

Germline and Somatic Mutations in Homologous Recombination Genes Predict Platinum Response and Survival in Ovarian, Fallopian Tube, and Peritoneal Carcinomas

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Abstract

Purpose: Hallmarks of germline *BRCA1/2*-associated ovarian carcinomas include chemosensitivity and improved survival. The therapeutic impact of somatic *BRCA1/2* mutations and mutations in other homologous recombination DNA repair genes is uncertain.

Experimental Design: Using targeted capture and massively parallel genomic sequencing, we assessed 390 ovarian carcinomas for germline and somatic loss-of-function mutations in 30 genes, including *BRCA1*, *BRCA2*, and 11 other genes in the homologous recombination pathway.

Results: Thirty-one percent of ovarian carcinomas had a deleterious germline (24%) and/or somatic (9%) mutation in one or more of the 13 homologous recombination genes: *BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*. Nonserous ovarian carcinomas had similar rates of homologous recombination mutations to serous carcinomas (28% vs. 31%, $P = 0.6$), including clear cell, endometrioid, and carcinosarcoma. The presence of germline and somatic homologous recombination mutations was highly predictive of primary platinum sensitivity ($P = 0.0002$) and improved overall survival ($P = 0.0006$), with a median overall survival of 66 months in germline homologous recombination mutation carriers, 59 months in cases with a somatic homologous recombination mutation, and 41 months for cases without a homologous recombination mutation.

Conclusions: Germline or somatic mutations in homologous recombination genes are present in almost one third of ovarian carcinomas, including both serous and nonserous histologies. Somatic *BRCA1/2* mutations and mutations in other homologous recombination genes have a similar positive impact on overall survival and platinum responsiveness as germline *BRCA1/2* mutations. The similar rate of homologous recombination mutations in nonserous carcinomas supports their inclusion in PARP inhibitor clinical trials. *Clin Cancer Res*; 20(3); 764–75. ©2013 AACR.

Introduction

Inherited mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) account for the majority of familial ovarian carcinoma (1). *BRCA1/2*, along with other genes in the Fanconi anemia (FA) pathway, play key roles in homologous recombina-

tion, the main mechanism that repairs double-strand DNA breaks. Other FA-*BRCA* genes have also been implicated in genetic susceptibility to ovarian carcinoma, including *BRIP1*, *RAD51C*, and *RAD51D* (1–4). Hallmarks of *BRCA1/2*-associated ovarian carcinomas include sensitivity to platinum chemotherapy, improved overall survival (5–10), and sensitivity to PARP inhibitors (PARPi; refs. 11, 12). In addition to *BRCA1/2*, mutations in other FA-*BRCA* genes may impact the homologous recombination function and increase sensitivity to DNA-damaging agents. *In vitro* studies demonstrate that deficiency in other homologous recombination proteins such as *ATM*, *CHEK1*, *CHEK2*, *NBN*, and *RAD51D* also confers sensitivity to PARPi (2, 13). PARPi are active agents in ovarian carcinomas from women with germline *BRCA1/2* mutations, but also in a subset of "sporadic" recurrent platinum-sensitive ovarian carcinomas (12). Indeed, The Cancer Genome

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org>).

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Translational Relevance

We have demonstrated that germline and somatic loss-of-function mutations in homologous recombination DNA repair genes are associated with significantly improved primary platinum sensitivity and overall survival. These better outcomes are applied to somatic as well as germline mutations and to genes other than *BRCA1* and *BRCA2*. Until now, many scientists assumed that homologous recombination deficiency occurs more commonly in high-grade serous ovarian carcinomas. However, we found that homologous recombination mutations occurred at similar frequency in nonserous carcinomas. It is known that ovarian carcinomas associated with germline *BRCA1* and *BRCA2* mutations have high response rates to treatment with PARP inhibitors. We hypothesize that ovarian carcinomas with mutations in homologous recombination genes other than *BRCA1* and *BRCA2* will also respond to PARP inhibitors and suggest that PARP inhibitor trials should target both nonserous and serous carcinomas.

Atlas (TCGA) reported homologous recombination defects in approximately 50% of high-grade serous ovarian carcinomas (14). The availability of PARPi as therapeutic agents adds incentive to better characterize this subset of ovarian carcinoma.

We hypothesize that somatic and germline mutations in a variety of FA-BRCA genes could identify those subjects with "sporadic" ovarian carcinoma in which cancers are sensitive to PARPi, and that these cases will have increased sensitivity to platinum chemotherapy and prolonged survival, as do individuals with germline *BRCA1/2* mutations. We, therefore, sought to determine the rate of germline and somatic mutations in 13 homologous recombination genes in a series of women with ovarian, fallopian tube, and peritoneal carcinoma, and to correlate the presence of these mutations with response to platinum-based chemotherapy and overall survival.

Results

A total of 367 individuals and 390 carcinomas were included in the study: 310 individuals with primary carcinoma, 34 with recurrent carcinoma, and 23 with a paired primary and recurrent carcinoma. Of the 367 subjects, 304 had ovarian carcinoma, 24 had fallopian tube carcinoma, 32 had peritoneal carcinoma, and 7 had synchronous ovarian and endometrial carcinomas. Table 1 provides characteristics of cases included in the study. Most cases were advanced stage (83%), of either serous histology or poorly differentiated adenocarcinoma (83%), and were optimally cytoreduced (66%, to <1 cm maximal residual tumor diameter) at the time of primary surgery. All primary carcinomas received platinum-based chemotherapy, with the exception of five stage I carcinomas. Targeted capture by BROCA baits and genomic sequencing yielded median 289-

fold coverage; the percentage of targeted bases at >10×>50× depth was 99% and 93%, respectively.

Overall homologous recombination mutation rate

Eighty-seven subjects (24%) had a germline homologous recombination mutation, and 32 subjects (9%) had a somatic homologous recombination mutation (Supplementary Table S1). Four subjects (1.1%) had both a germline and somatic homologous recombination mutation (Supplementary Table S2). Thus, the total proportion of subjects with at least one loss-of-function germline or somatic homologous recombination mutation was 31% (115 of 367; Fig. 1A). Of the 123 germline and somatic homologous recombination mutations, 68 (55%) occurred in *BRCA1*, 23 (19%) in *BRCA2*, and 32 (26%) in 11 other homologous recombination genes: *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*. Of the four cases with both germline and somatic homologous recombination mutations, one case had both a germline (816delGT) and somatic *BRCA1* mutation (del exons 1–2). In this case, the somatic mutation may represent the "second hit" inactivating the wild-type *BRCA1* allele. In the remaining three cases, the somatic mutation represented only a smaller fraction (20%–35%) of the DNA sequences in the neoplasm. Presumably, in these cases, the germline mutation was the driver and the somatic mutation was incidental.

