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ORIGINAL ARTICLE

Association of RAD51 with homologous recombination deficiency (HRD) and clinical outcomes in untreated triple-negative breast cancer (TNBC): analysis of the GeparSixto randomized clinical trial

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Background: Current genetic and genomic tests measuring homologous recombination deficiency (HRD) show limited predictive value. This study compares the performance of an immunohistology-based RAD51 test with genetic/genomic tests to identify patients with HRD primary triple-negative breast cancer (TNBC) and evaluates its accuracy to select patients sensitive to platinum-based neoadjuvant chemotherapy (NACT).

Patients and methods: This is a retrospective, blinded, biomarker analysis from the GeparSixto randomized clinical trial. TNBC patients received neoadjuvant paclitaxel plus Myocet®-nonpegylated liposomal doxorubicin (PM) or PM plus carboplatin (PMCb), both arms including bevacizumab. Formalin-fixed paraffin-embedded (FFPE) tumor samples were laid on tissue microarrays. RAD51, BRCA1 and γH2AX were quantified using an immunofluorescence assay. The predictive value of RAD51 was assessed by regression models. Concordance analyses were carried out between RAD51 score and tumor *BRCA* (t*BRCA*) status or genomic HRD score (Myriad myChoice®). Associations with pathological complete response (pCR) and survival were studied. Functional HRD was predefined as a RAD51 score $\leq$10% (RAD51-low).

Results: Functional HRD by RAD51-low was evidenced in 81/133 tumors (61%). RAD51 identified 93% t*BRCA*-mutated tumors and 45% non-t*BRCA* mutant cases as functional HRD. The concordance between RAD51 and genomic HRD was 87% [95% confidence interval (CI) 79% to 93%]. In patients with RAD51-high tumors, pCR was similar between treatment arms [PMCb 31% versus PM 39%, odds ratio (OR) 0.71, 0.23-2.24, $P = 0.56$]. Patients with RAD51-low tumors benefited from PMCb (pCR 66% versus 33%, OR 3.96, 1.56-10.05, $P = 0.004$; interaction test $P = 0.02$). This benefit maintained statistical significance in the multivariate analysis. Carboplatin addition showed similar disease-free survival in the RAD51-high [hazard ratio (HR) 0.40, log-rank $P = 0.11$] and RAD51-low (0.45, $P = 0.11$) groups.

Conclusions: The RAD51 test identifies tumors with functional HRD and is highly concordant with t*BRCA* mutation and genomic HRD. RAD51 independently predicts clinical benefit from adding Cb to NACT in TNBC. Our results support further development to incorporate RAD51 testing in clinical decision-making.

Key words: RAD51, HRD biomarkers, *BRCA1*, *BRCA2*, triple-negative breast cancer, platinum-based neoadjuvant chemotherapy, personalized medicine

INTRODUCTION

Triple-negative breast cancers (TNBCs) have an increased sensitivity to DNA-damaging agents such as platinum salts.[1-3] However, the toxicity derived from these agents and lack of conclusive correlation with survival outcomes raise concern about their use in the early disease setting.[4-6] Molecular

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and/or clinical biomarkers will help to guide treatment decisions.

The high sensitivity of TNBC to platinum-based chemotherapy can be explained by the high proportion of TNBCs harboring genetic or epigenetic alterations in the homologous recombination repair (HRR) pathway that lead to DNA double-strand break repair deficiency. These alterations mainly encompass germline mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) or hypermethylation of the *BRCA1* promoter. Other less-frequent alterations resulting in a similar phenotype include mutations in *PALB2, RAD51C* and *RAD51D* or epigenetic silencing of *RAD51C*. Identifying tumors harboring these diverse alterations represents a clinical challenge. Genomic scars and genomic signatures have demonstrated a high correlation with HRR gene alterations,[7,8] but neither *BRCA1/2* mutations nor genomic scars have succeeded in identifying the patient population that benefit from platinum agents.[2,5,9,10] Importantly, HRR deficient (HRD) tumors may evolve toward restoring HRR and acquire resistance to DNA-damaging agents. Consequently, these tumors would be misclassified according to their underlying genomic scar/signature.[11] In this sense, it has been proposed that a functional and dynamic measure of HRR is needed to more accurately establish the actual status of HRD. Here, we studied the feasibility and validity of quantifying RAD51 nuclear foci in untreated TNBC to establish its concordance with genetic/genomic HRD assays and to test its association with clinical outcomes.

