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First-in-human study of SBRT and adenosine pathway blockade to potentiate the benefit of immunochemotherapy in early-stage luminal B breast cancer: results of the safety run-in phase of the Neo-CheckRay trial

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ABSTRACT

Background Luminal B breast cancer (BC) presents a worse prognosis when compared with luminal A BC and exhibits a lower sensitivity to chemotherapy and a lower immunogenicity in contrast to non-luminal BC subtypes. The Neo-CheckRay clinical trial investigates the use of stereotactic body radiation therapy (SBRT) directed to the primary tumor in combination with the adenosine pathway inhibitor oleclumab to improve the response to neo-adjuvant immuno-chemotherapy in luminal B BC. The trial consists of a safety run-in followed by a randomized phase II trial. Here, we present the results of the first-in-human safety run-in.

Methods The safety run-in was an open-label, single-arm trial in which six patients with early-stage luminal B BC received the following neo-adjuvant regimen: paclitaxel q1w×12 → doxorubicin/cyclophosphamide q2w×4; durvalumab (anti-programmed cell death receptor ligand 1 (PD-L1)) q4w×5; oleclumab (anti-CD73) q2w×4 → q4w×3 and 3×8 Gy SBRT to the primary tumor at week 5. Surgery must be performed 2–6 weeks after primary systemic treatment and adjuvant therapy was given per local guidelines, RT boost to the tumor bed was not allowed. Key inclusion criteria were: luminal BC, Ki67≥15% or histological grade 3, MammaPrint high risk, tumor size≥1.5 cm. Primary tumor tissue samples were collected at three timepoints: baseline, 1 week after SBRT and at surgery. Tumor-infiltrating lymphocytes, PD-L1 and CD73 were evaluated at each timepoint, and residual cancer burden (RCB) was calculated at surgery.

Results Six patients were included between November 2019 and March 2020. Median age was 53 years, range 37–69. All patients received SBRT and underwent surgery 2–4 weeks after the last treatment. After a median follow-up time of 2 years after surgery, one grade 3 adverse event (AE) was reported: pericarditis with rapid resolution under corticosteroids. No grade 4–5 AE were documented. Overall cosmetical breast evaluation after surgery was 'excellent' in four patients and 'good' in two patients. RCB results were 2/6 RCB 0; 2/6 RCB 1; 1/6 RCB 2 and 1/6 RCB 3.

Conclusions This novel treatment combination was considered safe and is worth further investigation in a randomized phase II trial.

Trial registration number NCT03875573.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The neo-adjuvant treatment of early breast cancer encompasses the use of chemotherapy and immunotherapy. It is unknown if the preoperative addition of stereotactic body radiation therapy (SBRT) and adenosine blockade to immuno-chemotherapy is feasible in this setting.

WHAT THIS STUDY ADDS

⇒ First-in-human feasibility and safety measures of a novel combination treatment in early-stage breast cancer.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

⇒ The study paves the way for randomized phase II trials that will evaluate the efficacy of the combination of SBRT, adenosine pathway blockade and immunochemotherapy in the neo-adjuvant setting for early-stage breast cancer.

BACKGROUND

Immune checkpoint blockade (ICB) in combination with neoadjuvant chemotherapy (NACT) has been investigated in early-stage triple negative breast cancer (TNBC) and in estrogen receptor-positive, human epidermal growth factor receptor 2-negative (ER+/HER2-) breast cancer (BC). In TNBC, the addition of ICB to NACT resulted in a higher rate of pathological complete response (pCR) and improved long-term outcomes, such as event-free survival (EFS) or invasive disease-free survival (DFS).[1 2] Conversely, in ER+/HER2− BC, the addition of ICB to NACT yielded a more modest increase in pCR[3–5] and long-term outcome results are awaited. Generally, ER+/HER2− BC has a better overall prognosis than other BC subtypes, but the luminal B subtype is characterized by high-grade tumors, lower sensitivity to endocrine therapy, higher benefit to chemotherapy and a worse prognosis compared with patients with luminal A tumors.[6–8] High-risk luminal B BC can be identified through the use of gene expression signatures, such as the MammaPrint test.[8 9] More than 40% of patients with luminal B BC treated with NACT develop a local or distant recurrence within 5 years.[6–8] However, patients responding well to NACT present a better outcome,[6 7] highlighting the importance of developing novel pre-operative treatments.

Tumors can be classified according to their immunological contexture: inflamed cancer types are characterized by the presence of tumor-infiltrating lymphocytes (TILs), high CD8+ T cell density, and high programmed cell death receptor ligand 1 (PD-L1) positivity of tumor or immune cells. In these inflamed cancer types, improved overall long-term outcomes can be attained with immunotherapy in comparison to less inflamed tumors.[10] Likewise, differences in immune contexture between TNBC and ER+/HER2− BC can explain the different impact of the addition of ICB to NACT.[11] Therefore, novel priming strategies that enhance the immune response could increase the clinical benefit of ICB in ER+/HER2− BC.

In recent years, large amounts of preclinical evidence and clinical trials have described the synergistic effects on local and distant tumor control of combining radiation therapy (RT) with immunotherapy.[12–17] The enhanced immune response by adding RT is mediated by multiple mechanisms, including the upregulation of the major histocompatibility complex (MHC), activation of dendritic cells, induction of immunogenic cell death with release of neoantigens, enhanced antigen (cross) presentation, increased T-cell infiltration into the tumor and modulation of checkpoint expression.[18] In the Neo-CheckRay trial, three fractions of 8 Gy are delivered to the primary tumor concomitantly with preoperative ICB and NACT. In addition, the adenosine pathway blocker oleclumab, a monoclonal antibody targeting the cluster of differentiation 73 (CD73), was added with the intent to mitigate RT-induced immunosuppression. Preclinical evidence demonstrates that CD73 generates immunosuppressive adenosine in the tumor microenvironment (TME), and that the combination of RT with ICB and CD73 blockade could improve treatment response.[19 20]

The hypothesis behind the Neo-CheckRay trial is that in early-stage luminal B BC classified as high risk by the MammaPrint test, the innovative combination therapy of NACT-durvalumab-oleclumab-stereotactic body radiation therapy (SBRT) will increase response rates and improve long-term outcome. The trial is the first to investigate this novel treatment combination and consists of a safety run-in followed by a randomized phase II trial. Here, we report the results of the safety run-in.