Germline mutations

Ninety-four loss-of-function germline mutations were identified in the 367 subjects in 15 different genes. Eighty-seven subjects (24%) had 88 germline mutations in homologous recombination genes, whereas six (1.6%) subjects had mutations in nonhomologous recombination genes, including 3 in *TP53*, 1 in *MSH2*, 1 in *BUB1B*, and 1 in *MSH6*. The 88 germline loss-of-function mutations in 11 homologous recombination genes included 49 (56%) in *BRCA1*, 17 (19%) in *BRCA2*, and 22 (25%) in other homologous recombination genes: 2 (2%) in *BARD1*, 4 (4.5%) in *BRIP1*, 1 (1%) in *CHEK1*, 3 (3%) in *CHEK2*, 2 (2%) in *FAM175A*, 1 (1%) in *NBN*, 2 (2%) in *PALB2*, 3 (3%) in *RAD51C*, and 4 (4.5%) in *RAD51D* (Fig. 1B and Supplementary Table S1). One subject had germline mutations in both *MSH6* and *BRIP1*, as previously reported (1). One subject had a germline nonsense mutation in *RAD50* (p.Y625X). However, several other nonsense and frameshift mutations in *RAD50* are relatively common in the North American population, as reported on the exome variant server (<http://evs.gs.washington.edu/EVS/>, April 2013). Therefore, the clinical significance of inactivation of one *RAD50* allele is questionable and heterozygous *RAD50* mutations were not included in the homologous recombination-deficient category.

Somatic mutations

Thirty-two of 367 subjects (8.7%) had a total of 35 somatic loss-of-function mutations. The 35 mutations occurred in seven homologous recombination genes: 19

Table 1. Clinical characteristics and fraction with homologous recombination mutations

	All subjects (N)	Fraction with germline homologous recombination mutation ^a	Fraction with somatic homologous recombination mutation ^a
Median age, y	59	53	59
Range, y	27–89	34–75	29–80
Site			
Ovary	304	0.22	0.08
Fallopian tube	24	0.42	0.04
Peritoneal	32	0.22	0.03
Synch ov/endo	7	0.29	0.14
Histology			
High-grade ^b serous	249	0.26	0.05
Low-grade ^c serous	9	0.11	0
Poorly differentiated NOS	48	0.25	0.13
Clear cell	19	0.05	0.21
High-grade ^b endometrioid	20	0.15	0.10
Low-grade ^c endometrioid	6	0.17	0.17
Carcinosarcoma	12	0.25	0.08
Other ^d	4	0.25	0
Stage ^e			
I	36	0.17	0.14
II	19	0.16	0
III	255	0.24	0.08
IV	49	0.27	0.04
Cytoreduction ^f			
Optimal	243	0.23	0.09
Suboptimal	109	0.22	0.06
Total	367	0.24	0.08

NOTE: Synch ov/endo: cases classified pathologically as having two primary cancers arising from the ovary and endometrium. Abbreviation: NOS, not otherwise specified.

^aCases with both a germline and somatic homologous recombination mutation were included in the germline homologous recombination category.

^bGrades 2–3.

^cGrade 1.

^dOther = one malignant Brenner, one mucinous, and two mixed carcinomas.

^eStage was unknown for eight cases.

^fCytoreduction status was not available for 15 cases.

(54%) in *BRCA1*, 6 (17%) in *BRCA2*, 3 (9%) in *ATM*, 2 (6%) in *BRIP1*, 3 (9%) in *CHEK2*, 1 (3%) in *MRE11A*, and 1 (3%) in *RAD51C* (Fig. 1C). Supplementary Table S1 gives details of all deleterious germline mutations, somatic homologous recombination mutations, somatic *PTEN* mutations, and accompanying case characteristics. One subject had a *RAD50* gene rearrangement, which was excluded. 290 cases (79%) had a deleterious somatic *TP53* mutation. *TP53* mutations in serous carcinomas were limited to grade 2–3 carcinomas. However, *TP53* mutations were also observed in other histologies, including 3 of 19 clear cell, 11 of 26 endometrioid (including one grade 1 carcinoma), 10 of 12 carcinosarcoma, 1 of 1 malignant Brenner, and 1 of 2 mixed histologies.

The role of *PTEN* in homologous recombination deficiency is controversial, and we did not classify *PTEN*

mutations as homologous recombination deficient. Twenty-two cases (6%) had somatic *PTEN* loss-of-function mutations, including four that had an accompanying homologous recombination germline mutation and two that had an accompanying somatic homologous recombination mutation.

Nonserous histology

Sixty-one cases (17%) were of nonserous histology, including 19 clear cell, 26 endometrioid, 12 carcinosarcoma, 2 mixed with predominant endometrioid histology, 1 mucinous, and 1 malignant Brenner carcinoma. Seventeen of 61 (28%) nonserous cases had a deleterious germline or somatic homologous recombination mutation (Table 2). Similarly, 80 of 258 (31%) serous cases had a germline or somatic homologous recombination mutation