PATIENTS AND METHODS

Patients and treatments

Patients diagnosed with TNBC who participated in the GeparSixto trial (NCT01426880) were scheduled to receive paclitaxel (80 mg/m^2) plus nonpegylated liposomal doxorubicin (20 mg/m^2 Myocet; Teva Pharm, North Wales, PA), both administered q1w for 18 weeks, and bevacizumab (15 mg/kg q3w) during all chemotherapy cycles. Patients were randomized to receive simultaneously carboplatin (PMCb) at 2.0-1.5 area under the curve q1w for 18 weeks or no additional treatment (PM).[1] All patients provided written informed consent for trial participation and translational research. Ethical committee approval (119/20) from the University of Marburg, Germany was obtained.

Aims and endpoints

The primary endpoint of the GeparSixto study was pathological complete response (pCR) defined as ypT0 ypN0. Predefined secondary endpoints included disease-free survival (DFS) and overall survival (OS), and a predefined exploratory analysis of HRD biomarkers including germline and tumor mutation in *BRCA1/BRCA2* (g*BRCA* and t*BRCA*) and genomic HRD.[4,5,12] In this study we report an exploratory measure of HRD, namely the functional biomarker RAD51. Specifically, we aimed to investigate if the RAD51 score correlates with a genomic HRD score and with the t*BRCA* status. A secondary objective was to determine whether RAD51 before therapy initiation correlates with therapy outcome. pCR, DFS and OS endpoints were defined as previously described.[5]

Tumor BRCA status and genomic HRD score

Treatment-naïve formalin-fixed paraffin-embedded (FFPE) core biopsies were assessed retrospectively for mutations in *BRCA1* and *BRCA2* and for the genomic HRD score using Myriad myChoice® (Myriad Genetics Inc., Salt Lake City, UT) as specified.[5,12]

RAD51 immunofluorescence test

Treatment-naïve FFPE core biopsies were laid on a tissue microarray (TMA) format with one spot per patient. When available, to minimize tumor heterogeneity aspects, two half core biopsies from the same biopsy were placed within the same TMA spot, with representative tumor tissue, after intensive pathological assessment. Three-micrometer TMA sections were provided for analysis of RAD51 foci (as a functional readout of HRD), BRCA1 foci (as a mediator of HRR) and γH2AX foci (as a biomarker of endogenous double-strand DNA damage), with each biomarker counterstained with geminin (as a marker of the S/G2 cell cycle phase) and 4′,6-diamidino2-phenylindole (DAPI), as previously described.[13,14] The following primary antibodies were used for immunofluorescence: rabbit anti-RAD51 (Abcam, Cambridge, UK ab133534, 1:1000), mouse anti-BRCA1 (Santa Cruz Biotechnology Inc, Dallas, TX sc-6954, 1 : 50), mouse anti-γH2AX (Merk Millipore, Darmstadt, Germany #05-636, 1 : 200), mouse anti-geminin (Novocastra Laboratories Ldt, Newcastle upon Tyne, UK NCL-L, 1 : 60) and rabbit anti-geminin (Proteintech, Manchester, UK 10802-1-AP, 1 : 400). Goat anti-rabbit Alexa fluor 568, goat anti-mouse Alexa fluor 488, donkey anti-mouse Alexa fluor 568 and goat anti-rabbit Alexa fluor 488 (Invitrogen, Waltham, MA; 1 : 500) were used as secondary antibodies.

Scoring was carried out blindly to treatment and patient characteristics using live images and a 60× immersion oil lens in a Nikon (Amsterdam, Netherlands) Ti-Eclipse microscope. At least 40 geminin-positive cells were analyzed per core and the γH2AX score was used as quality check to ensure the presence of enough endogenous DNA damage to evaluate HRR functionality (cut-off 25% geminin-positive cells with γH2AX foci). RAD51 and BRCA1 scores were considered low or high based on the predefined cut-off of 10% geminin-positive cells with ≥5 RAD51 or BRCA1 nuclear foci.[15,16]

Statistical evaluation

Fisher's exact test was used to compare groups. Univariate and multivariate logistic regression models adjusted for prespecified variables were used to analyze the predictive value of biomarkers for pCR. DFS and OS were analyzed by the Kaplan—Meier method, log-rank tests and Cox regression models. Interaction with the treatment arm was assessed by including an interaction term into regression models. All confidence intervals (CIs) reported were 95%. All

tests were two-sided with a value of $P < 0.05$ considered statistically significant. Analyses were carried out using SPSS Software (Chicago, IL), version 25.

