JAMA Oncology | Original Investigation

Germline Mutation Status, Pathological Complete Response, and Disease-Free Survival in Triple-Negative Breast Cancer

Secondary Analysis of the GeparSixto Randomized Clinical Trial

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IMPORTANCE The GeparSixto trial provided evidence that the addition of neoadjuvant carboplatin to a regimen consisting of anthracycline, taxane, and bevacizumab increases pathological complete response (pCR) rates in patients with triple-negative breast cancer (TNBC). Whether *BRCA1* and *BRCA2* germline mutation status affects treatment outcome remains elusive.

OBJECTIVE To determine whether *BRCA1* and *BRCA2* germline mutation status affects therapy response in patients with TNBC.

DESIGN, SETTING, AND PARTICIPANTS This secondary analysis of a randomized clinical trial used archived DNA samples and cancer family history of 315 patients with TNBC enrolled between August 1, 2011, and December 31, 2012, in the GeparSixto trial. In all, 291 participants (92.4%) were included in this multicenter prospective investigation. DNA samples were analyzed for germline mutations in *BRCA1, BRCA2,* and 16 other cancer predisposition genes. The pCR rates between the carboplatin and noncarboplatin arms were compared. Genetic analyses were performed at the Center for Familial Breast and Ovarian Cancer in Cologne, Germany; data analysis, November 1 through December 31, 2015.

MAIN OUTCOMES AND MEASURES Proportion of patients who achieved pCR and disease-free survival after neoadjuvant treatment according to *BRCA1* and *BRCA2* germline mutation status. For pCR rates, the ypT0/is ypN0 definition was used as a primary end point.

RESULTS Of the 291 patients with TNBC, all were women; the mean (SD) age was 48 (11) years. The pCR rate in the carboplatin group was 56.8% (83 of 146) and 41.4% (60 of 145) in the noncarboplatin group (odds ratio [OR], 1.87; 95% CI, 1.17-2.97; $P = .009$). Pathogenic *BRCA1* and *BRCA2* germline mutations were present in 50 of the 291 patients (17.2%). In the noncarboplatin arm, the pCR rate was 66.7% (16 of 24) for patients with *BRCA1* and *BRCA2* mutations and 36.4% (44 of 121) for patients without (OR, 3.50; 95% CI, 1.39-8.84; $P = .008$). The high pCR rate observed in *BRCA1* and *BRCA2* mutation carriers (16 of 24 [66.7%]) was not increased further by adding carboplatin (17 of 26 [65.4%]). In contrast, carboplatin increased response rates in patients without *BRCA1* and *BRCA2* mutations: 66 of the 120 patients (55%) without *BRCA1* and *BRCA2* mutations achieved pCR in the carboplatin arm vs 44 of the 121 patients (36.4%) in the noncarboplatin arm (OR, 2.14; 95% CI, 1.28-3.58; $P = .004$). Patients without pathogenic *BRCA1* and *BRCA2* alterations showed elevated disease-free survival rates when carboplatin was added (without carboplatin, 73.5%; 95% CI, 64.1%-80.8% vs with carboplatin, 85.3%; 95% CI, 77.0%-90.8%; hazard ratio, 0.53; 95% CI, 0.29-0.96; $P = .04$).

CONCLUSIONS AND RELEVANCE Under the nonstandard GeparSixto polychemotherapy regimen, patients without *BRCA1* and *BRCA2* germline mutations benefited from the addition of carboplatin and those with *BRCA1* and *BRCA2* mutations showed superior response rates without additive effects observed for carboplatin.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01426880

JAMA Oncol. 2017;3(10):1378-1385. doi:10.1001/jamaoncol.2017.1007
Published online July 13, 2017.