MATERIALS AND METHODS

Eligible patients

Eligible patients were adult women with newly diagnosed early-stage BC with the following characteristics: ER+/HER2− BC as defined by the American Society of Clinical Oncology—College of American Pathologists guidelines[21]; previously untreated, nonmetastatic disease, which was defined as combined primary tumor (T) and regional lymph node (N) involvement, according to the American Joint Committee on Cancer staging criteria (seventh edition), T2-3N0 or T1b-3N1-3[22]; primary tumor size assessed on MRI≥1.5 cm; proliferation Index Ki67≥15% or histological grade 3; MammaPrint status high risk[8]; an Eastern Cooperative Oncology Group performance-status score of 0 or 1[23] and adequate organ function. Detailed eligibility criteria are listed in the online supplemental file 1.

Trial design

The safety run-in was an open-label, non-randomized neo-adjuvant trial that was conducted to evaluate treatment safety and feasibility before launching the randomized, phase II part of the Neo-CheckRay trial.[24] Six patients of the Jules Bordet Institute, Brussels, Belgium, had to be included in the safety run-in to receive q1w paclitaxel 80 mg/m² intravenous for 12 administrations (12 weeks) followed by q2w dose-dense doxorubicin-cyclophosphamide (ddAC) intravenous (60 mg/m² and 600 mg/m², respectively) for four administrations; the anti-PD-L1 antibody durvalumab 1500 mg intravenous q4w for five administrations and the anti-CD73 antibody oleclumab 3000 mg intravenous q2w for four administrations followed by q4w for three administrations (figure 1A). The use of glucocorticoids to avoid allergic reactions before chemotherapy and for the management of immune mediated adverse events (AE) was allowed. RT, delivered only to the primary tumor, was provided on week 4-5 at a dose of 3 fractions of 8 Gy on 3 consecutive days, immediately followed by the week 5 systemic treatment. Involved lymph nodes, elective nodal regions and the uninvolved ipsilateral breast were not treated with RT in the preoperative phase.

Patients underwent definitive surgery (breast conservation or mastectomy with sentinel lymph node evaluation and/or axillary dissection) within 2–6 weeks after the

