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RAD51-based homologous recombination deficiency is associated with treatment response and survival in early breast cancer

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Advances in breast cancer (BC) therapy are limited by the absence of well-established biomarkers for DNA-damage targeted treatments. We evaluated the predictive and prognostic value of homologous recombination repair (HRR) deficiency (HRD) by RAD51 nuclear foci and stromal tumour-infiltrating lymphocytes (TILs) in early-stage BC patients with suspected germline susceptibility. Among 291 patients, HRD by RAD51 was found in 78.4% of tumours, and 69.8% had low TILs (<30%). In 178 patients treated with neoadjuvant chemotherapy, pathologic complete response (pCR) was higher in those with HRD vs HRR-proficient (HRP) tumours (52.3% vs 36.4%); RAD51 remained independently associated with pCR ($p = 0.03$). Overall survival (OS) favoured HRD, with 5-year OS of 89.2% vs 82.8% in HRP ($p = 0.009$), with stronger evidence in triple-negative TILs-low disease ($p = 0.005$). These findings support RAD51-based HRD assessment as a predictive and prognostic biomarker that may guide treatment decisions in early-stage BC.

Multi-omics analysis has led to a deeper insight into breast cancer (BC) heterogeneity and subsequent drug development[1]. Nevertheless, those advances were not necessarily assisted by predictive and prognostic biomarkers to guide patient selection and stratification, critical for therapy de-escalation and expansion of targeted therapies.

Recent biomarkers in early BC include germline *BRCA1/BRCA2* (gBRCA1/2) mutational status and stromal tumour-infiltrating lymphocytes (TILs)[2,3]. *gBRCA1/2* pathogenic variants, associated with breast and ovarian cancer predisposition, are known to predict sensitivity to certain chemotherapy agents and poly(ADP) ribose polymerase inhibitors (PARPi)[2,4]. Across studies, BC patients carrying a *gBRCA1/2* pathogenic mutation achieve a higher pathologic complete response (pCR) rate to neoadjuvant chemotherapy (vs non-carriers) even in the absence of a platinum agent[4,5]. Furthermore, in the Phase III OlympiA trial a significant overall survival (OS) benefit was observed with 1-year of adjuvant olaparib vs placebo in high-risk HER2 negative early-stage *gBRCA1/2* BC patients (6-year OS = 87.5% vs 83.2%, HR = 0.72; 95% CI 0.56–0.93; $p = 0.009$)[6].

Nonetheless, *gBRCA1/2* testing alone does not capture the full spectrum of homologous recombination deficiency (HRD), which can arise from epigenetic silencing or alterations in other genes within the homologous recombination repair (HRR) pathway, nor recognise the dynamic changes in HRR functionality due to treatment pressure[7]. To address this, we and others have proposed the detection of nuclear RAD51 foci as a more accurate surrogate marker of functional HRD, and, thus, a candidate predictive biomarker of DNA-damaging agents' response, capable of reflecting tumour evolution before or after platinum or PARPi exposure[8–10].

Similarly, TILs have long been recognised as a predictive biomarker of chemotherapy response, with increased pCR rates observed among subjects with TILs high (≥30%) vs low (<30%), particularly in triple negative breast cancer (TNBC)[11,12]. Moreover, a large, pooled analysis on early-stage TNBC

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patients that did not receive any systemic treatment found that each 10% TILs increment was associated with improved distant relapse free survival (HR = 0.87 [0.84–0.90]), and OS (HR = 0.88 [0.85–0.91]), establishing TILs as an independent prognostic biomarker in TNBC[13].

Our study aims to evaluate the prognostic role of RAD51, in combination with TILs, in an HRD-enriched, early-stage BC population comprised of patients with TNBC and/or early-onset BC, or with known *gBRCA1/2* or *PALB2* mutations.

Results

Patients' characteristics

This multicentre, real-world study enrolled 291 early-stage BC patients, with clinical and pathological details summarized in Table 1 and Supplementary Fig. 1. The median age at diagnosis was 45 (26–78) years. Most patients were diagnosed with stage II disease (47.8%, 139/291) and TNBC subtype (70.4%, 205/291). Germline pathogenic variants were found in *BRCA1* (35.7%, 104/291), *BRCA2* (18.9%, 55/291), and *PALB2* (7.2%, 21/291).

Most patients (87%) received (neo)adjuvant chemotherapy; 61.2% (178/291) in the neoadjuvant setting, with 31.6% (91/178) receiving an anthracycline plus taxane neoadjuvant regimen, while 23.7% (69/178) received platinum-containing chemotherapy. In addition, 25.8% (75/291) received adjuvant chemotherapy. Table 1 summarizes the main (neo) adjuvant systemic treatments administered.