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Supplemental content

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The GeparSixto randomized clinical trial[1] assessed the efficacy of adding neoadjuvant carboplatin to a regimen of paclitaxel, non-pegylated doxorubicin hydrochloride, and targeted therapy for triple-negative breast cancer (TNBC) and *ERBB2*/*HER2* (OMIM 164870)-positive breast cancer. Targeted therapy included lapatinib and trastuzumab for *ERBB2*/*HER2*-positive breast cancer and bevacizumab for TNBC. Of the patients with TNBC, 90 of 158 (57%) achieved a pathological complete response (pCR) with carboplatin therapy compared with 67 of 157 patients (42.7%) without carboplatin therapy (P = .015; ypT0/is ypN0 definition).[1] Of the patients with *ERBB2*/*HER2*-positive tumors, 72 of 137 (52.6%) achieved a pCR with carboplatin compared with 67 of 136 patients (49.3%) without carboplatin (P = .58; ypT0/is ypN0 definition).[1] Thus, the addition of neoadjuvant carboplatin to the anthracycline and taxane-containing regimen substantially increased pCR rates in patients with TNBC but not in patients with *ERBB2*/*HER2*-positive breast cancer. The GeparSixto trial used a nonstandard neoadjuvant chemotherapy regimen that included low-dose doxorubicin and no cyclophosphamide. In the Cancer and Leukemia Group B (CALGB 40603 Alliance) trial, standard neoadjuvant chemotherapy (paclitaxel, dose-dense doxorubicin, and cyclophosphamide) of patients with TNBC revealed elevated pCR rates when carboplatin was added,[2] but an event-free survival benefit was not observed.[3]

The triple-negative tumor phenotype accounts for up to 17% of all breast cancers[4] and appears to be associated with a hereditary disease cause. Approximately 70% of breast cancers arising in *BRCA1* (OMIM 113705) mutation carriers and up to 23% of breast cancers in *BRCA2* (OMIM 600185) carriers are triple negative.[5] In line with these findings, mutational screening of TNBC cases for deleterious germline mutations in *BRCA1* and *BRCA2* revealed comparatively high mutation frequencies. While germline *BRCA1* and *BRCA2* mutations were found in 5.3% of all breast cancers according to The Cancer Genome Atlas,[6] a recent study showed that 11.2% of unselected TNBC cases had deleterious mutations in the *BRCA1* (8.5%) and *BRCA2* (2.7%) genes. Mutations in additional 15 non-*BRCA1* and *BRCA2* cancer predisposition genes were detected in 3.7% of the patients.[7]

BRCA1 and *BRCA2* are critical genes in the homologous recombination repair of double-stranded DNA breaks. Many of the other genes involved in homologous recombination repair are now recognized to also contribute to hereditary breast cancer risk and/or ovarian cancer risk, including *ATM*, *BRIP1*, *CHEK2*, *NBN*, *PALB2*, *RAD51C,* and *RAD51D*; limited evidence is available for *BARD1*, *FANCM*, *MRE11A,* and *RAD50*.[8-12] Among these genes, only *BRCA1*, *BRCA2,* and *PALB2* so far have been associated with the TNBC tumor phenotype.[8] Heterozygous germline inactivation of homologous recombination genes may be accompanied by a somatic inactivation of the second allele by mutation, loss of heterozygosity, or promoter methylation and result in a homologous recombination deficiency and limited DNA repair capacities of the tumor cells.[13] This functional role in DNA repair could be exploited in the treatment of homologous recombination-deficient cancers by targeting the tumors with drugs that create DNA damage that is highly reliant on these genes for repair.[14] There is increasing evidence that breast and ovarian cancers arising in *BRCA1* and *BRCA2* germline mutation carriers are associated with a better response to DNA-damaging treatment regimens.[9,15-19] These data prompted us to conduct this prospective-retrospective secondary analysis of the germline mutation status using archived DNA samples and cancer family history of patients with TNBC enrolled in the GeparSixto trial.

Key Points

Question Does *BRCA1* and *BRCA2* germline mutation status predict therapy response in patients with triple-negative breast cancer enrolled in the GeparSixto trial?

Findings In this secondary analysis of a randomized clinical trial of 291 patients with triple-negative breast cancer, patients with *BRCA1* and *BRCA2* mutations showed superior response rates, without additive effects observed for carboplatin. Patients without *BRCA1* and *BRCA2* germline mutations benefited from the addition of carboplatin to a regimen of paclitaxel, low-dose doxorubicin, and bevacizumab.

Meaning A less-intense treatment regimen might be considered for *BRCA1* and *BRCA2* mutation carriers, but further prospective studies are needed to identify the optimal regimen.

