myeloid suppressor cells(18)
- Functional T cell assays, incl. ELISPOT, proliferation, Bioplex cytokine profiling, multimers (19, 20)
- Test of T cell reactivity against the individual spectrum of tumor antigens in each patient´s tumor, identified through tumor sequencing and epitope prediction(21, 22)
- Tumor gene profiling, for monitoring tumor evolution during treatment(16, 23)
- Circulating tumor cells in peripheral blood(24, 25)

11.0 STATISTICS

11.1 Statistical analyses

A descriptive analysis of demographics, medical history, and clinical data will be performed.

The primary efficacy analysis will be a descriptive analysis of progression free survival (PFS) in the combination arm, compared to the control group. PFS is, defined as the time from randomization to the occurrence of disease

progression, as determined per iRECIST, or death from any cause, whichever occurs first. Data for patients without disease progression or death will be censored at the last tumor assessment date. Data for patients with a PFS event who missed two or more assessments scheduled immediately prior to the date of the PFS event will be censored at the last tumor assessment prior to the missed visits. If no tumor assessment was performed after randomization, data will be censored at the date of randomization +1 day. Clinical deterioration without objective radiological evidence will not be considered as documented disease progression. PFS will be compared between treatment arms with use of the log-rank test. The HR for disease progression or death will be estimated using a Cox proportional hazards model. The 95% CI for the HR will be provided. Kaplan-Meier methodology will be used, and Kaplan-Meier curves will be produced.

Overall survival (OS) will be calculated from time of randomization until death. Patients alive at the time of data analysis will be treated as censored. OS will be estimated by the Kaplan Meier method.

Exploratory analyses will be carried out to evaluate the data of the immunological and molecular analyses (e.g. biomarker studies) carried out. The statistical analyses will be dependent on the biological factors investigated and the analysis methodology used, and will be defined separately for each molecular study.

We expect to reach the data-driven time point for PFS-analysis (95% PFS in the control group) approximately 3 years after the study opens. If this is not met within 24 months after inclusion of the last patient, the PFS-analysis will be performed at this time point.

The data analysis will be performed on the ITT population (all randomized patients) and analyzed accordning to the following factors:

- Tumor PD-L1 status (BMS test protocol)
- Disease free interval: Less than 24 months versus >24 months between end of adjuvant chemotherapy and relapse (most LumB patients receive adjuvant chemo for 6 months before/after radical surgery; a short disease-free interval suggests aggressive disease).
- Prior chemotherapy against metastatic disease (no previous chemo vs. previous chemo). Chemotherapy given in the neoadjuvant/adjuvant setting is not to be considered in this analysis

Exploratory analyses will be carried out to evaluate data from translational studies. Here, statistical methods will be defined separately for each study, as advised by the statisticians.

11.2 Statistical considerations regarding sample size and randomization ratio

The phase II study cannot be powered to demonstrate a statistically significant ($p<0.05$) clinical effect. If the study suggests acceptable toxicity and potential clinical benefit, a larger randomized study will be warranted. We plan to conduct a phase II study with 75 patients (45 patients in the nivo-chemo arm, 30 patients in the chemo-only arm).

The number of 75 patients and the randomization ratio of 3:2 were based on the following considerations:

- **Expected PFS for the control group, receiving only chemotherapy:**

Months	Progression-free proportion
5	50%
12	25%
20	5%

- **Statistical power calculations for the primary endpoint PFS**
  A two-sided hypothesis test was performed with a 10% significance level and a desired power of 80%. The test was NOT performed to define a number of patients where a significant clinical effect ($p<0.05$) could be determined, as this is not the aim of a phase II trial. Rather, the calculation was done to illustrate what we can expect to observe within a realistic number of patients for a phase II trial and to inform the choice of randomisation ratio. For this purpose, we choice a significance level of 10% and performed the test for a sample size of 60, 75 and 80 patients. The calculation for 75 patients is given below (Table 4).

Randomisation	Surv1	Surv2	HR
1:1	0.05	0.208	0.52
2:3	0.05	0.198	0.54
1:2	0.05	0.198	0.54

Table 1: Statistical power with 75 patients
Surv1 is the survival probability in the control group at the end of the study. Surv2 is the survival probability in the experimental group. HR = hazard ratio, effect size of the experimental to the control group

The power calculation indicates that a **randomization ratio of 2:3** or 1:2 is preferable to 1:1. A ratio of 2:3 is chosen rather than 1:2 in order to increase the statistical power for collateral research analyses.

The biomarker research program aims at identifying which patients benefit from treatment and may inform the design of a subsequent randomized trial. The suggested number of patients will allow for meaningful statistical comparisons of biological/immunological data, and comparison with data from our previous studies and the OUS breast cancer biobank.

12.0 ETHICAL CONSIDERATIONS

12.1 Compliance with Laws and Regulations

This study will be conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting), in addition with the E.U. Clinical Trial Directive (2001/20/EC).

12.2 Informed Consent

The Informed Consent Form will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research. The investigator or authorized designee will explain to each patient the objectives of the exploratory research. Patients will be told that they are free to refuse to participate and may withdraw their specimens at any time and for any reason during the storage period. A separate, specific signature will be required to document a patient's agreement to allow any remaining specimens to be used for exploratory research. Patients who decline to participate will not provide a separate signature.

The Consent Forms must be signed and dated by the patient or the patient's legally clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate.

Patients must be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and IRB/EC policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the patient or the patient's legally authorized representative. All signed and dated Consent Forms must remain in each patient's study file or in the site file and must be available for verification by study monitors at any time.

12.3 Ethics Committee

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information will be submitted to the Ethics Committee, reviewed and approved according to EU and national regulations before the study is initiated. This is also valid for any amendments and/or a new version of the study protocol (Amended Protocol).

12.4 Confidentiality

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. This means that patient names are not included in data sets.

Patient medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of the Competent Authority, Sponsor, monitors, representatives, and collaborators, and the EC for each study site, as appropriate.

13.0 ADMINISTRATIVE AND REGULATORY DETAILS

13.1 Study Documentation

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol, protocol amendments, Informed Consent Forms, and documentation of EC and governmental approval.

13.2 Protocol Adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations.
All significant protocol deviations will be recorded and reported in the Clinical Study Report (CSR).

13.3 Monitoring

The investigator/site will be visited on a regular basis by the Clinical Study Monitor, who will assess compliance with the trial protocol and general principles of Good Clinical Practice. The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required.
Sponsor's representatives (e.g. monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

13.4 Audit and Inspections

Authorized representatives of a Competent Authority and Ethics Committee may visit the centres to perform inspections, including source data verification. Likewise the representatives from sponsor may visit the centres to perform an audit. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (ICH GCP), and any applicable regulatory requirements. The principal investigator at each study site will ensure that the inspectors and auditors will be provided with access to source data/documents.

13.5 Data Management

The Investigator or qualified designee is responsible for recording and verifying the accuracy of subject data. Detailed information regarding Data Management procedures for this protocol will be provided separately.

13.6 Publication Policy

Upon study completion and finalization of the study report the results of this study will either be submitted for publication and/or posted in a publicly assessable database of clinical study results. The results of this study will also be submitted to the Competent Authority and the Ethics Committee according to EU and national regulations.

14.0 LIST OF REFERENCES

1. Bezu L, Gomes-de-Silva LC, Dewitte H, Breckpot K, Fucikova J, Spisek R, et al. Combinatorial strategies for the induction of immunogenic cell death. Frontiers in immunology. 2015;6:187.
2. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med. 2007;13(9):1050-9.
3. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med. 2014;20(11):1301-9.
4. Kroemer G, Senovilla L, Galluzzi L, Andre F, Zitvogel L. Natural and therapy-induced immunosurveillance in breast cancer. Nat Med. 2015;21(10):1128-38.
5. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. Immunity. 2016;44(2):343-54.
6. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother. 2007;56(5):641-8.
7. Munzone E, Colleoni M. Clinical overview of metronomic chemotherapy in breast cancer. Nat Rev Clin Oncol. 2015;12(11):631-44.
8. Rau KM, Lin YC, Chen YY, Chen JS, Lee KD, Wang CH, et al. Pegylated liposomal doxorubicin (Lipo-Dox(R)) combined with cyclophosphamide and 5-fluorouracil is effective and safe as salvage chemotherapy in taxane-treated metastatic breast cancer: an open-label, multi-center, non-comparative phase II study. BMC cancer. 2015;15:423.
9. Jehn CF, Hemmati P, Lehenbauer-Dehm S, Kummel S, Flath B, Schmid P. Biweekly Pegylated Liposomal Doxorubicin (Caelyx) in Heavily Pretreated Metastatic Breast Cancer: A Phase 2 Study. Clin Breast Cancer. 2016;16(6):514-9.
10. Rossi D, Baldelli AM, Casadei V, Fedeli SL, Alessandroni P, Catalano V, et al. Neoadjuvant chemotherapy with low dose of pegylated liposomal doxorubicin plus weekly paclitaxel in operable and locally advanced breast cancer. Anti-cancer drugs. 2008;19(7):733-7.
11. Dellapasqua S, Mazza M, Rosa D, Ghisini R, Scarano E, Torrisi R, et al. Pegylated liposomal doxorubicin in combination with low-dose metronomic cyclophosphamide as preoperative treatment for patients with locally advanced breast cancer. Breast. 2011;20(4):319-23.
12. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med. 2015;373(1):23-34.
13. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373(2):123-35.
14. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet. 2014;384(9948):1109-17.
15. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13(11):674-90.
16. Kristensen VN, Lingjaerde OC, Russnes HG, Vollan HK, Frigessi A, Borresen-Dale AL. Principles and methods of integrative genomic analyses in cancer. Nat Rev Cancer. 2014;14(5):299-313.
17. Kleivi Sahlberg K, Bottai G, Naume B, Burwinkel B, Calin GA, Borresen-Dale AL, et al. A serum microRNA signature predicts tumor relapse and survival in triple-negative breast cancer patients. Clin Cancer Res. 2015;21(5):1207-14.
18. Hansen GL, Gaudernack G, Brunsvig PF, Cvancarova M, Kyte JA. Immunological factors influencing clinical outcome in lung cancer patients after telomerase peptide vaccination. Cancer Immunol Immunother. 2015;64(12):1609-21.

19. Kyte JA, Gaudernack G, Dueland S, Trachsel S, Julsrud L, Aamdal S. Telomerase Peptide Vaccination Combined with Temozolomide: A Clinical Trial in Stage IV Melanoma Patients. Clin Cancer Res. 2011;17(13):4568-80.
20. Kyte JA, Aamdal S, Dueland S, Inderberg EM, Sæbøe-Larsen S, Madsbu UE, et al. Immune response and long term clinical outcome in advanced melanoma patients vaccinated with tumor-mRNA transfected dendritic cells. Oncoimmunology. 2016:in press.
21. Bentzen AK, Marquard AM, Lyngaa R, Saini SK, Ramskov S, Donia M, et al. Large-scale detection of antigen-specific T cells using peptide-MHC-I multimers labeled with DNA barcodes. Nat Biotechnol. 2016.
22. Stronen E, Toebes M, Kelderman S, van Buuren MM, Yang W, van Rooij N, et al. Targeting of cancer neoantigens with donor-derived T cell receptor repertoires. Science. 2016.
23. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016.
24. Naume B, Synnestvedt M, Falk RS, Wiedswang G, Weyde K, Risberg T, et al. Clinical outcome with correlation to disseminated tumor cell (DTC) status after DTC-guided secondary adjuvant treatment with docetaxel in early breast cancer. J Clin Oncol. 2014;32(34):3848-57.
25. Seeberg LT, Waage A, Brunborg C, Hugenschmidt H, Renolen A, Stav I, et al. Circulating tumor cells in patients with colorectal liver metastasis predict impaired survival. Ann Surg. 2015;261(1):164-71.
26. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol. 2017;18(3):e143-e52.

15.0 ABBREVIATIONS

Abbreviation/Term	Definition
ACD	Acid Citrate Dextrose
AE	Adverse Event
AESI	Adverse Events of Special Interest
ALT	Alanine Aminotransferase
aPTT	activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
BML	Below Measurable Limit
BPI	Brief Pain Inventory
CA125	Cancer Antigen 125
CBC	Complete Blood Count
CEA	Carcinoembryonic Antigen
CNS	Central Nervous System
CR	Complete Response
CRF	Case Report Form (electronic/paper)
CRP	C-reactive Protein
CT	Computer Tomography
CTC	Circulating Tumor Cells
CTCAE	Common Terminology Criteria for Adverse Event
CTLA-4	Cytotoxic T Lymphocyte Antigen 4
CYTOF	CYtometry Time Of Flight
DNA	Deoxyribonucleic Acid
DOR	Duration of Objective Response
DR	Duration Of response
DRR	Durable tumor Response Rate
DRR	Durable response Rate
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EMA	European Medicines Agency
EORTC	European Organization for Research and Treatment of Cancer
ER	Estrogen Receptor
ERC	European Research Council
EU	European Union
FPI	First Patient In
FQ	Fatigue Questionnaire
GP	General Practician
HER2	Human Epidermal growth factor Receptor 2
HLA	Human Leukocyte Antigen
HR	Hazard Ratio

ICH	International Conference on Harmonization
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
IRB	Institutional Review Board
iRECIST	immune-modified Response Evaluation Criteria in Solid Tumors
ISH	In situ hybridization
iCPD	Confirmed progressive disease according to iRECIST
ITT	Interntion To Treat
IUD	Intrauterine device
iUPD	Unconfirmed progressive disease according to iRECIST
IV	Intravenous
LDH	Lactate Dehydrogenase
LVEF	Left Ventricular Ejection Fraction
MCID	Minimally Clinically Important Difference
MDSC	Myeloid-derived Suppressor Cells
MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NRS	Numerical Rating Scale
ORR	Overall Response Rate
OS	Overall Survival
OUS	Oslo University Hospital
PBMC	Peripheral Blood Mononuclear Cell
PD	Progressive disease
PD-1	Programmed Death 1
PD-L1	Programmed Death Ligand-1
PFS	Progression-free Survival
PI	Principle Investigator
PR	Progesterone Receptor
PRO	Patient Reported Outcome
QLQ	Quality of Life Questionnaire
RNA	Ribonuceleic Acid
SAE	Serious Adverse Event
SD	Stable Disease
SIA	Systemic Immune Activation
SLD	Sum of Longest Diameters
SMC	Safety Monitoring Committee
SNP	Single Nucleotide Polymorphism
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCR	T-Cell Receptor
TNBC	Triple Negative Breast Cancer
TNF	Tumor Necrosis Factor
TPA	Tissue Plasminogen Activator
TSH	Thyroid Stimulating Hormone

TTD	Time to Deterioration
ULN	Upper Limit of Normal

16.0 APPENDICES

16.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

16.2 Common Terminology Criteria for Adverse Events v 4.0

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html).

16.3 Response Evaluation Criteria in Solid Tumors

Selected sections from the Response Evaluation Criteria in Solid Tumors (RECIST), Version 1.1 are presented below, with slight modifications and the addition of explanatory text as needed for clarity.

16.3.1 Measurability of tumor at baseline

DEFINITIONS
At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

Measurable Tumor Lesions
Tumor Lesions. Tumor lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (CT/MRI scan slice thickness/interval no greater than 5 mm)
- 10-mm caliper measurement by clinical examination (lesions that cannot be accurately measured with calipers should be recorded as non-measurable)
- 20 mm by chest X-ray

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in the short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed. See also notes below on "Baseline Documentation of Target and Nontarget Lesions" for information on lymph node measurement.

Non-Measurable Tumor Lesions

Non-measurable tumor lesions encompass small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), as well as truly non-measurable lesions. Lesions considered truly non-measurable include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, peritoneal spread, and abdominal masses/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques.

Special Considerations Regarding Lesion Measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment, as outlined below.

Bone lesions:

- Bone scan, positron emission tomography (PET) scan, or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic blastic lesions, with identifiable soft tissue components, that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- Cystic lesions thought to represent cystic metastases can be considered measurable lesions if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

16.3.2 **Target lesions: specifications by methods of measurements**

Measurement of Lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

Method of Assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during study. Imaging-based evaluation should always be the preferred option.

Clinical Lesions. Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm in diameter as assessed using calipers (e.g., skin nodules).
For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is suggested.

Chest X-Ray. Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI. CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable.