RESULTS

Prevalence of functional HRD by RAD51

From 315 participants with TNBC in the GeparSixto trial, 259 tumor samples laid on TMAs were considered for biomarker analyses (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2021.09.003). Fifty-nine cases were excluded due to absence/insufficient tumor cells. RAD51, BRCA1 and γH2AX nuclear foci were successfully scored in 133/200 cores (67%). These 133 patients exhibited similar clinical and molecular characteristics as the full TNBC population ($N = 315$) in the GeparSixto trial (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2021.09.003).[5]

Functional HRD by RAD51 (RAD51-low score) was found in 81/133 tumors (61%) (Figure 1 and Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2021.09.003). The BRCA1 nuclear foci score (BRCA1 score)

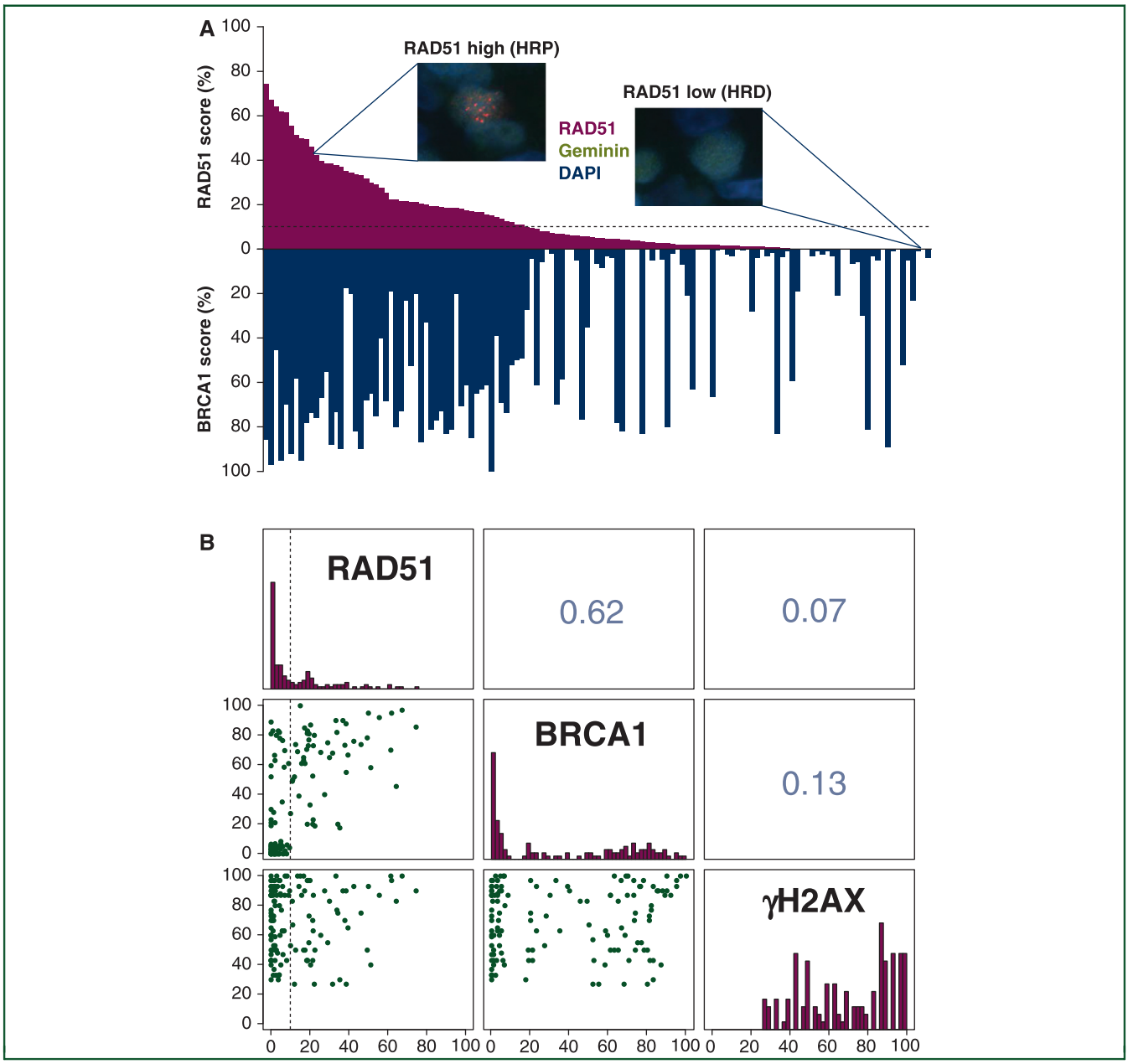


Figure 1. Analysis of homologous recombination deficiency (HRD) tumor biomarkers by immunofluorescence in tissue microarray (TMA) preparations of untreated triple-negative breast cancer (TNBC) patients.

(A) The percentages of geminin-positive tumor cells with RAD51 (upper blue bars) and BRCA1 (bottom green bars) nuclear foci in 133 samples are shown. Insert images depict representative geminin-positive cells with and without RAD51 foci. (B) Bivariate analysis showing the correlation between RAD51, BRCA1 and γH2AX scores. Pearson coefficients are used to estimate bivariate correlations. Endogenous DNA damage (γH2AX) is high (>25%) in all samples. Samples with low BRCA1 scores (<10%) likely result from