Methods

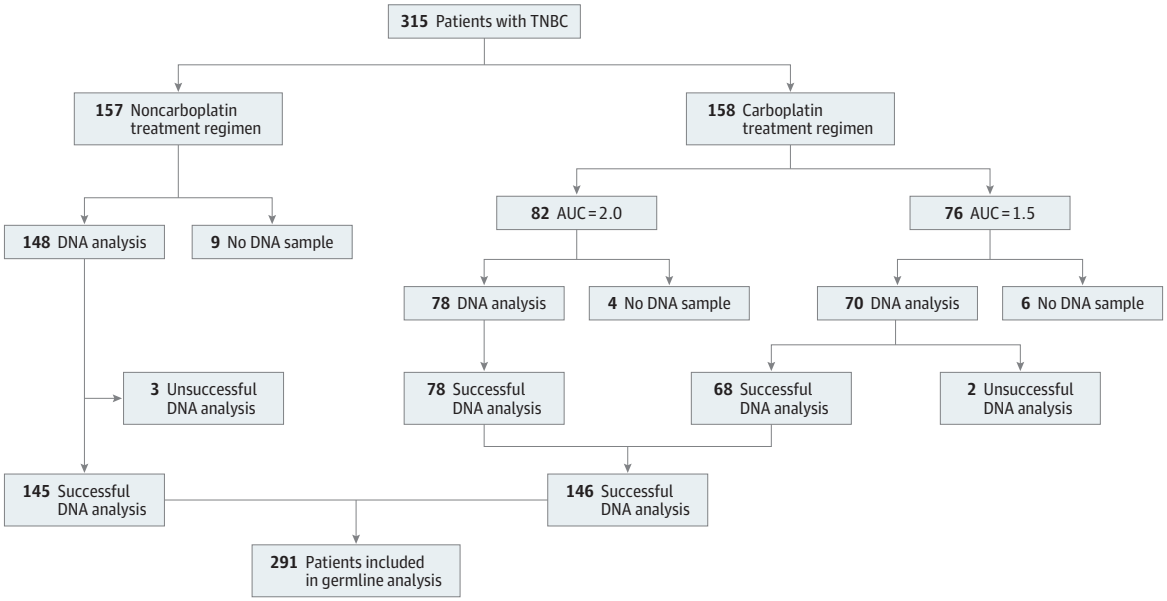
Study Design

The GeparSixto trial cohort, randomization process, clinical procedures, and statistical analyses were described in the initial trial publication.[1] Of the 315 patients with TNBC enrolled between August 1, 2011, and December 31, 2012, in the GeparSixto study, 24 (7.6%) were excluded from this secondary analysis because of unavailable or insufficient amounts of DNA samples (**Figure 1**). Genomic DNA samples isolated from venous blood samples were derived from the other 291 patients with TNBC (92.4%) and were successfully analyzed for germline mutations. The treatment regimen for these 291 patients is shown in Figure 1. Data on cancer family history for all 291 patients were available and were considered positive when the inclusion criteria of the German Consortium for Hereditary Breast and Ovarian Cancer for genetic germline testing were fulfilled (eTable 1 in the Supplement). Data analysis took place from November 1, 2015, to December 31, 2015. Ethical approval for this secondary analysis was granted by the ethics committee of the University of Cologne, and written informed consent was obtained from all patients.

Procedures

Genetic analyses were performed at the Center for Familial Breast and Ovarian Cancer in Cologne, Germany, the coordinating entity of the German Consortium for Hereditary Breast and Ovarian Cancer involved in diagnostic *BRCA1* and *BRCA2* germline testing since 1996.[20] The diagnostic pipeline is certified by the European Molecular Genetics Quality Network. Genomic DNA samples were isolated from venous blood

Figure 1. CONSORT Flow Diagram



All patients with triple-negative breast cancer (TNBC) were scheduled to receive paclitaxel, 80 mg/m^2, and non-pegylated liposomal doxorubicin, 20 mg/m^2, both given once a week for 18 weeks, as well as bevacizumab, 15 mg/kg, intravenously every 3 weeks simultaneously with all cycles. Patients who were randomly assigned to receive simultaneous carboplatin received the drug at a dose of 2.0 area under curve (AUC), once every week for 18 weeks. The carboplatin dose was reduced to 1.5 AUC after an interim safety analysis when approximately half of the patients were randomized.

samples using standard methods. All samples (n = 291) were screened for gross genomic aberrations in the *BRCA1* and *BRCA2* genes by multiplex ligation-dependent probe amplification (MLPA) using probe mixes (SALSA MLPA probe mixes P002 [*BRCA1*] and P045 [*BRCA2*]; MRC-Holland) according to the manufacturer's protocol. Data were analyzed using the Coffalyzer.Net software, version 140429.1057 (MRC-Holland). All *BRCA1* and *BRCA*2 deletions or duplications were verified using probe mixes (SALSA MLPA probe mixes P087 [*BRCA1*] and P077 [*BRCA2*]; MRC-Holland). In parallel, all samples were screened for the predominant pathogenic *BRCA1* and *BRCA2* mutations identified within the framework of the German Consortium for Hereditary Breast and Ovarian Cancer. For these analyses, a customized single-nucleotide polymorphism (SNP) genotyping assay (SNP Type Assay; Fluidigm) covering 90 distinct *BRCA1* and *BRCA2* alterations was established (eTable 2 in the Supplement). Specific target amplification was performed according to the assay manufacturer's potocol using 25 ng of genomic DNA. Samples and SNP type assay mixes were loaded on nanofluid chips (96.96 Dynamic Arrays; Fluidigm), run on a thermocycler (FC1-Cycler; Fluidigm), and analyzed using a fluorecence imager (EP1-System; Fluidigm). For variant calling, the SNP Genotyping Analysis Software, version 3.1.3 (Fluidigm) was used. All mutations identified by this approach were verified by Sanger sequencing.