If prior to enrollment it is known that a patient is unable to undergo CT scans with intravenous (IV) contrast due to allergy or renal insufficiency, the decision as to whether a noncontrast CT or MRI (without IV contrast) will be used to evaluate the patient at baseline and during the study should be guided by the tumor type under investigation and the anatomic location of the disease. For patients who develop contraindications to contrast after baseline contrast CT is done, the decision as to whether noncontrast CT or MRI (enhanced or nonenhanced) will be performed should also be based on the tumor type and the anatomic location of the disease and should be optimized to allow for comparison with the prior studies if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the patient should be considered not evaluable from that point forward. Care must be taken in measurement of target lesions on a different modality and interpretation of nontarget disease or new lesions since the same lesion may appear to have a different size using a new modality.

Ultrasound. Ultrasound is not useful in the assessment of lesion size and should not be used as a method of measurement.

Endoscopy, Laparoscopy, Tumor Markers, Cytology, Histology. The utilization of these techniques for objective tumor evaluation cannot generally be advised.

16.3.3 **Tumor response evaluation**

16.3.3.1 **Assessment of overall tumor burden and measurable disease**

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and to use this as a comparator for subsequent measurements. Measurable disease is defined by the presence of at least one measurable lesion, as detailed above.

16.3.3.2 **Baseline documentation of target and nontarget lesions**

When more than one measurable lesion is present at baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. This means in instances where patients have only one or two organ sites involved, a maximum of two lesions (one site) and four lesions (two sites), respectively, will be recorded. Other lesions (albeit measurable) in those organs will be recorded as non-measurable lesions (even if the size is > 10 mm by CT scan).

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and be representative of all involved organs, but additionally, should lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement, in which circumstance the next largest lesion that can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures that may be visible by imaging even if not involved by tumor. As noted above, pathological nodes that are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan, this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal, or coronal). The smaller of these measures is the short axis. For example, an abdominal node that is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered nontarget lesions. Nodes that have a short axis < 10 mm are considered nonpathological and should not be recorded or followed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum of diameters. If lymph nodes are to be included in the sum, then, as noted above, only the short axis is added into the sum. The baseline sum of diameters will be used as a reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease), including pathological lymph nodes, should be identified as nontarget lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present," "absent," or in rare cases "unequivocal progression."

In addition, it is possible to record multiple nontarget lesions involving the same organ as a single item on the Case Report Form (CRF) (e.g., "multiple enlarged pelvic lymph nodes" or "multiple liver metastases").

16.3.3.3 **Response criteria**

Evaluation of Target Lesions

This section provides the definitions of the criteria used to determine objective tumor response for target lesions.

- **Complete response (CR)**: disappearance of all target lesions
  Any pathological lymph nodes (whether target or nontarget) must have reduction in short axis to < 10 mm.

- **Partial response (PR)**: at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of diameters
- **Progressive disease (PD)**: at least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (nadir), including baseline.
  In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.
  The appearance of one or more new lesions is also considered progression.
- **Stable disease (SD)**: neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum on study

Special Notes on the Assessment of Target Lesions

Lymph Nodes. Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to < 10 mm on study. This means that when lymph nodes are included as target lesions, the sum of lesions may not be zero even if CR criteria are met since a normal lymph node is defined as having a short axis < 10 mm.

Target Lesions That Become Too Small to Measure. While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). However, sometimes lesions or lymph nodes that are recorded as target lesions at baseline become so faint on the CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being too small to measure. When this occurs, it is important that a value be recorded on the CRF as follows:

- If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm.
- If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned and below measurable limit (BML) should be ticked. (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well and BML should also be ticked.)

To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm, and, in that case, BML should not be ticked.

Lesions That Split or Coalesce on Treatment. When non-nodal lesions fragment, the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the coalesced lesion.

Evaluation of Nontarget Lesions

This section provides the definitions of the criteria used to determine the tumor response for the group of nontarget lesions. While some nontarget lesions may actually be measurable, they need not be measured and, instead, should be assessed only qualitatively at the timepoints specified in the protocol.

- **CR**: disappearance of all nontarget lesions and (if applicable) normalization of tumor marker level)
  All lymph nodes must be non-pathological in size (< 10 mm short axis).

- **Non-CR/Non-PD**: persistence of one or more nontarget lesion(s) and/or (if applicable) maintenance of tumor marker level above the normal limits
- **PD**: unequivocal progression of existing nontarget lesions
  The appearance of one or more new lesions is also considered progression.

Special Notes on Assessment of Progression of Nontarget Disease

When the Patient Also Has Measurable Disease. In this setting, to achieve unequivocal progression on the basis of the nontarget disease, there must be an overall level of substantial worsening in nontarget disease in a magnitude that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest increase in the size of one or more nontarget lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in nontarget disease in the face of SD or PR of target disease will therefore be extremely rare.

When the Patient Has Only Non-Measurable Disease. This circumstance arises in some Phase III trials when it is not a criterion of study entry to have measurable disease.
The same general concepts apply here as noted above; however, in this instance, there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in nontarget disease cannot be easily quantified (by definition: if all lesions are truly non-measurable), a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease, that is, an increase in tumor burden representing an additional 73% increase in volume (which is equivalent to a 20% increase in diameter in a measurable lesion). Examples include an increase in a pleural effusion from “trace” to “large” or an increase in lymphangitic disease from localized to widespread or may be described in protocols as “sufficient to require a change in therapy.” If unequivocal progression is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so; therefore, the increase must be substantial.

New Lesions
The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal, that is, not attributable to differences in scanning technique, change in imaging modality, or findings thought to represent something other than tumor (for example, some “new” bone lesions may be simply healing or flare of preexisting lesions). This is particularly important when the patient’s baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a “new” cystic lesion, which it is not.

A lesion identified during the study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

16.3.3.4 **Evaluation of response**

Timepoint Response (Overall Response)

It is assumed that at each protocol-specified timepoint, a response assessment occurs. Table 1 provides a summary of the overall response status calculation at each timepoint for patients who have measurable disease at baseline.

When patients have non-measurable (therefore nontarget) disease only, Table 2 is to be used.

Table 1 Timepoint Response: Patients with Target Lesions (with or without Nontarget Lesions)

Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR= complete response; NE= not evaluable; PD = progressive disease;
PR= partial response; SD = stable disease.

Table 2 Timepoint Response: Patients with Nontarget Lesions Only

Nontarget Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD [a]
Not all evaluated	No	NE
Unequivocal PD	Yes or no	PD
Any	Yes	PD

CR= complete response; NE= not evaluable; PD = progressive disease.

[a] "Non-CR/non-PD" is preferred over "stable disease" for nontarget disease since stable disease is increasingly used as an endpoint for assessment of efficacy in some trials; thus, assigning "stable disease" when no lesions can be measured is not advised.

Missing Assessments and Not-Evaluable Designation

When no imaging/measurement is done at all at a particular timepoint, the patient is not evaluable at that timepoint. If only a subset of lesion measurements are made at an assessment, usually the case is also considered not evaluable at that timepoint, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned timepoint response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and, during the study, only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

If one or more target lesions were not assessed either because the scan was not done or the scan could not be assessed because of poor image quality or obstructed view, the response for target lesions should be "unable to assess" since the patient is not evaluable. Similarly, if one or more nontarget lesions are not assessed, the response for nontarget lesions should be "unable to assess" except where there is clear progression. Overall response would be "unable to assess" if either the target response or the nontarget response is "unable to assess," except where this is clear evidence of progression as this equates with the case being not evaluable at that timepoint.

Table 3 Best Overall Response When Confirmation Is Required

Overall Response at First Timepoint	Overall Response at Subsequent Timepoint	Best Overall Response
CR	CR	CR
CR	PR	SD, PD, or PR [a]
CR	SD	SD, provided minimum duration for SD was met; otherwise, PD
CR	PD	SD, provided minimum duration for SD was met; otherwise, PD
CR	NE	SD, provided minimum duration for SD was met; otherwise, NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD, provided minimum duration for SD was met; otherwise, PD

PR	NE	SD, provided minimum duration for SD was met; otherwise, NE
NE	NE	NE

CR= complete response; NE= not evaluable; PD = progressive disease; PR = partial response;
SD= stable disease.

[a] If a CR is truly met at the first timepoint, any disease seen at a subsequent timepoint, even disease meeting PR criteria relative to baseline, qualifies as PD at that point (since disease must have reappeared after CR). Best response would depend on whether the minimum duration for SD was met. However, sometimes CR may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR, at the first timepoint. Under these circumstances, the original CR should be changed to PR and the best response is PR.

Special Notes on Response Assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to "normal" size (< 10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of "zero" on the CRF.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response; it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and nontarget disease as shown in Table 1, Table 2, and Table 3.

For equivocal findings of progression (e.g., very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment progression is confirmed, the date of progression should be the earlier date when progression was suspected.

In studies for which patients with advanced disease are eligible (i.e., primary disease still or partially present), the primary tumor should also be captured as a target or nontarget lesion, as appropriate. This is to avoid an incorrect assessment of complete response if the primary tumor is still present but not evaluated as a target or nontarget lesion.

16.4 Immune-modified Response Evaluation Criteria in Solid Tumors (iRECIST)

Conventional response criteria may not be adequate to characterize the anti-tumor activity of immunotherapeutic agents, which can produce delayed responses that may be preceded by initial apparent radiological progression, including the appearance of new lesions. Therefore, immune-modified response criteria have been developed (iRECIST) for the possibility of pseudo progression.

An updated consensus guideline for immune-modified Response Evaluation Criteria in Solid Tumors (iRECIST), was published on behalf of the RECIST working group March 2017. Theis guideline (xx) is to be used in the present protocol.

Tables 1 and 2 below show key points in iRECIST. Please see the published iRECIST guideline, with supplementary materials, for the complete criteria (26).

	RECIST 1.1	iRECIST
Definitions of measurable and non-measurable disease; numbers and site of target disease	Measurable lesions are ≥10 mm in diameter (≥15 mm for nodal lesions); maximum of five lesions (two per organ); all other disease is considered non-target (must be ≥10 mm in short axis for nodal disease)	No change from RECIST 1.1; however, new lesions are assessed as per RECIST 1.1 but are recorded separately on the case report form (but not included in the sum of lesions for target lesions identified at baseline)
Complete response, partial response, or stable disease	Cannot have met criteria for progression before complete response, partial response, or stable disease	Can have had iUPD (one or more instances), but not iCPD, before iCR, iPR, or iSD
Confirmation of complete response or partial response	Only required for non-randomised trials	As per RECIST 1.1
Confirmation of stable disease	Not required	As per RECIST 1.1
New lesions	Result in progression; recorded but not measured	Results in iUPD but iCPD is only assigned on the basis of this category if at next assessment additional new lesions appear or an increase in size of new lesions is seen (≥5 mm for sum of new lesion target or any increase in new lesion non-target); the appearance of new lesions when none have previously been recorded, can also confirm iCPD
Independent blinded review and central collection of scans	Recommended in some circumstances—eg, in some trials with progression-based endpoints planned for marketing approval	Collection of scans (but not independent review) recommended for all trials
Confirmation of progression	Not required (unless equivocal)	Required
Consideration of clinical status	Not included in assessment	Clinical stability is considered when deciding whether treatment is continued after iUPD

Table 1: Comparison of RECIST v1.1. and iRECIST, adapted from (26)

	Timepoint response with no previous iUPD in any category	Timepoint response with previous iUPD in any category*
Target lesions: iCR; non-target lesions: iCR; new lesions: no	iCR	iCR
Target lesions: iCR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iPR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iSD; non-target lesions: non-iCR/non-iUPD; new lesions: no	iSD	iSD
Target lesions: iUPD with no change, or with a decrease from last timepoint; non-target lesions: iUPD with no change, or decrease from last timepoint; new lesions: yes	Not applicable	New lesions confirm iCPD if new lesions were previously identified and they have increased in size (≥5 mm in sum of measures for new lesion target or any increase for new lesion non-target) or number; if no change is seen in new lesions (size or number) from last timepoint, assignment remains iUPD
Target lesions: iSD, iPR, iCR; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in the size of non-target disease (does not need to meet RECIST 1.1 criteria for unequivocal progression)
Target lesions: iUPD; non-target lesions: non-iCR/non-iUPD, or iCR; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in sum of measures ≥5 mm; otherwise, assignment remains iUPD
Target lesions: iUPD; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed based on a further increase in previously identified target lesion iUPD in sum of measures ≥5 mm or non-target lesion iUPD (previous assessment need not have shown unequivocal progression)
Target lesions: iUPD; non-target lesions: iUPD; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in previously identified target lesion iUPD sum of measures ≥5 mm, previously identified non-target lesion iUPD (does not need to be unequivocal), or an increase in the size or number of new lesions previously identified
Target lesions: non-iUPD or progression; non-target lesions: non-iUPD or progression; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of an increase in the size or number of new lesions previously identified

Table 2: Assignement of timepoint response with iRECIST, adapted from (26)

16.5 EORTC QLQ-C15-PAL (version 1)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: _____

Your birthdate (Day, Month, Year): _____

Today's date (Day, Month, Year): _____

_____

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
2.	Do you need to stay in bed or a chair during the day?	1	2	3	4
3.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
4.	Were you short of breath?	1	2	3	4
5.	Have you had pain?	1	2	3	4
6.	Have you had trouble sleeping?	1	2	3	4
7.	Have you felt weak?	1	2	3	4
8.	Have you lacked appetite?	1	2	3	4
9.	Have you felt nauseated?	1	2	3	4
10.	Have you been constipated?	1	2	3	4
11.	Were you tired?	1	2	3	4
12.	Did pain interfere with your daily activities?	1	2	3	4
13.	Did you feel tense?	1	2	3	4
14.	Did you feel depressed?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

15. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

16.6 Chalder Fatigue Questionnaire (FQ) and pain score

SPØRRESKJEMA FOR ICON-STUDIEN

Fatigue

Vi vil gjerne vite om du har følt deg sliten, svak eller i mangel av overskudd den siste måneden. Vennligst besvar ALLE spørsmålene ved å krysse av (X) for det svaret du synes passer best for deg. Vi ønsker at du besvarer alle spørsmålene selv om du ikke har hatt slike problemer. Vi spør om hvordan du har følt deg i det siste og ikke om hvordan du følte deg for lenge siden. Hvis du har følt deg sliten lenge, ber vi om at du sammenlikner deg med hvordan du følte deg sist du var bra. (Ett kryss på hver linje)

1. Har du problemer med at du føler deg sliten?	☐ Mindre enn vanlig	☐ Ikke mer enn vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
2. Trenger du mer hvile?	☐ Nei, mindre enn vanlig	☐ Ikke mer enn vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
3 Føler du deg søvnig eller døsig?	☐ Mindre enn vanlig	☐ Ikke mer enn vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
4. Har du problemer med å komme igang med ting?	☐ Mindre enn vanlig	☐ Ikke mer enn vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
5. Mangler du overskudd?	☐ Ikke i det hele tatt	☐ Ikke mer enn vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
6. Har du redusert styrke i musklene dine?	☐ Ikke i det hele tatt	☐ Ikke mer enn vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
7. Føler du deg svak?	☐ Mindre enn vanlig	☐ Som vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
8. Har du vansker med å konsentrere deg?	☐ Mindre enn vanlig	☐ Som vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
9. Forsnakker du deg i samtaler?	☐ Mindre enn vanlig	☐ Ikke mer enn vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
10. Er det vanskeligere å finne det rette ordet?	☐ Mindre enn vanlig	☐ Ikke mer enn vanlig	☐ Mer enn vanlig	☐ Mye mer enn vanlig
11. Hvordan er hukommelsen din?	☐ Bedre enn vanlig	☐ Ikke verre enn vanlig	☐ Verre enn vanlig	☐ Mye verre enn vanlig

Smerter

Vennligst sett ring rundt det tallet som best angir hvor sterke smerter du har hatt i gjennomsnitt de siste 24 timene.

Ingen smerter 0 1 2 3 4 5 6 7 8 9 10 **Verst tenkelige smerter**

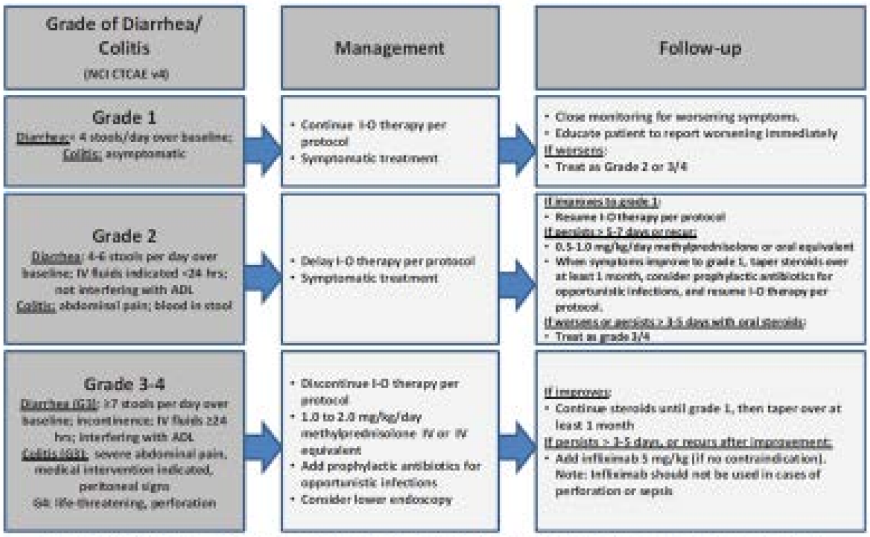
I hvor stor grad har behandling eller medisiner lindret smertene dine de siste 24 timene?
Vennligst sett en ring rundt det prosenttallet som viser hvor stor smertelindring du har fått.