Prevalence of HRD by RAD51 score

The RAD51 test was conducted in treatment naïve BC FFPE tumour samples; 78.4% (228/291) exhibited a low RAD51 score (≤10%) and were categorised as HRD, and 21.6% (63/291) exhibited a high RAD51 score (>10%) and were categorised as HRP. Patient characteristics were well balanced between the HRD and HRP groups in terms of age, BC stage and subtype, TILs extent, and prior platinum exposure, except for germline mutational status (Table 1). Figure 1A illustrates the distribution of RAD51 scores according to the germline pathogenic variant. Tumours with HRR gene mutations showed significantly lower RAD51 scores compared to the wild-type (WT) group ($p < 0.001$). The prevalence of HRD by RAD51 was 82.7% in *BRCA1*, 89.1% in *BRCA2* and 95.2% in *PALB2* tumours. In addition, 65.8% of patients without HRR pathogenic variants (WT group) had HRD tumours by RAD51; within this HRD WT subset, 93.2% were TNBC and 6.8% were luminal BC. In the overall patient cohort, the prevalence of HRD in luminal vs TNBC tumours was comparable (79.1% vs 78.0%, respectively; Fig. 1B).

Association of RAD51 status with TILs and pCR

Based on a 30% cut-off, 30.2% (88/291) of tumours were classified as TILs high and 69.8% (203/291) as TILs low. TILs extent was comparable in tumours with HRD and HRP by RAD51 score (TILs high in 30.7% of HRD tumours vs 28.6% of HRP tumours; Table 1). The proportion of tumours with RAD51 low and TILs high was higher in TNBC compared with luminal tumours (30.2 vs 9.3%; $p < 0.001$), consistent with the higher frequency of HRD and TILs high in TNBC vs luminal BC[14,15] (Fig. 1B).

The overall pCR rate in patients receiving neoadjuvant chemotherapy ($n = 178$) was 48.3% (95% CI 40.8–55.9), with rates of 52.3% (95% CI 43.4–61.0) in HRD tumours and 36.4% (95% CI 22.8–52.3) in HRP by RAD51.

In the multivariable analysis, HRD by RAD51 score (RAD51 low), but not TILs, was statistically associated with pCR after adjusting for clinicopathological factors and treatment (OR RAD51 low vs high 2.30, 95% CI 1.08–5.04; $p = 0.03$; Fig. 2A). The ability of RAD51 score to predict pCR was consistent across patient subgroups, regardless of clinical stage or type of neoadjuvant treatment (Fig. 2B).

Association of RAD51 status and TILs with overall survival

At data cutoff with a median follow-up of 5.7 years (95% CI 5.3–6.2), 47 death events (19.5%) were observed with an estimated 5-year OS of 87.8% (95% CI 83.8–91.9).

Table 1 | Patient and tumour characteristics

Characteristics	Overall population ($n = 291$); *n* (%)	RAD51 low ($n = 228$); *n* (%)	RAD51 high ($n = 63$); *n* (%)
Age, years			
Median (range)	45.1 yo (26–78)	45.1 yo (26–75)	44.9 yo (28–78)
Stage			
I	74 (25.4)	59 (25.9)	15 (23.8)
II	139 (47.8)	111 (48.7)	28 (44.4)
III	78 (26.8)	58 (25.4)	20 (31.7)
Histological type			
NST	104 (89.7)	80 (88.9)	24 (92.3)
Lobular	8 (6.9)	7 (7.8)	1 (3.8)
Other histology	4 (3.4)	3 (3.3)	1 (3.8)
Oestrogen receptor status			
Positive	83 (28.5)	65 (28.5)	18 (28.6)
Negative	208 (71.5)	163 (71.5)	45 (71.4)
HER2 status			
Positive	14 (4.8)	11 (4.8)	3 (4.8)
Negative	277 (95.2)	217 (95.2)	30 (95.2)
BC subtypes			
Luminal A	17 (5.8)	12 (5.3)	5 (7.9)
Luminal B	69 (23.7)	56 (24.6)	13 (20.6)
TNBC	205 (70.4)	160 (70.2)	45 (71.4)
Germline HRR mutations			
Wild type	111 (38.1)	73 (32.0)	38 (60.3)
BRCA1	104 (35.7)	86 (37.7)	18 (28.6)
BRCA2	55 (18.9)	49 (21.5)	6 (9.5)
PALB2	21 (7.2)	20 (8.8)	1 (1.6)
RAD51			
Low	228 (78.4)	228 (100.0)	0 (0)
High	63 (21.6)	0 (0)	63 (100.0)
TIL extent			
Low (0-29)	203 (69.8)	158 (69.3)	45 (71.4)
High (30-100)	88 (30.2)	70 (30.7)	18 (28.6)
Neoadjuvant chemotherapy	178 (61.2)	132 (57.9)	46 (73.0)
• Platinum-based regimen	69 (23.7)	52 (22.8)	17 (27.0)
• Anthracycline and taxane regimen	91 (31.6)	68 (29.8)	23 (36.5)
• Anthracycline-based regimen	1 (0.3)	1 (0.4)	0 (0)
• Taxane-based regimen	10 (3.4)	6 (2.6)	4 (6.3)
• Not reported	7 (2.4)	5 (2.2)	2 (3.2)
Adjuvant chemotherapy	75 (25.8)	59 (25.9)	16 (25.4)
• Platinum-based regimen	1 (0.3)	1 (0.4)	0 (0)
• Anthracycline and taxane regimen	48 (16.5)	35 (15.4)	10 (15.9)
• Anthracycline-based regimen	15 (5.1)	11 (4.8)	4 (6.3)
• Other agent or not reported	11 (3.8)	9 (3.9)	2 (3.2)
Anti-HER2 therapy	5 (1.7)	5 (2.2)	0 (0)
Immunotherapy	3 (1)	2 (0.9)	1 (1.6)
PARP inhibitor	2 (0.7)	2 (0.9)	0 (0)

