Supplementary Material

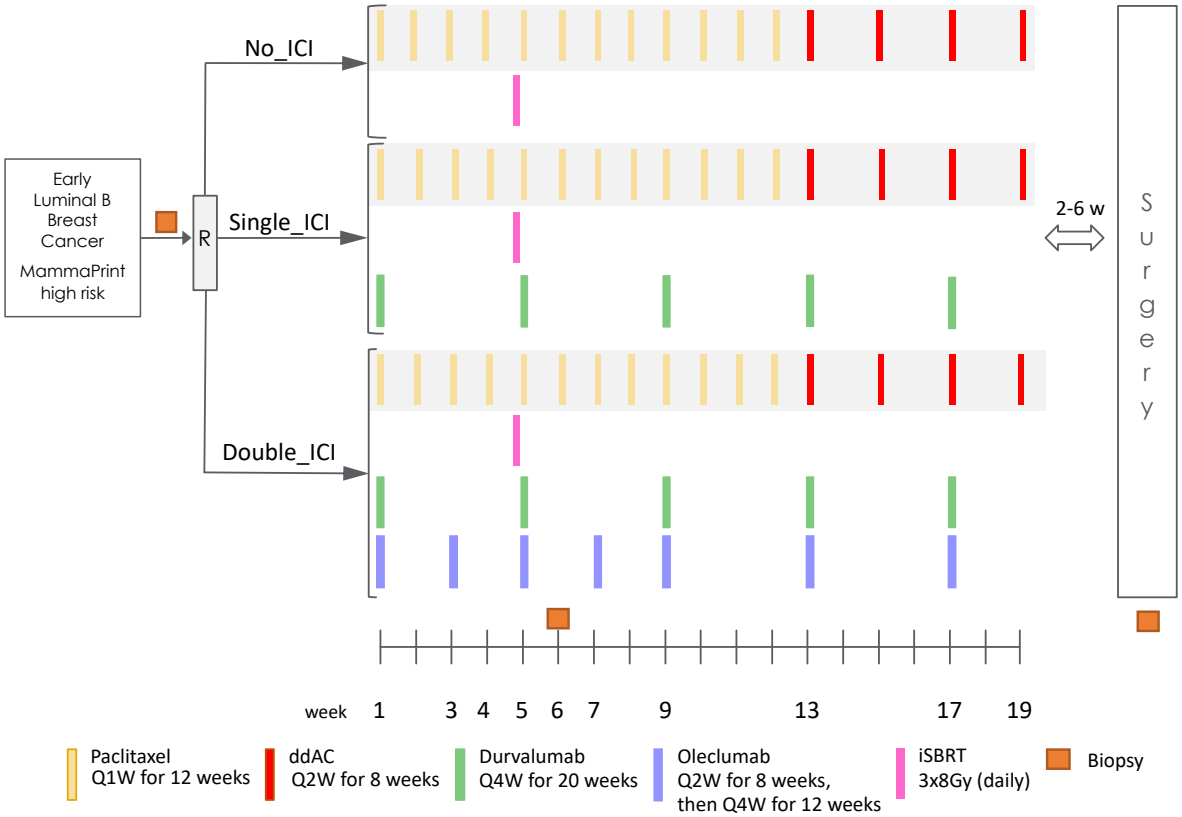
Neoadjuvant immune-modulating SBRT and anti-CD73 to increase response to anti-PD-L1 and chemotherapy in early ER+/HER2- breast cancer: primary endpoint and translational results from the randomized Neo-CheckRay trial.

De Caluwé A. et al.

July 2025

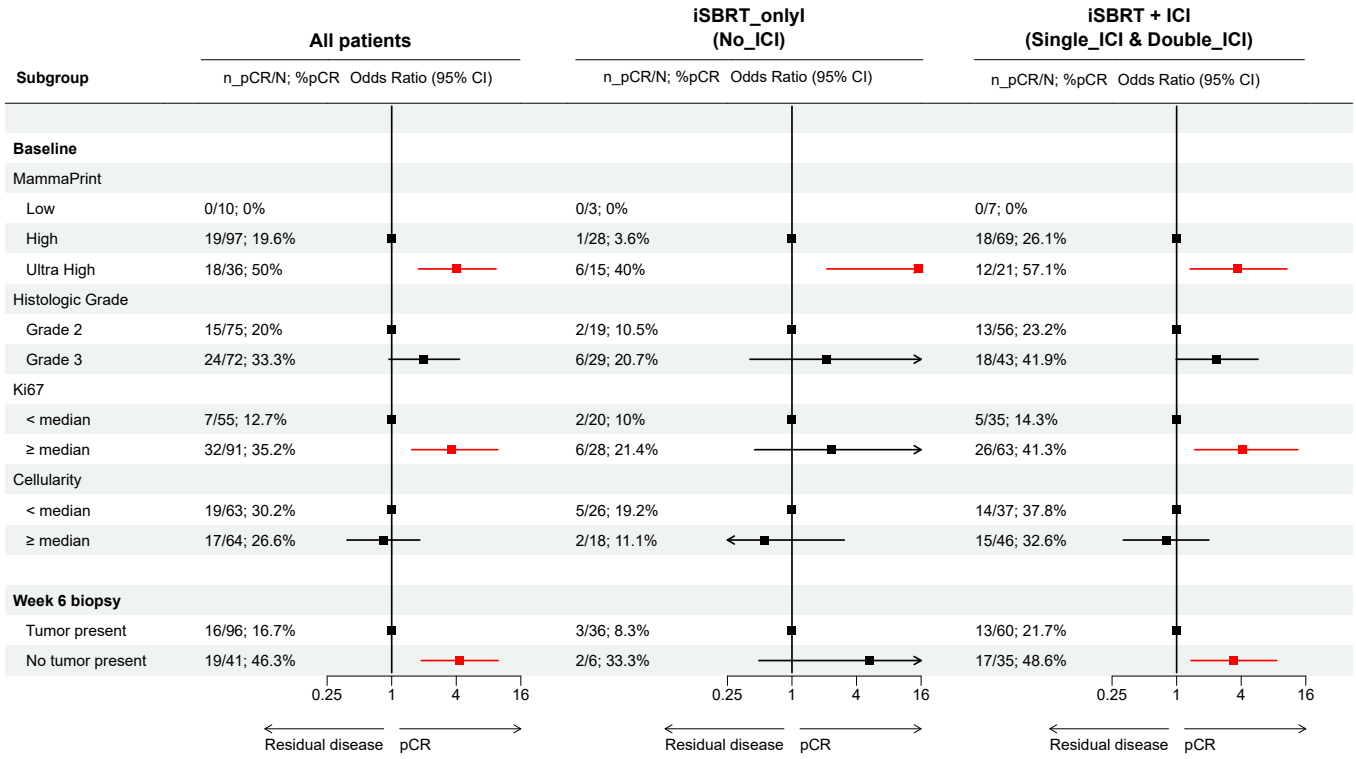
This document contains the supplementary figures and the detailed inclusion/exclusion criteria referenced in the main manuscript.

Supp. Fig. 1 | Trial design.