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

Ingen lindring **Fullstendig lindring**

GI Adverse Event Management Algorithm

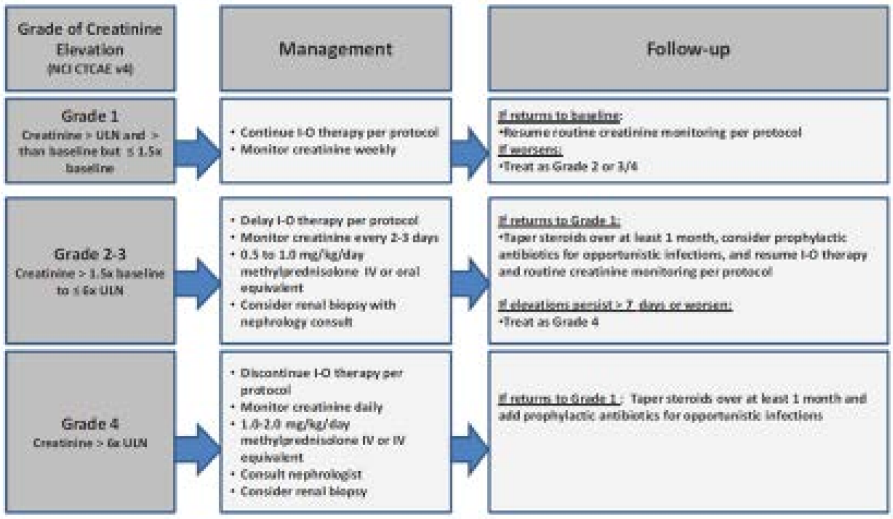
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

Grade of Pneumonitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and ID consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1.0 mg/kg/day methyl-prednisolone IV or oral equivalent • Consider bronchoscopy , lung biopsy	• Re-image every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics If not improving after 2 weeks or worsening: • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worsening hypoxia; Life-threatening	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider bronchoscopy, lung biopsy	If improves to baseline: • Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: • Add additional immunosuppression

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Hepatic Adverse Event Management Algorithm

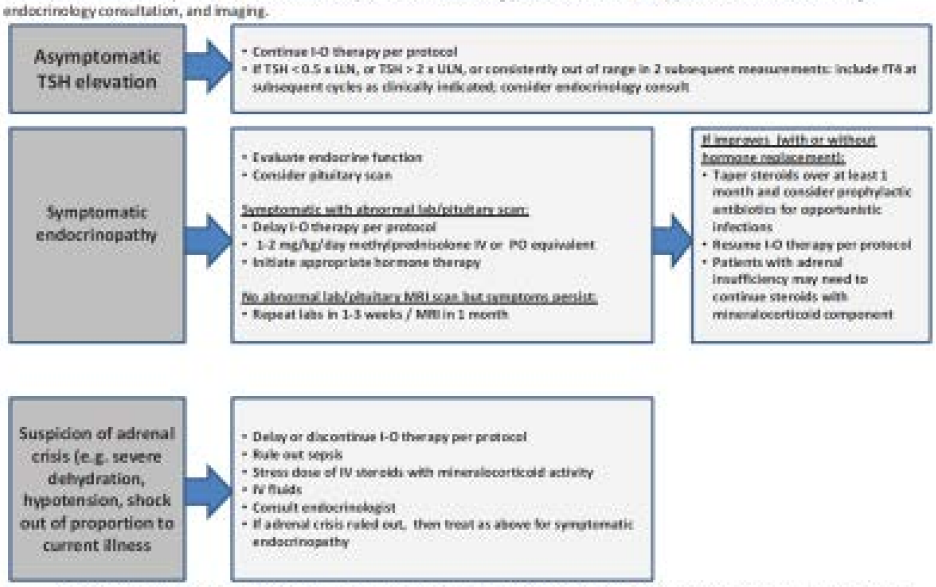
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

Grade of Liver Test Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 AST or ALT > ULN to 3.0 x ULN and/or T. bili > ULN to 1.5 x ULN	• Continue I-O therapy per protocol	• Continue LFT monitoring per protocol If worsens: • Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN	• Delay I-O therapy per protocol • Increase frequency of monitoring to every 3 days	If returns to baseline: •Resume routine monitoring, resume I-O therapy per protocol If elevations persist > 5-7 days or worsen : • 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
Grade 3-4 AST or ALT > 5 x ULN or T.bili >3 x ULN	• Discontinue I-O therapy* • Increase frequency of monitoring to every 1-2 days • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent** • Add prophylactic antibiotics for opportunistic infections • Consult gastroenterologist	If returns to grade 2: • Taper steroids over at least 1 month If does not improve in >3-5 days, worsens or rebounds: • Add mycophenolate mofetil 1 g BID • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN or T.bili ≤ 5 x ULN.
**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Endocrinopathy Management Algorithm

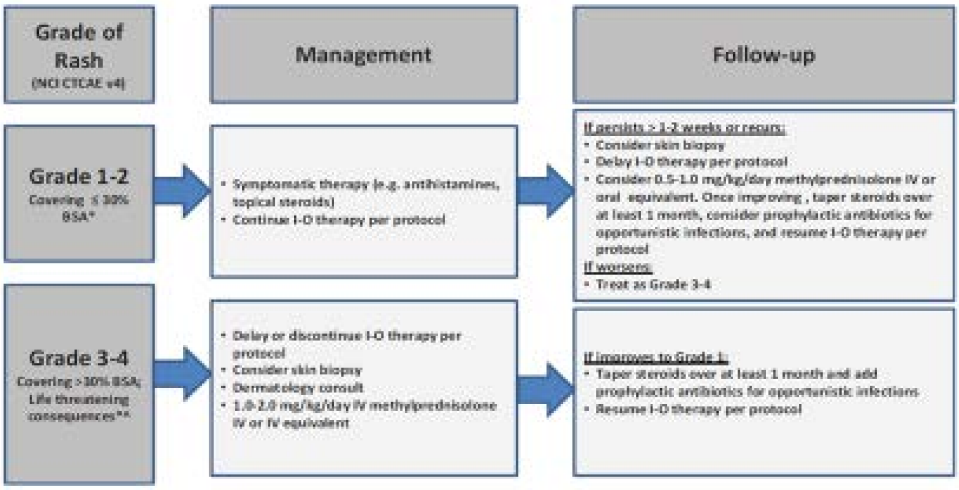
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

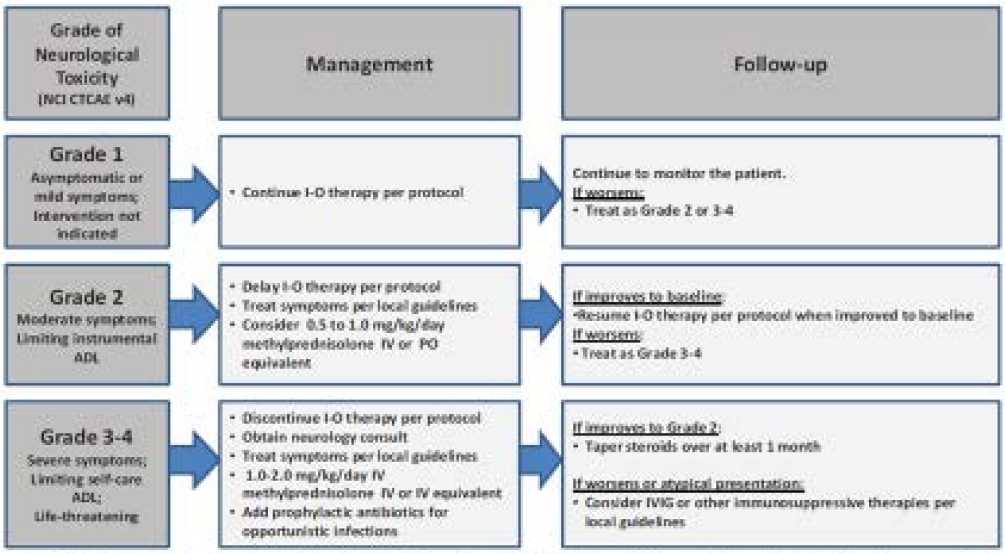
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.
^If SJS/TEN is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Statistical Analysis Plan

TRIAL FULL TITLE	ICON: a randomized phase IIb study evaluating immunogenic chemotherapy combined with ipilimumab and nivolumab in patients with metastatic hormone receptor positive breast cancer
SAP VERSION	v1.0
SAP VERSION DATE	05 APR 2022
TRIAL STATISTICIAN	Ragnhild Sørum Falk
Protocol Version (SAP associated with)	v4.3.1
TRIAL PRINCIPAL INVESTIGATOR	Jon Amund Kyte
SAP AUTHOR	Nikolai Kragøe Andresen

1 SAP Signatures

I give my approval for the attached SAP entitled "ICON: a randomized phase IIb study evaluating immunogenic chemotherapy combined with ipilimumab and nivolumab in patients with metastatic hormone receptor positive breast cancer" dated 05 APR 2022.

Statistician Reviewer

Name: Ragnhild S Falk

Signature: Ragnhild S Falk

Date: April 8, 2022

Principal Investigator

Name:

Signature: Jon A Kyte

Date: April 8, 2022

2 Table of Contents

3 Abbreviations and Definitions

AE	Adverse event
ANC	Absolute neutrophil count
BC	Breast cancer
CBR	Clinical benefit rate
CNS	Central nervous system
CR	Complete response by RECIST1.1
CTC	Circulating tumor cells
CTCAE	Common terminology criteria for adverse events
DR	Duration of response
DRR	Durable response rate
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
ER	Estrogen Receptor
EPO	Erythropoietin
FQ	Fatigue questionnaire
FAS	Full analysis set
HR	Hormone receptor
HR	Hazard ratio
HER2	Human epidermal growth factor receptor 2
IMP	Investigational medical product
LVEF	Left ventricular ejection fraction
LLN	Lower level normal
MCID	Minimally clinically important difference
MedDRA	Medical dictionary for regulatory activities
MUGA	Multi gated acquisition scan
NYHA	New York Heart Association
NRS	Numerical rating scale
ORR	Objective response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD-1	Programmed death-ligand 1
PFS	Progression free survival

PLD	Pegylated liposomal doxorubicin
PP	Per protocol
PR	Partial response
RECIST	Response evaluation criteria in solid tumors
iRECIST	Immunotherapy response evaluation criteria in solid tumors
SAP	Statistical analysis plan
SD	Stable disease
SOC	System organ class
TIS	Tumor inflammation signature
TTD	Time to deterioration
ULN	Upper Limit Normal

4 Introduction

4.1 Preface

Breast cancer (BC) is rarely curable after metastasis, and the therapeutic options are limited. Interestingly, the host immune response is strongly predictive for the effect of chemotherapy in subgroups of patients with breast cancer. In the present proposal, we aim at releasing the brake on the immune response by use of ipilimumab, which blocks CTLA-4 and may deplete regulatory T cells, combined with nivolumab (anti PD-1). Importantly, it is possible that non-responders to nivolumab/ipilimumab (nivo/ipi) can be turned responders by use of immunogenic chemotherapy. There is compelling evidence from animal studies, supported by data from humans, that some chemotherapeutic agents are immunogenic. Doxorubicin and cyclophosphamide have been shown to be particularly powerful inducers of immunogenic cell death. Both agents fulfil 5/5 criteria established for assessing the immunogenicity of different chemotherapeutic drugs (Table 1 in [2]). There is also strong evidence from humans, particularly in breast cancer, indicating that the clinical effect of doxorubicin and cyclophosphamide depends on the host immune response [3]. Further, these agents have been shown to induce a Type I interferon immune response in breast cancer [4, 5]. Taken together, there is a strong rationale for synergy between doxorubicin/cyclophosphamide and PD-1/CTLA-4 blockade [6].

In studies conducted at Oslo University Hospital and by a collaborator, we have observed that patients with hormone receptor positive breast cancer are highly diverse with regard to immune activation and up-regulation of inhibitory pathways, including PD1/PD-L1.

We will combine nivolumab and ipilimumab with established 1st choice chemotherapy in patients with metastatic hormone receptor (HR) positive breast cancer. We hypothesize that nivolumab/ipilimumab (nivo/ipi) may

i) potentiate the patient´s spontaneous anti-tumor immune response
ii) synergize with chemotherapeutic agents that induce immunological cell death

The prospect of clinical benefit from immunotherapy is probably best in patients that have not received multiple previous lines of chemotherapy, and we thus aim to bring the nivo/ipi combination into current early line regimes. Our chosen chemotherapeutic regime is a combination of antracyclin and cyclophosphamide, which is an acknowledged option. To facilitate rapid recruitment into the study, we suggest allowing for one previous line of chemotherapy, but with a requirement of good performance status (ECOG 0 or 1) and adequate organ function. Further, we suggest using the chemo drugs in a metronomic fashion (daily cyclophosphamide), rather than as high dose regimes administered every third week. We hypothesize that the metronomic regime will induce immunological cell death and counter T regulatory cells [7], while maintaining the leukocyte counts and the ability of the effector immune cells to respond. Indeed, a low-dose metronomic

cyclophosphamide regime has been used in several cancer vaccine studies, in order to counter regulatory T cells and myeloid suppressor cells . Finally, we will use liposomal doxorubicin (PLD; Caelyx), which minimizes the adverse effects of antracyclins on the heart and allows for continued treatment beyond the otherwise mandatory antracyclin limits. This is of particular importance for immunotherapy, where the aim is to induce long term disease remission. It is important to identify a chemo regime that can be continued for an extended period of time, in combination with nivo/ipi.

4.2 Scope of the analyses

These analyses will assess the safety and efficacy of combining nivolumab and ipilimumab in combination with immunogenic chemotherapy or alone in patients with metastatic hormone receptor (HR) positive/HER-2 negative breast cancer. These analyses will be presented in the final study report. Analyses of the main trial and the cross over part will be published as two separate reports.

5 Study Objectives and Endpoints

5.1 Study Objectives

Primary objectives
Assessment of toxicity of combined treatment with ipilimumab, nivolumab, pegylated liposomal doxorubicin and cyclophosphamide (ipi/nivo/chemo).

Assessment of progression-free survival (PFS) in ipi/nivo/chemo group compared to chemo only group

Secondary objectives
Assessment of clinical response in ipi/nivo/chemo group compared to chemo only group: Objective tumor response rate (ORR), duration of response (DR), durable tumor response rate (DRR; >6 months), clinical benefit rate (CBR), overall survival (OS).

Assessment of toxicity of ipi/nivo (without chemotherapy) in cross-over arm.

Assessment of ORR, DR, DRR, CBR, PFS and OS in cross-over arm receiving ipi/nivo (without chemotherapy).

Assessment of PD-L1 expression, mutation load and immune gene expression as biomarkers for clinical response

Comparison of clinical and biological response in molecular subtypes of breast cancer

Assessment of patient reported outcomes, as measured by the Chalder Fatigue Questionnaire (FQ), an 11 point Numerical Rating Scale (NRS) for pain intensity and EORTC QLQ-C15-PAL

5.2 Endpoints

Primary endpoints
The safety outcome measures will be evaluated in the Full Analysis Set (FAS) population, as follows:

i) Incidence, nature, and severity of adverse events graded according to NCI CTCAE v4.0
ii) Changes in vital signs, physical findings, and clinical laboratory results

The primary efficacy outcome measure is to be assessed in patients evaluable per protocol (PP), as follows:

i) PFS, defined as the time from randomization to the time of radiographic progression (as assessed by RECIST v1.1) or death from any cause during the study.

Secondary efficacy endpoints
The secondary efficacy outcome measures will be assessed in the *PP population, FAS population and in the PD-L1-positive subpopulation* as follows:

Overall survival (OS), defined as the time from the date of randomization to the date of death from any cause.

Objective tumor response rate (ORR), defined as the proportion of patients with an objective tumor response (either partial response (PR) or complete response (CR) using RECIST v1.1).