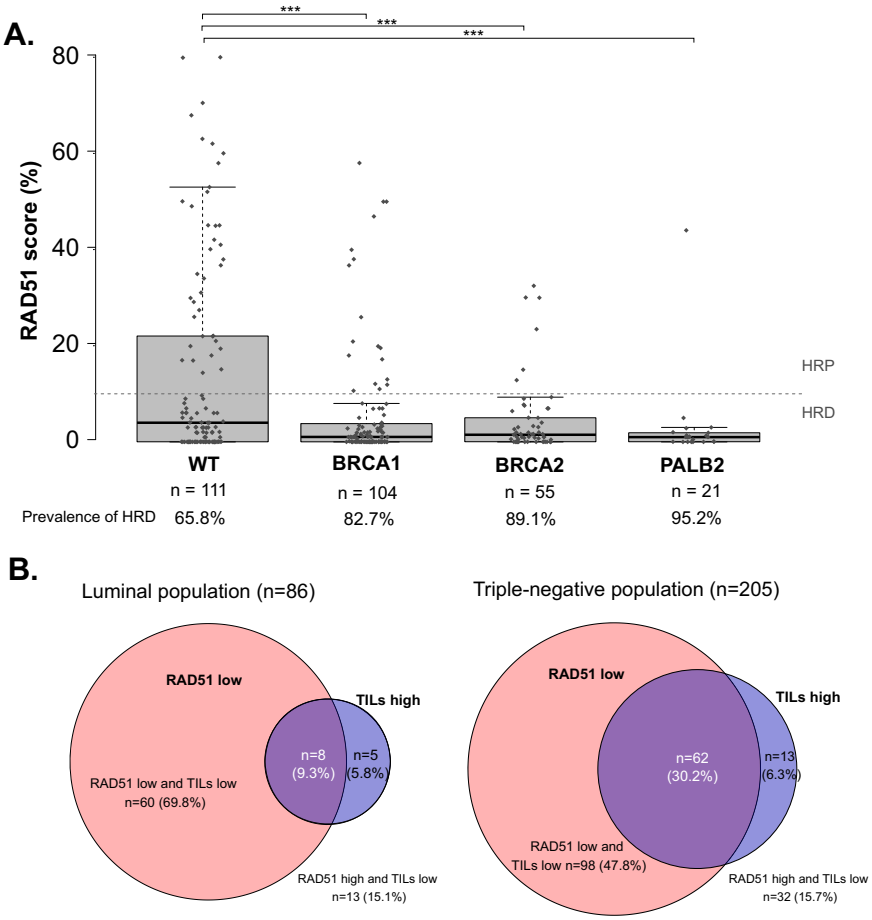

Fig. 1 | Prevalence of HRD by RAD51.
A Distribution of RAD51 scores according to germline pathogenic variant. Each point represents one tumour per patient. The horizontal dotted line indicates the 10% cut-off of RAD51 to determine HRD and HRP. WT, wild type for BRCA/PALB2. **B** Co-occurrence of HRD by RAD51 and high stromal TILs across breast cancer subtypes.

In the multivariable analysis, HRD by RAD51 was independently associated with improved OS (HR 0.44, 95% CI 0.23–0.85, $p = 0.01$), and stage III disease remained a strong predictor of worse OS (HR 4.11, 95% CI 2.27–7.45, $p < 0.01$; Fig. 3).

Patients with tumours harbouring HRD by RAD51 (RAD51 low) had a lower risk of death in comparison with those with HRP (RAD51 high) (HR = 0.42, 95% CI 0.22–0.80; $p = 0.009$). The 5-year OS for patients with tumours classified as HRD and HRP by RAD51 were 89.2% and 82.8%, respectively (Fig. 4A).

To fully assess the capacity of RAD51 to predict OS we examined those patients with TNBC ($n = 205$, Fig. 4B). 5-year OS was 85.4% for patients with TNBC and TILs high ($n = 75$). Among TNBC patients with TILs low ($n = 130$) RAD51 was able to identify subgroups with different outcomes (HR for HRP vs HRD by RAD51 = 0.27; 95% CI 0.11–0.67, $p = 0.005$; Fig. 4B). TNBC patients with TILs low and HRD by RAD51 had a 5-year OS of 88.9% vs 74.4% for patients with TNBC with TILs low and HRP.

Interestingly, within the subgroup of patients with no pCR after neoadjuvant chemotherapy ($n = 91$), those with HRD by RAD51 showed numerically better OS (80.1%) than those with HRP (73.6%, HR = 0.50; 95% CI 0.21–1.20, $p = 0.12$; Fig. 4C).