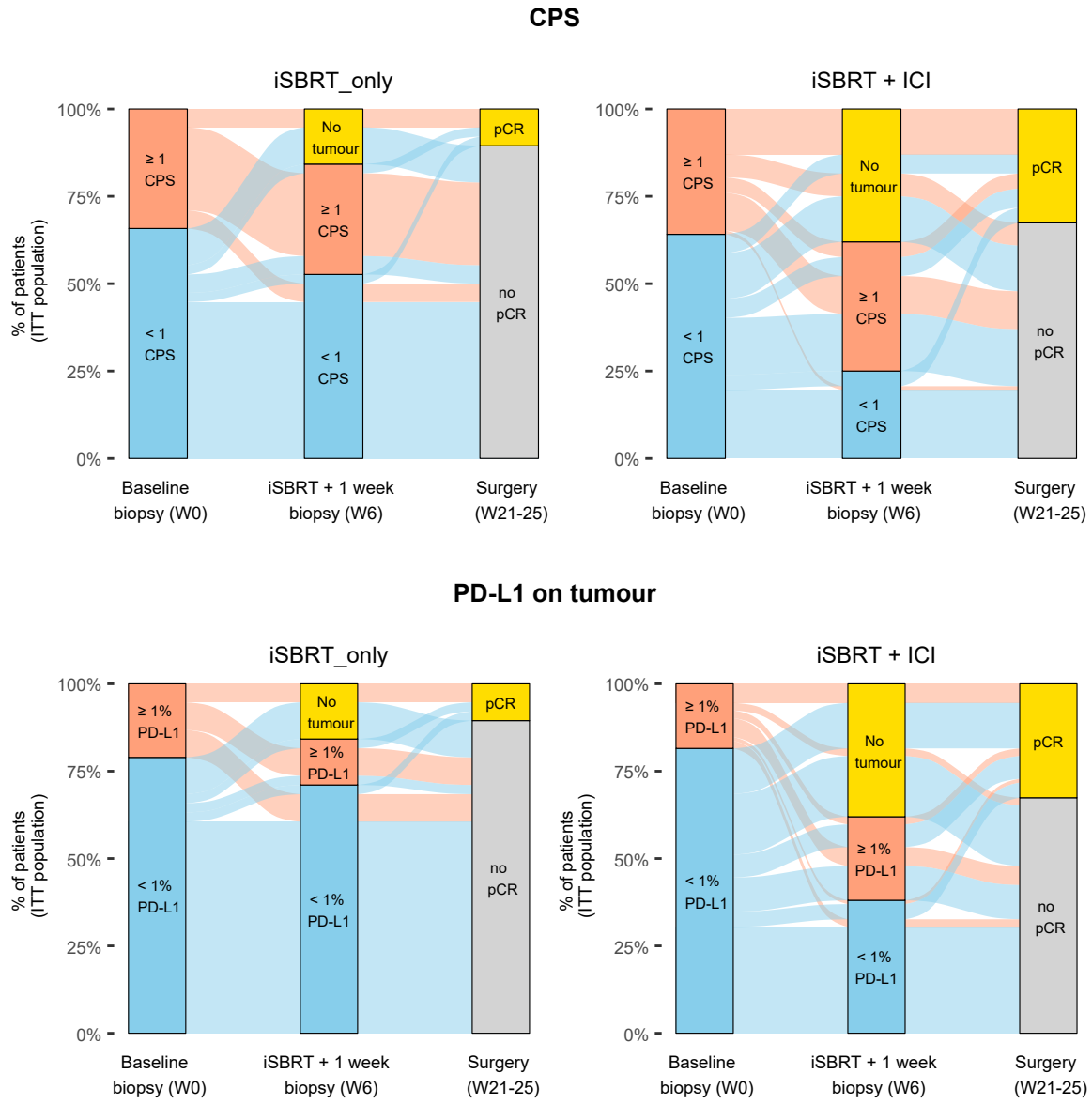
Supp. Fig. 1 | Trial design. Pre-operative systemic treatment consisted of q1w paclitaxel 80 mg/m² IV for 12 administrations (12 weeks) followed by q2w dose-dense doxorubicin-cyclophosphamide (ddAC) IV (60 mg/m² and 600 mg/m² respectively) for 4 administrations; the anti-PD-L1 antibody durvalumab 1500 mg IV q4w for 5 administrations and the anti-CD73 antibody oleclumab 3000 mg IV q2w for 4 administrations followed by q4w for 3 administrations. iSBRT was delivered daily immediately before the week 5 systemic treatment at a dose of 3 fractions of 8 Gy. The study design was previously published in De Caluwe et al, BMC Cancer, 2021. *Abbreviations*: iSBRT, immune-modulating stereotactic body radiation therapy; w, weeks.

Supp. Fig. 2 | Exploratory analysis of biomarker associations with pCR, presented as odds ratios in the ITT population (n=147).



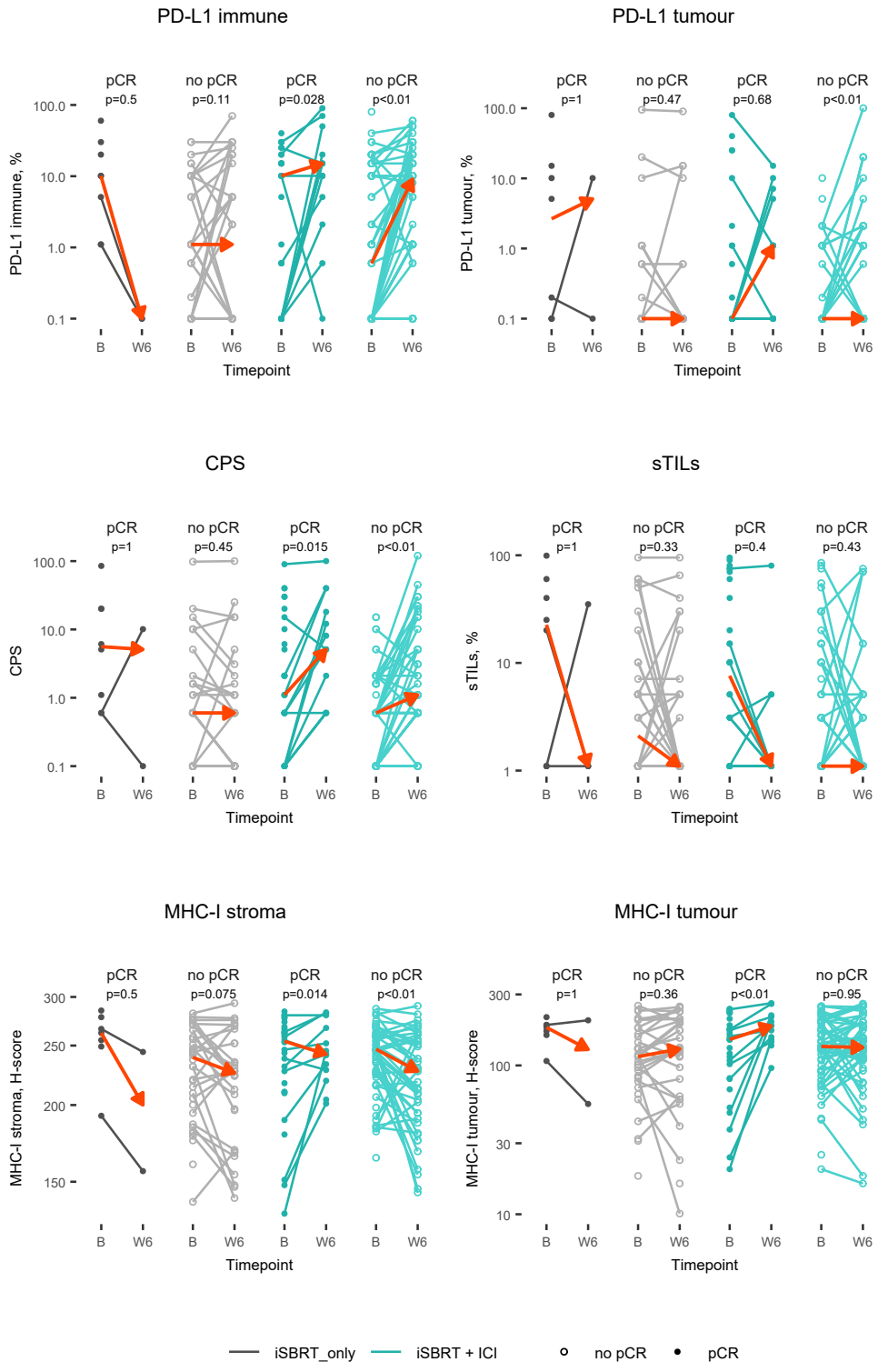
Supp. Fig. 2 | Exploratory analysis of biomarker associations with pCR, presented as odds ratios in the ITT population (n=147). *Abbreviations*: ITT, intention-to-treat; iSBRT, immune-modulating stereotactic body radiation therapy; OR, odds ratio; pCR, pathological complete response.