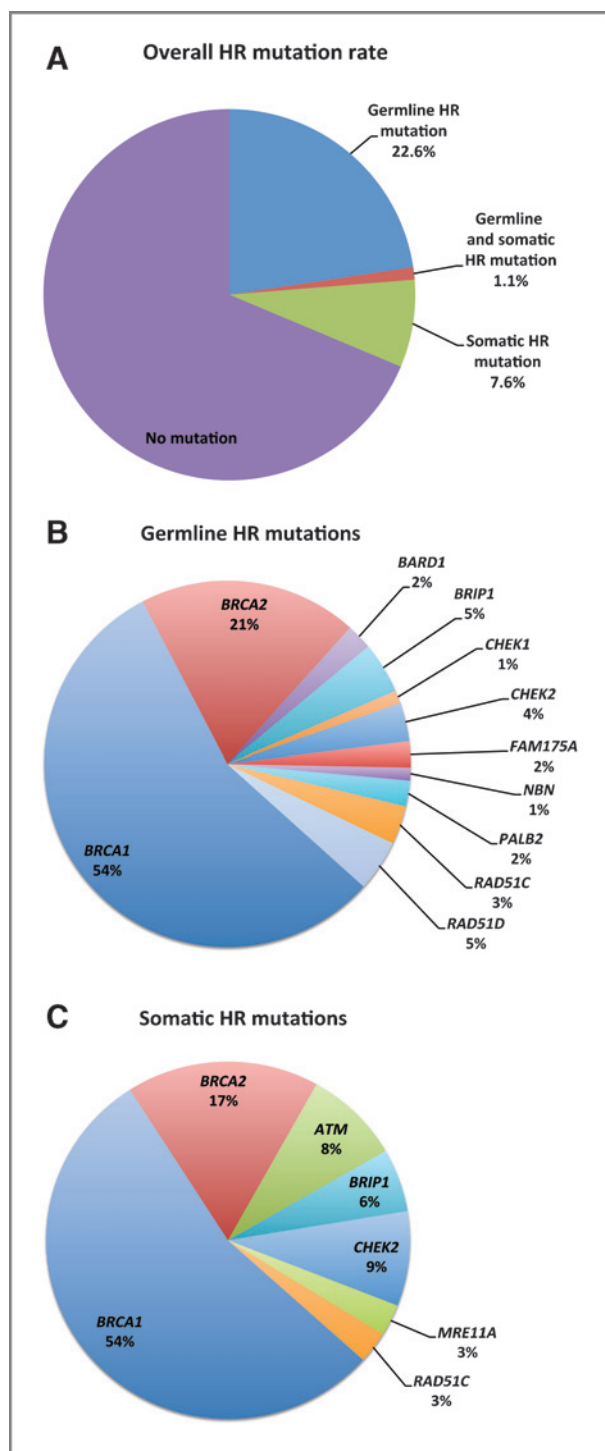


Figure 1. Mutation rates in homologous recombination (HR) genes. A, overall, 115 of 367 subjects (31.3%) had deleterious mutations in 13 homologous recombination genes: 83 (22.6%) with germline homologous recombination mutations, 28 (7.6%) with somatic homologous recombination mutations, and 4 (1.1%) with both germline and somatic homologous recombination mutations. Mutations were detected in every homologous recombination gene tested. B, 87 subjects (24%) had 88 germline mutations in 11 homologous recombination genes. Germline homologous recombination mutations included 49 (13.4%) in *BRCA1*, 17 (4.6%) in *BRCA2*, and 22 (6%) in other homologous

($P = 0.63$; Fig. 2A). Loss-of-function homologous recombination mutations were identified in almost every type of nonserous histology tested, including 5 of 19 (26%) clear cell, 7 of 26 (27%) endometrioid, 4 of 12 (33%) carcinosarcoma, and 1 of 1 (100%) malignant Brenner carcinoma (Fig. 2A). No homologous recombination mutations were identified in the one mucinous or two mixed histology carcinomas. Interestingly, 2 of 6 (33%) low-grade endometrioid carcinomas had homologous recombination mutations (Table 2). In the 9 subjects with low-grade serous carcinoma included in the study, one (11%) had a homologous recombination mutation. While there was a predominance of *BRCA1/2* mutations in serous cases, nonserous histologies had a wider distribution of mutations in genes other than *BRCA1/2* (Fig. 2B). In the nonserous carcinomas with germline or somatic homologous recombination mutations (collectively), 56% (10 of 18) of mutations were in genes other than *BRCA1/2*. In contrast, only 21% (18 of 85) of homologous recombination mutations in serous carcinomas had mutations in other homologous recombination genes ($P = 0.005$).

Primary platinum response

A total of 243 of 333 (73%) subjects with primary carcinoma had adequate clinical information available to define primary platinum response, with platinum sensitivity defined as the maintenance of complete response of 6 months or more after the completion of platinum therapy. The presence of a germline or somatic mutation in a homologous recombination gene was strongly associated with primary platinum sensitivity. Seventy-one of 85 (84%) primary carcinomas with a homologous recombination mutation (germline or somatic) demonstrated platinum sensitivity. In contrast, 95 of 158 (60%) carcinomas without an identified homologous recombination mutation had platinum sensitivity, and the remainder were either platinum resistant or refractory ($P = 0.0002$). Germline homologous recombination mutations and somatic homologous recombination mutations were each separately predictive of platinum sensitivity compared to cases without homologous recombination mutations: 49 of 61 (80%) cases with a germline mutation were platinum sensitive ($P = 0.005$), and 22 of 24 (92%) carcinomas with a somatic mutation were platinum sensitive ($P = 0.003$; Fig. 3). Although platinum sensitivity was correlated with optimal cytoreduction ($P < 0.00001$), carcinomas with homologous recombination mutations and those without homologous recombination mutations had similar rates of optimal cytoreduction (67% vs. 66%; $P = 0.43$).

recombination genes, including *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*. C, 32 carcinomas (8.7%) had a total of 35 somatic mutations in 7 homologous recombination genes. Somatic homologous recombination mutations included 19 (5.2%) in *BRCA1*, 6 (1.6%) in *BRCA2*, and 10 (2.7%) in other homologous recombination genes, including *ATM*, *BRIP1*, *CHEK2*, *MRE11A*, and *RAD51C*.

Table 2. Nonserous cases with homologous recombination mutations

Histology	ID	Grade	Germline homologous recombination mutation ^a	Somatic homologous recombination mutation(s) ^a	Somatic <i>PTEN</i> mutation(s) ^a
Clear cell	UW400	3	<i>CHEK2</i> p.S428F		
	UWf14	3		<i>BRCA2</i> p.S368X	
	UW420	3		<i>CHEK2</i> del exons 1–7	
	UW408	3		<i>ATM</i> c.5441delT	<i>PTEN</i> c.678delC
	UW358	3		<i>BRCA1</i> 1135insA <i>BRIP1</i> c.3260insA <i>MRE11A</i> c.1196insTT	<i>PTEN</i> c.968insA
Endometrioid	UW383	1	<i>BRIP1</i> p.R798X		<i>PTEN</i> c.955delACTT
	UW381	1		<i>ATM</i> c.3284(+1)G>C splice	
	UW131	2		<i>BRCA2</i> p.R2494X	
	UW341	2		<i>BRCA1</i> dup exons 21–24	
	UW165	3	<i>BRCA1</i> 187delAG		
	UWf2	3	<i>BRCA2</i> 3034delAAAC		
	UW132	3	<i>RAD51D</i> c.580delA		<i>PTEN</i> c.389delG
Carcinosarcoma	UWf77	3	<i>BRCA1</i> 2080delA		
	UW435	3	<i>FAM175A</i> c.1106insG		
	UW407	3	<i>RAD51C</i> c.706(–2) A>G splice		
	UW124	3		<i>BRCA1</i> 3481delA	
Malignant Brenner	UW96	3	<i>FAM175A</i> c.1106insG		

^a*BRCA1* and *BRCA2* mutations were annotated using BIC designation (<http://research.nhgri.nih.gov/bic/>; reference sequences: *BRCA1* GenBank U14680; *BRCA2* GenBank U43746); all other mutations were annotated using HGVS nomenclature.