Durable response rate (DRR), defined as the proportion of patients with an objective tumor response lasting at least 6 months, according to RECIST v1.1.

Clinical benefit rate (CBR), defined as the proportion of patients with an objective tumor response or with stable disease lasting at least 6 months.

Duration of objective response (DOR) among patients with an objective response, according to RECIST v1.1.

PFS in the FAS population and PD-L1-positive subpopulation assessed by RECIST v1.1

PFS, ORR, DRR, CBR and DOR assessed by iRECIST.

PFS, ORR, DRR, CBR and DOR for patients after cross-over, assessed by RECIST v1.1.

PFS, ORR, DRR, CBR and DOR for patients after cross-over, assessed by iRECIST.

OS for patients in the cross-over part, defined as the time from cross over cycle 1 day 1 to death from any cause.

Other Secondary/Exploratory Outcome Measures
Assessment of immunological response

Identification of biomarkers for clinical response, toxicity and immune response, including assessment of PD-L1 expression, mutation load and immune gene expression

Characterization of tumor evolution and changes in immunological milieu induced by the immune/chemo combination therapy, as compared to chemo only, and by ipi/nivo (without chemo) in the cross-over arm

Development in FQ score. The analyses will include time to deterioration (TTD) in the FQ score, defined by a minimally clinically important difference (MCID) of ≥ 3 points. The maximum total FQ

score is 33 points. For mean score, a separate analysis will be performed for subjects with a baseline FQ score ≥ 21 points

Development in NRS pain intensity score. The analyses will include TTD in the pain intensity score, defined by a minimally clinically important difference (MCID) of ≥ 2 points (scale 0-10). For mean score, a separate analysis will be performed for subjects with a baseline score for ≥ 4 points.

Mean changes and TTD in in item 15 (quality of life score) of the EORTC QLQ-C15-PAL, defined by a MCID ≥ 20 points at patient individual level. A change of ≥10 points is considered to be of clinical importance at group level. The development of other scales and items of QLQ-C15-PAL will also be recorded.

6 Study Methods

6.1 General Study Design and Plan

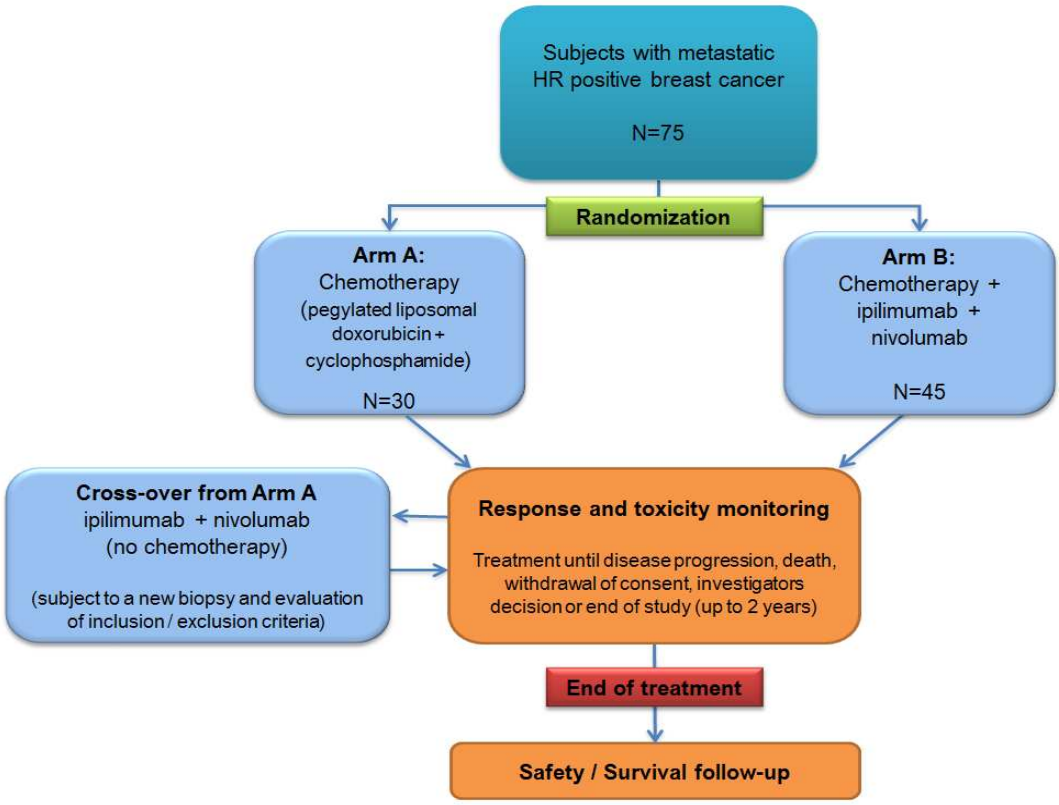
This is an open label randomized exploratory phase IIb study evaluating the safety and efficacy of combining nivolumab and ipilimumab with immunogenic chemotherapy in subjects with metastatic HR positive breast cancer. The Investigational Medicinal Products (IMPs) are nivolumab, ipilimumab, pegylated liposomal doxorubicin (PLD) and cyclophosphamide.

The trial will randomize 75 patients in to two arms (with randomization 2:3 in favour of arm B):

- Arm A: Chemotherapy only (pegylated liposomal doxorubicin + cyclophosphamide)
- Arm B: Chemotherapy + ipilimumab and nivolumab

Cross-over from arm A is allowed. The patients in arm A are offered ipilimumab + nivolumab (without chemotherapy) after disease progression, or treatment discontinuation due to toxicity, if considered not in need of immediate chemotherapy. Patients with aggressive and widespread disease, and acceptable tolerability for chemotherapy, should be recommended chemotherapy rather than cross-over to ipi/nivo therapy. Patients in arm A that have left the ICON study due to disease progression or treatment discontinuation due to toxicity, may be re-admitted to the cross over arm if they have received a maximum of one more line of chemotherapy after leaving the ICON study.

Study design:



6.2 Inclusion-Exclusion Criteria and General Study Population

(ICH E3;9.3. ICH E9;2.2.1)

7.2.1 Inclusion criteria

1. Metastatic hormone receptor positive breast cancer (primary or recurrent), defined as estrogen receptor (ER) positive >1% in metastatic biopsy (archival material or study biopsy) or cytology and HER2 negative in the last biopsy or cytology evaluable for HER2. HER2-analysis is to be perfomed according to national criteria.
2. Adequate core or excisional study biopsy of a tumor lesion. Lesions in previously irradiated areas may only be used for the biopsy if the lesion has appeared or progressed after radiation. No anti-tumor treatment is allowed between the time point for biopsy and study entry.
3. Measurable metastatic disease according to RECIST
4. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
5. Signed Informed Consent Form
6. Women or men aged ≥ 18 years
7. A minimum of 12 months from adjuvant/neoadjuvant chemotherapy with antracyclins to relapse of disease.
8. A maximum of one previous line with chemotherapy in the metastatic setting
9. Chemotherapy is considered as preferred treatment
10. Previous endocrine and targeted therapy is allowed
11. No use of systemic corticosteroids at study entry

12. Female subject of childbearing potential should have a negative urine or serum pregnancy within 7 days prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required
13. Female subjects of childbearing potential should agree to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of < 1% per year, during the treatment period and for at least 5 months after the last dose of study therapy.
14. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 7 months after the last dose of study therapy
15. Able to swallow and retain orally administered medication
16. Adequate organ function as defined in Table 1

Table 1 Adequate Organ Function Laboratory Values

System	**Laboratory Value**
Hematological	
Absolute neutrophil count (ANC)	≥1.00 x10^9/L
Lymphocyte count	≥0.50 x10^9/L
Platelets	≥75,000 / mcL
Hemoglobin	≥8 g/dL without transfusion or EPO dependency (within 10 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥40 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** **≤** 5 X ULN for subjects with liver metastases
Albumin	≥25 g/L
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

7.2.2 Exclusion criteria

The subject must be excluded from participating in the trial if the subject has/is:

1. Malignancies other than breast cancer within 5 years prior to randomization, with the exception of those with a negligible risk of metastasis or death and treated with expected

curative outcome (such as adequately treated carcinoma in situ of the cervix or basal or squamous cell skin cancer)

2. Spinal cord compression not definitively treated with surgery and/or radiation, or previously diagnosed and treated spinal cord compression without evidence that disease has been clinically stable for > 2 weeks prior to randomization
3. Known CNS disease, except for asymptomatic CNS metastases, provided all of the following criteria are met:
    a. Measurable disease outside the CNS
    b. Asymptomatic for CNS disease > 4 weeks
    c. No ongoing requirement for corticosteroids as therapy for CNS disease
    d. No radiation of brain lesions within 2 weeks prior to randomization
    e. No leptomeningeal disease
4. Uncontrolled pleural effusion, pericardial effusion, or ascites. Patients with indwelling catheters (e.g., PleurX®) are allowed
5. Uncontrolled tumor-related pain. Patients requiring narcotic pain medication must be on a stable regimen at study entry. Symptomatic lesions (e.g., bone metastases or metastases causing nerve impingement) amenable to palliative radiotherapy should be treated prior to randomization. Asymptomatic metastatic lesions whose further growth would likely cause functional deficits or intractable pain (e.g., epidural metastasis that is not presently associated with spinal cord compression) should be considered for loco-regional therapy if appropriate prior to randomization
6. Ionized calcium > 1.2 x UNL. The use of bisphosphonates is allowed
7. Pregnant or breastfeeding
8. Evidence of significant uncontrolled concomitant disease that could affect compliance with the protocol or interpretation of results, including significant liver disease (such as cirrhosis, uncontrolled major seizure disorder, or superior vena cava syndrome)
9. Significant cardiovascular disease, such as New York Heart Association (NYHA) cardiac disease (Class II or greater), myocardial infarction within 3 months prior to randomization, unstable arrhythmias, or unstable angina Patients with a known left ventricular ejection fraction (LVEF) < 40% will be excluded. Patients with known coronary artery disease, congestive heart failure not meeting the above criteria, or LVEF < 50% must be on a stable medical regimen that is optimized in the opinion of the treating physician, in consultation with a cardiologist if appropriate
10. Severe infection within 21 days prior to randomization, requiring hospitalization
11. Received oral or IV antibiotics within 1 week prior to Cycle 1, Day 1. Patients receiving routine antibiotic prophylaxis (e.g., to prevent chronic obstructive pulmonary disease exacerbation or for dental extraction) are eligible
12. Major surgical procedure within 21 days prior to randomization or anticipation of the need for a major surgical procedure during the course of the study other than for diagnosis. Placement of central venous access catheter(s) is not considered a major surgical procedure and is therefore permitted
13. A history of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins
14. Known hypersensitivity to any of the components of the investigational products
15. A history of autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxin, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment. Patients with eczema, psoriasis, lichen simplex chronicus or vitiligo with

dermatologic manifestations only (e.g., no psoriatic arthritis) are permitted provided that they meet all of the following conditions:

a. Rash must cover less than 10% of body surface area.
b. Disease is well controlled at baseline and only requiring low potency topical steroids
c. No acute exacerbations of underlying condition within the last 12 months (not requiring PUVA [psoralen plus ultraviolet A radiation], methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, high potency or oral steroids)

16. Undergone allogeneic stem cell or solid organ transplantation
17. A history of idiopathic pulmonary fibrosis or idiopathic pneumonitis, or evidence of active pneumonitis on screening chest CT scan. History of radiation pneumonitis in the radiation field (fibrosis) is permitted
18. A positive test for HIV
19. Active hepatitis B (defined as having a positive hepatitis B surface antigen [HBsAg] test at screening) or hepatitis C. Patients with past hepatitis B virus (HBV) infection or resolved HBV infection (defined as having a negative HbsAg test and a positive antibody to hepatitis B core antigen [anti-HBc] antibody test) are eligible. Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction (PCR) is negative for HCV RNA
20. Active tuberculosis
21. Currently receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment
22. Received treatment with immune checkpoint modulators, including anti−CTLA-4, anti−PD-1, or anti−PD-L1 therapeutic antibodies
23. Received treatment with systemic immunostimulatory agents (including but not limited to interferons or IL-2) within 4 weeks or five half-lives of the drug (whichever is shorter) prior to randomization
24. Received treatment with systemic corticosteroids or other systemic immunosuppressive medications (including but not limited to prednisone, dexamethasone, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti−tumor necrosis factor [TNF] agents) within 2 weeks prior to randomization, or anticipated requirement for systemic immunosuppressive medications during the trial
    a. Patients who have received acute, low-dose, systemic immunosuppressant medications (e.g., a one-time dose of dexamethasone for nausea) may be enrolled in the study
    b. Patients with a history of allergic reaction to IV contrast requiring steroid pre-treatment should have baseline and subsequent tumor assessments performed using MRI
    c. The use of inhaled corticosteroids for chronic obstructive pulmonary disease, mineralocorticoids (e.g., fludrocortisone) for patients with orthostatic hypotension, and low-dose supplemental corticosteroids for adrenocortical insufficiency are allowed
25. Received anti-cancer therapy (medical agents or radiation) within 2 weeks prior to study Cycle 1, Day 1. Palliative radiotherapy for bone lesions is allowed up to 7 days before start of therapy.
26. A history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating Investigator
27. Known psychiatric or substance abuse disorders that would interfere with cooperation and the requirements of the trial

28. Received a live vaccine within 30 days of planned start of study therapy, or is expected to receive such a vaccine while on therapy
    a. *Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.*
29. Any reason why, in the opinion of the investigator, the patient should not participate

7.2.3 Cross-over criteria

Inclusion criteria 11-16 must be met at the time of cross-over. ECOG status of ≤2 is required. Maximum one line of chemotherapy after discontinuation of treatment in arm A. New tests for HIV/HCV/HBV, INR, aPTT and lipase are not required. Patients with anticipated requirement for systemic immunosuppressive medications during the trial are not eligible, with the exemptions listed under "exclusion criteria". If the patient has CNS disease, the following criteria must be met:

- No ongoing requirement for corticosteroids as therapy for CNS disease
- No radiation of brain lesions within 7 days prior to start of ipi/nivo therapy

6.3 Randomization and Blinding

Randomization is automated by the trials eCRF system (Viedoc). Patients are randomized in a ratio of 2:3 in favor of arm B. Randomization was stratified on tumor PD-L1 status (positive/negative) until protocol 4.0 was implemented January 2019, after this time point randomization was unstratified.