Supp. Fig. 3 | Biomarkers dynamics between week 6 and baseline: CPS and PD-L1 on tumour (ITT population, n=147).



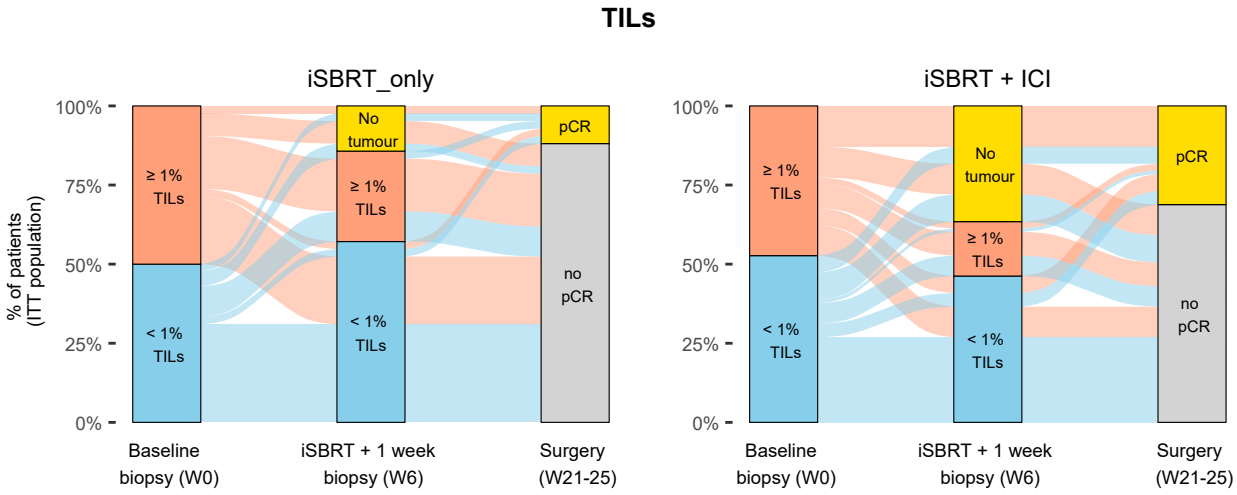
Supp. Fig. 3 | Biomarkers dynamics between week 6 and baseline: CPS and PD-L1 on tumour (ITT population, n=147). The CPS was calculated by dividing the number of PD-L1–positive cells (tumour cells, lymphocytes, macrophages) by the total number of viable tumour cells, then multiplying by 100. PD-L1 on tumour is the percentage of tumoral cells that are positive for PD-L1. iSBRT alone is the No_ICI arm (iSBRT+NACT), and iSBRT + ICI is the Single_ICI and Double_ICI combined. *Abbreviations*: CPS, combined positive score; ITT, intention-to-treat; pCR, pathological complete response.

Supp. Fig. 4 | Biomarkers dynamics between baseline and week 6 (ITT population, n=147).



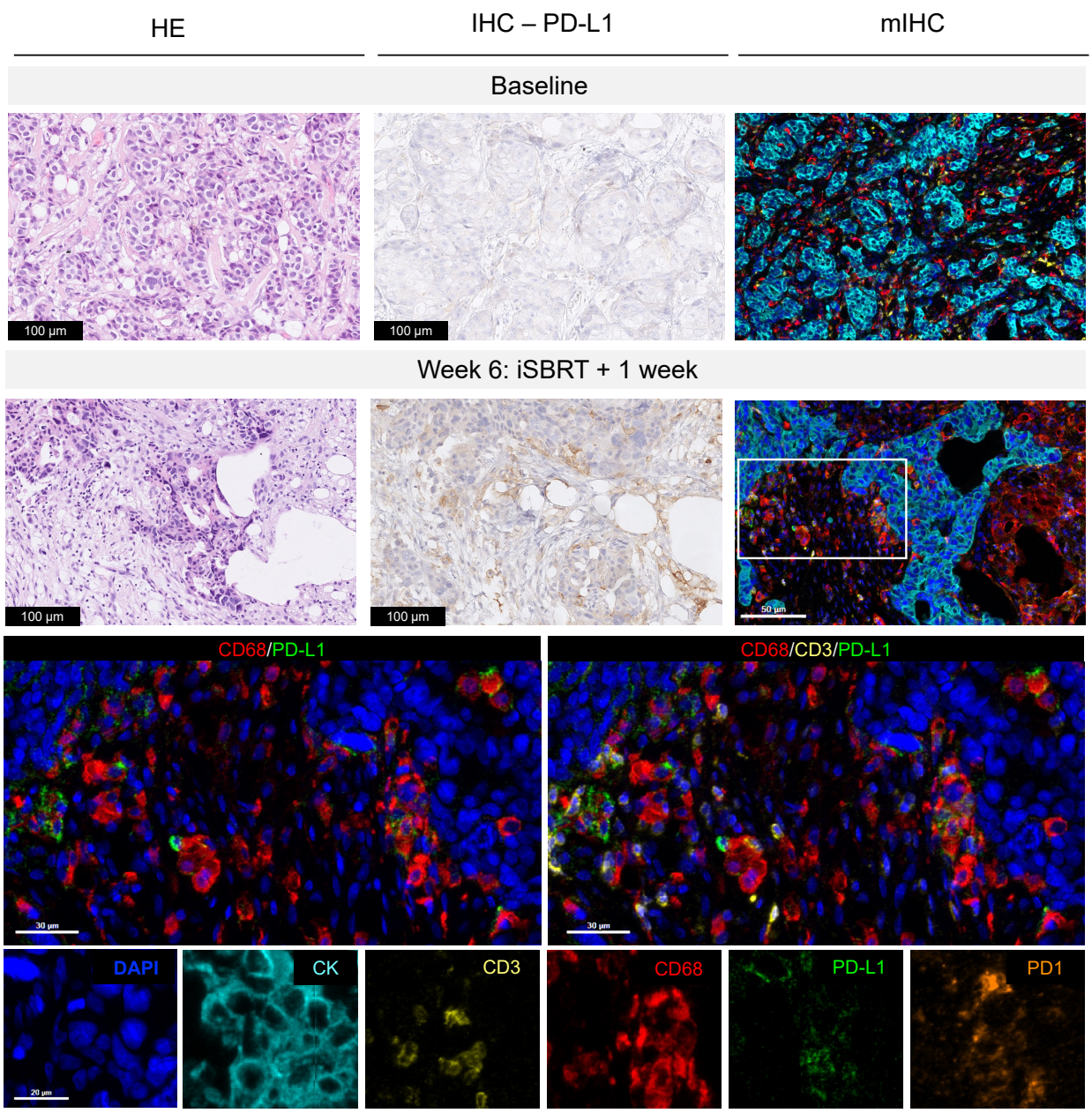
Supp. Fig. 4 | Biomarkers dynamics between baseline and week 6 (ITT population, n=147). Early biomarker dynamics between baseline and week 6 biopsy (1 week after iSBRT).) The red arrow depicts the change in the median between baseline and week 6. P-values were calculated using the Wilcoxon Signed-Rank Test, with paired samples originating from the same patient. *Abbreviations*: CI, confidence interval; ICI, immune checkpoint inhibitor; iSBRT, immune-modulationg stereotactic body radiation therapy; NACT, neo-adjuvant chemotherapy; pCR, pathological complete response

Supp. Fig. 5 | Exploratory biomarker dynamics: TILs (ITT population, n=147).