All samples that tested negative for pathogenic *BRCA1* and *BRCA2* mutations by MLPA/SNP type assay (n = 250) were subsequently analyzed by next-generation sequencing covering the entire coding regions and exon-flanking sequences (±25 nucleotides) of *BRCA1*, *BRCA2*, and 16 non-*BRCA1* and *BRCA2* cancer predisposition genes (*ATM* [OMIM 607585], *BARD1* [OMIM 601593], *BRIP1* [OMIM 605882], *CDH1* [OMIM 192090], *CHEK2* [OMIM 604373], *FANCM* [OMIM 609644], *MRE11A* [OMIM 600814], *NBN* [OMIM 602667], *PALB2* [OMIM 610355], *PTEN* [OMIM 601728], *RAD50* [OMIM 604040], *RAD51C* [OMIM 602774], *RAD51D* [OMIM 602954], *STK11* [OMIM 602216], *TP53* [OMIM 191170], and *XRCC2* [OMIM 600375]).[8] For next-generation sequencing, a customer-tailored gene panel protocol optimized for 200 ng of genomic DNA was used (SureSelect^{XT} Target Enrichment for Illumina Paired-End Multiplexed Sequencing; Agilent Technologies). Sequencing was performed using a sequencing platform (HiSeq 2000; Illumina). Bioinformatic analyses were carried out using the VARBANK, version 2.10 pipeline of the Cologne Center for Genomics. A detailed description of the variant calling is given in eTable 3 in the Supplement. Variant classification was performed in accordance with the regulations of the international ENIGMA consortium (https://enigmaconsortium.org).

Outcomes

The primary outcome of this biomarker study was the proportion of patients who achieved a pCR and disease-free survival (DFS) after neoadjuvant treatment according to *BRCA1* and *BRCA2* germline mutation status and family history (eTable 1 in the Supplement). Regarding the pCR rates, the ypT0/is ypN0 definition was used as a primary end point and the more stringent ypT0 ypN0 definition as a secondary end point.[21] Disease-free survival was defined according to the description by Hudis and colleagues[22] as time in months from randomization until any invasive locoregional (ipsilateral breast, local/regional

Table 1. pCR Rates According to *BRCA1* and *BRCA2* Germline Mutation Status and Treatment Arm

Type of Treatment	pCR[a]		Mutant vs Wild-type *BRCA*		pCR[b]		Mutant vs Wild-type *BRCA*	
	Yes	No	OR (95% CI)	*P* Value	Yes	No	OR (95% CI)	*P* Value
Noncarboplatin arm, No. (%)								
Overall (n = 145)	60 (41.4)	85 (58.6)			52 (35.9)	93 (64.1)		
Mutant (n = 24)	16 (66.7)	8 (33.3)	3.50 (1.39-8.84)	.008	12 (50.0)	12 (50.0)	2.03 (0.84-4.91)	.12
Wild-type (n = 121)	44 (36.4)	77 (63.6)			40 (33.1)	81 (66.9)		
Carboplatin arm, No. (%)								
Overall (n = 146)	83 (56.8)	63 (43.2)			77 (52.7)	69 (47.3)		
Mutant (n = 26)	17 (65.4)	9 (34.6)	1.55 (0.64-3.74)	.33	16 (61.5)	10 (38.5)	1.55 (0.65-3.68)	.32
Wild-type (n = 120)	66 (55.0)	54 (45.0)			61 (50.8)	59 (49.2)		

Abbreviations: OR, odds ratio; pCR, pathological complete response.

[a] Using ypTO/is ypNO definition.

[b] Using ypTO ypNO definition.