Finally, in the subset of patients with residual disease we further analysed the combination of TILs and RAD51 score to determine if the latter could identify a subgroup of patients with worse prognosis among the TILs low group. Indeed, patients with residual disease classified as TILs low and HRP by RAD51 showed the worst outcomes with a 5-year OS of 70.0% vs 78.5% for those with TILs low and HRD by RAD51, and 85.9% for TILs high (Fig. 4D).

Discussion

To unlock the full potential of precision oncology, drug development needs to be supported by reliable biomarkers to guide therapy decisions. In our study, we analysed the prognostic value of HRD assessed by RAD51 in addition to TILs in an HRD-enriched early-stage BC cohort. We describe that functional HRD by RAD51 is significantly associated with improved OS vs HRP (HR = 0.42, 95% CI 0.22–0.80; $p = 0.009$). Notably, RAD51 was able to stratify risk subgroups within the TNBC TILs low population (HR = 0.27, 95% CI, 0.11–0.67; $p = 0.005$). In fact, in this real-world study TNBC patients with HRD by RAD51 and TILs low showed superior OS compared to those with TNBC TILs high (88.9% vs 85.4%, respectively), suggesting RAD51 prognostic value beyond TILs extent.

As expected, tumours associated with HRR gene mutations exhibited higher HRD rates by RAD51 compared to the HRR WT group ($p < 0.001$). However, 15% of the *gBRCA1/2* mutant population were HRP by RAD51, raising the hypothesis that some tumours might not have biallelic inactivation[8]. Interestingly, over 60% of *gBRCA/PALB2* non-mutated tumours were HRD by RAD51. This result highlights that germline status alone may be insufficient to identify HRD, underscoring the contribution of other genetic and epigenetic alterations in HRR disruption.

In addition, given that over 50% of early-stage TNBC and 24% of early-onset BC exhibit HRD beyond *gBRCA1/2* pathogenic variants[8,14,16], RAD51 could serve as a broader marker for HRD, identifying patients who may benefit from targeted therapies such as PARPi. Furthermore, in our work, low RAD51 was significantly associated with pCR, consistent with previous reports[8,9]. Thus, using RAD51 as a predictive biomarker for pCR and PARPi sensitivity could further support treatment de-escalation strategies.

Interestingly, the prevalence of HRD by RAD51 was comparable between TNBC and luminal subtypes due to the enrichment of patients carrying a gBRCA1/2 or *gPALB2* pathogenic variant in the luminal BC group. This emphasizes that HRD is not exclusive to TNBC and may also play a role in luminal BC, namely in early-onset BC.

Regarding TILs, we observed no significant difference in TILs enrichment between HRD and HRP tumours, consistent with a pooled