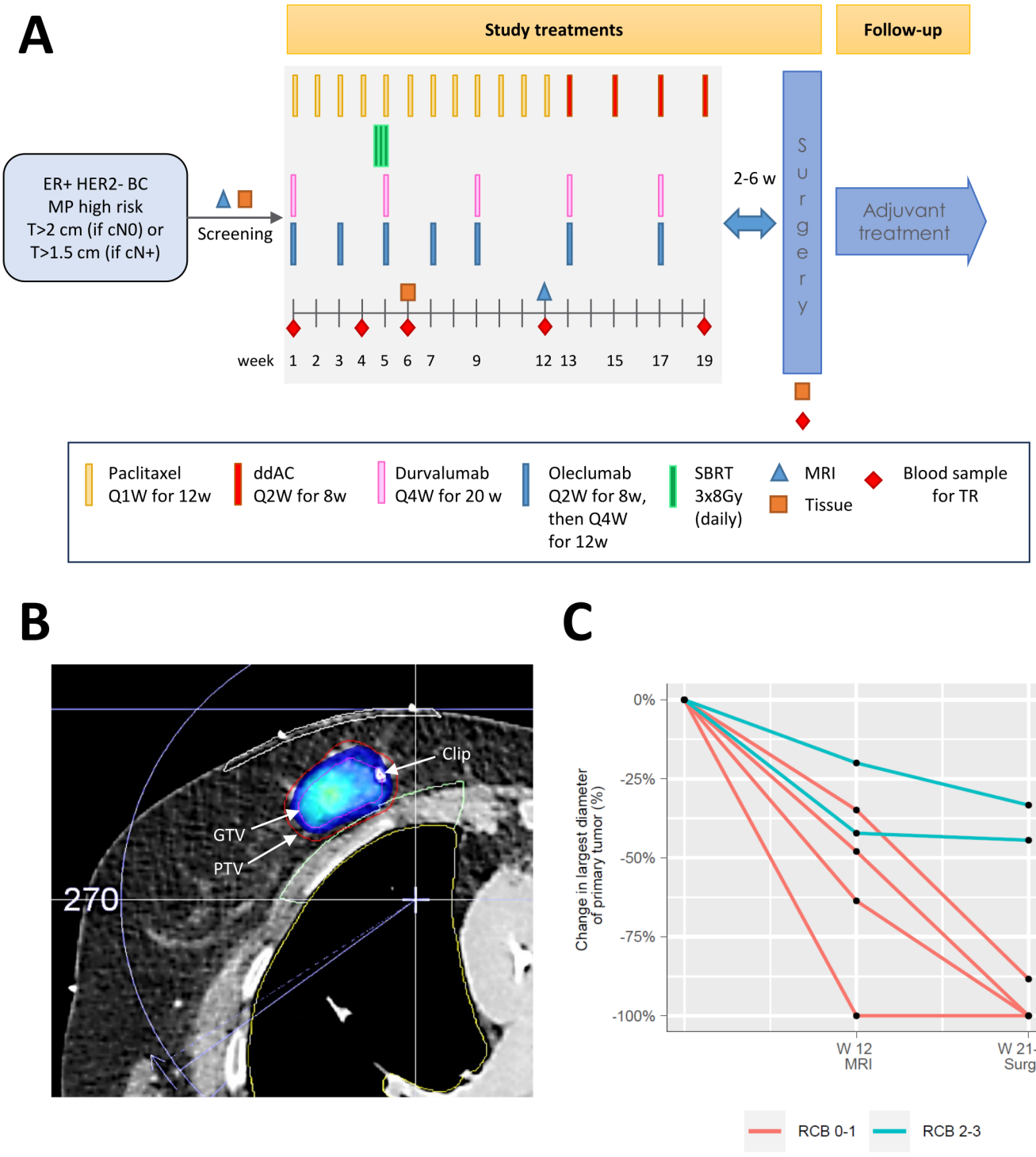

Figure 1 (A) Trial design of the Neo-CheckRay safety run-in. Preoperative systemic treatment consisted of q1w paclitaxel 80 mg/m² intravenous for 12 administrations (12 weeks) followed by q2w dose-dense doxorubicin-cyclophosphamide (ddAC) intravenous (60 mg/m² and 600 mg/m², respectively) for four administrations; the anti-PD-L1 antibody durvalumab 1500 mg intravenous q4w for five administrations and the anti-CD73 antibody oleclumab 3000 mg intravenous q2w for four administrations followed by q4w for three administrations. SBRT was delivered daily immediately before week 5 at a dose of 3 fractions of 8 Gy. BC, breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; SBRT, stereotactic body radiation therapy; TR, translational research. (B) Representative example of contours and dosimetry of SBRT treatment of the primary BC in the right breast. The contours are the GTV (gross tumor volume) in magenta, the PTV (planning target volume) in red, the lung in yellow, the chest wall in light green and the skin in white. The clip is one of the three clips that were implanted to guide SBRT using daily CBCT (cone beam CT). The color wash depicts the volume that receives a minimum dose of 3×8 Gy (blue) to 3×9 Gy (yellow). (C) Change of largest diameter of primary tumor at week 12 MRI and at surgery in comparison to baseline. RCB, residual cancer burden.

last neoadjuvant treatment cycle. Whenever indicated, patients received adjuvant RT to the breast, chest wall and lymph node regions according to local standards of care for the fractionation schedule. An RT boost to the tumor bed was not allowed, as the preoperative RT was considered as an anticipated boost. Durvalumab and oleclumab were discontinued after surgery. Adjuvant endocrine treatment was given according to standard of care.

The primary objective of the safety run-in was to evaluate the safety of the novel neo-adjuvant combination of systemic treatments with SBRT as this was never tested before. The objectives of the safety run-in were (1) the feasibility of delivering 3×8 Gy to the primary tumor (2) the occurrence of immune related or RT related toxicities; (3) the feasibility of delivering a sufficient dose of standard chemotherapy (paclitaxel and ddAC) and (4) the feasibility of performing surgery within 2 to 6 weeks after the last neo-adjuvant treatment.

Safety measures

An interval of seven calendar days was mandatory between the enrolment of each new patient to assess for unexpected acute AE requiring halting further inclusions and treatments. The first RT fraction of each patient could at the earliest be given 48 hours after the last fraction given to the previous patient. In case of any grade 3/4 RT-related acute AE (such as radiation dermatitis or radiation pneumonitis) occurring at any time before surgery, the dose per fraction for all fractions of the next patients would be reduced by 1 Gy.

RT was delivered by high precision SBRT. During SBRT planning, care was taken to minimize the dose to the heart, the lungs, the chest wall, the skin and the draining lymph nodes (figure 1B). To allow for optimal image guidance during SBRT treatment, at least three MRI-compatible fiducials were placed at the borders of the primary tumor. Image guidance during treatment was performed by daily cone beam CT and online markers-based matching.

Prespecified conditions should be met before the phase II randomized Neo-CheckRay trial could be launched. All six patients in the safety run-in should have undergone SBRT and not more than one out of six patients may have experienced an immune related or RT-related AE of special interest (details in the supplement section 2.1 and online supplemental figure S1). All patients should have undergone surgery within maximum 7 weeks after the last treatment and all patients should have received at least 75% of the planned dose of paclitaxel and dose-dense AC. Lastly, an independent data monitoring committee (IDMC) was consulted before making the final decision to proceed with the phase II randomized trial.

Assessments

AE were monitored throughout the trial and for 30 days after surgery, whereafter patients entered the follow-up period. AE were graded according to the Common Terminology Criteria for AE (CTCAE), version 5.0 of the National Cancer Institute.[25] Immune-mediated AE were determined based on a prespecified list of Medical Dictionary of Regulatory Activities terms.[26] Clinical assessments, including physical examination of the breast and the lymph nodes, were performed every 2 weeks during the treatment with paclitaxel and every 4 weeks during treatment with ddAC. Breast cosmesis was monitored via clinical examinations at baseline, week 9 and before surgery. Global cosmetic evaluation was performed and graded by the treating oncologist as 'poor', 'fair', 'good' and 'excellent'. Breast MRI was performed at baseline and after the 12 weeks of paclitaxel before starting the ddAC regimen.

Residual disease was measured using the residual cancer burden (RCB) index on the surgical specimen. RCB is calculated as a continuous index combining pathological measurements of the primary tumor (size and cellularity) and lymph node metastases (number and size). RCB 0 was defined as pCR and RCB 1 as minimal residual disease.[27] Follow-up for cosmetic outcome, disease status and survival was scheduled every 3 months for the first 2 years after surgery, then every 6 months during 3 years.

Biomarkers

Core biopsies of the primary tumor were performed at baseline and 1 week after SBRT (week 6). Tissues from the surgical resection specimen were also collected. Blood samples were collected before treatment (baseline), at week 4, at week 6 (1 week after radiation therapy) and at surgery (between week 21 and week 25).

Biomarkers assessed on the tumor sample included TILs, PD-L1, CD73 and MHC-I immunohistochemical (IHC) expression on formalin-fixed paraffin embedded full-face tissue sections (4 µm; cut within 24 hours). IHC were performed on Ventana Benchmark XT automated staining instrument (Ventana Medical Systems). To quantify the IHC staining in sections, the slides were scanned and visualized at high resolution using an NDPI viewer (Hamatsu NDP.view, version 2.5).