either *BRCA1* mutation or *BRCA1* epigenetic silencing, and all show low RAD51 scores. The dotted line indicates the prespecified cut-off of 10% for RAD51. DAPI, 4,6-diamidino2-phenylindole; HRD, HRR deficiency; HRP, HRR proficiency.

was low in 43% of tumors, all of them with low RAD51 values. γH2AX was high in all tumors, showing that treatment-induced DNA damage is not required to score RAD51 in primary TNBC.

Concordance analyses of RAD51 with tBRCA mutations and genomic HRD tests

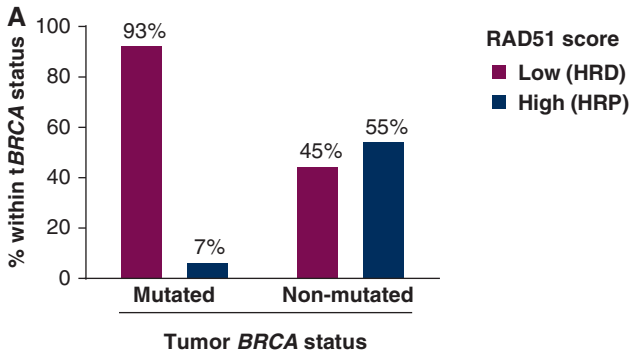
The RAD51 biomarker identified 93% (95% CI 76% to 99%) of t*BRCA*-mutated tumors and 45% (95% CI 34% to 56%) of the non-t*BRCA* mutants as harboring functional HRD (Figure 2A and C). RAD51 identified 86% of tumors with genomic HRD and 90% with genomic HRR proficiency (HRP) (Figure 2B and C).

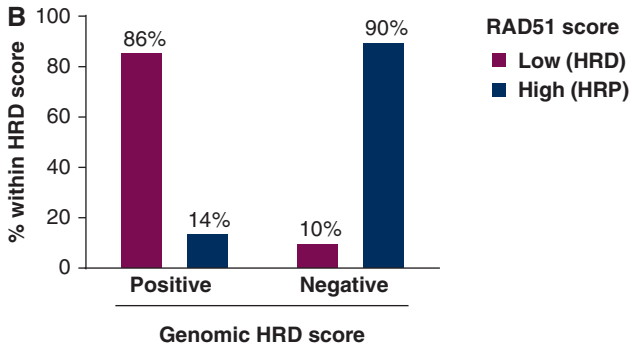
Overall, RAD51 and genomic HRD were 87% (95% CI 79% to 93%) concordant (Figure 2C). These results demonstrate the feasibility of the RAD51 test in untreated FFPE TNBC samples and its high degree of concordance with t*BRCA* mutations and genomic HRD tests.

Association of RAD51 with patient, tumor characteristics and clinical outcomes

As already observed for genomic HRD status,[5] HRD based on RAD51 was associated with younger patients' age, node-negative disease at diagnosis, g*BRCA* mutation and a higher family risk for developing breast and/or ovarian cancer (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2021.09.003). Of note, RAD51 identified 22/24 (92%) of g*BRCA* carriers.