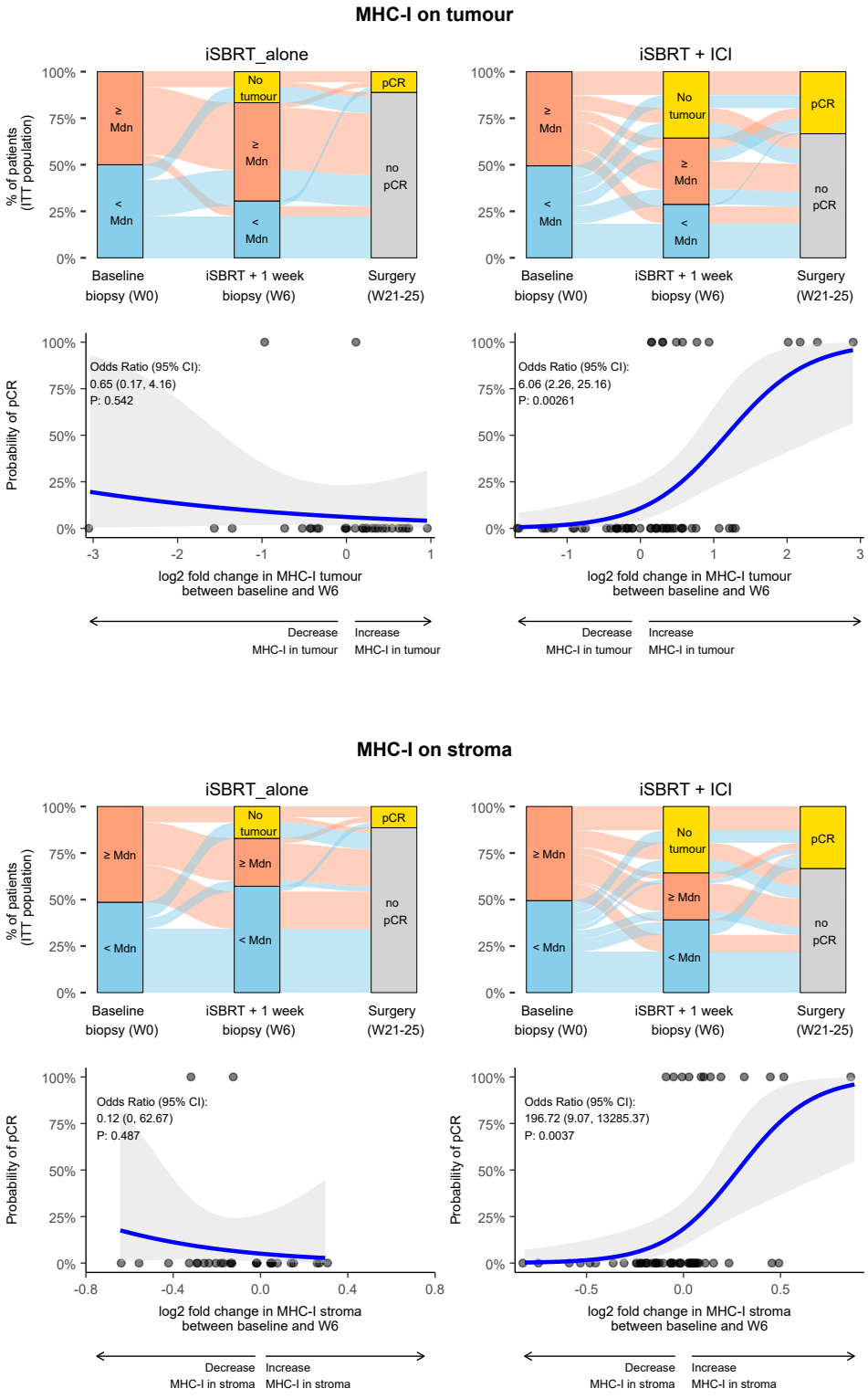
Supp. Fig. 5 | Exploratory biomarker dynamics: TILs (ITT population, n=147). The percentage of TILs was quantified on haematoxylin and eosin-stained slides according to the recommendations of the International TILs Working Group. iSBRT without ICI is the No_ICI arm (iSBRT+NACT), and iSBRT with ICI is the Single_ICI (No_ICI+durvalumab) and Double_ICI combined (No_ICI + durvalumab + oleclumab). *Abbreviations*: TILs, tumor infiltrating lymphocyes; ITT, intention-to-treat; pCR, pathological complete response.

Supp. Fig. 6 | Example of Multiplex IHC comparing baseline and week 6 (iSBRT + 1 week).



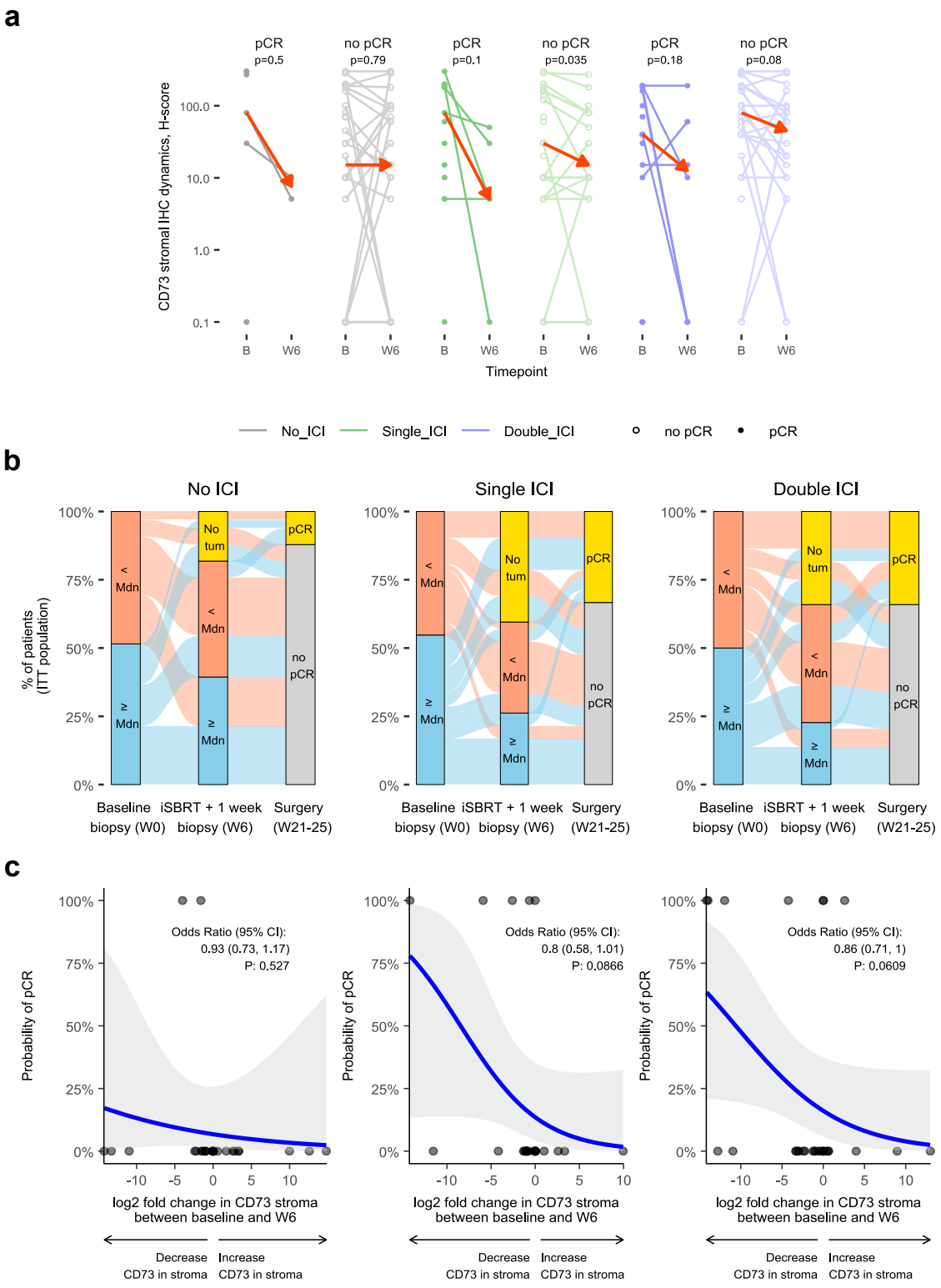
Supp. Fig. 6 | Example of Multiplex IHC comparing baseline and week 6 (iSBRT + 1 week). In this example, the baseline and iSBRT + 1 week samples provide from the same patient. Multiplex images in the third row are magnified views of the white inset shown in the second row. The multplex imaging demonstrates that the PD-L1 expression at week 6 is primarily localized to the CD68 positive macrophages. *Abbreviations*: CD, cluster of differentiation; DAPI, 4′,6-diamidino-2-phenylindole; HE, haematoxylin and eosin; IHC, immunohistochemistry; iSBRT, immune-modulating stereotactic body radiation therapy; mIHC, multiplex immunohistochemistry; PD-(L)1, programmed death (ligand) 1.