MammaPrint and BluePrint status were assessed at baseline, MammaPrint was further subdivided into high risk (MammaPrintHigh1 or MP1; from 0 to −0.56) or ultra-high risk (MammaPrintHigh2 or MP2; from −0.57 to −1.0).[28]

Stromal TILs were scored using hematoxylin and eosin (H&E) stained tumor sections following the 2014 guidelines from the International Tumor Infiltrating Lymphocyte Working Group.[29]

PD-L1 IHC expression was assessed with the Ventana PD-L1 (SP263) assay. PD-L1 was quantified separately in the epithelial and stromal compartment and calculated as the percentage of stained cells divided by the number of total cells within each compartment.

CD73 IHC expression was performed using Abcam EPR6115 clone. The expression was quantified as a histological score (H-score) calculated as percentage of stained cells (cancer cells or fibroblasts) multiplied by the intensity of staining in epithelium and stromal compartments, separately. The intensity of staining was graded from 0 to 4. The discrimination between the two compartments was

verified by α-SMA staining, using the method described by Magagna *et al*.[30] Representative baseline CD73 IHC expression examples are shown in online supplemental figure S3.

MHC-I IHC expression was performed with a validated mouse antihuman monoclonal antibody HC-10.[31 32] MHC-I expression was quantified as a histological score (H-score) calculated as the percentage of stained cells multiplied by the intensity of staining (graded from 0 to 4) and multiplied by 100. MHC-I expression was assessed in the epithelial and stromal compartments. Examples of baseline MHC-I intensity scores with corresponding TIL scores are provided in online supplemental figure S4.

RESULTS

Patients and treatment

Six patients were enrolled in the safety run-in of the NeoCheckRay trial between November 2019 and March 2020, and received the combination of chemotherapy–durvalumab–oleclumab–SBRT followed by surgery. Baseline clinicopathological characteristics are reported in table 1. Median follow-up was 25.5 months (range, 24.1–28.4) for safety, cosmetical and efficacy analyses. Systemic treatments as defined per protocol were delivered to all patients, except for 1 cycle of paclitaxel in 1 patient that was not given due to grade II paclitaxel-induced neuropathy. SBRT was given to six patients at a dose of 24 Gy in three fractions (3×8 Gy). Median time from the end of the systemic therapy until surgery was 23 days (range, 16–29). Clinical and radiologic evaluation did not reveal disease progression, breast inflammation or pseudoprogression during the preoperative phase. At time of surgery, four patients underwent breast conserving surgery and two underwent mastectomy. All patients underwent the type of surgery that was foreseen at

Table 1 Baseline characteristics

		N (%)
Age	Median (range)	53 (37–69)
	< 50	3 (50%)
	≥ 50	3 (50%)
ECOG performance status	0	6 (100%)
BMI	Median (range)	25.3 (21.9–30.3)
Smoking	Non-smoker	5 (83%)
	Smoker	1 (17%)
Clinical tumor size	< 2 cm	1 (17%)
	2–5 cm	5 (83%)
Number of clinical positive nodes	0	3 (50%)
	1–3	3 (50%)
Disease stage	Stage I	1 (17%)
	Stage II	5 (83%)
Histological grade	Grade 1	0
	Grade 2	1 (17%)
	Grade 3	5 (83%)
Histological subtype	NST	6 (100%)
Ki-67	Median (range)	40% (25–95)
ER Allred score	4–8	6 (100%)
PR Allred score	4–8	5 (83%)
	0–3	1 (17%)
MammaPrint result	High risk	6 (100%)
MammaPrint numeric score	Median (range)	−0.380 (−0.825 to −0.134)
MammaPrint high subtype	MP High 1 (0 to −0.56)	5 (83%)
	MP High 2 (−0.57 to −1.0)	1 (17%)
BluePrint result	Luminal	6 (100%)
	Basal	0
	HER2	0

BMI, body mass index; ECOG, Eastern Cooperative Oncology Group.

Table 2 Adverse events

	Any grade	Grade≥3
	Number of patients (%)	
Any adverse event	6 (100)	1 (16.7%)
Treatment-related adverse event*	6 (100)	0
Fatigue	6 (100)	0
Peripheral sensory neuropathy	6 (100)	0
Nail discoloration	4 (66.6)	0
ALT elevation	2 (33.3)	0
AST elevation	2 (33.3)	0
Anemia	2 (33.3)	0
Dry skin	2 (33.3)	0
Epistaxis	2 (33.3)	0
Headache	2 (33.3)	0
Hot flush	2 (33.3)	0
Stomatitis	2 (33.3)	0
Immune-mediated adverse events	4 (66.6)	1 (16.7%)
Pericarditis	1 (16.7)	1 (16.7%)
Rash	2 (33.3)	0
Conjunctivitis	1 (16.7)	0
Face rash	1 (16.7)	0
Hand dermatitis	1 (16.7)	0
Hyperthyroidism	1 (16.7)	0
Hypothyroidism	1 (16.7)	0
Related to SBRT	1 (16.7)	0
Breast pain	1 (16.7)	0
Related to surgery	2 (33.3)	0
Infection on mastectomy site	1 (16.7)	0
Lymphoedema	1 (16.7)	0
Post procedural infection	1 (16.7)	0

* Listed for all the adverse events of grade ≥ 3 and for all grades if occurrence was > 1. Complete list of adverse events can be found in online supplemental table S1.
SBRT, stereotactic body radiation therapy.

time of diagnosis, namely, no initially planned breast conserving surgery had to be converted to a mastectomy. Axillary surgery consisted of a sentinel lymph node biopsy in 50% (n=3) and of an axillary lymph node dissection in 50% (n=3).

Safety and cosmesis

AE of grade≥3 occurred in one out six patients (16.7%). Fatigue, peripheral sensory neuropathy, alopecia and nail discoloration were the most common treatment-related AE of any grade (table 2). Immune-mediated AE of any grade occurred in four patients (66.6%); immune-mediated AE of ≥grade 3 occurred in one patient (16.7%). All surgery-related and SBRT-related AE were grade≤2 (table 2). No serious adverse events (SAE) were reported in the preoperative phase and only one SAE occurred (16.7%) in the postoperative phase, namely a treatment-related grade 3 pericarditis.