6.4 Study Assessments

Trial Period:	**Screening Phase**		**Treatment Phase**					**Post-Treatment**		
Treatment Cycle (C)/Title: (Each cycle is 2 weeks)	Informed consent (Visit 1)	Main Study Screening (Visit 2)	To be repeated (see footnotes for details) C1	 C2	 C3	 C4	Treatment discontinuation	Safety Follow-up	Patients not progressed during treatment Follow Up Visits	Patients progressed during treatment Follow Up Visit
Scheduling Window (Days)[a]:		-21 to -1	± 3	± 3	± 3	± 3	At time of discon	30 days[q] post discon ± 7 days	Every 12 weeks ± 7 days post discon for 12 months, or until disease progression	16 weeks ± 10 days post discon
Administrative Procedures										
Informed Consent[b]	x									
Inclusion/Exclusion Criteria		x								
Demographics and Medical History		x								
Prior and Concomitant Medication Review[c]		x	x	x	x	x	x	x		
Trial Treatment Administration			x	x	x	x				
Post-study anticancer therapy status									x	x
Clinical Procedures/Assessments										
Review Adverse Events[d,e]		x	x	x	x	x	x	x[e]	x[e]	x[e]
Full Physical Examination		x	x[f]					x		
Directed Physical Examination				x	x	x	x		x	x
Vital Signs and Weight		x	x	x	x	x	x	x	x	x
ECOG Performance Status		x	x	x	X	x	x	x	x	x
Electrocardiogram (ECG)		x	x[f]							
LVEF assessment		x[f]								
Laboratory Procedures										
Pregnancy Test – Urine or Ser–m-HCG[g]		x	x[g]		x		x	x		
INR and aPTT[h]		x								
CBC with Differential[i]		x	x	x	x	x	x	x	x	x
Comprehensive Serum Chemistry Panel[i]		x	x	x	x	x	x	x	x	x
Urine analysis[j]		x	x[f]				x	x		
FT4 and TSH, anti TPO[j]		x	x[f]				x	x	x	x
MUC-1, CA 125, CEA, amylase		x	x[f]		x		x		x	x
HIV/HCV/HBV-tests, lipase		x								
Efficacy Measurements										
Tumor Imaging[i, l]		x	x[f]				x		x	
Biobanking										
Tumor Biopsy[m]		x			x		x			
PBMC collection[n]		(x)[n]	x[n]	x			x		x[k]	x

Plasma and serum sample[o]			x[o]	x[o]	x[o]	x[o]	x		x[k]	x
Urine sample		x	x[f]				x			
Faeces sample		x	x[f]							
CTC collection (only if sufficient resources)			x[f]		x		x			
Patient Reported Outcomes										
FQ, NRS, EORTC QLQ-C15-PAL[p]			x				x		x	x

a. General, assessments/procedures are to be performed on Day 1 and prior to the first dose of treatment for each cycle unless otherwise specified. Treatment cycles are 2 weeks; however the treatment cycle interval may be increased due to toxicity according to the dose modification guidelines provided in section 10.2.

b. Written consent must be obtained prior to performing any protocol specified procedure. Results of a test performed prior to the subject signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame in the protocol. Subject number will be assigned when the study informed consent is signed.

c. Prior medications – Record all medications taken within 30 days of screening visit. Concomitant medications – Enter new medications started during the trial through the Safety Follow-up visit. Record all medications taken for AEs.

d. AEs and laboratory safety measurements will be graded per NCI CTCAE version 4.0. All AEs, whether gradable by CTCAE or not, will also be evaluated for seriousness.

e. After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. After initiation of study drug, all adverse events will be reported until 30 days after the last dose of study treatment or until initiation of another anti-cancer therapy, whichever occurs first. After this period, investigators should report any serious adverse events and adverse events of special interest that are believed to be related to prior treatment with study drug. The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all serious adverse events considered to be related to study drug or trial-related procedures until a final outcome can be reported.

f. The full clinical examination is to be replaced by a directed clinical examination at C1 day 1. FT4, TSH, anti-TPO and urin analyses are not to be performed at C1 day 1, but at screening and at day 1 of C5 and every 4th cycle thereafter. MUC-1, CA 125, CEA and amylase analyses are not to be performed at C1 day 1, but at screening and at day 1 of C3 and ever[y] 2nd cycle thereafter.Tumor assessment is not to be performed at C1 day 1, but at screening, and as indicated in footnote i and l. For cross-over patients the tumor assessment at the discontinuation visit will serve as screening assessment. Urine sampling for the biobank is only to be performed at screening, day 1 of C5 and at time of progression. CTC sampling is scheduled at day 1 of C1 (or screening), day 1 of C3 and ToP, with option for additional time points. ECG is to be performed at screening (not C1), C5 day 1 and every 4th cycle thereafter. Left ventricular ejection fraction is to be measured at screening, cyclus 9 and every 8th week thereafter, if administration of pegylated liposomal doxorubicin is continued. Faeces sampling is only to be performed at screening and at C5. LVEF may be measured by Multi Gated Acquisition Scan (MUGA) or echocardiography.

g. Women of childbearing potential must have a negative serum pregnancy test result ≤7 days prior to the first dose of nivolumab. A serum or urine pregnancy test (investigator's discretion) must be performed ≤3 days prior to Day 1 of every 2nd cycle thereafter during the treatment phase. A serum pregnancy test must be performed at the End of Treatment visit.

h. Coagulation factors (PT/INR and aPTT) should be tested as part of the screening procedures for all subjects. Any subject receiving anticoagulant therapy should have coagulation factors monitored closely throughout the trial.

i. Tumor assessments performed as standard of care prior to obtaining informed consent and within 21 days of Cycle 1, Day 1 may be used rather than repeating tests. All measurable and evaluable lesions should be assessed and documented at the screening visit. Radiologic imaging performed during the screening period should consist of 1) CT of the chest/abdomen/pelvis, alternatively MRI 2) bone scan (MRI, PET scan or scintigraphy), and 3) any other imaging studies (CT neck, plain films, etc.) as clinically indicated by the treating physician. No anti-tumor treatment is allowed between the time point for baseline radiological scans and start of study therapy. The same radiographic procedures and technique must be used throughout the study for each patient (e.g., if the patient had CT chest/abdomen/pelvis performed during screening, then she/he should subsequently undergo CT performed using the same radiologic protocol throughout the remainder of the study). Tumor assessments will be performed at baseline, every 8 weeks from C1 day1 (± 1 week) for the first 12 months following randomization, and every 12 weeks (± 10 days) thereafter, with additional scans as clinically indicated. A CT of the chest/abdomen/pelvis must be performed at every scheduled evaluation in all patients. In patients without bone lesions at screening, a bone scan must as a minimum be performed at every second scheduled evaluation. If iUPD is detected, a new radiological scan should be performed after 4-8 weeks, in accordance with iRECIST. Tumor response will be evaluated using both iRECIST criteria and RECIST v1.1. In the absence of disease progression per iRECIST,

tumor assessments should continue regardless of whether patients discontinue study treatment, unless they withdraw consent or the study is terminated by the Sponsor, whichever occurs first.

j. Unresolved abnormal labs that are drug related AEs should be followed until resolution. Labs do not need to be repeated after the end of treatment if labs are within normal range.

k. For patients not progressed during treatment, plasma/serum and PBMC collection to be performed at first FU visit only (12 weeks after discontinuation). The Sponsor may ask for additional blood /PBMCs in selected cases.

l. In subjects who discontinue study therapy without confirmed disease progression, a radiologic evaluation should be performed at the time of treatment discontinuation (i.e., date of discontinuation ± 4 weeks). If a previous scan was obtained within 4 weeks prior to the date of discontinuation, then a scan at treatment discontinuation is not mandatory. Radiological assessments performed as standard of care can replace the tumor scans at follow-up visits, if performed ± 4 weeks of the scheduled time point.

m. Fresh frozen and FFPE tumor biopsies before start of treatment (mandatory), 4 weeks from C1 day 1 (± 5 days), 6 months from C1 day 1 (± 10 days) and at time of treatment discontinuation (fine needle aspiration is not sufficient). The prestudy biopsy may be obtained any time after signed informed consent. Archival tumor tissue can be used instead of pretreatment biopsy, but must be obtained within three months of Cycle 1, Day 1. No anti-tumor treatment is allowed between the time point for biopsy and study entry. If the archival biopsy does not include fresh frozen tumor, a new pre-treatment biopsy for preservation as fresh-frozen material is mandatory.

n. PBMC; at day 1 of C1 (or screening) and day 1 of C2, C5, C9, C13, C25, time of progression and the FU visit 12/16 weeks after discontinuation. 100 ml ACD blood to be drawn at C1day1/screening. 70 ml ACD blood to be drawn at all later time points. Samples for PBMC collection should always be taken before infusion.

o. Plasma and serum to be collected at day 1 and day 2 of C1 and C5, and at the day of nivolumab injection (day 1) of C2, C3, C4, C6, C9, C13, C25,time of progression and the FU visit 12/16 weeks after discontinuation.

p. PRO forms to be completed at day 1 (+/- 7 days) of C1, C5, C9, C13, C25, C39 and at time of progression and the FU visit 12/16 weeks after discontinuation. The forms should be completed prior to the evaluation visit with the study doctor. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. The safety visit may be combined with the EOT-visit, if new anti-cancer therapy is started within 10 days of EOT. If a patient is not able to come to the study center for the safety visit or later follow-up visits, every effort should be made to conduct these visits by phone, combined with information from and tests at the local hospital.

7 Sample Size

The phase II study cannot be powered to demonstrate a statistically significant ($p<0.05$) clinical effect. If the study suggests acceptable toxicity and potential clinical benefit, a larger randomized study will be warranted. We plan to conduct a phase II study with 75 patients (45 patients in the nivo-chemo arm (arm B), 30 patients in the chemo-only arm (arm A)).

The number of 75 patients and the randomization ratio of 3:2 were based on the following considerations:

- A two sided hypothesis test with a 10% significance level
- Power of 80%
- Randomization 2:3 in favor of the experimental arm (arm B).
- Expected PFS in the control group (arm A) after 20 months 5%
- Hazard ratio between the treatment groups: 0.54, this corresponds to an expected PFS in the experimental arm of 20% after 20 months.

8 General Analysis Considerations

8.1 Timing of Analyses

The PFS-analysis will be performed when 70 PFS events have occurred in the PP population. If this time point is not met within 24 months after inclusion of the last patient, the PFS-analysis will be performed at this time point. The database locking process prior to analysis will be performed according to our internal SOP “Research Support Services SOP - DM 06 Database Lock”.

8.2 Analysis Populations

Participant Analysis Set	Description
Full analysis set (FAS)	The FAS is defined as a modified Intention To Treat population (ITT): all patients that have started therapy with at least one of the IMPs, and where data on the relevant endpoint is obtained. Safety will be evaluated in the FAS population.
Per protocol (PP) population	All randomized participants in arm A and B that where evaluated for tumor response, received ≥2 doses of PLD and total of ≥700 mg cyclophosphamide and in addition for arm B ≥1 dose of ipilimumab and ≥2 doses of nivolumab. The primary efficacy analysis will be performed in the PP population.
PD-L1 positive FAS population	Patients in the FAS population with PD-L1 positive tumors. PD-L1 positive tumors are defined as tumors that are assessed as positive by immunohistochemistry using the manufacturers specified cut off. If multiple tumor samples are available, patients with any PD-L1 positive tumor specimen will be categorized as PD-L1 positive.
PD-L1 positive PP population	Patients in the PP population with PD-L1 positive tumors. PD-L1 positive tumors are defined as tumors that are assessed as positive by immunohistochemistry using the manufacturers specified cut off. If multiple tumor samples are available, patients with any PD-L1 positive tumor specimen will be categorized as PD-L1 positive.
Full analysis set cross over (FAS-CO)	The FAS-CO is defined as a modified Intention To Treat population (ITT): all patients that have started therapy in the cross over part of the trial with at least one of the IMPs, and where data on the relevant endpoint is obtained. Safety will be evaluated in the FAS-CO population.
Per protocol population cross over (PP-CO)	Cross over patients that where evaluated for tumor response at any time point after start of nivolumab and ipilimumab and received ≥1 dose of ipilimumab and ≥ 2 doses of nivolumab
PD-L1 positive FAS-CO population	Patients in the FAS-CO population with PD-L1 positive tumors. PD-L1 positive tumors are defined as tumors that are assessed as positive by immunohistochemistry using the manufacturers specified cut off. If multiple tumor samples are available, patients with any PD-L1 positive tumor specimen will be categorized as PD-L1 positive.
PD-L1 positive PP-CO	Patients in the PP population with PD-L1 positive tumors.

population	PD-L1 positive tumors are defined as tumors that are assessed as positive by immunohistochemistry using the manufacturers specified cut off. If multiple tumor samples are available, patients with any PD-L1 positive tumor specimen will be categorized as PD-L1 positive.

8.3 Covariates and Subgroups

Exploratory efficacy analyses in the PP and PP-CO population will be performed using the following pre-defined factors:

- Tumor PD-L1 status (positive vs negative).
- Disease free interval between end of (neo)adjuvant chemotherapy or surgery, whichever was last, and relapse (less than 5 years vs ≥ 5 years).
- Time from diagnosis of metastatic disease to start of therapy in the ICON-study (less than 2 years vs ≥ 2 years).
- Prior chemotherapy against metastatic disease (no previous chemo vs. previous chemo). Chemotherapy given in the neoadjuvant/adjuvant setting is not to be considered in this analysis
- *Sites of metastases:*
  - «liver» (yes/no)
  - «bone» (yes/no)
  - «lung» (yes/no)
  - «lymph nodes» (yes/no)
  - «Central nervous system» (yes/no)
  - Number of metastatic sites (0-3 vs >3)
- *Molecular breast cancer profiles:*
  - Intrinsic breast cancer subtype by PAM50 subtype (luminal A, luminal B, HER-2 enriched and basal like). For patients with more than one sample analysed and discordant subtypes the latest sample will be used for the subgroup analysis.
  - Immune gene profile (e.g. Tumor Inflammation Signature)
  - Other gene expression-derived subgroups (not determined at the time of writing)

Additional variables and cut offs may be included in the analyses.

8.3.1 Multi-center Studies

The study is conducted in five centers Oslo university hospital (NOR), Stavanger university hospital (NOR), Kristiansand hospital (NOR), Institute Jules Bordet (BE) and CHU UCL Namur (BE). Data from the different centers will be combined and no comparative analyses between centers will be performed.

8.4 Missing Data

(ICH E3; 9.7.1, 11.4.2.2. ICH E9;5.3. EMA Guideline on Missing Data in Confirmatory Clinical Trials)

Progression and survival data:
Missing progression dates:

- Progression free survival (PFS) is defined as the time from randomization to progression or death from any cause, whichever occurs first. Patients without disease progression or death will be censored at the date of last tumor assessment.
- If no tumor assessment was performed after randomization, data will be censored at randomization date +1 day.
- Data for patients with a PFS event who missed two or more assessments scheduled immediately prior to the date of the PFS event will be censored at the last tumor assessment prior to the missed visits.

Missing survival follow up data:

- Patients with no recorded date of death will be censored at the latest survival follow up or if lost to follow up, the last visit registered in the eCRF.

Missing data for sub-grouping variables (section 8.3):

- Patients with missing PD-L1 data, breast cancer subtype (PAM50) or immune profile will be handled as a separate group "unknown" in the sub group analysis and baseline characteristics.
- Patients with missing date (day or month) of last (neo-)adjuvant treatment, primary diagnosis, local relapse or time of metastatic disease will be handled the following way: If only the month is known the 15th day of the month will be used, if only the year is known the date 01 JUL will be used. If the year is missing the data will be handled as missing and the number of missing data will be reported.
- Patients with confirmed stage IV disease within 3 months of initial diagnosis will be grouped as "Stage IV" from initial diagnosis.

Missing patient related outcome assessments:

Chalder Fatigue questionnaire (FQ) score, NRS pain intensity score and EORTC QLQ-C15-PAL is assessed at the following time points baseline, C5, C9, C13, C25, C39 and EOT.

- EORTC QLQ-C15-PAL assessments with a single missing item in the pain scale (item 5 and 12) will be imputed by "assuming that the missing items have values equal to the average of those items which are present for that respondent" if at least one item is completed, as suggested by the scoring manual. Other missing items will be treated as missing.
- Patients that have a missing baseline assessment (other than above) will be removed from the time to deterioration (TTD) and mean change analysis.
- For patients that are missing a single form, but have complete forms on the assessment before and after, the least favorable assessment of the two will be imputed at that time point.
- Patients that missed two consecutive assessments will be censored from the time to deterioration analysis at the last assessment registered.

8.5 Interim Analyses and Data Monitoring (as applicable)

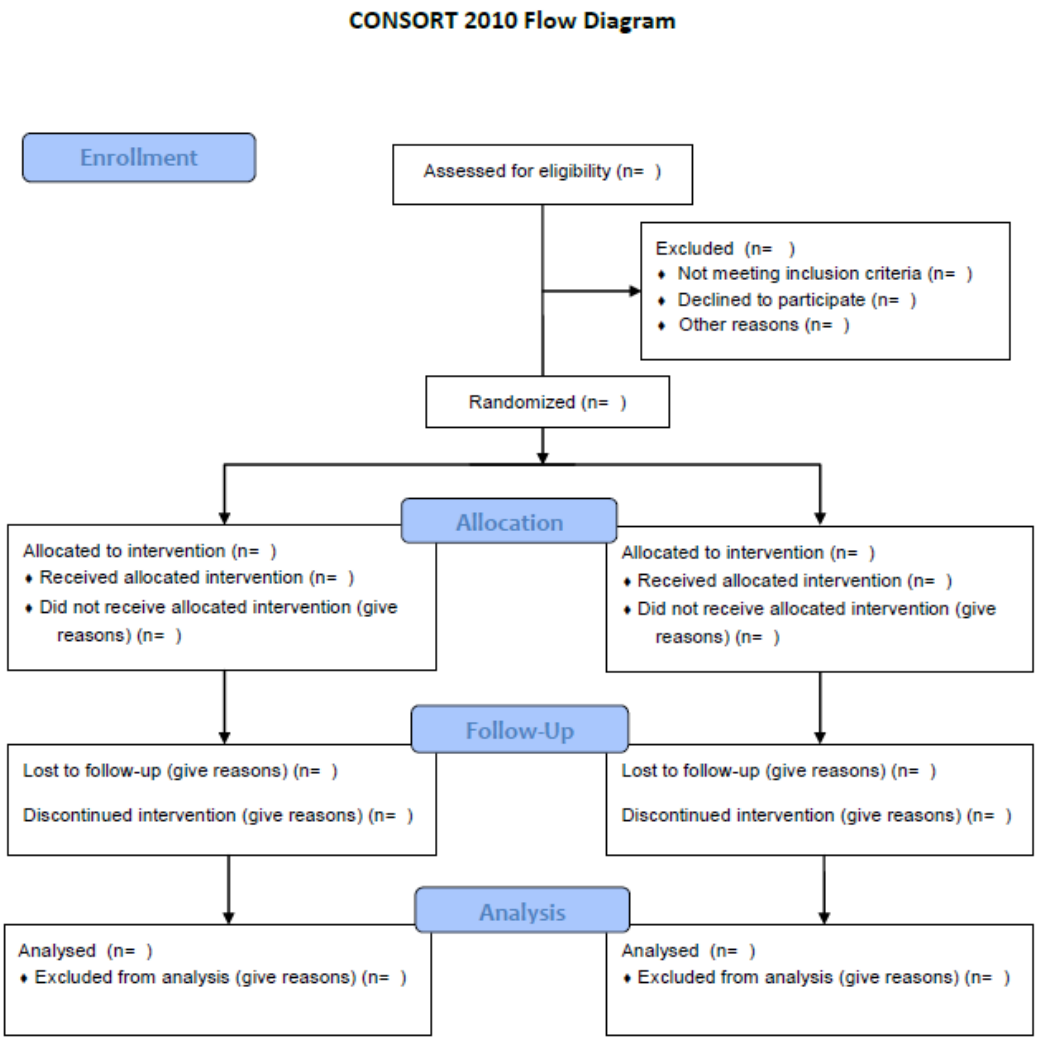
Not applicable

8.6 Multiple Testing

No formal hypothesis testing will be made in this data analysis, thus no adjustment for multiple testing will be performed.