Table 2. Comparison of pCR Rates by Treatment Arms and by *BRCA1* and *BRCA2* Germline Mutation Status

Type of Treatment in Cb vs NonCb Arm	pCR[a]		pCR[b]	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Cb vs nonCb, overall	1.87 (1.17-2.97)	.009	2.00 (1.25-3.19)	.004
Cb vs nonCb, mutant	0.94 (0.29-3.05)	.92	1.29 (0.46-3.56)	.63
Cb vs nonCb, wild-type	2.14 (1.28-3.58)	.004	2.23 (1.31-3.80)	.003

Abbreviations: Cb, carboplatin; OR, odds ratio; pCR, pathological complete response.

[a] Using ypTO/is ypNO definition.

[b] Using ypTO ypNO definition.

lymph nodes) recurrence of disease, any invasive contralateral breast cancer, any distant recurrence of disease, any secondary malignant neoplasm, or death from any cause, whichever occurs first. Disease progression under therapy was not considered as an event for DFS. Patients without an event (236 of 291 [81%]) were censored at the date of their last contact with the GeparSixto study.

Statistical Methods

The Pearson χ^2 test was used to compare pCR rates between groups. Univariate logistic regressions were performed to estimate odds ratios (ORs) and 95% CIs. Multivariate logistic regressions adjusting for baseline variables (age, tumor stage, nodal status, grading, Ki67 staining level, and BRCA risk according to family history) were performed, including interaction between mutation status and carboplatin treatment. The Kaplan-Meier product-limit method was used to estimate DFS. A Cox proportional hazards model was used to estimate hazard ratios (HRs) and 95% CIs, with a 2-sided Wald *P* value.

Results

pCR Rates in the Study Cohort

Detailed data on cancer family history and blood-derived DNA samples were available from 291 of 315 patients (92.4%) with TNBC enrolled in the GeparSixto trial. Of the 291 patients with TNBC, 100% were women, with a mean (SD) age of 48 (11) years. Compatible with the data on the initially published entire cohort (n = 315), the pCR rate (ypTO/is ypNO definition) in the carboplatin group was 56.8% (83 of 146 patients) and was 41.4% (60 of 145) in the noncarboplatin group (OR, 1.87; 95% CI, 1.17-2.97; *P* = .009; **Table 1** and **Table 2**), with differences reaching levels of significance in the multivariable analyses (OR, 2.08; 95% CI, 1.19-3.63; *P* = .01; eTable 4 in the Supplement).

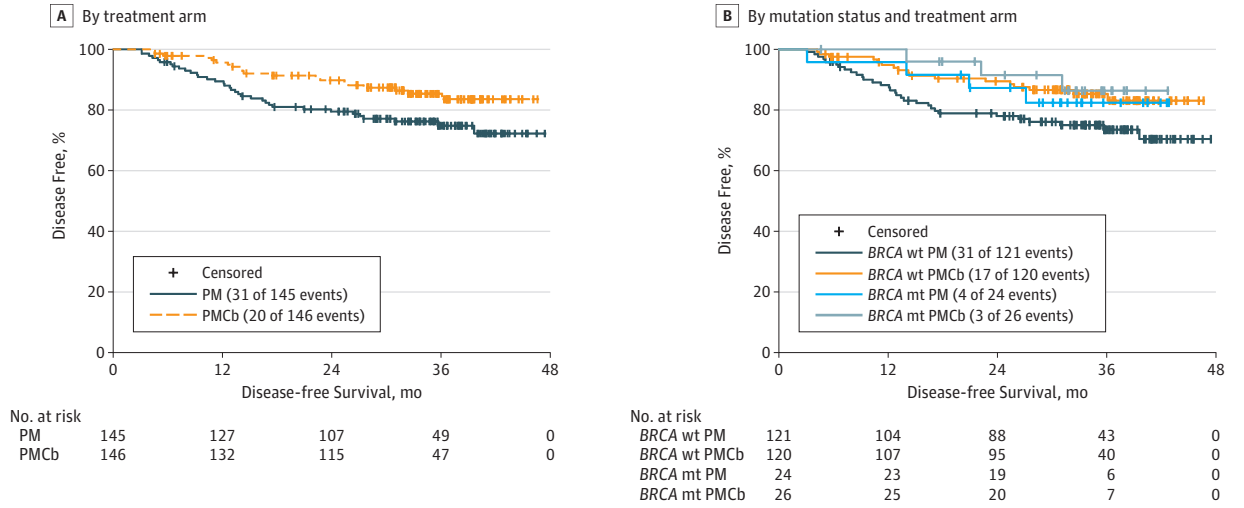
Germline Mutation Spectrum in the Study Cohort