The pericarditis was diagnosed after the patient developed left chest pain and dyspnea New York Heart Association grade 2. Echocardiogram revealed a normal left ventricular ejection fraction but a small pericardial effusion. Chest CT angiography and cardiac MRI corroborated the diagnosis of pericarditis. Coronary angiography was normal. Considering the absence of physiological consequences, this AE could be classified as grade 2 according to CTCAE v5.0 but was finally classified as grade 3 following the hospitalization to rule out a non-ST elevation myocardial infarction. After treatment with corticosteroids, the pericarditis improved to a grade 1 (asymptomatic with disappearance of chest pain) in less than 24 hours and the patient subsequently fully recovered. At the time of pericarditis diagnosis, a grade 1 troponin increase was measured, which normalized after treatment with corticosteroids. The pericarditis was most likely related to durvalumab and less likely to RT even though the BC was left-sided. Of note, the mean heart dose of the sum of preoperative SBRT and postoperative RT was less than 1 Gy; this low dose could be achieved because the patient was treated using deep inspiration breath hold and IMRT (intensity-modulated RT). However, a pericarditis caused by the combination of durvalumab with oleclumab and a very low heart RT dose cannot be fully excluded.

For the four out of six patients who underwent breast conserving surgery, global cosmetic evaluation was excellent (n=3) and good (n=1), after a median follow-up of 25 months after surgery.

Launch of phase II randomized trial

The safety results were reviewed by an IDMC on September 24, 2020. Based on the safety results, the IDMC recommended to proceed with the phase II randomized trial using the same systemic treatment and SBRT doses as used during the safety run-in. Minor changes were made to the protocol, including (1) the addition of a cortisol and adrenocorticotropic hormone (ACTH) assay to the laboratory tests to detect adrenal insufficiency and (2) standardized breast photography during the preoperative phase and the follow-up phase to evaluate breast cosmesis using bcct.core software.[33] The first patient was randomized in the phase II trial in June 2021. The design of the phase II randomized trial is available in the supplements (online supplemental figure S2).

Response and biomarkers

Five patients were classified high risk (MammaPrintHigh1), and one patient was ultra-high risk (MammaPrintHigh2). PD-L1 IHC and CD73 IHC were positive in tumors samples from three patients. One tumor sample was fully inflamed with high levels of TILs (50%) located in the tumor and stroma compartments whereas the other samples had low TILs (≤5%). CD73 staining in epithelial score was very low and was mainly observed in the stroma.

Out of six patients, two demonstrated a complete pathological response (RCB 0), two had a near pCR (RCB 1), one a RCB 2 and one a RCB 3. Out of the three cN+ patients, one converted into an ypN0. Breast MRI during the preoperative phase on week 12 revealed a reduction of the largest tumor diameter in all patients, with a tendency of less reduction in RCB 2/3 patients (figure 1C).

The week 6 biopsy (1 week after SBRT) revealed an absence of invasive tumor in the two patients that subsequently achieved a pCR, whereas the patients with RCB-1 (case #3 and #4) demonstrated an increase in TILs at week 6 in comparison to baseline (figure 2). Conversely, in the two patients not achieving a RCB 0/1 (case #5 and #6), the TILs decreased or remained stable in the week 6 biopsy. Similar results are observed for MHC-I expression correlations between baseline and week 6, with an increase in epithelial MHC-I expression at week 6 in a patient achieving a good response classified as RCB 0/1. Representative stained images of H&E and MHC-I IHC expression are shown in figure 3. Evolution of PD-L1, CD73, and MHC-I IHC expression in the epithelial and stromal compartments at the three tissue sample timepoints (baseline, week 6 and surgery) are illustrated in online supplemental figure S5.

DISCUSSION

In this trial, we investigated the feasibility and first-in-human safety of the preoperative use of SBRT to the primary breast tumor to prime the immune response in combination with NACT and the systemic immunotherapies durvalumab and oleclumab in luminal B BC. The combination of NACT+SBRT has been previously investigated by Bondiau *et al* in a phase 1 dose escalating trial.[34] Because the safe dose of SBRT in combination with NACT+ICB was unknown, the safety run-in was designed to allow SBRT dose reductions by steps of 1 Gy in case of toxicity. Overall results of this safety run-in showed that the combination is feasible, and AE were manageable. AE were mostly grade 1–2 with only one grade 3 toxicity in six treated patients. There were no SBRT-related grade 3–4 AEs and chemotherapy was given as planned. There were no surgery delays or postoperative complications. With a median follow-up time of more than 2 years after surgery, cosmetic results were good to excellent. Assessment of long-term cosmetic side effects (at 5, 10 years and longer term) are foreseen.

This trial was developed to improve the efficacy of NACT in luminal B BC because 40% of patients with early-stage luminal B BC treated with NACT develop local or distant recurrent disease within 5 years, highlighting the need for more effective treatments.[6 7] The benefit of the addition of ICB to NACT in early-stage luminal B BC was investigated in the KEYNOTE-756 trial and CheckMate-7FL. First pCR results of both trials were presented at ESMO 2023 : an absolute increase in pCR of respectively 8.5% and 10.5% was demonstrated; EFS results of both trials are awaited. Phase II results have been published in early-stage luminal B BC, such as the I-SPY2 trial demonstrating that the addition of pembrolizumab to NACT in luminal B BC increased pCR from 13% to 30%.[3] The increase in pCR indicates that the combination of ICB with NACT might induce long-term benefit in luminal B BC, especially if novel synergistic combinations can further increase the effect of ICB in luminal B BC. A hypothesis for the lower pCR rates in luminal B BC in comparison to TNBC is the less inflamed TME, characterized by a lower presence of TILs and lower PD-L1 expression.[35 36] In TNBC and HER2+BC, increased TIL levels are associated with response to chemotherapy and immunotherapy, and with increased survival, whereas in luminal BC the prognostic value of increased TIL levels is not fully established.[11] However, selected luminal B BC can be highly proliferative tumors with higher TILs, for which ICB could be effective.[37] Several innovative approaches aiming to increase the efficacy of ICB in immune cold tumors by priming the immune response and enhancing the conversion to a more inflamed state using specialized agents are in development.[38 39] In the Neo-CheckRay trial, RT in combination with an adenosine targeting agent was used to elicit an immune response and was delivered with an ICB and NACT in early luminal B BC.