9 Summary of Study Data

9.1 Subject Disposition



A consort diagram like above will be produced using the following variables from the eCRF:

- Subjects *assessed for eligibility* = patients that signed the ICF and started the screening process. These patients have all been given a trial ID in the eCRF and will be exported directly. Patients *assessed for eligibility* more than once, will be counted as a single subject.
- Patients *excluded* is the number of patients that were assessed for eligibility, but not randomized.

- The number of *randomized* subjects = patients that have completed the randomization module in the eCRF. All randomized patients have been *allocated to intervention*.
- Patients *lost to follow-up* = patients where survival data cannot be obtained.
- *Discontinued intervention* reason will be reported from "End of trial treatment" form in the eCRF.
- The number of patients *analyzed* = the number of patients analyzed in both the FAS and PP population.

9.2 Derived variables

Survival and response variables:

- Progression free survival: PFS = Date of progression or death from any cause (whichever comes first) - Randomization date
    - In the cross over part of the trial the start of the PFS interval is defined from cycle 1/day1:
      PFS = Date of progression or death from any cause (whichever comes first) – Date of Day 1/Cycle 1
    - Patients who discontinue follow up without progression will be censored at the date of the last registered radiological assessment.

- Overall survival: OS = Date of death – Randomization date
    - In the cross over part of the trial the start of the OS interval is defined as cycle 1/day 1:
      OS = Date of death – date of cycle 1/day 1
    - Patients alive at analysis will be censored at the date of the last registered survival follow up.

- Clinical benefit rate (CBR): the proportion of patients in the analyzed population with best overall response "PR" or "CR", or with stable disease (SD) lasting at least until the 6 month evaluation (performed at week 24 +/- 10 days). This means that patients where PD was first recorded at the 6 month evaluation will be considered to have clinical benefit.
  Duration of stable disease = Date of progression – Date of randomization
  Separate assessments will be done using both RECIST1.1 and iRECIST criteria.

- Duration of response (DOR): is defined as the interval from response was first documented (CR/iCR or PR/iPR) to either progression of disease or death from any cause, whichever comes first.
  DOR = Date of progression (or death) – Date response first documented
  Patients with an ongoing response at last follow up visit will be censored at this time point.

- Durable response rate (DRR): is the proportion of patients in the analyzed population with an objective response lasting at least 6 months.
  DRR = n with DOR ≥ 6 months/N

EORTC QLQ-C15-PAL scores:

- The EORTC QLQ-C15-PAL questionnaire score will be computed using the EORTC scoring manual.

9.3 Protocol Deviations

Protocol deviations that could affect the primary or secondary endpoints will be reported case by case.

9.4 Demographic and Baseline Variables

Baseline characteristics will be presented. A separate table for the single armed cross over part of the trial will be produced. For the cross over patients Cycle 1/day 1 will be defined as the baseline time point. Continuous variables will be presented with median and range, categorical variables presented as numbers with percentage in each group.

Potential variables to be described:

- Age, continous
- Sex
- Disease stage at initial diagnosis (Stage I-IV, patients with confirmed stage IV disease within 3 months of initial diagnosis will be grouped as “Stage IV”).
- ECOG score
- Metastatic sites: each organ grouped separately
- Number of metastatic sites: 0-3 vs >3
- Previous neoadjuvant or adjuvant chemotherapy, yes/no
- Previous adjuvant radiotherapy, yes/no
- Number of previous lines of chemotherapy in metastatic disease
- Type of prior metastatic chemotherapy
- Previous anthracyklin treatment in metastatic setting
- Prior CDK4/6 inhibitor therapy, yes/no
- The number of previous lines of endocrine therapy in metastatic setting presented as median with range and 0, 1, 2, ≥3.
- Ongoing bone resorption inhibitor treatment
- PD-L1 status: “positive”, “negative”, “unknown”
- Molecular subtype by PAM50
- Estrogen receptor expression 1-10% in metastasis, yes/no
- Time from initial diagnosis to randomization, continuous
- Time from diagnosis of advanced disease to randomization, continuous
- Time from last (neo)adjuvant treatment or surgery to randomization, continuous
- Time from last (neo)adjuvant antracycline/cyclophosphamide treatment or surgery to advanced diagnosis, continuous
- Study site

In addition for cross over patients:

- Objective response to treatment in Arm A, yes/no
- Clinical benefit to treatment in arm A, yes/no

Additional baseline variables may be presented.

9.5 Concurrent Illnesses and Medical Conditions

- Concurrent illnesses and medical conditions will be coded by MedDRA and listed by subject. This data will not be published.

- Previous (neo)adjuvant and metastatic breast cancer treatment, both will be summarized by treatment group and in the total patient population. The number and percentages of subjects that have received any previous treatment will be reported as well as the number and percentage of subjects that have received each drug class.

9.6 Treatment Compliance

Compliance for all IMPs are registered in the eCRF by study cycle under the treatment administration form. Administration of pegylated liposomal doxorubicin, nivolumab and ipilimumab are documented in hospital records and the administrated dose is registered in the eCRF. The compliance assessment for cyclophosphamide tablets is based on pill counts and is registered in the treatment administration form.

9.7 Adverse events

All adverse events and serious adverse events will be recorded from initiation of trial treatment until 30 days after the last dose of study drug or until the initiation of another anticancer therapy, whichever occurs first. After this period, investigators should report serious adverse events and adverse events of special interest that are believed to be related to prior treatment with study drug. Patients are actively followed for treatment related serious adverse events or adverse events of special interest until week 16 after treatment discontinuation. In addition serious adverse events that are related to any protocol specific intervention that occur prior to initiation of therapy will also be recorded.

Adverse events (AE) are coded using the Medical Dictionary for Regulatory Activities (MedDRA) and graded for severity using NCI Common Terminology Criteria for Adverse Events CTCAE 4.0.

Drug related AEs are those that are categorized in the eCRF as *possibly*, *probable* or *definite* related to the IMP by the investigator. For serious adverse events, relation to study drug is based on the causality assessment provided by the medical monitor.

Adverse events leading to drug discontinuation are: AEs where action taken due to AE is “Drug permanently discontinued” (serious adverse events or events of special interest) or where reason for treatment discontinuation is registered as an adverse event (non-serious events).

Adverse events recorded in arm A that are ongoing at cross over C1/D1 will be recorded as baseline symptoms in the cross over part of the trial.

Adverse events occurring after start of treatment in the cross over part are summarized separately. These events will not be counted in the summary for patients in arm A of the main trial, unless the event is considered related to previous treatment in arm A. Events that are considered possibly related to both arm A treatment and the cross over treatment will be included the adverse event summary for both of the groups.

Adverse events of special interest for this study include the following conditions which may be suggestive of an autoimmune disorder:

- Immune related Pneumonitis
- Immune related Colitis
- Immune related adrenal insufficiency

- Immune related Hepatitis
- Immune related Hypothyroidism
- Immune related Hyperthyroidism
- Immune related Pancreatitis
- Immune related Diabetes Mellitus
- Immune related Nephritis
- Severe treatment related cutaneous reactions
- Immune related Hypophysitis

10 Efficacy Analyses

10.1 Primary Efficacy Analysis

Analysis of PFS in the combination arm, compared to the control group in the PP population.

- Kaplan Meier curves will be produced.
- Comparison between the treatment arms will be given by HR for progression or death with 95% confidence intervals using the Cox proportional hazards model.
- Present the proportion of patients with progression or death within 20 months after randomization

10.2 Secondary Efficacy Analyses

10.2.1 Secondary Analyses of Primary Efficacy Endpoint

Stratification on the factors listed under 8.3. will be performed.

- A Forest plot for selected variables will be produced.

A comparison of PFS assessed by RECIST v1.1 in the

- FAS population
- PD-L1-positive PP population
- PD-L1 positive FAS population

In addition, the following PFS analysis will be performed on all populations

- PFS analysis without censoring at missing scans
- PFS analysis using iRECIST criteria

Kaplan Meier curves will be produced. Comparison between the treatment arms will be given by HR for progression or death with 95% confidence intervals using the Cox proportional hazards model.

10.2.2 Analyses of Secondary Endpoints

Comparison of OS between the treatment groups will be performed in the

- PP population
- FAS population
- PD-L1 positive PP population
- PD-L1 positive FAS population

Kaplan Meier curves will be produced. Comparison between the treatment arms will be given by HR for death with 95% confidence intervals using the Cox proportional hazards model.

Comparison of ORR between the treatment groups as assessed by RECIST v1.1 will be performed in the

- PP population
- FAS population
- PD-L1 positive PP population
- PD-L1 positive FAS population

The absolute number of patients with an objective response and the proportion with confidence intervals in each arm will be reported. A summary table of best objective response by treatment arm assessed by RECIST v1.1 will be produced.

Comparison of DRR between the treatment groups as assessed by RECIST v1.1 will be performed in the

- PP population
- FAS population
- PD-L1 positive PP population
- PD-L1 positive FAS population

The absolute number of patients with a durable response and the proportion with confidence intervals in each arm will be reported.

Comparison of CBR between the treatment groups as assessed by RECIST v1.1 will be performed in the

- PP population
- FAS population
- PD-L1 positive PP population
- PD-L1 positive FAS population

The absolute number of patients with clinical benefit and the proportion with confidence intervals in each arm will be reported.

Comparison of DOR between the treatment groups as assessed by RECIST v1.1 will be performed in the

- PP population
- FAS population
- PD-L1 positive PP population
- PD-L1 positive FAS population

Median duration of response, range and confidence intervals will be presented.

Comparison of ORR, DRR, CBR and DOR assessed *by iRECIST* will be performed in the

- PP population
- FAS population
- PD-L1 positive PP population
- PD-L1 positive FAS population

Data will be presented as above. A summary table of best overall response by arm assessed by iRECIST will be provided.

For the *cross over part* of the trial the following endpoints will be assessed by both RECIST v1.1 and iRECIST for the PP-CO, FAS-CO, PD-L1 positive PP-CO and PD-L1 positive FAS-CO population:

- PFS
    - Kaplan Meier curve will be produced.
- OS
    - Kaplan Meier curve will be produced

- ORR, DRR and CBR
    - The absolute number of patients and the proportion with confidence interval will be reported.
    - A summary table of best overall response and clinical benefit assessed by both RECIST v1.1 and iRECIST will be produced
- DOR
    - Median duration of response and range with confidence interval will be presented.

10.3 Handling repeated randomizations

In the case of a subject being randomized to both treatment arms, the subject will be included in the safety analyses of both arms. Regarding the efficacy assessment, the patient will be included in the FAS in both arms, and in the PP population in each arm if the PP criteria is fulfilled. The subject will be censored in the efficacy analyses for the first arm, as follows: PFS will be censored at the date of last tumor assessment, or at randomization +1 day if no tumor assessment is performed. OS will be censored at the date of the second randomization.

A sensitivity analysis will be performed to evaluate the effect of repeated randomizations on the primary efficacy endpoint, which is PFS in the PP population. In this analysis, the subjects will be censored for PFS as in the primary analysis above, but excluded from the PP population after the second randomization. Kaplan Meier curves will be produced and a comparison between the treatment arms will be given by HR for progression or death with 95% confidence intervals using the Cox proportional hazards model.

10.4 Exploratory Efficacy Analyses

Hazard ratio (HR) for death in arm B versus arm A with 95% confidence intervals will be calculated for each of the subgroups defined in section 8.3 using the Cox proportional hazard model. Forest plot of selected variables will be produced.

Kaplan-Meier curves will be produced for PFS and OS in the subgroups defined by selected variables defined in section 8.3.

Additional exploratory analyses may be performed. The statistical analyses will be dependent on the factors investigated and will be defined separately.

11 Safety Analyses

11.1 Extent of Exposure

The following parameters will be summarized for each IMP in the FAS population by each treatment arm and the cross over group:

- Mean dose intensity for each IMP, as a percentage of the full dose as defined in the protocol.
- Mean cumulative dose for each IMP
- Median duration of study therapy, defined as the date of the last treatment with IMP minus randomization date, including range

11.2 Adverse Events

When producing summary tables of adverse events repeated adverse events will be counted only once at the preferred term level for each subject with the worst CTCAE grade registered. Safety will be evaluated in the FAS population. Events are presented by preferred term and system organ class (SOC).

Adverse events

- The number of subjects with at least one grade ≥ 3 adverse event, by treatment group
- The number of subjects with at least one serious adverse event, by treatment group
- Overall summary of *all adverse events* by worst CTCAE grade (grade 1-5, total)
- Overall summary of *all adverse events* by worst CTCAE grade (any grade, grade 3-4, grade 5)
- Overall summary of *all treatment* related adverse events by worst CTCAE grade (any grade, grade 3-4, grade 5)
- Summary of *all adverse events* occurring *with a frequency ≥5%* within any arm by worst CTCAE grade (any grade, grade 3-4, grade 5)
- Summary of *all treatment related adverse events* occurring *with a frequency ≥5%* within any arm by worst CTCAE grade (any grade, grade 3-4, grade 5)
- Overall summary of all *non-serious adverse events* occurring *with a frequency ≥5%* within any arm by worst CTCAE grade (any grade, grade 3-4, grade 5)
- Other cutoffs may be used for producing summary tables

Adverse events of special interest

- Overall summary of all *adverse events of special interest* by worst CTCAE grade, by treatment group

Adverse events leading to discontinuation of study therapy

- Overall summary by worst CTCAE grade, by treatment group

Adverse events leading to dose modification of study therapy

- Overall summary by worst CTCAE grade, by treatment group

11.3 Deaths, Serious Adverse Events and other Significant Adverse Events

Deaths

All deaths will be summarized by treatment group. The following tables will be produced:

- All deaths, reason for death
- Deaths within 30 days of last dose of IMP, reason for death
- Deaths within 90 days of last dose of IMP, reason for death

Serious adverse events

- The number of subjects with at least one SAE, by treatment arm
- Overall summary of SAEs by worst CTCAE grade (grade 1-5, total)
- Overall summary of SAEs by worst CTCAE grade (any grade, grade 3-4, grade 5)
- Overall summary of treatment related SAEs by worst CTCAE grade (any grade, grade 3-4, grade 5)

11.4 Pregnancies (As applicable)

Pregnancy tests are performed regularly throughout the trial. Pregnancies occurring within the treatment phase or the 16 week follow up period will be reported.

11.5 Clinical Laboratory Evaluations

Specific normal limits for laboratory values on each site are registered in the CRF system. If laboratory values are to be presented, they will be presented in relation to the upper and lower limit of normal at each center. Only clinically significant laboratory abnormalities are recorded as adverse events.

11.6 Prior and Concurrent Medications (As applicable)

Prior and concurrent medications will be recorded in the CRF system and coded by ATC.

Summary of patients receiving immune modulating medication for management of immune mediated adverse events with oral or intravenous corticosteroids with a dose exceeding 20 mg prednisolone or equivalent for >1 week or other classes of immunosuppressant therapy (e.g., tumor necrosis factor alpha inhibitors, mycophenolate mofetil, tacrolimus or vedolizumab).