We assessed whether the observed association with homologous recombination mutations and platinum sensitivity was driven by the large number of *BRCA1/2* germline mutations, which have previously been associated with improved survival and platinum responsiveness (5–10). As expected, germline *BRCA1/2* mutations were associated with platinum sensitivity in 38 of 47 (81%) cases ($P = 0.01$, vs. no germline or somatic homologous recombination mutation). However, the presence of a germline mutation in any non-*BRCA1/2* homologous recombination gene or the presence of any homologous recombination somatic mutation (including *BRCA1/2*) also predicted platinum sensitivity, with 33 of 38 (87%) carcinomas exhibiting platinum sensitivity ($P = 0.002$, compared with cases with no germline or somatic homologous recombination mutation). The majority of these subjects had somatic *BRCA1/2* mutations. The relatively smaller number of subjects with other homologous recombination mutations limits analysis, but 14 of 18 (78%) carcinomas with a non-*BRCA1/2* homologous recombination mutation (germline or somatic) were platinum sensitive, compared with 61% of carcinomas without germline or somatic homologous recombination mutations ($P = 0.14$). Subjects who had both a *BRCA1/2* mutation and another homologous recombination mutation were excluded from these analyses.

The impact of *PTEN* deficiency on platinum response is unknown. Twelve primary carcinomas had isolated *PTEN* mutations and complete clinical information available; 8 carcinomas (67%) were platinum sensitive. Similarly, 89 of

148 (60%) carcinomas without mutations (no mutations in *PTEN* or homologous recombination genes) were platinum sensitive ($P = 0.8$).

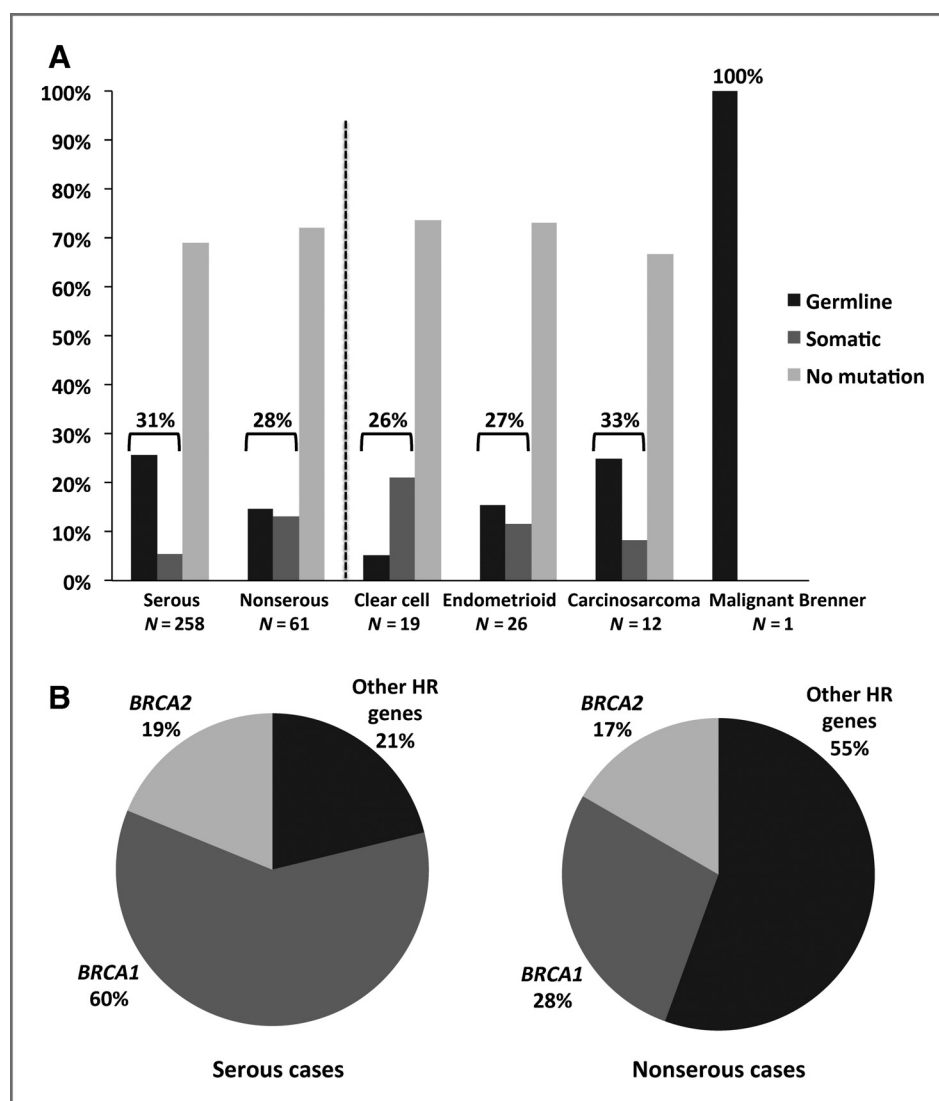
Platinum response at recurrence

We assessed whether the presence of a homologous recombination mutation in a recurrent carcinoma predicted platinum sensitivity for that recurrence. Of note, 45 of 57 (79%) recurrent carcinomas had complete clinical information allowing the determination of platinum sensitivity for that recurrence. Of 29 recurrent carcinomas without germline or somatic homologous recombination mutations, only 7 (24%) remained platinum sensitive. Similarly, of 16 recurrent carcinomas with a homologous recombination mutation, 5 (31%) remained platinum sensitive ($P = 0.73$). Therefore, homologous recombination mutations were more successful at predicting platinum sensitivity at primary treatment than at relapse. However, our recurrent cancers represented a range of clinical scenarios and this question should be reevaluated in a more uniform setting, such as at first recurrence.