The accuracy of pCR as a surrogate of long-term outcomes is less well established in patients with luminal BC compared with HER2-positive and TNBC.[6 40] In a recent pooled analysis assessing the value of RCB in patients treated with NACT (without immunotherapy), it was demonstrated that, in patients with luminal phenotype, those with RCB 0 or 1 had similar EFS, whereas in patients with HER2-positive and TNBC, patients achieving RCB-0 (pCR) had a significantly increased EFS compared with patients with residual disease of any extent.[41] In luminal BC, data from the neoadjuvant I-SPY2 trial demonstrated that the correlation between the pathological response at surgery and long-term outcomes appears to be more accurate in patients with a higher MammaPrint risk score.[42 43] Furthermore, in the era of neoadjuvant immunotherapy combined with NACT, the correlation between non-pCR and unfavorable long-term outcome is less obvious. In the KEYNOTE-522 trial evaluating the addition of pembrolizumab to NACT in patients with early TNBC,[1] patients with residual disease had better outcomes when treated with pembrolizumab than placebo whereas patients achieving a pCR had an excellent 3-year EFS regardless of the treatment arm. In the phase II GeparNUEVO study, the addition of durvalumab to NACT in early TNBC resulted in a nonsignificant improvement in pCR, but in a significant improvement in the 3-year DFS and OS.[2] These observations suggest that the addition of ICB in the neoadjuvant setting may present a long-term benefit, even without an increase in pCR. In luminal BC, insufficient data are currently available to assess whether or not ICB can increase EFS in case of residual disease. The ongoing phase III KEYNOTE-756 trial evaluating the addition of pembrolizumab to NACT in patients with high-risk luminal BC adopted pCR and