11.7 Other Safety Measures

NA

12 Pharmacokinetics (As Applicable)

NA

13 Other Analyses

13.1 Patient reported outcomes

Patient reported outcomes (FQ score, NRS pain intensity score and EORTC QLQ-C15-PAL) are assessed at C1, C5, C9, C13, C25, C39 and at the treatment discontinuation visit.

Time to deterioration analyses will be done using Kaplan-Meier methodology. Deterioration is defined as the time point where the change in score from baseline is equal to or greater than the MCID defined in chapter 6.2. Kaplan-Meier curves with deterioration-free survival will be produced. Comparison between the treatment arms will be given by HR for deterioration with 95% confidence intervals using the Cox proportional hazards model.

Mean score will be calculated at each time point and plotted with 95% confidence intervals for each treatment group. Separate FQ and NRS analyses will be performed for participants with a baseline

FQ score of ≥ 21 points or a baseline NRS score ≥ 4.

Change from baseline in the global health status of the EORTC QLQ-C15-PAL will be calculated at each assessment. A plot of mean change with 95% confidence intervals for each treatment group will presented.

Separate plots of patient related outcomes will be produced for the cross over part of the trial.

14 References

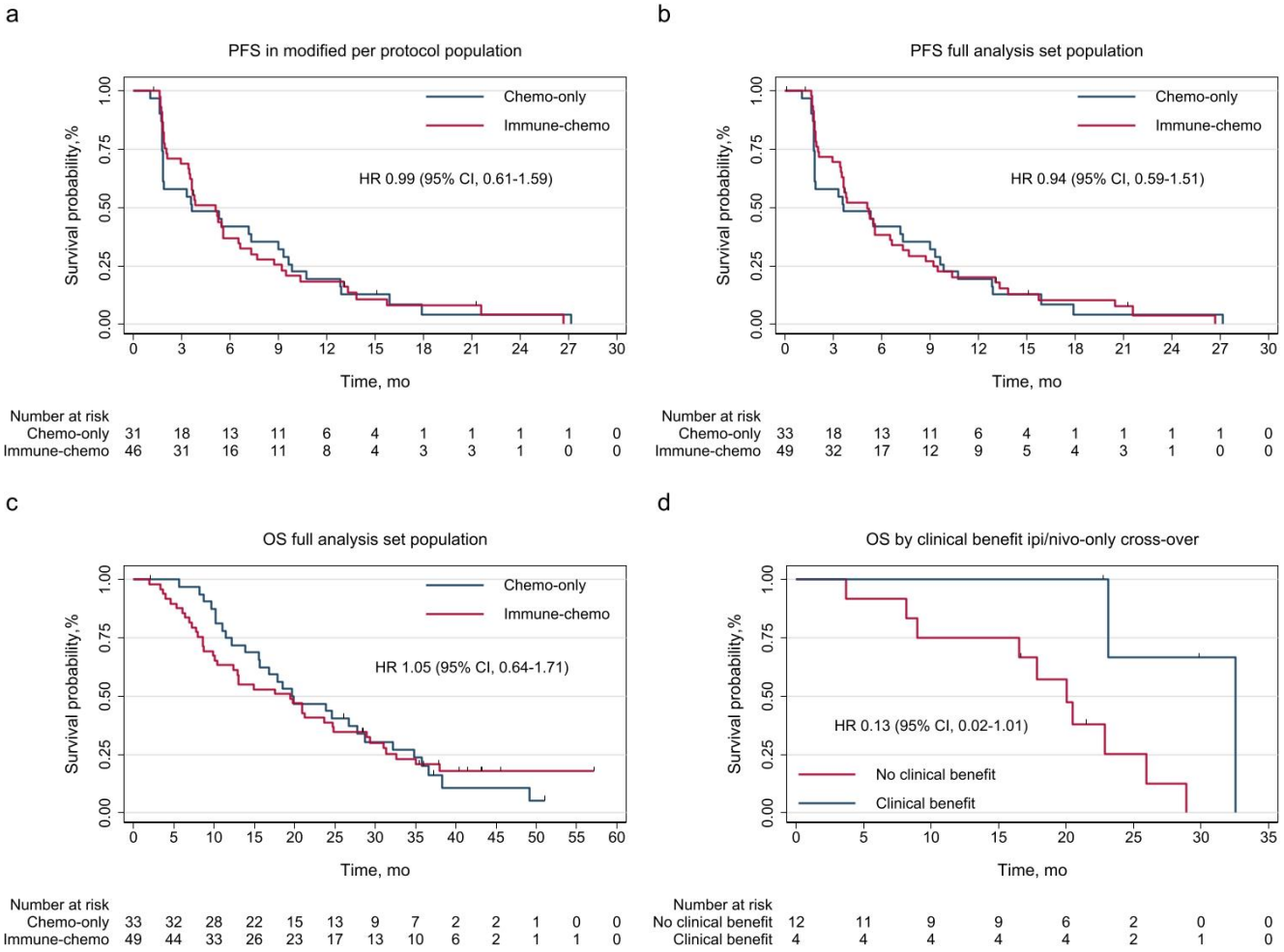
1. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, Congdon KL, Reap EA, Archer GE, Desjardins A *et al*: **Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients**. *Nature* 2015, **519**(7543):366-369.
2. Bezu L, Gomes-de-Silva LC, Dewitte H, Breckpot K, Fucikova J, Spisek R, Galluzzi L, Kepp O, Kroemer G: **Combinatorial strategies for the induction of immunogenic cell death**. *Frontiers in immunology* 2015, **6**:187.
3. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P *et al*: **Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy**. *Nat Med* 2007, **13**(9):1050-1059.
4. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, Vitale I, Goubar A, Baracco EE, Remedios C *et al*: **Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy**. *Nat Med* 2014, **20**(11):1301-1309.
5. Kroemer G, Senovilla L, Galluzzi L, Andre F, Zitvogel L: **Natural and therapy-induced immunosurveillance in breast cancer**. *Nat Med* 2015, **21**(10):1128-1138.
6. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, Yamazaki T, Poirier-Colame V, Newton A, Redouane Y *et al*: **Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy**. *Immunity* 2016, **44**(2):343-354.
7. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, Solary E, Le Cesne A, Zitvogel L, Chauffert B: **Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients**. *Cancer Immunol Immunother* 2007, **56**(5):641-648.

We would like to acknowledge the Cambridge University Hospitals Clinical Trials Unit for the development of this SAP template (version CCTU/TPLV2), which has been modified by the Michigan Institute for Clinical & Health Research (MICHR).

Supplemental Material

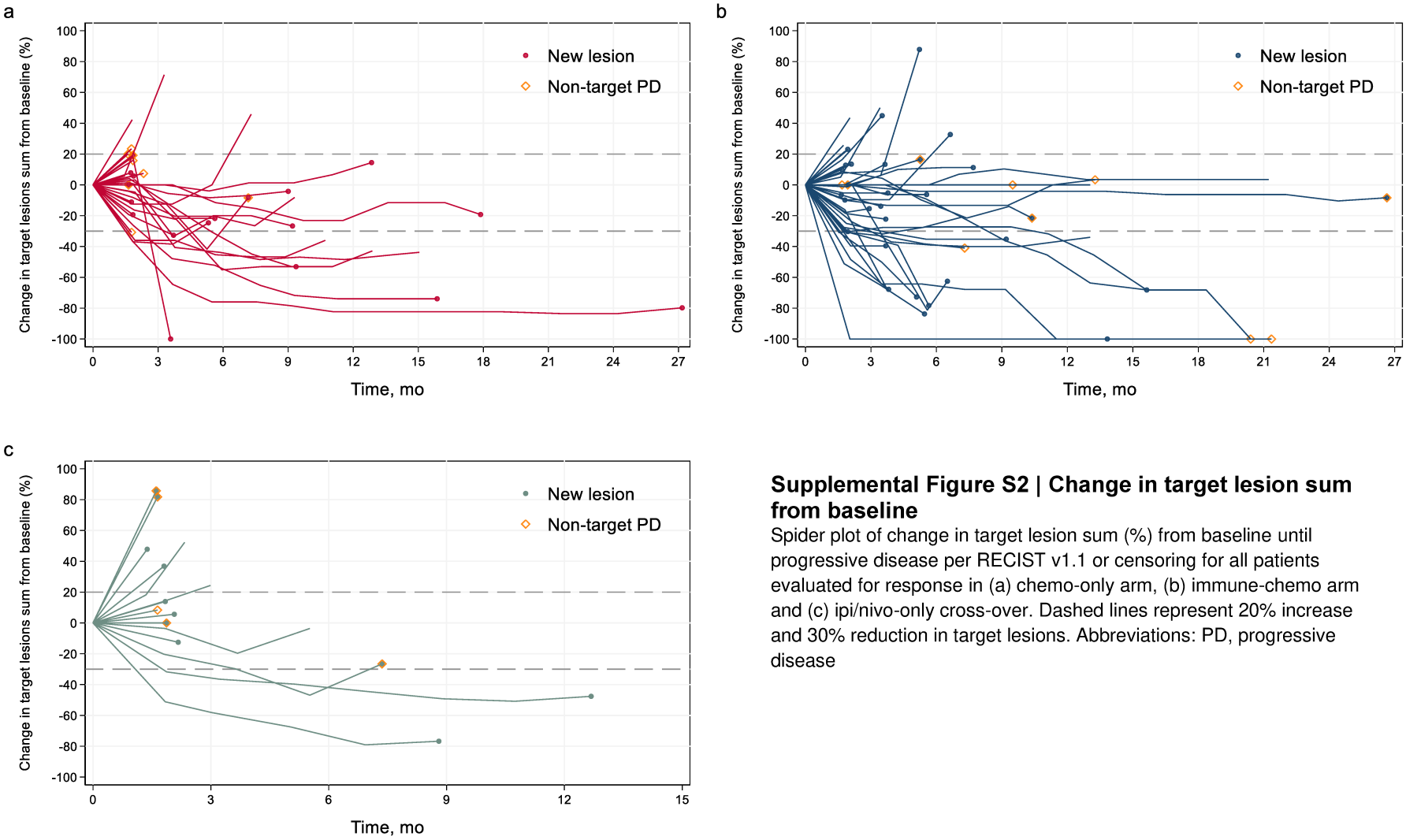
Ipilimumab and nivolumab combined with anthracycline-based chemotherapy in metastatic hormone receptor-positive breast cancer - A randomized, open-label, phase 2b trial

Andresen NK, *et al. J Immunother Cancer* 2024; 12:e007990. doi: 10.1136/jitc-2023-007990



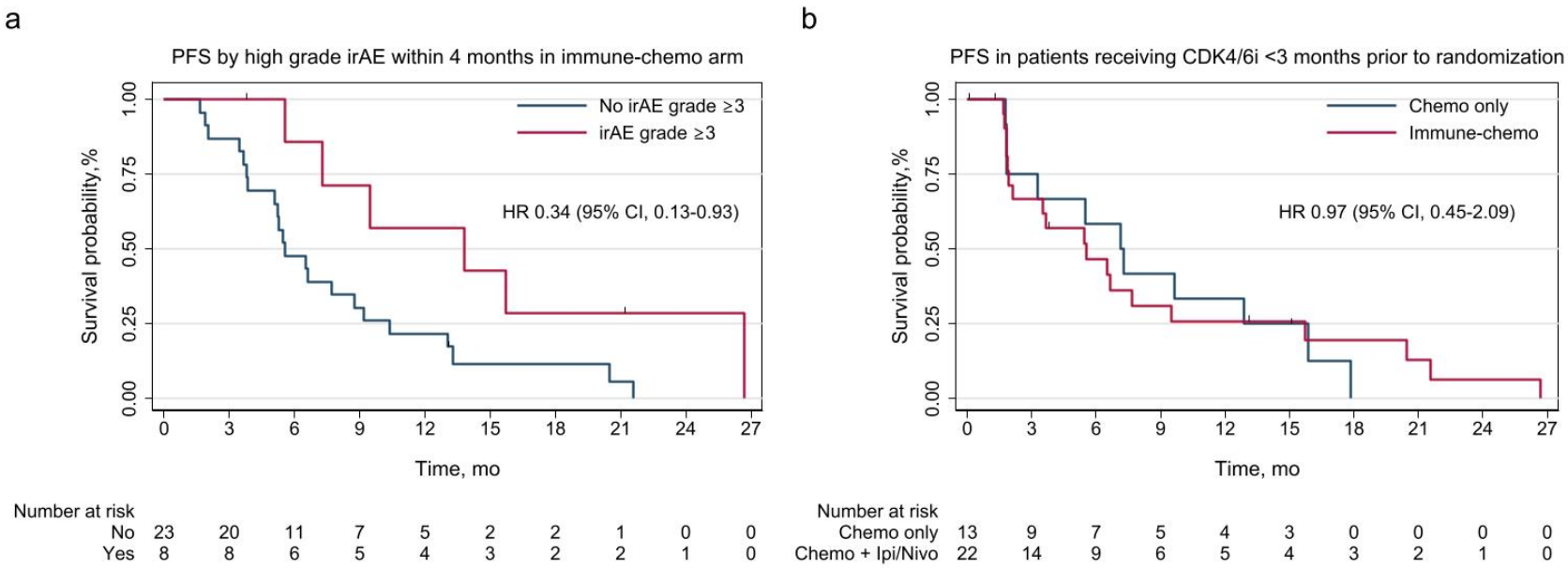
Supplemental Figure S1 | Kaplan-Meier plots of survival outcomes

(a) Sensitivity analysis assessing the effect of having one patient randomized to both treatment arms on the primary endpoint progression-free survival. The Kaplan-Meier plot shows progression-free survival in a modified per protocol population, where the patient that was randomized to both treatment arms is excluded from the chemo-immune arm (the second randomization). (b) Progression-free survival and (c) overall survival in the full analysis set population. (d) Overall survival by clinical benefit in the ipi/nivo-only cross-over arm. The hazard ratios are presented with a 95% confidence interval. Abbreviations: PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ipi, ipilimumab; nivo, nivolumab



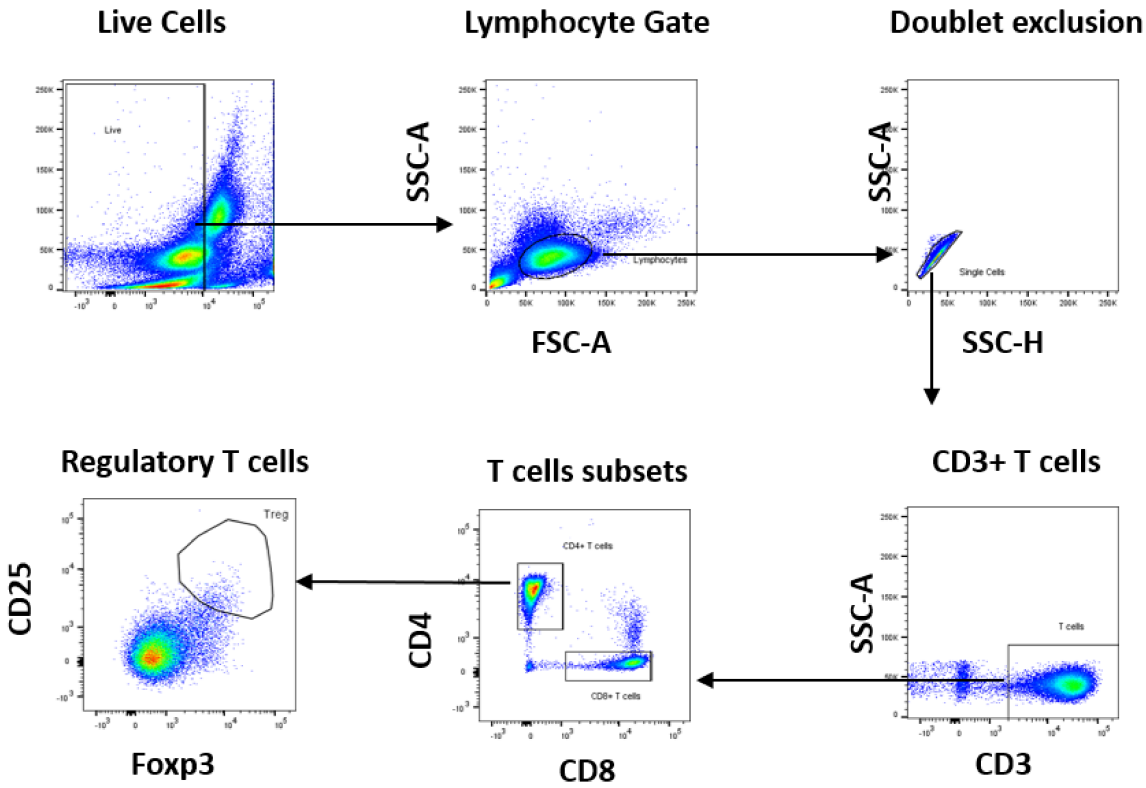
Supplemental Figure S2 | Change in target lesion sum from baseline

Spider plot of change in target lesion sum (%) from baseline until progressive disease per RECIST v1.1 or censoring for all patients evaluated for response in (a) chemo-only arm, (b) immune-chemo arm and (c) ipi/nivo-only cross-over. Dashed lines represent 20% increase and 30% reduction in target lesions. Abbreviations: PD, progressive disease



Supplemental Figure S3 | Exploratory efficacy analyses

The Kaplan-Meier plot in (a) presents a landmark analysis of PFS for patients in the immune-chemo arm with or without a CTCAE v4.0 grade ≥3 immune-related adverse event within 4 months from randomization (*N*= 31). Patients with treatment discontinuation within 4 months (*N*= 16) were excluded from the analysis to reduce the risk of immortal time bias. (b) Presents PFS in the sub-population of patients receiving treatment with CDK4/6 inhibitors <3 months prior to randomization. The hazard ratios are presented with a 95% confidence interval. Abbreviations: PFS, progression-free survival; CDK4/6i, cyclin-dependent kinase 4 and 6 inhibitor; CTCAE, common terminology criteria for adverse events; irAE, immune-related adverse event; HR, hazard ratio; CI, confidence interval.