Overall survival

The presence of a germline or somatic homologous recombination gene mutation was associated with significantly better overall survival for women with stage II–IV carcinomas compared with cases without homologous recombination mutations ($P = 0.0006$; HR, 0.6; 95% confidence interval (CI), 0.4–0.8; Fig. 4A). The following

Figure 2. Homologous recombination (HR) gene mutation rates by histology. A, homologous recombination gene mutations were identified at similar frequencies in nonserous and serous carcinomas. Seventeen of 61 (28%) nonserous cases and 80 of 258 (31%) serous cases had a deleterious germline or somatic homologous recombination mutation ($P = 0.63$). Homologous recombination gene mutations were identified in almost every type of nonserous histology tested, including 5 of 19 (26%) clear cell, 7 of 26 (27%) endometrioid, 4 of 12 (33%) carcinosarcoma, and 1 of 1 (100%) malignant Brenner carcinoma. No mutations were identified in the one mucinous or two mixed histology carcinomas. B, whereas serous carcinomas had a predominance of mutations in *BRCA1* and *BRCA2*, the nonserous carcinomas had a much wider distribution of mutations in other homologous recombination genes. In nonserous carcinomas with homologous recombination mutations, 56% of mutations were in other homologous recombination genes, compared with only 21% of mutations in serous carcinomas ($P = 0.005$).



additional characteristics were significantly related to overall survival: age ($P = 0.01$), optimal versus suboptimal cytoreduction ($P = 0.001$), and stage ($P = 0.0005$). In a multivariate model including these four characteristics, only the presence of a homologous recombination mutation ($P = 0.006$) and stage ($P = 0.0009$) remained significantly associated with overall survival, whereas optimal cytoreduction was of borderline significance ($P = 0.06$) and age was no longer significant ($P = 0.22$). Subjects with germline homologous recombination mutations had a median survival of 66 months, compared with 59 months for subjects with somatic homologous recombination mutations and 41 months for subjects without a homologous recombination mutation (Fig. 4B). Survival in subjects with germline homologous recombination mutations was significantly better than subjects without homologous recombination mutations ($P = 0.001$). Survival in cases with somatic mutations was similar to germline mutation carriers, but did not reach statistical significance when

compared with cases without homologous recombination mutations ($P = 0.09$).

Germline *BRCA1/2* mutations were associated with improved overall survival (median 70 months) compared with subjects without homologous recombination mutations ($P = 0.001$; HR, 0.5; 95% CI, 0.4–0.8). Subjects with a germline mutation in homologous recombination genes other than *BRCA1/2* or any homologous recombination somatic mutation (including somatic *BRCA1/2* mutations) also had improved survival compared with subjects without homologous recombination mutations, with a median survival of 59 versus 41 months ($P = 0.05$; HR, 0.7; 95% CI, 0.5–1.0; Fig. 4C).

To assess the association of *PTEN* mutations with clinical outcomes, we compared survival in subjects with somatic homologous recombination mutations, somatic *PTEN* mutations, and no mutations (no mutations in either *PTEN* or in homologous recombination genes). Subjects who had both a homologous recombination mutation and a *PTEN*

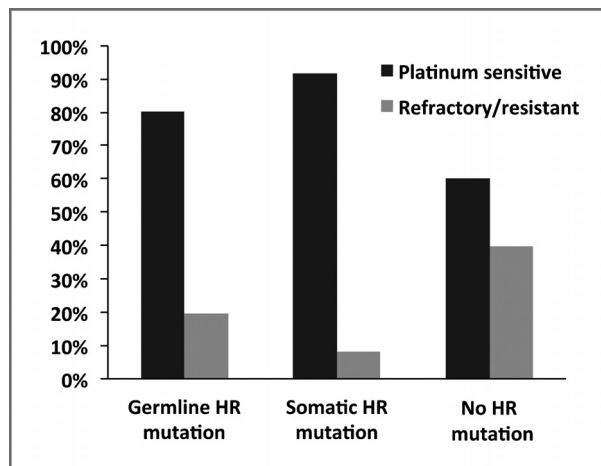


Figure 3. Germline homologous recombination (HR) mutations and somatic homologous recombination mutations were each predictive of platinum sensitivity compared with cases without homologous recombination mutations: 49 of 61 (80%) cases with a germline mutation ($P = 0.008$), and 22 of 24 (92%) carcinomas with a somatic mutation ($P = 0.003$) were platinum sensitive ($P = 0.003$). In contrast, only 95 of 158 (60%) carcinomas without an identified homologous recombination mutation had primary platinum sensitivity.

mutation were excluded from the analyses. Median overall survival in subjects with *PTEN* mutations was 25.5 months, significantly worse than the 42 months for cases with no mutations ($P = 0.007$; HR, 2.2; 95% CI, 1.4–7.4), and 59 months for somatic homologous recombination mutations. As only 12 subjects had isolated *PTEN* mutations, these findings require confirmation in larger studies. Although *PTEN* mutation carriers had similar rates of suboptimal cytoreduction compared with cases without mutations (42% vs. 31%, respectively), a higher proportion of *PTEN* subjects had stage IV disease (50% vs. 11%, $P = 0.002$), which might account for the worse survival. The small number of subjects with *PTEN* mutations preclude multivariate analysis.

Primary–recurrent pairs

Next-generation sequencing can identify somatic mutations that may guide personalized treatment decisions. However, the stability of somatic mutations over time is unclear. It is unknown whether a biopsy should be performed at the time of recurrence to guide treatment decisions, or if archived primary carcinoma tissue could be used. We therefore sought to evaluate neoplastic evolution in paired primary and recurrent ovarian carcinomas based on significant alterations in read ratios of the target mutation. Because comparing the differences in somatic alterations between carcinomas is difficult due to differences in neoplastic purity between samples, we were very conservative in calling differences between the primary and recurrent carcinoma. A pair was considered discordant if a somatic mutation was present in one carcinoma of the pair and absent in the other.

Twenty-three subjects had a paired primary and recurrent carcinoma specimen (Supplementary Table S3). All subjects

had at least one mutation present in one or both specimens. Twelve subjects had germline mutations: 8 in *BRCA1*, 2 in *BRCA2*, 1 in *PALB2*, and 1 in *FAM175A*. Two pairs had somatic homologous recombination mutations: one with a somatic *BRCA1* mutation and one with five different somatic mutations in the primary carcinoma. A total of 20 pairs had a somatic *TP53* mutation.