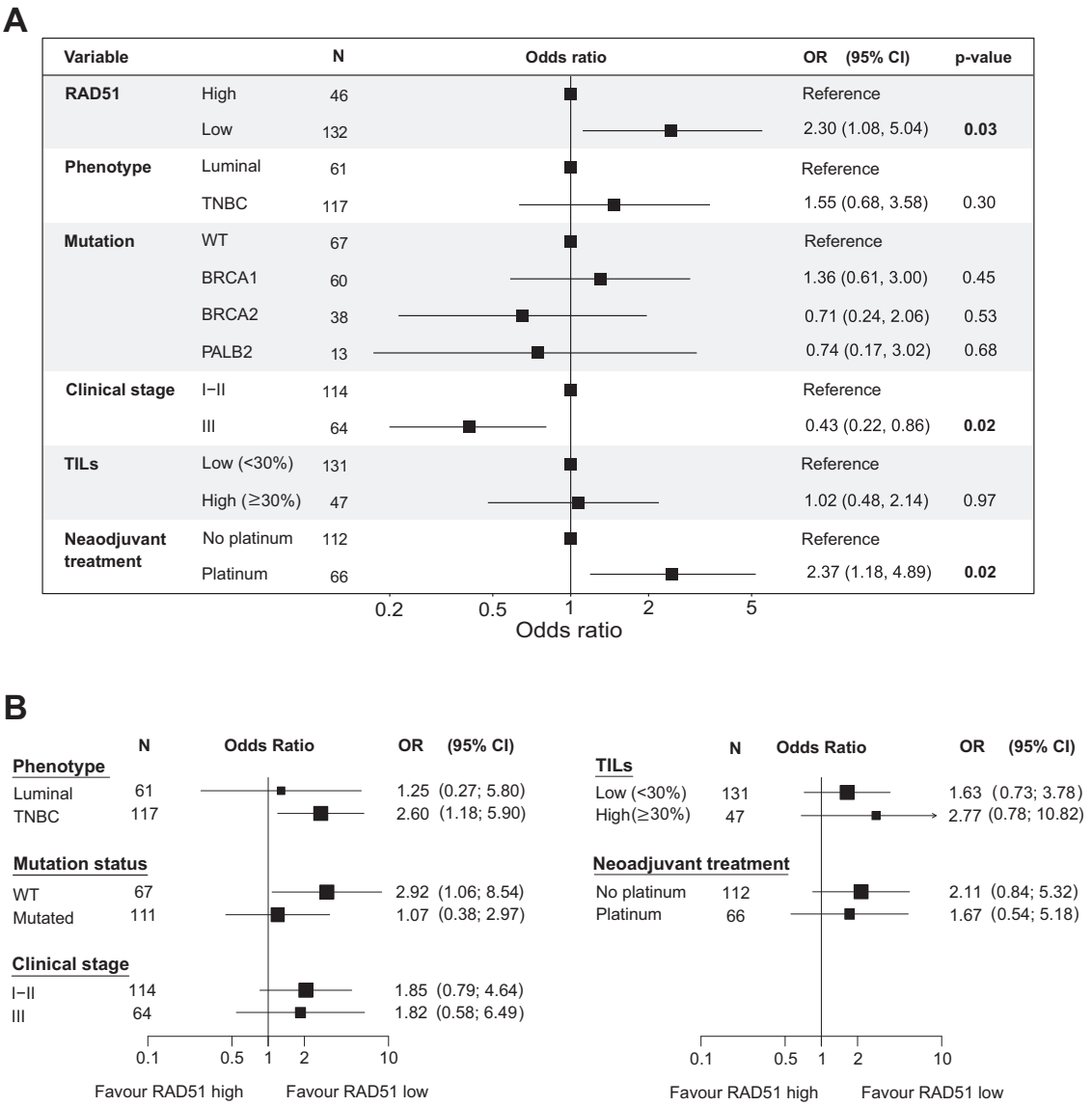

Fig. 2 | RAD51 as predictive biomarker of pathological complete response. A Multivariable analysis for the pathological complete response (pCR) endpoint in 178 patients. **B** Univariable analysis of RAD51 status and pCR within relevant patient subgroups.

analysis of 161 TNBC patients treated with neoadjuvant chemotherapy, where genomic HRD and tumour *BRCA1/BRCA2* mutation status were not associated with TILs density[17]. These findings show that these biomarkers (TILs and HRD status) are not overlapping. In this regard, recent studies have proposed the incorporation of TILs into the TNBC AJCC staging system[18,19]. Combining RAD51 and TILs could refine TNBC risk stratification, identifying a highly chemotherapy-sensitive group with a better prognosis, defined as HRD by RAD51 and TILs-high, where de-escalating strategies would be suitable for evaluation. In contrast, patients with HRP by RAD51 and TILs-low represent a worst-outcome group that may benefit from novel therapies.

This study presents several limitations, such as the heterogeneity of the patient population, the relatively low prevalence of tumours with TILs high, the minimal exposure to immunotherapy or PARPi as compared to nowadays standards, and its retrospective nature. Additionally, most patients received (neo)adjuvant chemotherapy, limiting to fully determine whether RAD51-assessed HRD is prognostic, independent of its predictive value for chemotherapy benefit. A validation analysis in a prospective trial will help to clarify this. Finally, TILs were not predictive of pCR in this cohort, likely due to the small number of TILs high cases and the enrichment of tumours with HRD (78.4%), which is strongly associated with pCR.

To our knowledge, this is the first study evaluating the prognostic role of functional HRD by RAD51 in early-stage BC. Our work proposes the combination of RAD51 scoring with TILs to identify a high-risk group, particularly in TNBC, where new therapies should be tested.

In conclusion, this study underscores the prognostic value of functional HRD by RAD51 beyond TILs extent. Incorporating RAD51-based HRD assessment in early-stage BC, particularly in TNBC, could enhance patient stratification and support individualized treatment decisions.

Methods

Study population

Eligible patients were female individuals diagnosed with early-stage BC meeting at least one of the following criteria: (a) a previously known germline pathogenic variant in *BRCA1*, *BRCA2*, or *PALB2*; (b) triple-negative breast cancer (TNBC); or (c) early-onset BC, defined as ≤35 years old at diagnosis. Patients diagnosed between January 2006 and December 2022 at Vall d'Hebron University Hospital (Spain), six Italian hospitals affiliated with the Gruppo Oncologico Italiano di Ricerca Clinica, or Institut Jules Bordet (Belgium) were included. Additional eligibility requirements included the availability of both written informed consent and diagnostic, treatment-naïve tumour samples for molecular analysis. Patients with