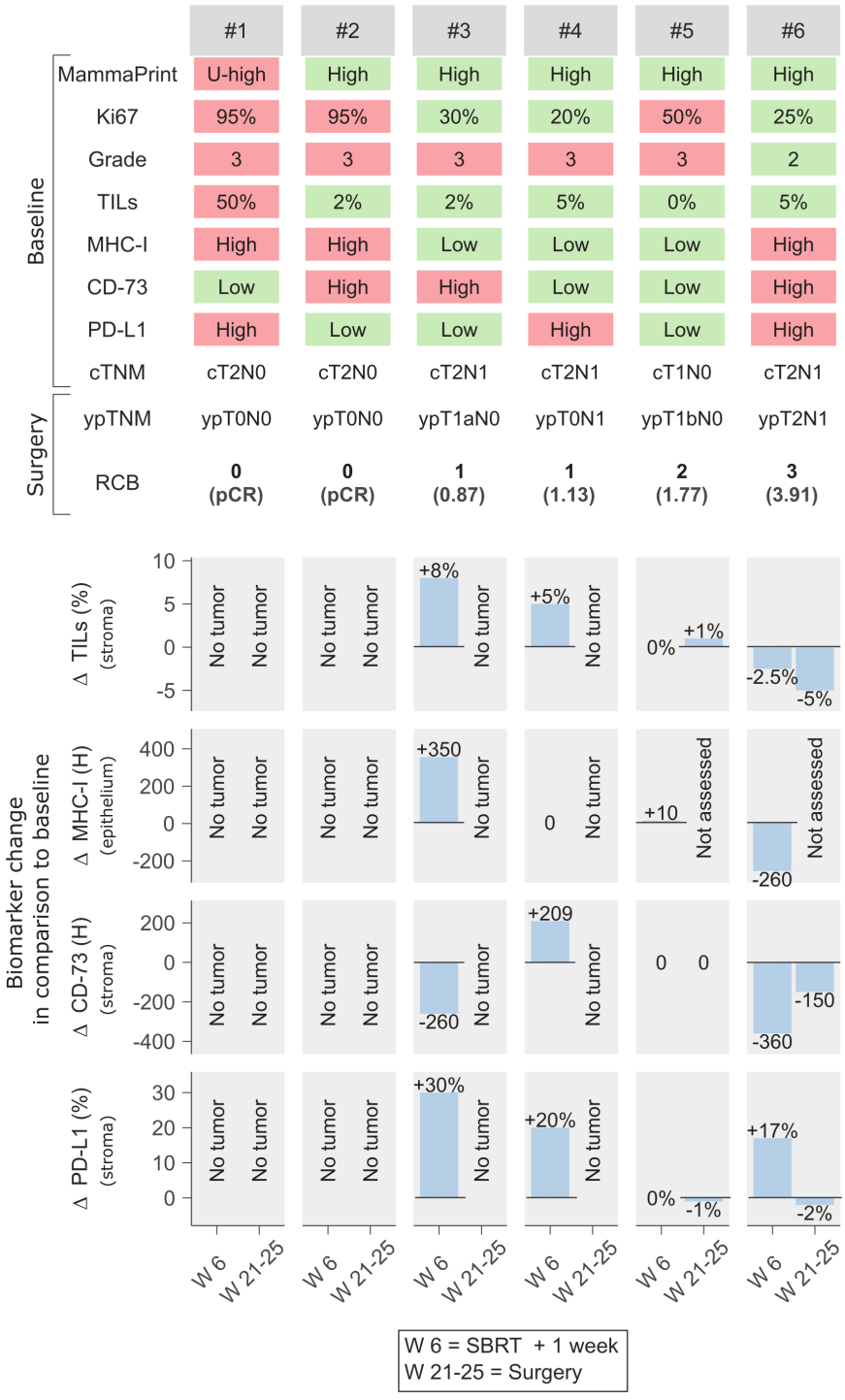

Figure 2 Overview of baseline biomarkers and comparisons with baseline at two on-study timepoints: 1 week after stereotactic body radiation therapy (SBRT; week 6) and at surgery (weeks 21–25). Results at surgery were measured using ypTNM and the residual cancer burden (RCB) score. The categorical RCB score is provided in bold, and the continuous RCB score is shown between parentheses, unless in case of RCB 0 for which pathological complete response (pCR) is indicated. RCB is calculated as a continuous index combining pathological measurements of the primary tumor (size and cellularity) and lymph node metastases (number and size). TIL levels, CD73 and PD-L1 expression were measured in the stroma; major histocompatibility complex (MHC)-I expression was estimated in the epithelial compartment. Epithelial expression of PD-L1 and CD73, and stromal expression of MHC-I, at the different timepoints, can be found in the online supplemental figure S5. MHC-I and CD73 results are shown using the H-score (H). Baseline MHC-I, CD73 and PD-L1 'high' were defined as measurements above their median. The delta in biomarker expression between baseline—week 6 and between baseline—weeks 21–25 was calculated by subtracting the baseline value from the week 6 and weeks 21–25 expression. Details on the biomarker expression measurements and definition of the H-score are available in the Materials and Methods section. CD73, cluster of differentiation 73; H, H-score; PD-L1, programmed death-ligand 1; TILs, tumor-infiltrating lymphocytes; U-high, ultra-high; W 6, week 6; W 21–25, weeks 21 to 25.

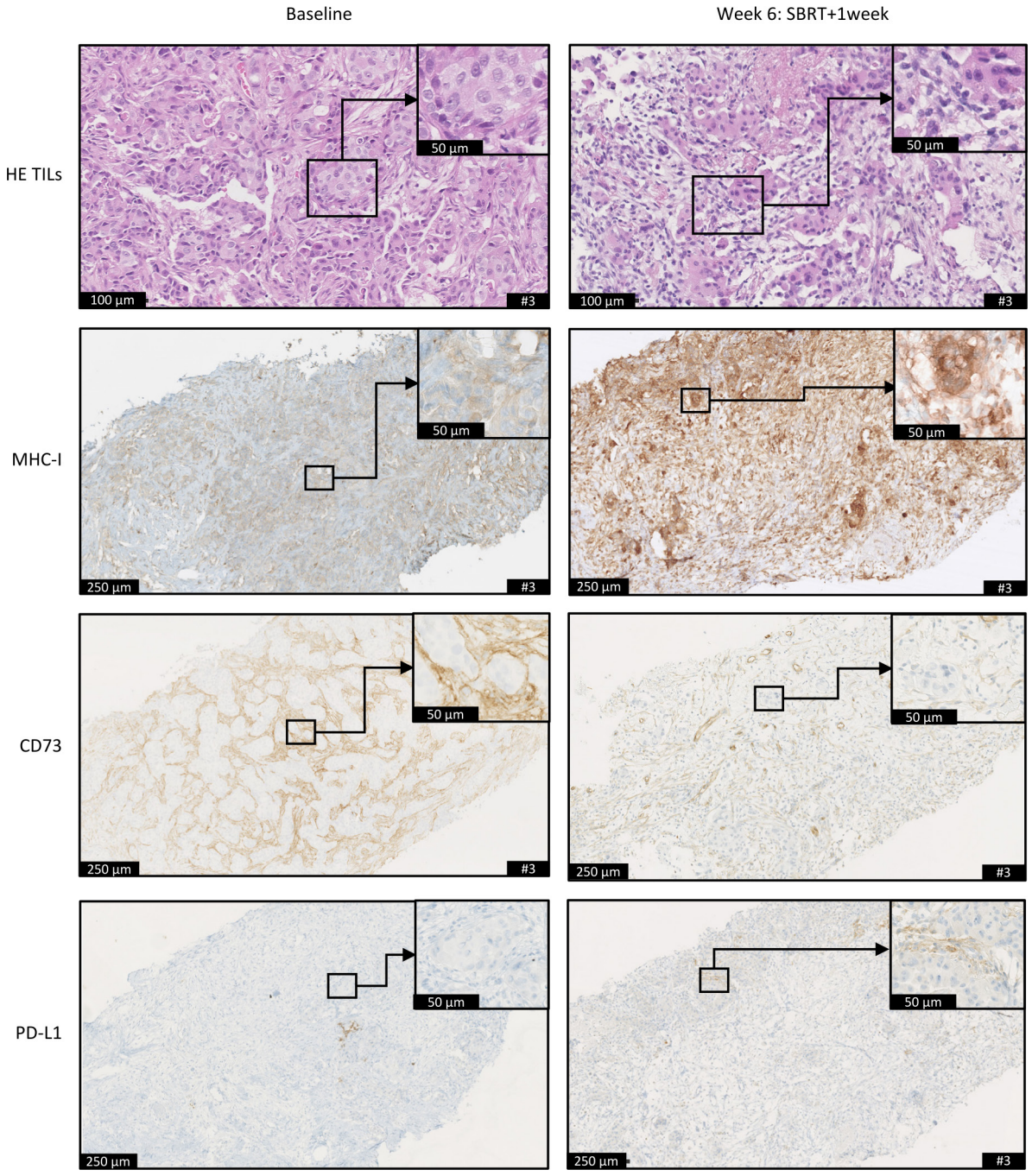

Figure 3 representative examples of H&E, MHC-I, CD73 and PD-L1 staining at baseline and 1 week after SBRT (week 6). All samples originate from patient #3. In this example, comparison of H&E slides between baseline and week 6 showed an increase in TILs and a reduction in tumor cellularity. Comparison of MCH-I and PD-L1 positivity show an increase in the biomarker expressions between baseline and week 6, whereas for CD73 a decrease is seen. CD73, cluster of differentiation 73; MHC-I, major histocompatibility complex type I; PD-L1, programmed death-ligand 1; SBRT, stereotactic body radiation therapy.

EFS as dual primary endpoints and its results will probably help to answer questions regarding the relationship between pCR and EFS in luminal BC.