Supp. Fig. 7 | Exploratory biomarker dynamics: MHC-I on tumour and on stroma (ITT population, n=147).



Supp. Fig. 7 | Exploratory biomarker dynamics: MHC-I on tumour and on stroma (ITT population, n=147). Alluvial showing the dynamic changes in MHC-I on tumour and on stroma between baseline, iSBRT + 1 week (week 6) and at surgery. MHC-I was assessed with an H-score on the tumour and on the stroma separately. H-scores at baseline were separated in two groups: < median (< Mdn) and ≥ the median (≥ Mdn). The median value at baseline was kept to separate the H-score at week 6. Logistic regression analysis assessed the correlation between the H-score and pCR. *Abbreviations*: iSBRT, immuno-modulating stereotactic radiation therapy; Mdn, median; MHC-I, major histocompatbility cluster I, pCR, pathologic complete response, W6, week 6.

Supp. Fig. 8 | Exploratory biomarker dynamics: CD73 (ITT population, n=147).



Supp. Fig. 8 | Exploratory biomarker dynamics: CD73 (ITT population, n=147). **a**, Change in expression of stroma CD73 (H-score) between baseline (B) and iSBRT + 1 week (week 6, W6). The red arrow depicts the change in the median between baseline and week 6. P-values were calculated using the Wilcoxon Signed-Rank Test, with paired samples originating from the same patient. **b**, Alluvial showing the changes in stromal CD73 experssion (H-score) between baseline, iSBRT + 1 week (week 6) and at surgery. H-scores at baseline were separated in two groups: < median (< Mdn) and ≥ the median (≥ Mdn). The median value at baseline was used to separate the H-score at week 6. **c**, Logistic regression analysis assessed the correlation between the H-score and pCR. *Abbreviations*: iSBRT, immuno-modulating stereotactic radiation therapy; Mdn, median; pCR, pathologic complete response, W6, week 6.

Supp. Fig. 9 | Collected and analyzed tumour samples on pre-treatment (baseline) and post-treatment (week 6) for the exploratory analyses. *Abbreviations:* MP; MammaPrint, MA; MicroArray.

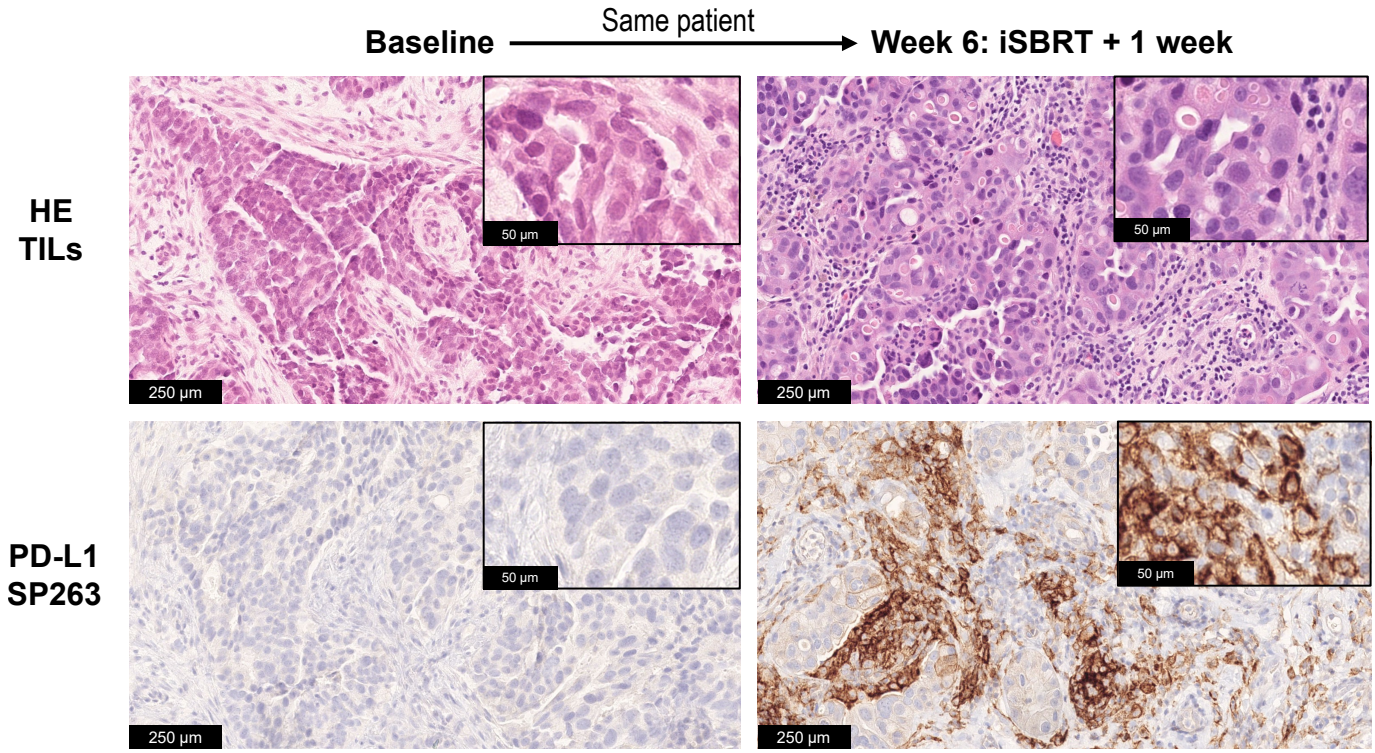
Biomarkers analyses in the overall population (n=147)