The pCR in patients with RAD51-high tumors was similar between the treatment arms (PMCb 31% versus PM 39%, OR = 0.71, 95% CI 0.23-2.24, $P = 0.56$) (Figure 3A and B). In contrast, patients with RAD51-low tumors significantly benefited from PMCb (pCR PMCb 66% versus PM 33%, OR = 3.96, 95% CI 1.56-10.05, $P = 0.004$). The RAD51 test was able to significantly discriminate tumors sensitive to carboplatin (interaction test, $P = 0.02$). This benefit maintained statistical significance also in the multivariate





C

Number of cases (% of group)		Tumor *BRCA* status		Genomic HRD score	
		Mutated	Non-mutated	Positive	Negative
RAD51 score	Low (HRD)	27 (93%)	36 (45%)	56 (86%)	3 (10%)
	High (HRP)	2 (7%)	44 (55%)	9 (14%)	27 (90%)
Concordance rate		65%		87%	
Cohen's kappa		0.35		0.72	
P value		<0.001		<0.001	

Figure 2. Association of the RAD51 score with t*BRCA* status and genomic homologous recombination deficiency (HRD) score.
(A and B) Bars represent the percentage of cases in each indicated group. (C) Summary table with the number of individual cases (and percentage within each column group) as well as statistics showing the concordance between RAD51 and genetic/genomic HRD biomarkers.