We screened for deleterious germline mutations in *BRCA1*, *BRCA2,* and 16 non-*BRCA1* and *BRCA2* cancer predisposition genes. Besides homologous recombination and other DNA repair genes, cancer predisposition genes not belonging to the DNA repair machinery (*CDH1, PTEN, STK11,* and *TP53*), all of which were associated with rare cancer predisposition syndromes, were included.[8] Pathogenic *BRCA1* mutations were present in 43 of 291 cases (14.8%), *BRCA2* mutations were found in 7 of 291 cases (2.4%), and another 10 cases (3.4%) carried deleterious non-*BRCA1* and *BRCA2* gene mutations (*BARD1* [n = 1], *FANCM* [n = 2], *MRE11A* [n = 1], *NBN* [n = 2], *RAD50* [n = 2], *RAD51C* [n = 1], and *XRCC2* [n = 1]). Overall, 50 of 291 patients (17.2%) with TNBC carried germline *BRCA1* and *BRCA2* alterations, and 10 of 291 patients (3.4%) carried deleterious alterations in other putative cancer predisposition genes (eTable 3 in the Supplement). Because of the small number of patients with deleterious mutations in non-*BRCA1* and *BRCA2* genes (n = 10) and the unclear association of these genes with the TNBC tumor phenotype, we refrained from calculating pCR rates for this small and heterogeneous subgroup and considered these patients as *BRCA1* and *BRCA2*-negative.

pCR Rates According to *BRCA1* and *BRCA2* Mutation Status

For 23 of 50 patients (46%) with pathogenic *BRCA1* and *BRCA2* mutations, a disease onset before age 40 years was observed, compared with only 42 of 241 patients (17.4%) without deleterious *BRCA1* and *BRCA2* alterations (eTable 5 in the Supplement). An association with a positive *BRCA1* and *BRCA2* mutation status was also observed for family history: 31 of 50

Figure 2. Kaplan-Meier Analysis of Disease-Free Survival



A, Disease-free survival by treatment arm. B, Disease-free survival by *BRCA1* and *BRCA2* mutation status and treatment arm. mt Indicates mutant; PM, paclitaxel and myocet; PMCb, paclitaxel, myocet, and carboplatin; and wt, wild-type.

patients (62%) carrying pathogenic *BRCA1* and *BRCA2* mutations reported a positive family history, compared with 79 of 241 patients (32.8%) without (eTable 5 in the Supplement). No significant associations were observed between *BRCA1* and *BRCA2* mutation status and tumor stage at baseline, nodal status at baseline, grading, or Ki67 staining level (eTable 5 in the Supplement). In the noncarboplatin arm, 16 of 24 patients (66.7%) with *BRCA1* and *BRCA2* mutation showed a pCR, compared with only 44 of 121 patients (36.4%) without *BRCA1* and *BRCA2* mutations (OR, 3.50; 95% CI, 1.39-8.84; $P = .008$; Table 1). Adding carboplatin did not enhance overall pCR rates in the subgroup of germline *BRCA1* and *BRCA2* mutation carriers: 17 of 26 patients (65.4%) carrying the *BRCA1* and *BRCA2* mutations achieved a pCR with adjuvant carboplatin therapy compared with 16 of 24 patients (66.7%) without carboplatin therapy (Table 1). In an interaction test, the interaction between the mutation and carboplatin revealed nonsignificant results (OR, 0.68; 95% CI, 0.17-2.68; $P = .58$; eTable 4 in the Supplement). The overall increased pCR rate with carboplatin therapy appeared to be driven by elevated response rates in patients with TNBC not carrying germline *BRCA1* and *BRCA2* mutations: patients with TNBC without pathogenic *BRCA1* and *BRCA2* alterations showed a 36.4% response rate (44 of 121 patients), which increased to 55% (66 of 120) when carboplatin was added to the regimen (OR, 2.14; 95% CI, 1.28-3.58; $P = .004$; Tables 1 and 2).