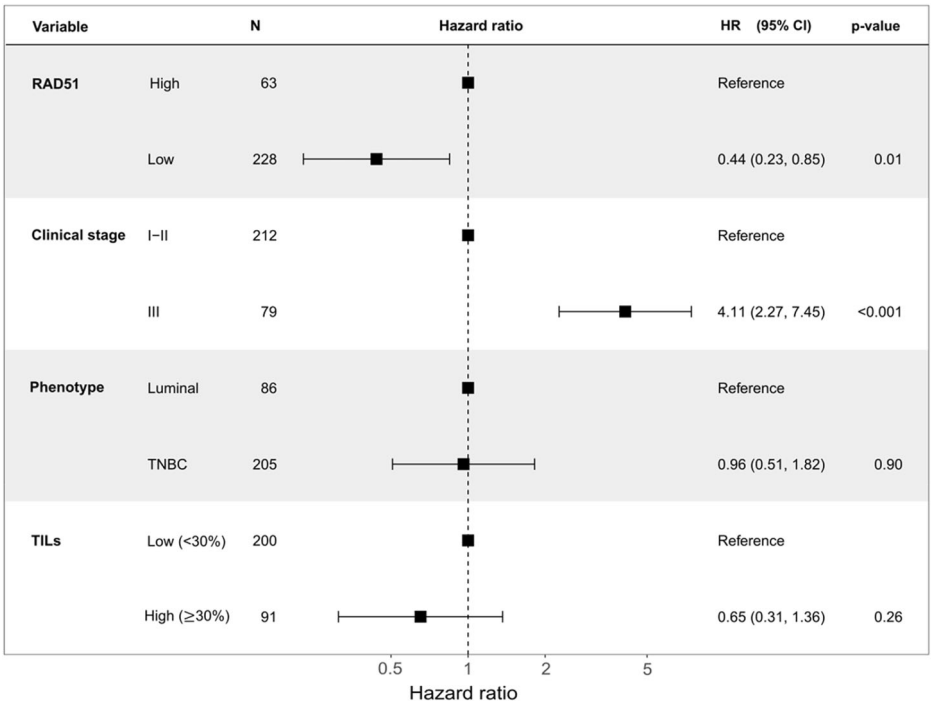

Fig. 3 | RAD51 as a prognostic biomarker. Multivariable analysis for overall survival (OS) in the entire study cohort ($n = 291$).

metastatic disease at diagnosis or without germline testing results for *BRCA1*, *BRCA2*, or *PALB2* were excluded.

The study protocol was approved by the Research Ethics Committees of Vall d'Hebron University Hospital, the six participating GOIRC hospitals, and Institut Jules Bordet. All procedures were performed in accordance with the ethical standards of the Declaration of Helsinki.

Clinical data collection included age, TNM stage using the American Joint Committee on Cancer (AJCC) 6th edition, nuclear grade, oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status, treatments received and survival follow-up. Tumour samples were analysed at the Vall d'Hebron Institute of Oncology (VHIO), University of Parma and Institut Jules Bordet between 2006 and 2023. Nuclear grade, ER, PR and HER2 were determined locally. TNBC was defined as ER and PR negative (<1%) and HER2-negative in accordance with the 2013 ASCO-CAP HER2 testing guidelines[20]. pCR was defined as the absence of residual invasive carcinoma in the surgical specimen, both in breast tissue and lymph nodes.

HRD status by RAD51 score

The immunofluorescence-based RAD51 test was performed on formalin-fixed paraffin-embedded (FFPE) tumour samples as described in Castroviejo-Bermejo et al.[21]. The following primary antibodies were used: rabbit anti-RAD51 (Abcam ab133534, 1:1000, RRID: AB_2722613), mouse anti-geminin (NovoCastra NCL-L, 1:100 in PDX samples, 1:60 in patient samples, RRID: AB_563738), rabbit anti-geminin (ProteinTech 10802-1-AP, 1:400, RRID: AB_2110945), mouse anti-BRCA1 (Abcam ab16780, 1:200, RRID: AB_2259338), mouse anti-phospho-H2AX (Millipore #05-636, 1:200, RRID: AB_2755003). Goat anti-rabbit Alexa fluor 568 (Invitrogen; 1:500), goat anti-mouse Alexa fluor 488 (Invitrogen; 1:500), donkey anti-mouse Alexa fluor 568 (Invitrogen; 1:500), and goat anti-rabbit Alexa fluor 488 (Invitrogen; 1:500) were used as secondary antibodies[21]. Biomarker scoring (γH2AX and RAD51) was performed onto life images using a 60X-immersion oil lens[21]. One hundred geminin-positive cells from at least three representative areas of each sample were analysed and biomarker scores were calculated as the percentage of geminin-positive cells with 5 or more nuclear foci. Samples with γH2AX score <25% or with <40 geminin-positive cells were not included in the analysis, as considered without sufficient endogenous DNA damage or tumour cells in the S/G2-phase of the cell cycle, respectively, to assess the HRD status. A pre-validated cut-off of RAD51 was used to categorise tumours as functional HRD if RAD51 ≤ 10% (RAD51 low) and homologous recombination proficient (HRP) if RAD51 > 10% (RAD51 high)[22].

TILs analysis

Stromal TILs were scored quantitatively on haematoxylin and eosin stained FFPE tumour sections as the percentage of stromal cells (i.e. fraction of total stromal nuclei that represent mononuclear inflammatory cell nuclei) within the borders of the invasive tumour, as recommended in Salgado et al.[23]. For tumours with heterogeneous TILs, median values were calculated from multiple counts from different tumour areas. The cut-off of 30% was used to classify tumours in TILs high (≥30%) and low (<30%), according to Salgado's criteria[23].

Germline sequencing

All patients performed germline breast and ovarian cancer gene-panel sequencing under routine clinical protocols, including *BRCA1, BRCA2, PALB2, ATM, CHEK2, RAD51C and RAD51D* genes. Analysis of point mutations and copy number variations was performed. Variants were interpreted according to ACMG guidelines[24].