Out of two pairs with somatic homologous recombination mutations, one pair was considered concordant. UW124 had a *BRCA1* 3481delA somatic mutation with a similar percentage mutant allele in the primary (73%) and recurrent (80%) carcinomas. The pair also had a somatic *TP53* mutation (p.R273C) present in 80% of DNA sequences in the primary and 82% in the recurrent carcinoma. The other pair (UW358) had four different somatic homologous recombination mutations (*BRCA1* 1135insA, *BRCA1* 3650insT, *BRIP1* 3260insA, and *MRE11A* 1196insTT), and was considered discordant (Supplementary Table S3). The *BRCA1* 3650insT mutation was present in the recurrent carcinoma (7% mutant allele), but not detected in the primary carcinoma. Whether this represents a new mutation acquired after initial therapy, or whether it was initially present at very low levels at diagnosis and then selected for at recurrence is unknown. The *BRIP1* mutation was present in the primary carcinoma (23% mutant allele), but was absent in the recurrent sample.

Twenty pairs had somatic *TP53* mutations. Two pairs were discordant, whereas 18 were concordant. UW440 had a p.G245D mutation present only in the primary carcinoma, and a c.994(–1)G>A splice site mutation present only in the recurrent carcinoma. A germline *BRCA1* mutation was present in both samples, precluding a sample mix-up. UW406 had a p.R248W mutation detected in 65% of DNA sequences in the primary carcinoma and was not detected in the recurrent carcinoma.

Nineteen pairs had complete clinical information about the platinum sensitivity for both the primary and recurrent carcinoma. Recurrent carcinomas with homologous recombination mutations developed platinum resistance at a similar rate to those without homologous recombination mutations; 3 of 10 recurrent carcinomas (30%) with homologous recombination mutations remained platinum sensitive at recurrence compared with 4 of 9 (44%) without homologous recombination mutations. However, these comparisons are limited by the heterogeneity of the recurrent carcinomas, which included first recurrences to up to fourth recurrence and intervals from the primary to recurrent carcinoma ranging from 11 to 84 months.

Discussion

Our study demonstrates that both germline and somatic loss-of-function mutations in genes in the FA–BRCA pathway predict higher rates of platinum sensitivity and better overall survival in primary ovarian carcinoma. The improved primary platinum sensitivity associated with germline and somatic homologous recombination mutations is consistent with *in vitro* data that cells with defective homologous

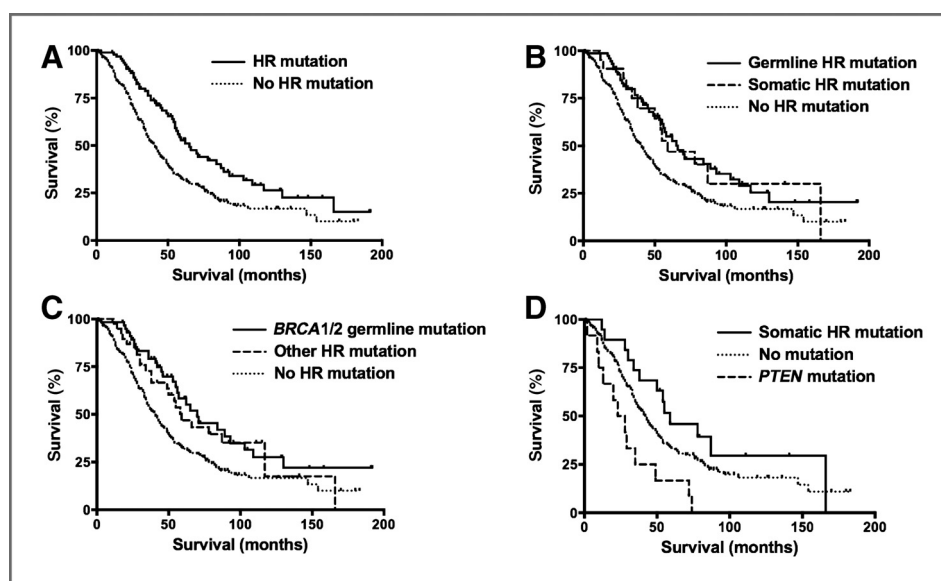


Figure 4. Overall survival by genetic status. A, the presence of a homologous recombination (HR) gene mutation was associated with an improved overall survival compared with cases without homologous recombination mutations (median overall survival 66 vs. 41 months, $P = 0.006$; HR, 0.6; 95% CI, 0.4–0.8). Homologous recombination gene mutations were significantly related to overall survival after accounting for the covariates age, stage, and optimal cytoreduction ($P = 0.006$). B, overall survival in subjects with germline homologous recombination mutations was significantly better than subjects without homologous recombination mutations (median 66 months vs. 41 months, $P = 0.001$). Overall survival in cases with somatic mutations (median 59 months) was similar to germline mutation carriers, but these differences did not reach statistical significance when compared with cases without homologous recombination mutations ($P = 0.09$). C, subjects with germline *BRCA1/2* mutations had an improved overall survival compared with subjects without homologous recombination mutations (median 70 vs. 41 months; $P = 0.001$; HR, 0.5; 95% CI, 0.4–0.8). Subjects with a germline mutation in homologous recombination genes other than *BRCA1/2* or any homologous recombination somatic mutation (including *BRCA1/2* mutations) also had improved survival (median 59 months; $P = 0.05$; HR, 0.7; 95% CI, 0.5–1.0). D, median overall survival in subjects with *PTEN* mutations was 25.5 months, significantly shorter than for cases with no homologous recombination or *PTEN* mutations (median 42 months; $P = 0.007$; HR, 2.2; 95% CI, 1.4–7.4), and cases with somatic homologous recombination mutations (median 59 months).

recombination are more sensitive to agents that induce double-strand DNA breaks (15). Women with germline homologous recombination mutations had significantly longer overall survival (median 66 months versus 41 months) than subjects without germline or somatic homologous recombination mutations ($P = 0.001$), consistent with previous studies demonstrating longer survival in women with germline *BRCA1/2* mutations (5–7, 15). Importantly, somatic *BRCA1/2* mutations and germline and somatic mutations in homologous recombination genes other than *BRCA1/2* were also associated with improved survival and platinum sensitivity ($P = 0.05$). To the best of our knowledge, we are the first to correlate the presence of mutations in other homologous recombination genes in ovarian carcinomas with clinical outcomes. The improved overall survival observed in germline homologous recombination mutation carriers may be due to not only improved response to platinum-based chemotherapy, but also the retention of platinum sensitivity through multiple recurrences, which has previously been reported for *BRCA1/2* carriers (8). We predicted that somatic mutations would be less stable over time due to clonal selection and, therefore, would have less impact on overall survival than germline mutations. However, overall survival in cases with somatic mutations (median, 59 months; HR, 0.6) was also improved, and was similar to germline mutation carriers, but did not reach statistical significance when compared with cases without homo-

logous recombination mutations due to smaller numbers ($P = 0.09$).