	NACT-iSBRT ("No_IO") **48** allocated to ITT							NACT-iSBRT-durvalumab ("Single_IO") **51** allocated to ITT							NACT-iSBRT-durvalumab-oleclumab ("Double_IO") **48** allocated to ITT						
	TILs	**PD-L1**	**Ki67**	**MHC-I**	**CD73**	**MP**	**MA**	**TILs**	**PD-L1**	**Ki67**	**MHC-I**	**CD73**	**MP**	**MA**	**TILs**	**PD-L1**	**Ki67**	**MHC-I**	**CD73**	**MP**	**MA**
Pre-treatment (Baseline)																					
No biopsy available/Not done	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Insufficent material or no tumor on slide	-	-	-	5	5	2	2	2	-	1	5	5	2	2	-	-	-	5	5	0	0
Failed staining	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Evaluable	**48/48**	**48/48**	**48/48**	**43/48**	**43/48**	**46/48**	**46/48**	**49/51**	**51/51**	**50/51**	**46/51**	**46/51**	**49/51**	**49/51**	**48/48**	**48/48**	**48/48**	**43/48**	**43/48**	**48/48**	**48/48**
On-treatment (Week 6)																					
Biopsy unavailable or biopsy not performed	6	6	6	6	6	-	-	2	2	2	2	2	-	-	2	2	2	2	2	-	-
Representative tissue biopsy, absence of tumour cells	6	6	6	6	6	-	-	19	19	19	19	19	-	-	16	16	16	16	16	-	-
Insufficient tissue available for biomarker testing	-	2	3	4	6	-	-	-	2	3	3	4	-	-	-	-	-	-	-	-	-
Failed staining	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-
Evaluable	**36/48**	**32/48**	**32/48**	**32/48**	**30/48**	**-**	**-**	**30/51**	**28/51**	**27/51**	**27/51**	**26/51**	**-**	**-**	**30/48**	**29/48**	**29/48**	**30/48**	**30/48**	**-**	**-**

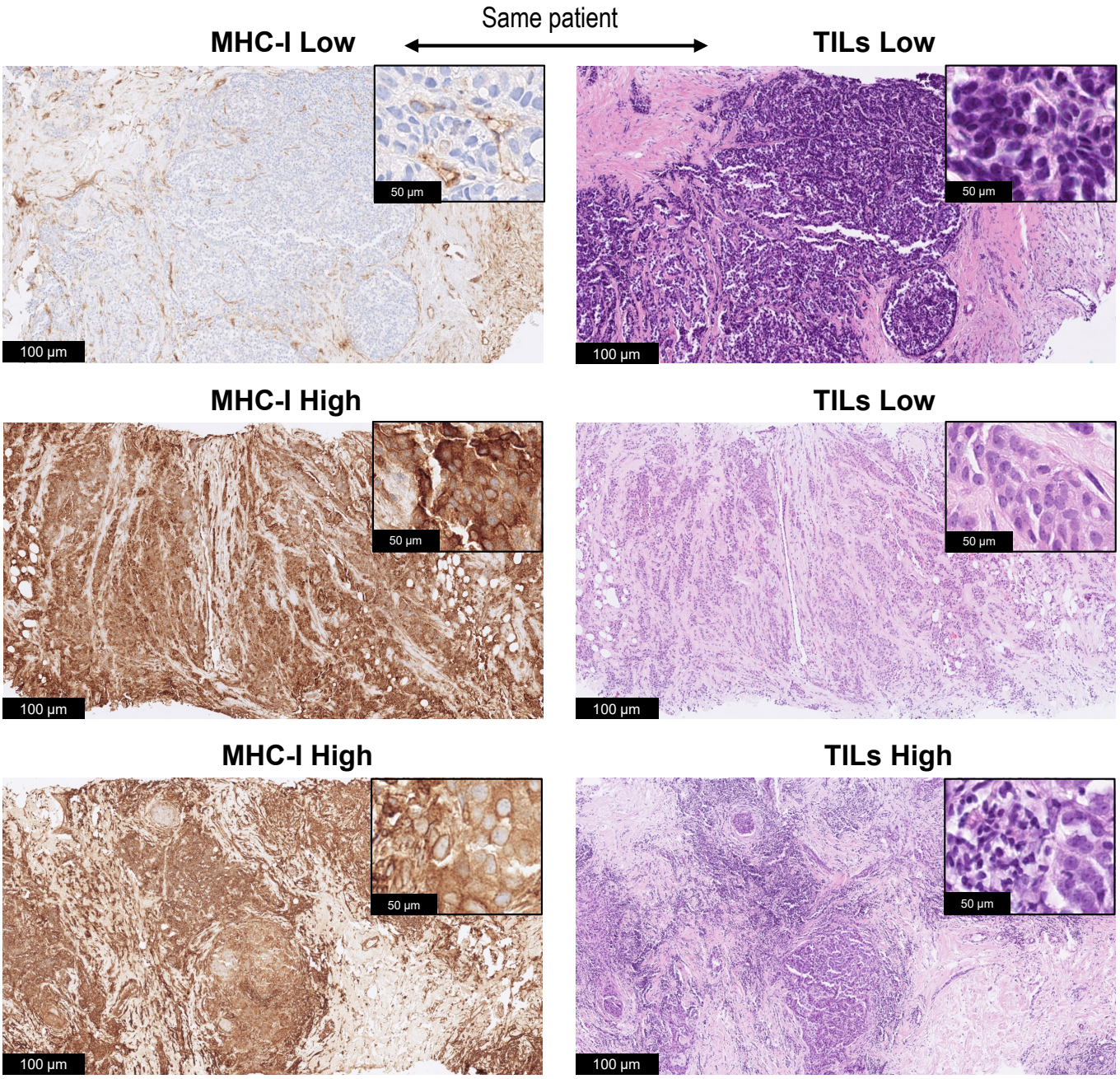
Supp. Fig. 10 | List of antibodies used for IHC in the exploratory biomarker analyses (Ventana Benchmark platform).

Target	Clone	Isotype	Company	Catalog	Dilution	Antigen Retrieval	Incubation Conditions	Amplification	Detection Kit
PD-L1	SP263	Rabbit monoclonal (IgG)	Ventana Roche	790-4905	Ready to use	Tris/Borate/ EDTA buffer pH 8,4	16 min at 36°C	No	OptiView DAB
Ki67	30-9	Rabbit monoclonal (IgG)	Ventana Roche	790-4286	Ready to use	Tris/Borate/ EDTA buffer pH 8,5	16 min at 36°C	No	UltraView DAB
CD73	D7F9A	Rabbit monoclonal (IgG)	Cell Signaling	13160T	1:400	Citrate buffer pH 6	32 min at 36°C	Yes	UltraView DAB
MHC-I	EMR8-5	Rabbit monoclonal (IgG)	Abcam	ab70328	1:1000	Citrate buffer pH 7	32 min at 36°C	Yes	UltraView DAB

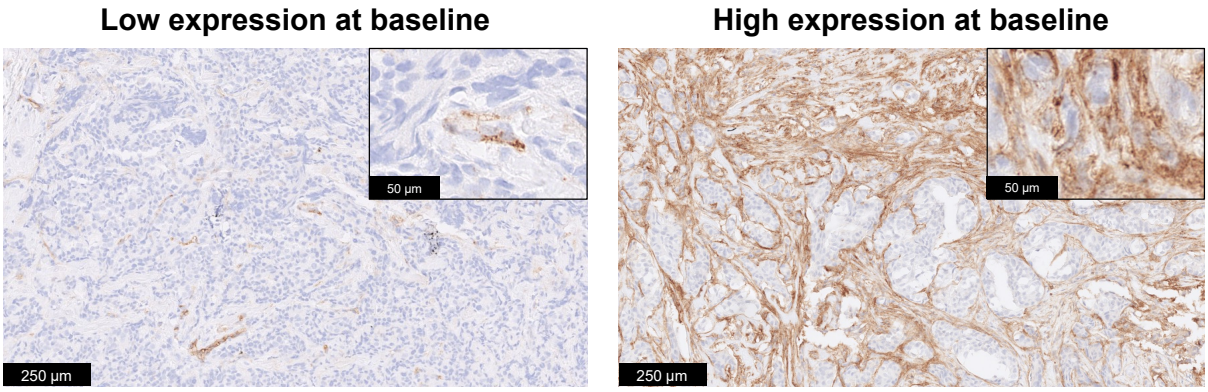
Supp. Fig. 11 | Examples of H&E and PD-L1 IHC at baseline and 1 week after iSBRT.