Supplemental Figure S4 | Flow cytometry gating strategy for peripheral blood mononuclear cell phenotyping
Dot plots of peripheral blood mononuclear cells (PBMC) from a representative patient showing the gating strategy used to identify the different T cell populations by flow cytometry. Populations identified are: $CD4^+$ T cells ($CD3^+CD4^+CD8^-$), $CD8^+$ T cells ($CD3^+CD4^-CD8^+$), regulatory T cells were defined as $CD3^+CD4^+CD25^{hi}Foxp3^+$. Abbreviations: PBMC, peripheral blood mononuclear cell

	Chemotherapy only (*N*= 33)				Chemotherapy plus ipi/nivo (*N*= 49)				Ipi/nivo-only cross-over (*N*= 16)			
Adverse event	**All grades (*N* / %)**		**Grade ≥3 (*N* / %)**		**All grades (*N* / %)**		**Grade ≥3 (*N* / %)**		**All grades (*N* / %)**		**Grade ≥3 (*N* / %)**	
Fatigue	16	48 %	1	3 %	27	55 %	4	8 %	6	38 %	0	0 %
Lymphocyte count decreased	15	45 %	7	21 %	32	65 %	18	37 %	0	0 %	0	0 %
Rash	13	39 %	3	9 %	28	57 %	9	18 %	6	38 %	2	13 %
Nausea	16	48 %	0	0 %	26	53 %	2	4 %	2	13 %	0	0 %
Constipation	18	55 %	1	3 %	16	33 %	0	0 %	0	0 %	0	0 %
Stomatitis	12	36 %	1	3 %	20	41 %	1	2 %	2	13 %	0	0 %
Hypothyroidism	1	3 %	0	0 %	23	47 %	0	0 %	2	13 %	0	0 %
PPE[a]	10	30 %	0	0 %	16	33 %	2	4 %	0	0 %	0	0 %
Neutrophil count decreased	10	30 %	5	15 %	11	22 %	3	6 %	1	6 %	0	0 %
Musculoskeletal pain	2	6 %	0	0 %	11	22 %	0	0 %	5	31 %	0	0 %
Diarrhea	1	3 %	0	0 %	13	27 %	0	0 %	3	19 %	0	0 %
Upper respiratory tract infection	8	24 %	0	0 %	8	16 %	1	2 %	1	6 %	0	0 %
Abdominal pain	7	21 %	0	0 %	8	16 %	2	4 %	1	6 %	0	0 %
Urinary tract infection	3	9 %	1	3 %	12	24 %	1	2 %	0	0 %	0	0 %
Pyrexia	2	6 %	0	0 %	8	16 %	2	4 %	4	25 %	0	0 %
Alopecia	4	12 %	0	0 %	8	16 %	0	0 %	1	6 %	0	0 %
Hyperthyroidism/thyroiditis	0	0 %	0	0 %	11	22 %	1	2 %	1	6 %	0	0 %
Neuropathy peripheral	6	18 %	0	0 %	5	10 %	2	4 %	1	6 %	0	0 %
Vertigo	2	6 %	0	0 %	8	16 %	0	0 %	1	6 %	0	0 %
Gastroesophageal reflux disease	3	9 %	0	0 %	7	14 %	0	0 %	0	0 %	0	0 %
Pruritus	2	6 %	0	0 %	3	6 %	0	0 %	5	31 %	0	0 %
Hemoglobin decreased	5	15 %	1	3 %	4	8 %	1	2 %	0	0 %	0	0 %
Insomnia	1	3 %	0	0 %	7	14 %	0	0 %	1	6 %	0	0 %
Cough	3	9 %	0	0 %	4	8 %	0	0 %	1	6 %	0	0 %
Decreased appetite	3	9 %	0	0 %	4	8 %	1	2 %	1	6 %	0	0 %
Infusion related reaction	4	12 %	0	0 %	4	8 %	0	0 %	0	0 %	0	0 %
Lung infection	0	0 %	0	0 %	8	16 %	6	12 %	0	0 %	0	0 %

Skin infection	3	9 %	0	0 %	5	10 %	0	0 %	0	0 %	0	0 %
Headache	4	12 %	0	0 %	3	6 %	0	0 %	0	0 %	0	0 %
Hypophysitis/Adrenal insufficiency	0	0 %	0	0 %	5	10 %	5	10 %	2	13 %	0	0 %
Oedema	2	6 %	0	0 %	4	8 %	0	0 %	0	0 %	0	0 %
Back pain	3	9 %	1	3 %	2	4 %	1	2 %	0	0 %	0	0 %
Conjunctivitis	2	6 %	0	0 %	2	4 %	0	0 %	1	6 %	0	0 %
Ejection fraction decreased	2	6 %	0	0 %	3	6 %	0	0 %	0	0 %	0	0 %
Hepatitis	0	0 %	0	0 %	3	6 %	3	6 %	2	13 %	1	6 %
Hyperglycemia	3	9 %	1	3 %	2	4 %	1	2 %	0	0 %	0	0 %
Pneumonitis	0	0 %	0	0 %	4	8 %	1	2 %	1	6 %	0	0 %
Procedural pain	2	6 %	1	3 %	2	4 %	1	2 %	1	6 %	0	0 %
Tooth infection	2	6 %	1	3 %	3	6 %	0	0 %	0	0 %	0	0 %
Skin fissures	3	9 %	0	0 %	2	4 %	0	0 %	0	0 %	0	0 %
Alanine aminotransferase increased	0	0 %	0	0 %	2	4 %	1	2 %	2	13 %	0	0 %
Dysphagia	3	9 %	0	0 %	1	2 %	0	0 %	0	0 %	0	0 %
Dyspnea	0	0 %	0	0 %	4	8 %	1	2 %	0	0 %	0	0 %
Dysuria	0	0 %	0	0 %	4	8 %	0	0 %	0	0 %	0	0 %
Ear discomfort	1	3 %	0	0 %	3	6 %	0	0 %	0	0 %	0	0 %
Febrile neutropenia	1	3 %	1	3 %	2	4 %	2	4 %	1	6 %	1	6 %
Hypokalemia	0	0 %	0	0 %	4	8 %	0	0 %	0	0 %	0	0 %
Influenza like illness	1	3 %	0	0 %	2	4 %	0	0 %	1	6 %	0	0 %
Muscle spasms	1	3 %	0	0 %	3	6 %	0	0 %	0	0 %	0	0 %
Pain in extremity	1	3 %	0	0 %	1	2 %	0	0 %	2	13 %	0	0 %
Platelet count decreased	0	0 %	0	0 %	4	8 %	1	2 %	0	0 %	0	0 %
Post procedural hemorrhage	2	6 %	0	0 %	2	4 %	1	2 %	0	0 %	0	0 %
Rhinitis	3	9 %	0	0 %	0	0 %	0	0 %	1	6 %	0	0 %
Vomiting	1	3 %	0	0 %	3	6 %	0	0 %	0	0 %	0	0 %
Weight decreased	1	3 %	0	0 %	2	4 %	0	0 %	1	6 %	0	0 %
Colitis	0	0 %	0	0 %	2	4 %	2	4 %	1	6 %	1	6 %
Depression	3	9 %	0	0 %	0	0 %	0	0 %	0	0 %	0	0 %

Flushing	1	3 %	0	0 %	2	4 %	0	0 %	0	0 %	0	0 %
Nail disorder	1	3 %	0	0 %	2	4 %	0	0 %	0	0 %	0	0 %
Oral candidiasis	0	0 %	0	0 %	3	6 %	0	0 %	0	0 %	0	0 %
Pleural effusion	0	0 %	0	0 %	3	6 %	1	2 %	0	0 %	0	0 %
Visual impairment	3	9 %	0	0 %	0	0 %	0	0 %	0	0 %	0	0 %
Vulvovaginal discomfort	1	3 %	0	0 %	2	4 %	0	0 %	0	0 %	0	0 %
Xeroderma	2	6 %	0	0 %	1	2 %	0	0 %	0	0 %	0	0 %
Arthritis	1	3 %	0	3 %	2	4 %	0	0 %	0	0 %	0	0 %
Fracture	1	3 %	0	0 %	2	4 %	2	4 %	0	0 %	0	0 %
Amylase increased	1	3 %	1	3 %	1	2 %	1	2 %	0	0 %	0	0 %
Aspartate aminotransferase increased	0	0 %	0	0 %	1	2 %	1	2 %	1	6 %	0	0 %
Dysgeusia	0	0 %	0	0 %	2	4 %	0	0 %	0	0 %	0	0 %
Dysphonia	1	3 %	0	0 %	1	2 %	0	0 %	0	0 %	0	0 %
Hemorrhoids	0	0 %	0	0 %	2	4 %	0	0 %	0	0 %	0	0 %
Hepatocellular injury	1	3 %	0	0 %	1	2 %	0	0 %	0	0 %	0	0 %
Hypercalcemia	1	3 %	0	0 %	1	2 %	0	0 %	0	0 %	0	0 %
Hyperhidrosis	1	3 %	0	0 %	1	2 %	0	0 %	0	0 %	0	0 %
Hypertension	0	0 %	0	0 %	2	4 %	1	2 %	0	0 %	0	0 %
Hypoglycemia	0	0 %	0	0 %	1	2 %	0	0 %	1	6 %	1	6 %
Lipase increased	0	0 %	0	0 %	2	4 %	2	4 %	0	0 %	0	0 %
Neck pain	2	6 %	0	0 %	0	0 %	0	0 %	0	0 %	0	0 %
Oropharyngeal pain	2	6 %	0	0 %	0	0 %	0	0 %	0	0 %	0	0 %
Periorbital oedema	0	0 %	0	0 %	2	4 %	0	0 %	0	0 %	0	0 %
Tinnitus	1	3 %	0	0 %	0	0 %	0	0 %	1	6 %	0	0 %
Toothache	2	6 %	0	0 %	0	0 %	0	0 %	0	0 %	0	0 %
Tumor pain	1	3 %	0	0 %	0	0 %	0	0 %	1	6 %	0	0 %
Type 1 diabetes mellitus	0	0 %	0	0 %	2	4 %	2	4 %	0	0 %	0	0 %
Urinary incontinence	0	0 %	0	0 %	2	4 %	0	0 %	0	0 %	0	0 %
Abdominal distension	1	3 %	0	0 %	1	2 %	0	0 %	0	0 %	0	0 %

Conduction disorder	1	3 %	0	0 %	1	2 %	0	0 %	0	0 %	0	0 %
Renal failure	1	3 %	0	0 %	1	2 %	1	2 %	0	0 %	0	0 %

Supplemental Table S1 | Adverse events occurring in ≥2 patients

All adverse events in the FAS population occurring in ≥2 patients. Adverse events are presented by preferred term and listed by total frequency in the study population. Repeated adverse events in the same subject are counted only once at the preferred term level in the treatment arm and presented by the highest recorded CTCAE v4.0 grade. [a]Palmar-plantar erythrodysaesthesia. Abbreviations: CTCAE, common terminology criteria for adverse events; ipi, ipilimumab; nivo, nivolumab

Population	Treatment group	*N*	ORR		CBR		DRR		DOR	
PP	Chemo-only	31	29 %	(16.1-46-6)	48 %	(32.0-65.2)	19 %	(9.2-36.3)	7.4 months	(3.7-11.3)
	Immune-chemo	47	32 %	(20.4-46.2)	55 %	(41.3-68.6)	13 %	(6.0-25.2)	5.5 months	(2.8-10.3)
FAS	Chemo-only	33	27 %	(15.1-44.2)	46 %	(29.8-62.0)	18 %	(8.6-34.4)	7.4 months	(3.7-11.3)
	Immune-chemo	49	31 %	(19.5-44.5)	53 %	(39.4-66.3)	12 %	(5.7-24.2)	5.5 months	(2.8-10.3)
PD-L1+ FAS	Chemo-only	10	30 %	(10.8-60.3)	50 %	(23.7-76.3)	30 %	(10.8-60.3)	11.3 months	(7.4-12.2)
	Immune-chemo	19	32 %	(15.4-54.0)	53 %	(31.7-72.7)	11 %	(2.9-31.4)	4.2 months	(1.8-7.2)

Supplemental Table S2 | Summary of response variables

Summary of response variables presented in the PP population, FAS population and in the PD-L1 positive FAS population. The proportions of patients with objective response, clinical benefit (objective response or stable disease until radiological assessment at week 24 ±10 days) and durable response are presented with confidence intervals calculated using the Wilson score method. The duration of response is presented as the median duration in months with interquartile range. Abbreviations: PP, per protocol; FAS, full analysis set; PD-L1, programmed death-ligand 1; ORR, objective response rate; CBR, clinical benefit rate; DRR, durable response rate; DOR, duration of response.

Biomarkers ipi/nivo-only cross-over	Clinical benefit *N*= 4	No clinical benefit *N*= 12
PD-L1 expression (IHC, SP142)		
Positive	1 (25)	4 (33)
Negative	3 (75)	8 (67)
Estimated TMB (mut/Mb)	1.7 (1.0-7.7)	2.2 (0.7-9.5)
Tumor infiltrating lymphocytes (TIL)		
High (2-3)	1 (25)	2 (17)
Low (0-1)	3 (75)	10 (83)
Change in TIL score		
Any increase	3 (75)	1 (8)
Stable/reduced	0	5 (42)
Missing	1 (25)	6 (50)
PD-L1 gene expression		
≥ Median	1 (25)	7 (58)
< Median	3 (75)	4 (33)
Missing	0	1 (8)
Tumor Inflammation Signature		
≥ Median	1 (25)	7 (58)
< Median	3 (75)	4 (33)
Missing	0	1 (8)
Treg gene signature expression		
≥ Median	2 (50)	6 (50)
< Median	2 (50)	5 (42)
Missing	0	1 (8)
PAM50 subtype		
Luminal A	0	3 (25)
Luminal B	4 (100)	7 (58)
HER-2 enriched	0	1 (8)
Missing	0	1 (8)

Supplementary Table S3 | Biomarker assessment in the ipi/nivo-only cross-over

Data is presented as *N* (%) for all categorical measures and median (range) for estimated TMB, by clinical benefit (*N*= 4) vs no clinical benefit (*N*= 12) in the ipi/nivo-only cross-over cohort. Tumor infiltrating lymphocytes (TILs) were scored from 0-3 in H&E stained slides and categorized as low (score 0 and 1) or high (score 2 and 3) infiltration. Baseline TILs were assessed in a study biopsy taken prior to ipi/nivo-only and after start of treatment in the chemo-only arm. In patients with available on-study biopsies the change in TIL score was calculated as any change (TIL score 0-3) from baseline to 4 weeks after start of ipi/nivo-only. PD-L1 expression was assessed by IHC in pre-study biopsies with the SP142 clone scoring PD-L1 expression on immune cells. Patients with more than one sample available were categorized as PD-L1 positive if any of the samples were positive. Tumor Inflammation Signature and the PAM50 subtype were obtained from bulk RNA isolated from the most recent pre-study biopsy available and analyzed with the nCounter BC360 gene expression assay. Abbreviations: PD-L1, programmed death-ligand 1; IHC, immunohistochemistry; TIL, tumor infiltrating lymphocytes; TMB, tumor mutational burden; Treg, regulatory T cell; H&E, hematoxylin and eosin