Interestingly, subjects with somatic *PTEN* mutations had significantly worse overall survival (median 25.5 months) than subjects without any mutations (42 months) or with somatic mutations in homologous recombination genes (59 months). *PTEN* mutations also did not correlate with primary platinum sensitivity. Because of the small number of subjects with *PTEN* mutations, these findings require confirmation. The impact of *PTEN* deficiency on homologous recombination is debated. Although some studies suggest that carcinoma cells with defective *PTEN* have reduced RAD51-dependent homologous recombination and are sensitive to PARPi (16–18), other studies failed to demonstrate these findings (19, 20). The worse overall survival of cases with *PTEN* mutations in our study suggests that *PTEN* and homologous recombination mutations are not similar predictors of outcomes in ovarian carcinomas.

Overall, 31% of ovarian carcinomas had a deleterious germline (24%) and/or somatic (9%) mutation in one of the following 13 homologous recombination DNA repair pathway genes: *BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*. We identified mutations in every homologous recombination gene included on our panel, and the mutation rate would likely be higher if more genes in the homologous recombination pathway were queried.

Although the majority of both germline and somatic homologous recombination mutations were in *BRCA1* or *BRCA2* (74%), 26% occurred in other homologous recombination genes. We hypothesize that individuals with mutations (either somatic or germline) in homologous recombination genes other than *BRCA1/2* will also have increased response rates to PARPi, as they do to platinum chemotherapy. However, not all genes may be equally important in therapeutic response. Functional assays to determine which of these alterations actually cause PARPi sensitivity, animal models with various genetic defects, and clinical trials that correlate homologous recombination mutation status with PARPi response are needed to optimally develop biomarkers of PARPi responsiveness.

Contrary to popular dogma that only high-grade serous ovarian carcinomas are likely to be homologous recombination deficient, we found homologous recombination gene mutations (germline and somatic) to be equally common in carcinomas with nonserous histologies. Mutations in homologous recombination genes were present in 17 of 61 (28%) nonserous carcinomas and were identified in nearly every histology subtype tested, including clear cell, endometrioid, and carcinosarcoma. Although nonserous cases had some *BRCA1/2* mutations, they had a greater proportion of mutations in other homologous recombination genes, with two thirds of germline homologous recombination mutations in genes other than *BRCA1/2*. Therefore, nonserous ovarian carcinomas also have a meaningful risk of hereditary breast and ovarian carcinoma, but identification necessitates evaluation with a larger panel of ovarian cancer susceptibility genes. Our findings contrast with those of a recent study of 131 women with nonmucinous ovarian carcinoma, which found that germline *BRCA1/2* mutations were exclusively associated with high-grade serous histology, but evaluated only 23 nonserous cases (21). The identification of three germline *BRCA1/2* mutations in our nonserous cases is unlikely secondary to misclassification, as all of our nonserous cases underwent a recent centralized pathology review by a single gynecologic pathologist blinded to genetic status. Our findings may influence clinical trial design, as most PARPi trials have selected high-grade serous carcinomas as their focus (12). Given the similar homologous recombination mutation rate, we suggest that PARPi trials should include a variety of ovarian carcinoma histologies.

It is interesting to compare our germline mutation results with those of TCGA, which performed exome sequencing in 316 women with high-grade serous ovarian carcinoma (14). Among the 249 women with high-grade serous carcinoma in our series, there were 53 germline *BRCA1/2* mutations, a germline mutation rate of 21%. In contrast, TCGA reported 47 germline *BRCA1/2* mutations in 316 women: 14%, after subtracting the three reported occurrences of *BRCA2* p.K3326X, a benign polymorphism found in 1% of the general population (22). TCGA's lower germline *BRCA1/2* mutation rate is likely due to cohort selection bias. Many participating IRBs required consenting living patients, and thus contributed cases were biased toward

deceased patients and new enrollees, but away from long-term survivors. As *BRCA1/2* mutation carriers with ovarian carcinoma have improved overall survival (5–7), the lower number of long-term survivors included in TCGA may have negatively impacted their overall germline *BRCA1/2* mutation rate.

It is more difficult to compare our overall homologous recombination deficiency rate with that of TCGA given significant differences in methodology. TCGA reported homologous recombination defects in approximately 50% of high-grade serous cases (14), but included a wide variety of genomic alterations that we did not assess, including *BRCA1* hypermethylation, *EMSY* amplification or mutation, and *RAD51C* hypermethylation, which in aggregate comprised 22% of their homologous recombination deficiency. *BRCA1* methylation did not impact overall survival in TCGA or in a previous study by our group (23), and the impact of *RAD51C* methylation is unknown. Furthermore, *EMSY* amplification, which is thought to silence *BRCA2*, is associated with worse survival, opposite to the expected association for homologous recombination deficiency (24, 25). In addition, TCGA assessed many homologous recombination genes that we did not assess, counted all missense mutations as deleterious, and included somatic *PTEN* mutations as homologous recombination deficient. Furthermore, TCGA did not assess germline mutations in homologous recombination genes other than *BRCA1/2*. Therefore, other than the cases with germline and somatic *BRCA1/2* mutations, we have likely identified a different subset of ovarian carcinomas to be homologous recombination deficient than were classified as such by TCGA.

We analyzed 23 paired primary and recurrent ovarian carcinomas to evaluate the stability of somatic mutations over time. The vast majority of somatic *TP53* mutations were concordant, although two were not. *TP53* is thought to be a driver event in ovarian carcinogenesis, and it is possible that *TP53* mutations are more stable over time compared with other mutations. As only two paired cases had somatic homologous recombination mutations, we are unable to generalize on the stability of homologous recombination somatic mutations during treatment. We and others have shown that germline *BRCA1/2* mutations can "revert" to wild-type sequence in recurrent ovarian carcinoma (26–30), and we presume that somatic homologous recombination mutations would be under a similar high negative selection pressure during multiple rounds of chemotherapy. Obtaining tissue biopsies of recurrent ovarian carcinoma at uniform time-points in the treatment setting is critical to understanding clonal progression. These studies would determine when obtaining a biopsy at recurrence is needed and when archived primary carcinoma tissue can be used to guide personalized treatment decisions.