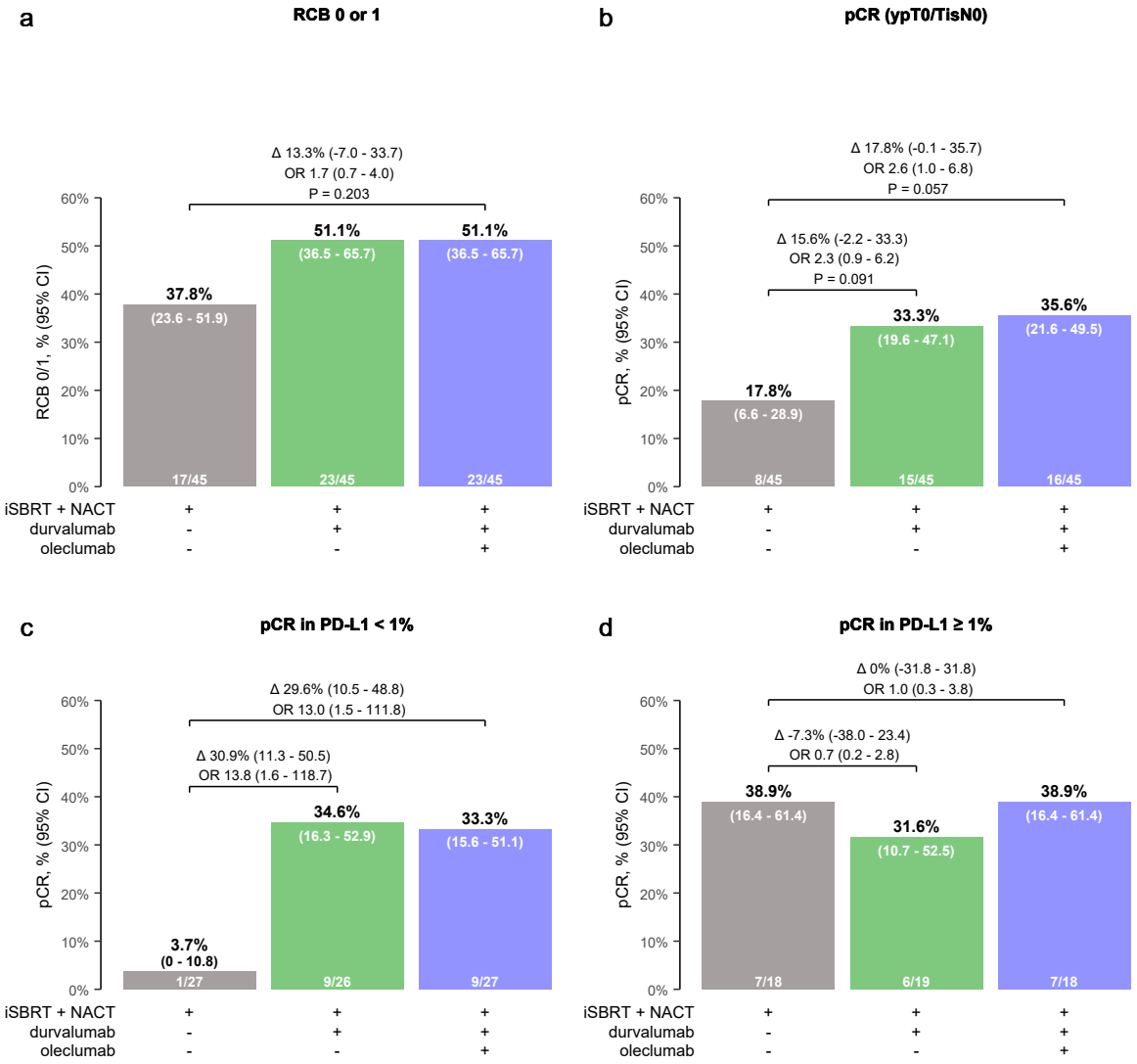
Supp. Fig. 12 | Examples of different H-scores for MHC-I with its corresponding H&E.



Supp. Fig. 13 | Examples of different H-scores for CD73.



Supp. Fig. 14 | RCB and pCR in the population excluding MammaPrint low risk patients and excluding patients without result at surgery (sensitivity analysis).



Supp. Fig. 14 | RCB and pCR in the population excluding MammaPrint low risk patients and excluding patients without result at surgery (sensitivity analysis). This population includes MammaPrint status Unknown and MammaPrint High Risk, however excludes patients without known result at surgery. The PD-L1 IC (immune cell) score was prospectively and centrally determined by the proportion of PD-L1-expressing immune cells, with 1% cutoff, assessed using the VENTANA SP263 IHC assay. **a**, bar plots depicting RCB 0/1, and **b**, pCR rates in the overall and by subgroups defined by **c**, PD-L1 negative and **d**, PD-L1 positive. *Abbreviations*: iSBRT, immune-modulating stereotactic body radiation therapy; pCR, pathological complete response; PD-L1, programmed death ligand receptor 1; RCB, residual cancer burden.

Detailed inclusion and exclusion criteria

Inclusion criteria

Subjects must meet all the following criteria to be eligible for this study:

1. Age ≥ 18 years old
2. Female
3. ECOG performance status ≤ 1
4. Weight ≥ 35 kg.
5. Histological diagnosis of invasive breast adenocarcinoma that is estrogen receptor-positive (ER-positive) and HER2- negative, as per the updated American Society of Clinical Oncology (ASCO) - College of American Pathologists (CAP) guidelines and performed according to local testing. In addition, only tumours with Proliferation Index Ki67 ≥ 15% **or** histology grade III are accepted.
6. Agreement to perform new study related biopsies to provide tissue samples.
7. MammaPrint genomic High Risk index according to centralised testing, except for specific conditions as mentioned below. MammaPrint will only be tested for luminal B breast tumours with either Proliferation Index Ki67 ≥ 15% or histology grade III tumours. (Testing to be done during screening period).

MammaPrint result status at time of termination of all other screening procedures:

- *MammaPrint is High Risk*: subject may be randomized.
- *MammaPrint is Low Risk*: subject cannot be randomized.
- *MammaPrint result is not yet known*:

  If the MammaPrint result is not known at time of termination of all other screening procedures, the investigator is allowed to randomize the subject and start study treatment without waiting for the result of the MammaPrint in the following situations:

<table>
<tr><td></td><td></td><td>Age < 50 years</td><td>Age ≥ 50 years</td></tr>
<tr><td rowspan="2">Ki67 ≤ 20%

and grade II</td><td>cN0</td><td colspan="2" rowspan="2">Wait for MammaPrint result</td></tr>
<tr><td>cN+</td></tr>
<tr><td rowspan="2">Ki67 > 20 %

or grade III</td><td>cN0</td><td>Allowed not to wait for MammaPrint result</td><td>Wait for MammaPrint result</td></tr>
<tr><td>cN+</td><td colspan="2">Allowed not to wait for MammaPrint result</td></tr>
</table>

  If the subject is randomized in a situation without known MammaPrint result and the MammaPrint result proves to be Low Risk after randomisation, the subject will stay in the study.

- *MammaPrint result is unevaluable or is technically impossible*:

The sponsor should be contacted as soon as possible to discuss the inclusion of the concerned subject. Under specific medical conditions and breast cancer disease characteristics, the medical team of the sponsor can accept that the site continues the screening process and randomisation of the subject. There will be maximum 5% of non-evaluable MammaPrint results among enrolled patients.