Statistical analysis

A descriptive analysis of all variables included in the study was performed. The pCR endpoint was defined as the proportion of patients who received neoadjuvant treatment with ypT0/isN0 at surgery. pCR rates were expressed with absolute values and percentages along with 95% confidence interval (95% CI) using the Clopper-Pearson method. Multivariable logistic regression models were used to investigate the association for each variable with pCR in terms of odds-ratios (ORs) with 95% CI. Additionally, the association of RAD51 score (high vs low) with pCR was also evaluated in several clinically relevant subgroups of patients. Overall survival (OS) was defined from diagnosis to death from any cause. OS were estimated using the Kaplan–Meier method. To assess the prognostic value of RAD51 score, univariable Cox proportional-hazard models were used to obtain hazard ratios (HRs) with 95% CIs in the overall population and within specific subgroups of interest. Missing at random values were imputed using the chained equations via the mice R package (Supplementary Table 1). Age and

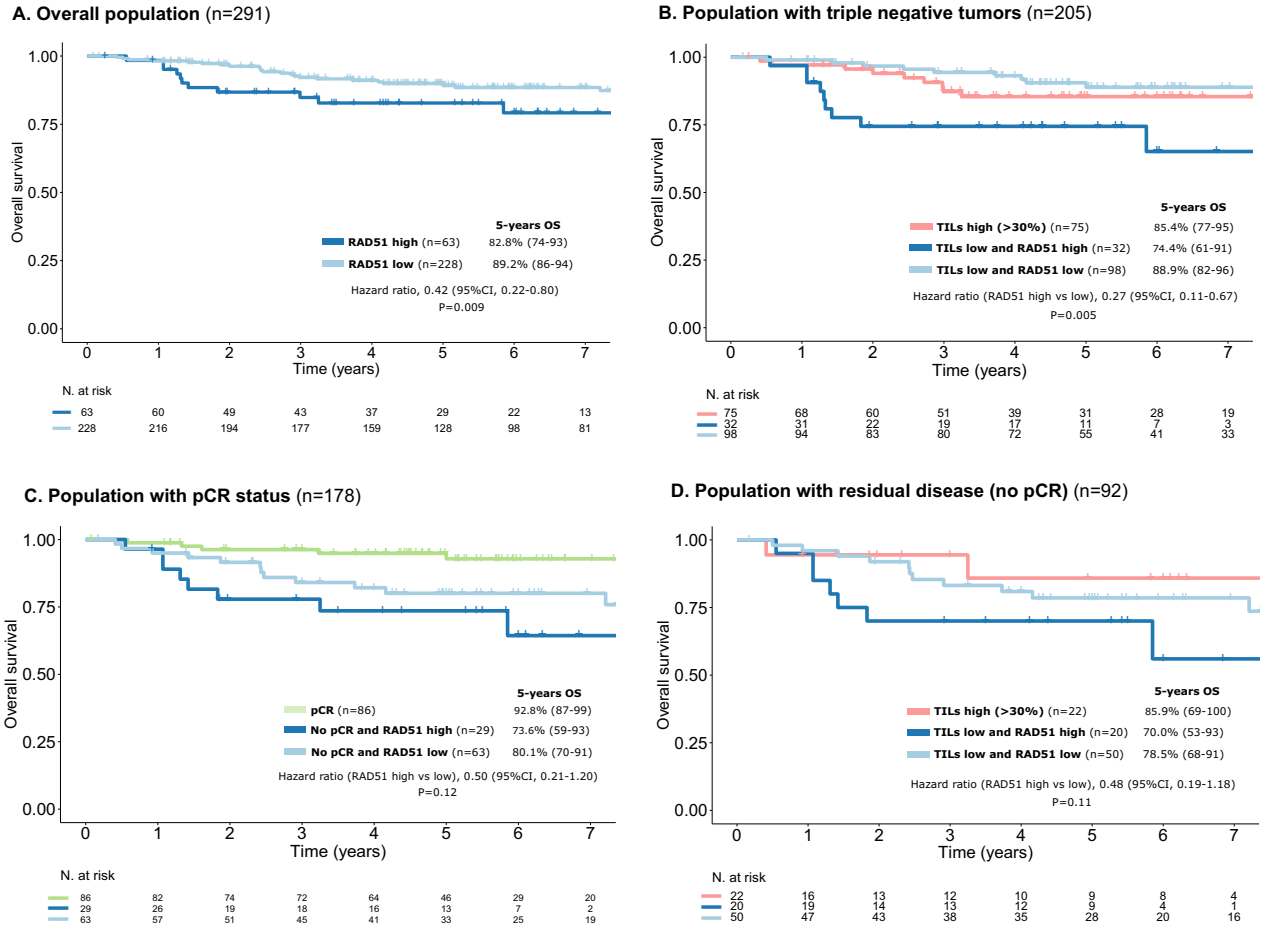

Fig. 4 | Kaplan–Meiers of overall survival. A Association of RAD51 with overall survival in the entire study cohort ($n = 291$). **B** Association of RAD51 and TILs with overall survival in patients with triple negative breast cancer ($n = 205$). **C** Association of RAD51 with overall survival in patients with pCR status after neoadjuvant chemotherapy ($n = 178$). **D** Association of RAD51 and TILs with overall survival in patients with residual disease after neoadjuvant chemotherapy ($n = 92$).