RT has been shown to induce T cell priming via antigenic release and by activation of the innate immunity.[44 45] Pivotal preclinical work showed that a fractionation schedule of three fractions of 8 Gy given daily induces a better activation of the cGAS-STING pathway in comparison to treatments with a higher dose per fraction.[14] Clinical trials, however, evaluating the 3×8 Gy regimen in the metastatic setting were largely disappointing and yielded negative results.[46–48] RT was typically administered to 1–3 metastases whereas the remaining metastatic load was not treated with RT. In the neoadjuvant setting, the situation is fundamentally different with RT being exclusively directed towards the primary cancer. Clinical results in this setting have been more encouraging,[16 49] with emerging signs for an abscopal effect in involved lymph nodes when RT is delivered to the primary cancer.[17] As such,

the use of RT to prime the immune response is not a one-size-fits-all solution, and its use and fractionation should be carefully tailored to the primary cancer and the disease stage.[50] For luminal BC, preclinical models capturing key immunobiological features of human ER+BC reveal RT to be highly effective in ICB-resistant ER+BC. RT can change disease progression patterns by improving local and distant tumor control when given concomitantly with ICB.[12 51]

Sparing the draining lymph nodes when delivering RT to the primary cancer in combination with immunotherapy may be important to achieve favorable long-term results.[52] This lymph node-sparing approach is applied in the Neo-CheckRay trial by delineating the draining lymph node areas (including the involved lymph nodes) and avoiding them from SBRT delivery to reduce incidental nodal radiation dose.[53] By consequence, the nodal response in node-positive patients is an important secondary endpoint of the Neo-CheckRay trial because it enables the measurement of the tumoral response outside the irradiated volume, hence being an indicator of the systemic response to treatment.

Importantly, the effect of RT is not only immunogenic as RT can at the same time induce immunosuppressive changes, underscoring the need of investigating approaches that shift the overall RT effect from immunosuppressive to proimmunogenic.[54] Oleclumab is a human immunoglobulin monoclonal antibody that selectively binds to and inhibits the ectonucleotidase activity of CD73, thereby inhibiting the conversion of proinflammatory ATP into immunosuppressive adenosine.[55] In preclinical models, RT has been shown to upregulate CD73 expression on epithelial cells and to increase adenosine levels in the TME. RT might therefore act synergistically with anti-CD73 through the prevention of adenosine-mediated immunosuppression[20] and CD73 blockade in combination with RT and ICB could improve response rates.[56–58] In addition to its role as radiation-induced checkpoint inhibitor, targeting CD73 could possibly attenuate the late side effects of RT through inhibition of radiation induced fibrosis.[59 60]

Neoadjuvant treatment followed by complete surgical resection of the tumor allows comparisons of tumor biopsies at multiple time points with the surgical specimen. In the Neo-CheckRay trial, core biopsies of the primary tumor are performed at baseline, 1 week after SBRT (week 6) and tumor samples are also available from surgery, enabling analyses of the short-term changes induced by RT–ICB–oleclumab in comparison to baseline and in comparison, to the residual tumor at surgery. Our preliminary analyses on the tumor samples from the safety run-in suggest that RT could increase TILs and MHC-I expression. Further analyses in the larger cohorts of the randomized phase II part of the trial will allow us to gain a better insight on the impact of the SBRT and adenosine pathway inhibition on the TME and the immune response in luminal B BC.

The role of preoperative RT in combination with immunotherapy is under investigation in a few other trials in BC.[61] The '*Pre-operative pembrolizumab with radiation therapy in early stage TNBC*' (PEARL trial, NCT03366844) investigates two cycles of pembrolizumab combined with 3×8 Gy to the primary breast tumor followed by 3 weeks pause before initiating NACT regimen per treating oncologist choice. The RT was not given concomitantly with chemotherapy. The first results after including 50 patients and 1-year follow-up was presented at the 2021 San Antonio Breast Cancer Symposium (not yet published), showing this regimen to be feasible and achieving 74% RCB 0/1.[62] The *'Converting HR+Breast Cancer Into an Individualized Vaccine'* (CBCV) trial (NCT03804944–recruiting) is a 4-arms study consisting of (1) RT 3×8 Gy + hormonal treatment (HT); (2) RT/HT+ pembrolizumab; (3) RT/HT+ CDX-301; (4) RT/HT+ pembrolizumab + CDX-301. The *'Pre-op Pembro+Radiation Therapy in Breast Cancer'* (P-RAD) trial (NCT04443348-recruiting) has as main objective the identification of the optimal dose of preoperative RT when combined with pembrolizumab and chemotherapy. In this trial, node-positive, luminal or TNBC patients will receive neoadjuvant pembrolizumab combined with NACT, and patients will be randomized between 1/no RT, 2/low dose RT boost (3×3 Gy to the primary cancer) and 3/high dose RT boost (3×8 Gy). The coprimary endpoints of this trial are the level of TILs and the rate of pCR in the lymph nodes.

In conclusion, the Neo-CheckRay first-in-human safety run-in demonstrates that the combination of NACT, ICB, oleclumab and SBRT 3×8 Gy to the primary tumor is feasible with encouraging results at surgery and with a manageable toxicity profile in early luminal B BC. This novel combination is under further investigation in an ongoing phase II trial to evaluate its efficacy.

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Contributors ADC is the study chair and acts as the guarantor of the trial. ADC has full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish. Clinical trial conceptualization and design: ADC, ER, LB, MI, MP and PP. Clinical trial development and conduct: ADC. Writing original draft of the manuscript: ADC and LB. Reviewing and editing of the manuscript: AD, CP, CS, DVG, ER, MI, MP and PP. Clinical investigators: AG, IV, MI and LB. Medical monitoring: EA and GNM (research medical fellows). Study medical physicist: CV. Study pathologists: DL and RS. Study radiologist: SD. Study radiation oncologist: ADC. Study statistician: MPa. Translational research analyses: ADC, CS, LB, LC, RS and ZD. All the authors approved the final version of this manuscript.

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