8. Tumour size:
    - If subject is cN0: tumour size ≥ 2 cm, as determined by MRI imaging.
    - If subject is cN1, cN2 or cN3: tumour size ≥ 1.5 cm, as determined by MRI imaging.

    The requirement for an MRI is not applicable in the case of medical contraindications to perform MRI (e.g., obesity or claustrophobia). In this situation, tumour evaluations should be performed by ultrasound.

9. Multifocal, multicentric unilateral or bilateral breast adenocarcinoma tumours are allowed provided that all biopsied foci are ER+/HER2- according to local testing and all foci can receive SBRT treatment within the defined dosimetry constraints. In some cases, a separate biopsy of every focus is not mandatory, but only if every of the following conditions are present:
    - small focal lesion
    - lesion near the main primary cancer from which a biopsy was taken.
    - the investigator and the radiologist consider the lesion to be clearly related to the main primary breast cancer from which a biopsy was taken.
    - the lesion will be removed during the same lumpectomy than the main primary breast cancer.

    For bilateral, multifocal or multicentric disease, the site selected for pre-treatment biopsy should correspond to the site of largest measurable disease meeting eligibility criteria. The location of tumour biopsy site (laterality, quadrant, position from the nipple and type of imaging modality to guide biopsy) should be collected (see Appendix 2).

10. Serum pregnancy test (for subjects of childbearing potential) negative within 2 weeks prior to first dose of study administration.
11. Women of childbearing potential must agree to use 1 highly effective method of contraception (see protocol section 6.9.1) during the screening period, during the study and at least 12 months after the last administration of study treatment. It is strongly recommended for the male partner of a female subject to also use male condom plus spermicide throughout this period.
12. Adequate bone marrow function as defined below:
    - Absolute neutrophil count ≥1500/µL, i.e. $1.5x10^9$/L
    - Haemoglobin ≥ 9.0 g/dL
    - Platelets ≥100000/µL, i.e. $100x10^9$/L
13. Adequate liver function as defined below:
    - Serum total bilirubin ≤ 1.5 x ULN. In case of known Gilbert's syndrome ≤ 3 x UNL is allowed
    - AST (SGOT) ≤ 3.0 x ULN
    - ALT (SGPT) ≤ 3.0 x ULN

14. Adequate renal function as defined below:
    - Creatinine ≤ 1.5 x UNL or eGFR≥40ml/min/1.73m²
15. Adequate coagulant function as defined below:
    - International Normalized Ratio (INR) ≤ 1.5 x ULN
16. Completion of all necessary screening procedures within 28 days prior to randomisation (except if written differently).
17. Willingness to provide tissue and blood samples for immuno-monitoring and translational research activities.
18. Left ventricular ejection fraction (LVEF) ≥ 50%. LVEF performed in routine is accepted if done within 6 months prior to beginning of screening.
19. Signed Informed Consent form (ICF) obtained prior to any study related procedure.

Inclusion criterion for phase II only (all phase II subjects):

20. Tumour sample provided for central PD-L1 IHC assessment. (Testing done during screening period).

Inclusion criterion applicable to FRANCE only (all safety run-in and phase II subjects):

21. Affiliated to the French Social Security System (applicable only to subjects treated in France)

Exclusion criteria

Subjects meeting one of the following criteria are not eligible for this study:

1) Pregnant and/or lactating women.
2) Subject with a significant medical, neuro-psychiatric, substance abuse or surgical condition, currently uncontrolled by treatment, which, in the principal investigator's opinion, may interfere with completion of the study.
3) TNM stage cT4 breast cancer including inflammatory breast cancer.
4) Presence of any distant metastasis.
5) Contra-indication for treatment by paclitaxel, doxorubicin or cyclophosphamide, or known allergy to any tested substances or any excipients (e.g., chemotherapy or immunotherapy formulations). Contra-indication for subjects with known sensitivity to acetaminophen/paracetamol, diphenhydramine, or equivalent antihistamine (this is a contra-indication for treatment with oleclumab).
6) Previously known contra-indication for treatment by radiation therapy such as rare genetic disorders associated with DNA repair disorders such as ataxia-telangiectasia (A-T), Nijmegen Breakage Syndrome (NBS) and Fanconi anaemia.
7) Active or prior documented autoimmune disease (including inflammatory bowel disease, celiac disease, Wegener's granulomatosis) within the past 3 years. NOTE: Subjects with childhood atopy or asthma, vitiligo, alopecia, Grave's disease, Hashimoto's thyroiditis, or psoriasis not requiring systemic treatment (within the past 2 years) are not excluded
8) Prior malignancy active within the previous 5 years, except for localised cancers that are considered to have been cured and in the opinion of the investigator present a low risk for recurrence. Examples include basal or squamous cell skin cancer, superficial bladder cancer, or carcinoma in situ of the cervix or breast.

9) Known history of, or any evidence of active, non-infectious pneumonitis.

10) Active infection including:

- Tuberculosis (TB) (clinical evaluation that includes clinical history, physical examination and radiographic findings, and TB testing in line with local practice)
- Hepatitis B (known positive HBV surface antigen (HBsAg) result). Subjects with a past or resolved HBV infection (defined as the presence of hepatitis B core antibody [anti-HBc] and absence of HBsAg) are eligible.
- Hepatitis C. Subjects positive for hepatitis C (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV RNA.

11) Significant cardiovascular disease, such as New York Heart Association cardiac disease (Class II or greater), myocardial infarction, transient ischemic attack, or stroke within the previous 3 months, unstable arrhythmias, and/or unstable angina.

12) Medical condition requiring current systemic anticoagulation, or a history of congenital hypercoagulable condition. Subjects taking aspirin at doses < 325 mg per day are eligible if prothrombin time is within the institutional range of normal. Use of local anticoagulation for port maintenance is permitted.

13) Subjects with history of venous thrombosis in the past 12 months prior to the scheduled first dose of study treatment (oleclumab).

14) Diabetes mellitus Type 1 or poorly controlled Type 2 diabetes mellitus defined as a screening haemoglobin A1C ≥ 8 % or a fasting plasma glucose ≥ 160 mg/dL (or 8.8 mmol/L)

15) Any live (attenuated) vaccine within 30 days of planned start of study therapy.

16) Prior systemic immunosuppressive medication (excluding corticosteroids) within 30 days of planned start of study therapy.

17) Prior radiation therapy to the ipsilateral breast.

18) Prior immunotherapy, including tumour vaccine, cytokine, anti-CTLA4, PD-1/PD-L1, including durvalumab, blockade or similar agents.

19) Concomitant use of other investigational drugs.

20) Any unresolved toxicity NCI CTCAE Grade ≥2 from previous anticancer therapy except for alopecia, vitiligo, and the laboratory values defined in the inclusion criteria. Subjects with Grade ≥2 neuropathy will be evaluated on a case-by-case basis after consultation with the Study Physician. Subjects with irreversible toxicity not reasonably expected to be exacerbated by treatment with durvalumab or oleclumab may be included only after consultation with the Study Physician.

21) Uncontrolled intercurrent illness, including but not limited to, ongoing or active infection, symptomatic congestive heart failure, uncontrolled hypertension, unstable angina pectoris, cardiac arrhythmia, interstitial lung disease, serious chronic gastrointestinal conditions associated with diarrhoea, or psychiatric illness/social situations that would limit compliance with study requirement, substantially increase risk of incurring AEs or compromise the ability of the subject to give written informed consent.

22) 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.

23) Prior organ transplantation.

24) Subjects with urinary outflow obstruction.

Exclusion criterion applicable to FRANCE only (all safety run-in and phase II subjects):

25) Vulnerable persons according to the article L.1121-6 of the CSP, adults who are the subject of a measure of legal protection or unable to express their consent according to article L.1121-8 of the CSP.