histological type had more than 20% of missing data and were not included in the multivariable analysis. The threshold for statistical significance was defined as 0.05 (two-sided). All statistical analyses were performed using R statistical software.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due to ethical restrictions and patient confidentiality but are available from the corresponding author [J.B.] on reasonable request. Data requests will be reviewed by the institutional data access committee at Vall d'Hebron Institute of Oncology or AOU Parma or Institut Bordet to ensure compliance with ethical and legal obligations. A data transfer agreement will be required.

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Author contributions

B.P. and I.P. wrote the main manuscript and G.V. prepared all figures and tables. A.L.G., V.S., A.M. and J.B. lead the research and obtained research funding. C.S., C.T., M.C., D.B., M.M., M.V.D., M.L., G.Z., A.S., C.C., E.C., L.C., C.S. and K.W.G. included their patients in the trial and contributed to the trial conceptualization. S.T.E., R.M., B.B. and A.B. performed and analysed germline testing for hereditary breast cancer. B.P., A.L.G. and N.C. performed RAD51 assay and TIL assessment on the samples. N.C., S.S., E.M.S. and A.B. performed TIL assessment on patients' samples. O.S. and E.R. were involved in data management and ethical committee's approval. All authors have read and approved the manuscript.

Competing interests

Alba Llop-Guevara, Violeta Serra and Judith Balmana are co-authors of a patent related to this work (WO2019122411A1, pending). Alba Llop-Guevara and Violeta Serra are current employees of AstraZeneca. Benedetta Pellegrino reports advisory board from Daiichi-Sankyo, other support from Lilly, Pfizer, Novartis and Gilead; and personal fees from MSD outside the submitted work. Isabel Pimentel reports advisory board functions from AstraZeneca and received honoraria and/or travel support from MSD, Novartis, Pfizer, AstraZeneca and Gilead. Judith Balmana received honoraria from MSD and AstraZeneca. Laura Cortesi reports honoraria from Astra Zeneca, Pfizer, MSD, Novartis, Gilead, Roche and Diichi-Sankyo. Laura Cortesi reports a relationship with AstraZeneca, Merck, Daiichi Sankyo and Pfizer that includes board membership and consulting or advisory. Cinzia Solinas reports travel grants from Lilly and Ipsen, and speaker honoraria from Bayer. Guillermo Villacampa received a speaker's fee from Pfizer, MSD, GSK and Pierre Fabrer, and held an advisory role with AstraZeneca and received consultant fees from Reveal Genomics. Gabriele Zoppoli reports honoraria from Menarini Stemline, travel grants from Menarini Stemline and Novartis. Cristina Cruz reports honoraria and/or travel support from Novartis, Daichi Sanchyo, Roche, Lilly and Gilead. Cristina Saura reports consulting fees, honoraria or meeting/travel support from AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Daiichi Sankyo, Eisai, Genentech,Gilead, Lilly, MediTech, Menarini, MSD Spain, Novartis, Pfizer, Philips Healthcare, Pharmalex, Pierre Fabre, Puma Biotechnology, Roche, Seagen, Synthon and Zymeworks. Matteo Lambertini reports advisory role for Roche, Lilly, Novartis, AstraZeneca, Pfizer, Seagen, Gilead, MSD, Pierre Fabre, Menarini and Exact Sciences; receiving speaker honoraria from Roche, Lilly, Novartis, Pfizer, Sandoz, Libbs, Daiichi Sankyo, Takeda, Ipsen, Menarini and AstraZeneca; receiving travel grants from Gilead, Roche, and Daiichi Sankyo; receiving research funding (to his institution) from Gilead; and having non-financial interests as a member of the national council of the Italian Association of Medical Oncology (AIOM). Maria Vittoria Dieci reports personal fees and/or advisory board functions from Novartis, Lilly, Seagen, Exact Science, Pfizer, Daiichi Sankyo, Gilead, Roche, MSD, AstraZeneca. She also reports travel/accommodation support from Roche and Gilead, licensing Fees, listed as co-inventor in patent applications for HER2 DX. Research Grant from Roche. Antonino Musolino reports Consulting or Advisory Role from Lilly, Eisai Europe, Daiichi Sankyo, Astra Zeneca, Novartis. Research Funding from Lilly and travel support from Pfizer. Sara Torres-Esquius, Nicoletta Campanini, Sara Simonetti, Chiara Tommasi, Olga Serra, Matilde Corianò, Daniela Boggiani, Maria Michiara, Roberta Minari, Beatrice Bortesi, Elena Rapacchi, Alessio Schirone, Chiara Casarini, Elisabetta Cretella, Enrico Maria Silini, Karen Willard-Gallo and Anais Boisson have no conflicts of interest to declare related to this work.

Additional information

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