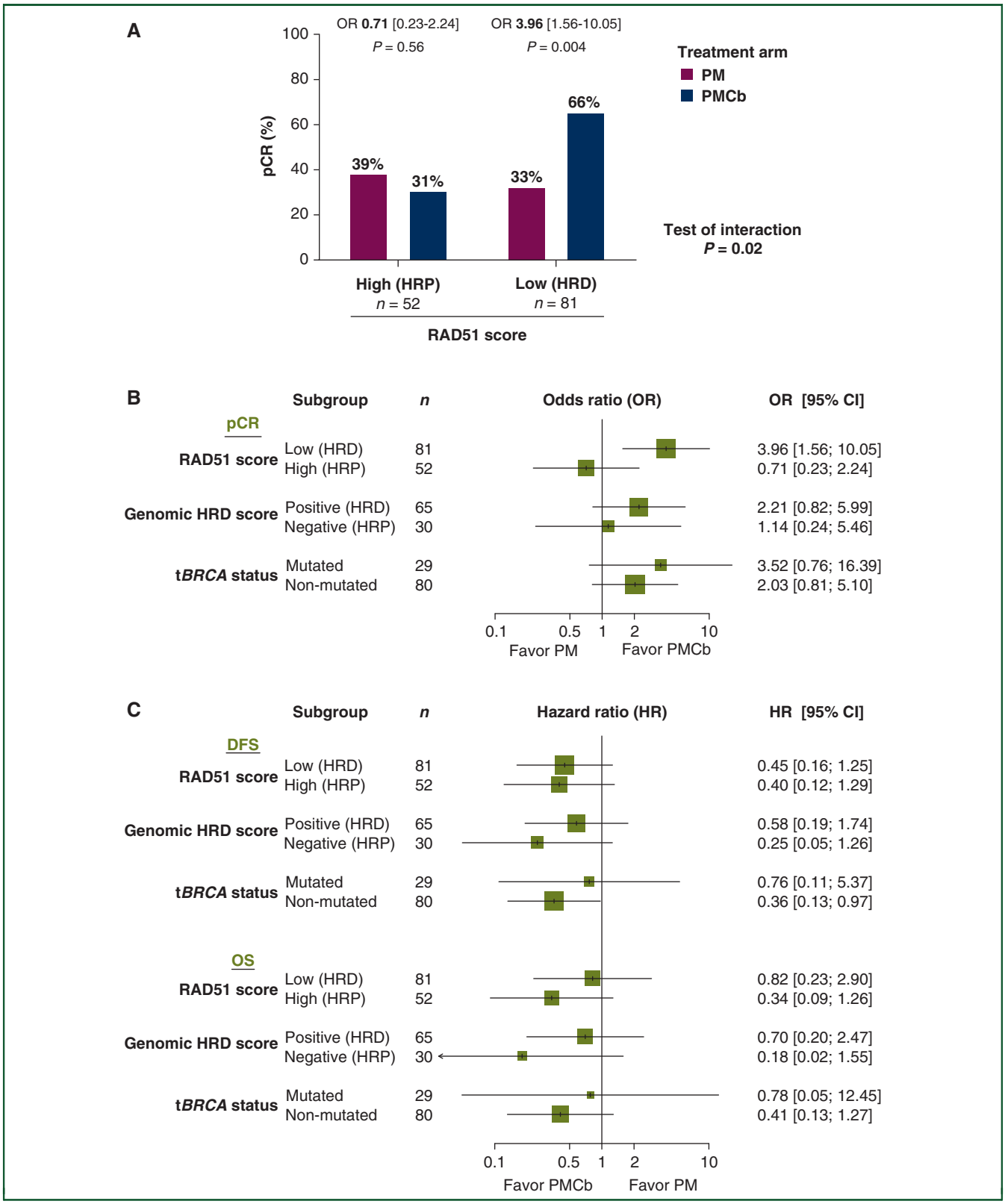


Figure 3. Association of HRD biomarkers with clinical outcome.
(A) Response (ypT0 ypN0) to treatment by RAD51-based HRD in patients treated with paclitaxel plus Myocet-nonpegylated liposomal doxorubicin (PM) or PM plus carboplatin (PMCb). (B) Difference in pathological complete response (pCR) between PM or PMCb for the indicated biomarkers. (C) Difference in disease-free survival (DFS) and overall survival (OS) between PM or PMCb for the indicated biomarkers. All results are based on univariate analyses.

analysis after adjustment for predefined clinicopathological variables (OR = 7.52, 95% CI 2.21-25.61, $P = 0.001$) (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2021.09.003). In terms of DFS, the benefit of adding carboplatin was similar in RAD51-high (HR = 0.40, 95% CI 0.12-1.29, log-rank $P = 0.11$) and RAD51-low (HR = 0.45, 95% CI 0.16-1.25, log-rank $P = 0.11$) groups (Figure 3C and Supplementary Figure S3A, available at https://doi.org/10.1016/j.annonc.2021.09.003). Regarding OS, no statistically significant association was found from the addition of carboplatin in RAD51-high (HR = 0.34, 95% CI 0.09-1.26, log-rank $P = 0.09$) and RAD51-low (HR = 0.82, 95% CI 0.23-2.90, log-rank $P = 0.76$) tumors (Figure 3C and Supplementary Figure S3B, available at https://doi.org/10.1016/j.annonc.2021.09.003).

DISCUSSION

In this substudy of the GeparSixto trial in TNBC, the immunofluorescence-based RAD51 test is highly concordant with genetic and genomic tests, and capable of identifying tumors that benefit from addition of carboplatin in terms of pCR.

Functional HRD, defined as RAD51-low scores, was evidenced in 61% of tumors. Prior reported prevalence of HRD in primary TNBC, based on genomic HRD and/or *BRCA1/2* status, has ranged between 40% and 70%.[9,17-19] Interestingly, RAD51 was able to detect a high proportion of non-t*BRCA*-mutated cases with HRD, likely encompassing tumors with mutations in other HRR genes or with epigenetic silencing of the pathway. The discordant t*BRCA* mutation cases with RAD51 foci likely reflect the population of *BRCA1/2*-associated cancers that do not harbor biallelic inactivation.[20] RAD51 and genomic HRD were highly concordant (87%) in this cohort. The discordant cases could be likely attributed to (a) genomic HRD tests identifying tumors with high genomic instability, but not of HRR origin; (b) primary tumors that might have restored HRR as part of the tumor evolution; or (c) tumor heterogeneity not captured in the TMA setting.

Previous studies have demonstrated a pCR benefit of adding carboplatin as part of the neoadjuvant treatment in TNBC.[1-3] However, no significant interaction between tumor mutations or genomic HRD status and carboplatin treatment benefit could be established. In the current study, patients with RAD51-low tumors benefited from adding carboplatin compared to RAD51-high tumors, as shown by the pCR and a significant test of interaction ($P = 0.02$). This finding was independent from other clinicopathological variables ($P = 0.001$ in the multivariate analysis), supporting the utility of RAD51 as a predictive biomarker for carboplatin-based therapy in TNBC. Similarly, RAD51 was shown to be predictive of response to poly(ADP-ribose) polymerase inhibition in early TNBC[16] and, therefore, strengthens the evidence of RAD51 as a functional biomarker of HRD and a candidate test to personalize medicine. With regard to survival outcome, there was no indication that RAD51-low status predicted benefit of carboplatin, on top of paclitaxel, liposomal doxorubicin and bevacizumab.