pCR Rates According to Cancer Family History

Patients with TNBC without a family history of cancer (181 of the 291 patients), a subgroup not enriched for *BRCA1* and *BRCA2* mutation carriers (19 of 181 patients [10.5%]; eTable 5 in the Supplement), showed significantly elevated pCR rates in the carboplatin arm, which increased from 37% (34 of 92 patients) without carboplatin to 53.9% (48 of 89) with carboplatin (OR, 2.0; 95% CI, 1.10-3.62; $P = .02$; eTable 6 in the Supplement). Thus, the absence of a positive cancer family history is associated with carboplatin response in this trial. In all, 110 of 291 patients (37.8%) with TNBC reported a positive cancer family history (eTable 6 in the Supplement). Patients with a positive cancer family history, a subgroup enriched for *BRCA1* and *BRCA2* germline mutation carriers (31 of 110 [28.2%]; eTable 5 in the Supplement), showed comparatively high response rates in the noncarboplatin arm (26 of 53 [49.1%]), which moderately increased to 61.4% (35 of 57) when carboplatin was added (OR, 1.65; 95% CI, 0.77-3.53; $P = .19$; eTable 6 in the Supplement). Of note, 9 of 10 patients carrying non-*BRCA1* and *BRCA2* germline mutations did not show a cancer family history, and 4 of 10 patients achieved a pCR (eTable 7 in the Supplement).

DFS Rates According to Treatment and *BRCA1* and *BRCA2* Mutation Status

With a median follow-up of 35 months, superior DFS rates were observed in the carboplatin group vs the noncarboplatin group (HR, 0.55; 95% CI, 0.32-0.95; $P = .03$; **Figure 2**A). These data were compatible with the early DFS rates described for the entire TNBC cohort (n = 315).[23] Patients with TNBC without pathogenic *BRCA1* and *BRCA2* alterations showed elevated DFS rates when carboplatin was added to the treatment regimen: without carboplatin, 73.5%; 95% CI, 64.1%-80.8%; with carboplatin, 85.3%; 95% CI 77.0%-90.8% (HR, 0.53; 95% CI, 0.29-0.96; $P = .04$; Figure 2B). Regardless of the treatment regimen, the DFS rate was generally high in *BRCA1* and *BRCA2* mutation carriers, with differences separated by study arm that did not reach levels of significance: without carboplatin, 82.5%; 95% CI, 59.6%-93.1%; with carboplatin, 86.3%; 95% CI, 63.1%-95.4% (Figure 2B). We observed a significant correlation of pCR rates with DFS rates (log-rank $P < .001$) irrespective of the *BRCA1* and *BRCA2* mutation status (eFigure in the Supplement).

Discussion

In the noncarboplatin arm, this investigation suggests superior pCR rates in patients with germline *BRCA1* and *BRCA2* mutations compared with patients without *BRCA1* and *BRCA2* mutations, with differences translating into clinical benefit when considering the respective DFS rates. This finding may be the result of better treatment response of *BRCA1* and *BRCA2* mutation carriers to either of the chemotherapeutic agents used in the noncarboplatin arm. The primary mechanism of action of doxorubicin is thought to be via DNA intercalation and stabilization of the topoisomerase IIa/DNA complex, ultimately promoting the formation of single-stranded and double-stranded DNA breaks.[24,25] Thus, it appears plausible that *BRCA1* and *BRCA2* mutation carriers achieve higher response rates under therapy with antracyclines because of limited DNA repair capacities of the tumors. On the basis of initial data presented by the randomized Triple Negative Trial,[26] *BRCA1* and *BRCA2* mutation status is unlikely to be correlated with therapy response to docetaxel. The Triple Negative Trial included patients with metastatic disease, and pretreatment may have changed their responsiveness to chemotherapeutic agents. However, the trial did not suggest that the differences observed between *BRCA1* and *BRCA2* mutation carriers and noncarriers in the noncarboplatin arm were driven by treatment with taxane. Bevacizumab, a vascular endothelial growth factor inhibitor, has been shown to elevate pCR rates in patients with TNBC (OR, 1.36; 95% CI, 1.11-1.66).[27] Vascular endothelial growth factor expression in tumors of *BRCA1* and *BRCA2* mutation carriers is demonstrably higher than in sporadic tumors.[28] Concordantly, an investigation of the GeparQuinto trial recently revealed higher pCR rates in response to bevacizumab therapy for *BRCA1* and *BRCA2* mutation carriers than for noncarriers. However, a superior outcome of survival probability could not be demonstrated.[29]

Because the TNBC tumor phenotype is closely associated with hereditary breast cancer,[5] the use of platinum agents has received a new impetus. The cytotoxic actions of platinum drugs are mediated by covalent binding of platinum to DNA, interfering with DNA replication and transcription and ultimately inducing cell death.[30] It seems likely that partially processed cross-links cause replication fork stalling when encountered by the DNA replication machinery during S phase, which may degenerate into double-stranded DNA breaks.[31] Thus, tumor cells with limited DNA repair capacities are hypersensitive against platinum, as demonstrated in preclinical studies.[32] In a recent neoadjuvant trial, platinum-based chemotherapy was shown to be highly effective in *BRCA1* germline mutation carriers: a total of 50 of 82 patients (61%) with TNBC experienced a pCR following cisplatin single-agent therapy.[15] The high sensitivity of *BRCA1* and *BRCA2* mutation carriers to platin-based chemotherapy is in line with data in the metastatic or recurrent locally advanced setting in which carboplatin monotherapy revealed significantly higher response rates in *BRCA1* and *BRCA2* mutation carriers than in patients without *BRCA1* and *BRCA2* mutations.[26] In summary, carboplatin therapy has been proven effective in *BRCA1* and *BRCA2* mutation carriers.

The GeparSixto treatment regimen for TNBC cases included 2 DNA-damaging compounds, doxorubicin and carboplatin, both of which challenge the DNA repair machinery. This finding could explain why, in germline mutation carriers, the addition of carboplatin to the treatment regimen does not further increase pCR or DFS rates above those observed for the combination of paclitaxel, doxorubicin, and bevacizumab. Given that the more intense regimen significantly increases hematological and nonhematological adverse effects in the GeparSixto trial,[1] our findings may have implications for personalized therapy regimens that consider *BRCA1* and *BRCA2* germline mutation status. Because of the lack of additive effects observed in this study and the finding that elevated pCR rates translate into a clinical benefit,[21] a less intense therapy regimen might be considered for *BRCA1* and *BRCA2* germline mutation carriers.

Limitations

This study has several limitations. First, the GeparSixto trial was not powered for long-term end points such as DFS and overall survival. Second, there was a small number of patients carrying *BRCA1* and *BRCA2* (n = 50), especially non-*BRCA1* and *BRCA2* gene mutations (n = 10). Thus, additional trials are necessary to assess the clinical benefit of a combination use of DNA-damaging compounds, especially in *BRCA1* and *BRCA2* mutation carriers.

Conclusions

In contrast to the GeparSixto trial, the CALGB 40603 trial revealed that the addition of carboplatin to a standard neoadjuvant chemotherapy (paclitaxel, dose-dense doxorubicin, and cyclophosphamide) did not result in an event-free survival benefit.[2,3] The GeparSixto trial used a nonstandard neoadjuvant chemotherapy regimen, including low-dose doxorubicin and no cyclophosphamide. Thus, the differences in response rates between the GeparSixto and CALGB 40603 trials might be caused by the different doxorubicin (low dose vs dose dense) and/or cyclophosphamide exposures. It would be interesting to stratify the CALGB 40603 response rates by *BRCA1* and *BRCA2* mutation status. It appears likely that *BRCA1* and *BRCA2* mutation carriers show superior response rates following standard neoadjuvant chemotherapy, while patients without *BRCA1* and *BRCA2* mutations may benefit from the addition of carboplatin. Byrski and colleagues[15,33] suggested that a chemotherapy regimen with doxorubicin and cyclophosphamide or platinum may result in the highest benefit for *BRCA1* germline mutation carriers. This suggestion is based on a limited number of patients, but future trials may evaluate this hypothesis. Under the nonstandard GeparSixto polychemotherapy regimen, however, patients without *BRCA1* and *BRCA2* germline mutations benefit from the addition of carboplatin while those with *BRCA1* and *BRCA2* mutations show superior response rates without additive effects observed for carboplatin. Additional prospective studies stratified by *BRCA1* and *BRCA2* mutation status are needed to elucidate the effect of carboplatin in polychemotherapy regimens.

ARTICLE INFORMATION

Accepted for Publication: February 24, 2017.

Published Online: July 13, 2017. doi:10.1001/jamaoncol.2017.1007

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Obtained funding: Gerber, Schmutzler.
Administrative, technical, or material support: Hahnen, Lederer, Kröber, Schneeweiss, Denkert, Fasching, Blohmer, Jackisch, Paepke, Gerber, Huober, Rhiem, Costa, Altmüller, Nürnberg, Untch, von Minckwitz, Schmutzler.
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Conflict of Interest Disclosures: Drs Loibl and von Minckwitz report that their institution has received research funding from Amgen, AstraZeneca, Celgene, Hexal, Novartis, Pfizer, Roche, and Teva. No other disclosures were reported.

Funding/Support: The molecular genetic analyses were supported by grant No. 110837 from the German Cancer Aid.

Role of the Funder/Sponsor: The funding source had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We are thankful to all patients who participated in this study.

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