This substudy has some limitations. It evaluated 133/315 patients participating in the GeparSixto trial. In addition, the GeparSixto trial was not powered to demonstrate benefits in survival endpoints and the control arm used a nonconventional treatment regimen. A validation analysis in a randomized and powered study for survival outcomes may help to clarify this.

Conclusion

This study supports the clinical validity of the RAD51 assay as a functional HRD test and a predictive biomarker of response to carboplatin in untreated TNBC.

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(CD). All remaining authors have declared no conflicts of interest.

REFERENCES

1. von Minckwitz G, Schneeweiss A, Loibl S, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. *Lancet Oncol*. 2014;15:747-756.
2. Loibl S, O'Shaughnessy J, Untch M, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): a randomised, phase 3 trial. *Lancet Oncol*. 2018;19:497-509.
3. Sikov WM, Berry DA, Perou CM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). *J Clin Oncol*. 2015;33:13-21.
4. Hahnen E, Lederer B, Hauke J, et al. Germline mutation status, pathological complete response, and disease-free survival in triple-negative breast cancer: secondary analysis of the GeparSixto randomized clinical trial. *JAMA Oncol*. 2017;3:1378-1385.
5. Loibl S, Weber KE, Timms KM, et al. Survival analysis of carboplatin added to an anthracycline/taxane-based neoadjuvant chemotherapy and HRD score as predictor of response-final results from GeparSixto. *Ann Oncol*. 2018;29:2341-2347.
6. Sikov WM, Berry DA, Perou CM, et al. Event-free and overall survival following neoadjuvant weekly paclitaxel and dose-dense AC +/− carboplatin and/or bevacizumab in triple-negative breast cancer: outcomes from CALGB 40603 (Alliance). *Cancer Res*. 2016;76(suppl 4): Abstract nr S2-05.
7. Abkevich V, Timms KM, Hennessy BT, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer*. 2012;107:1776-1782.
8. Davies H, Glodzik D, Morganella S, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat Med*. 2017;23:517-525.
9. Tutt A, Tovey H, Cheang MCU, et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. *Nat Med*. 2018;24:628-637.
10. Isakoff SJ, Mayer EL, He L, et al. TBCRC009: a multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. *J Clin Oncol*. 2015;33:1902-1909.
11. Watkins JA, Irshad S, Grigoriadis A, Tutt ANJ. Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. *Breast Cancer Res*. 2014;16:211.
12. Timms KM, Abkevich V, Hughes E, et al. Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. *Breast Cancer Res*. 2014;16:475.
13. Cruz C, Castroviejo-Bermejo M, Gutierrez-Enriquez S, et al. RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. *Ann Oncol*. 2018;29:1203-1210.
14. Castroviejo-Bermejo M, Cruz C, Llop-Guevara A, et al. A RAD51 assay feasible in routine tumor samples calls PARP inhibitor response beyond BRCA mutation. *EMBO Mol Med*. 2018;10:e9172.
15. Graeser M, McCarthy A, Lord CJ, et al. A marker of homologous recombination predicts pathologic complete response to neoadjuvant chemotherapy in primary breast cancer. *Clin Cancer Res*. 2010;16:6159-6168.
16. Eikesdal HP, Yndestad S, Elzawahry A, et al. Olaparib monotherapy as primary treatment in unselected triple negative breast cancer. *Ann Oncol*. 2021;32:240-249.
17. Telli ML, Timms KM, Reid J, et al. Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. *Clin Cancer Res*. 2016;22:3764-3773.
18. Chopra N, Tovey H, Pearson A, et al. Homologous recombination DNA repair deficiency and PARP inhibition activity in primary triple negative breast cancer. *Nat Commun*. 2020;11:2662.
19. Bertucci F, Ng CKY, Patsouris A, et al. Genomic characterization of metastatic breast cancers. *Nature*. 2019;569:560-564.
20. Jonsson P, Bandlamudi C, Cheng ML, et al. Tumour lineage shapes BRCA-mediated phenotypes. *Nature*. 2019;571:576-579.