In summary, germline and somatic mutations in homologous recombination genes are present in almost one third of ovarian carcinomas and predict a better response to primary platinum chemotherapy and improved overall survival. We hypothesize that individuals with these mutations will also have increased response rates to PARPi.

Clinical trials of PARPi that fully characterize genetic status will be needed to confirm this hypothesis. Notably, non-serous ovarian carcinomas have an equal rate of homologous recombination mutations relative to serous carcinomas, but with a higher fraction of those mutations in genes other than *BRCA1/2*.

Materials and Methods

Study subjects

Women with ovarian, fallopian tube, or primary peritoneal carcinoma who underwent surgery at the University of Washington (Seattle, WA) or at Swedish Hospital (Seattle, WA), and provided informed consent approved by the human subjects divisions of the IRB were eligible for the study. Subjects were prospectively enrolled at diagnosis and not selected for age or family history. We excluded carcinomas identified at the time of risk-reducing surgery performed due to genetic risk. Clinical information was retrieved from medical records. Genomic DNA was extracted from peripheral blood mononuclear cells (germline DNA) and from frozen or formalin-fixed paraffin-embedded sections from areas with 60% or greater neoplastic cellularity. Library construction, hybridization, and massively parallel sequencing were performed as previously described (1). The 30-gene panel (Supplementary Table S4) was designed to include all known breast and ovarian cancer genes, as well as additional homologous recombination-related genes most integral to the FA-BRCA pathway. Assessment for germline mutations was previously reported for 216 subjects in 21 genes (1); these cases were assessed for nine additional genes using the 30-gene panel. A small subset of samples previously underwent testing for germline mutations in other homologous recombination genes (such as *FAM175A* and *CHEK1*), and when these were identified, they were also reported. In addition, a total of 243 subjects had germline (lymphocyte) DNA assessed for mutations in *RAD51D*, either through Sanger sequencing (reported previously in 216 subjects; ref 31) or through targeted capture and genomic sequencing (27 subjects).

All cases with nonserous histology were reviewed by a dedicated gynecologic pathologist (M.H. Rendi). Cases with high-grade endometrioid histology as well as those with mixed or uncertain histology were also reviewed by a second gynecologic pathologist (R.L. Garcia) and a consensus diagnosis was obtained for each case. Cases with mixed histology were only considered nonserous if the predominant histology (>50%) was not serous.

Mutation analysis

The BROCA panel identifies all classes of mutations, including single-base substitutions, small insertions and deletions, and large gene rearrangements (32). Sequence alignment and variant calling were performed against the reference human genome (UCSC hg19) as previously described (1). Each variant was annotated with respect to gene location and predicted function in human genome variation society (HGVS) nomenclature. *BRCA1* and *BRCA2* mutations were annotated using the designations used by

the Breast Cancer information Core (BIC; <http://research.nhgri.nih.gov/bic/>, *BRCA1* GenBank U14680; *BRCA2* GenBank U43746); all other mutations were annotated using HGVS nomenclature. Deletions and duplications of exons have been detected in normal cells by a combination of relative read depth and split read algorithms, as described previously (32, 33). For carcinoma samples, changes in copy number state were identified using similar normalized read depth approach, but incorporating normal mixture modeling via expectation maximization (34), which allowed the detection of copy number state changes that were present in a proportion of the complex population of cells in the tumor sample. Using this approach, we were able to detect the amplification and deletion of entire genes and of small copy number variations (CNV) within the loci down to single exon resolution (approximately 200 bp).

For all suspected loss-of-function variants, PCR amplification and Sanger sequencing was performed both on lymphocyte-derived (germline) and neoplastic DNA to confirm and classify the mutation as somatic or germline. Only missense variants previously demonstrated to be deleterious were included. There was no minimum threshold for the variant reads for somatic mutations as long as they validated with Sanger sequencing. For somatic large gene rearrangements or CNVs, any gene-disrupting intragenic deletion or duplication was considered deleterious. Homozygous whole gene deletions were considered deleterious; hemizygous whole gene deletions (i.e., loss of heterozygosity) were excluded. CNVs were validated using PCR amplification and Sanger sequencing when breakpoints could be identified. If breakpoints were not clear, CNVs were validated using quantitative PCR.

Mutation analysis was performed on the paired primary and recurrent carcinomas using an alternate pipeline tailored to detect somatic mutations in clinical cancer specimens, as described by Pritchard and colleagues (35). The percentage of mutant allele present in DNA sequences was compared in the paired primary and recurrent carcinoma using read ratios (variant reads/total reads) and also using fluorescent peak ratios from Sanger sequencing for each target mutation. Each primary-recurrent pair was classified as concordant or discordant based on alterations in read ratios of the target mutation between the primary and recurrent carcinoma. Read ratios were only used for comparison when both carcinomas had adequate total number of reads (>100); in two cases with poor depth of coverage, fluorescent peak ratios from Sanger sequencing were used instead. A pair was considered discordant if a mutation was present in only one carcinoma of the pair and absent in the other.

Statistical analysis

In cases with paired primary and recurrent samples, individuals were counted only once and the primary carcinoma of the pair was used for analysis (unless otherwise specified). When subjects had both a germline and a somatic homologous recombination mutation, they were included in the germline homologous recombination mutation

group and not the somatic mutation group for analyses. Significance of contingency tables was analyzed with a χ^2 or Fisher exact test. Primary platinum sensitivity was defined by a complete response during adjuvant chemotherapy and clinical remission for at least 6 months after the completion of chemotherapy. Primary platinum resistance was defined as progressive disease on platinum therapy, less than a complete response to platinum therapy, or progression within 6 months of completing platinum therapy. To classify platinum responsiveness in recurrent carcinomas, the actual response to platinum-based chemotherapy for that recurrence was used, and not the previous interval since previous chemotherapy. If the subject's most recent treatment did not include platinum and if her previous interval between treatment and progression was 6 months or more, then she was considered nonevaluable for platinum sensitivity. If that interval was less than 6 months or if she was previously classified as platinum resistant, then that recurrence was considered platinum resistant.

Survival analyses were performed using the methods of Kaplan and Meier; differences were assessed using the log rank test. Significant variables for survival were used as covariates in a multivariate model. Overall survival was calculated from time of diagnosis to death. Survival data were censored for living patients at time of last follow-up.

All *P* values were two-tailed, with α set at 0.05. GraphPad Prism software was used for all statistical analyses.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Germline and Somatic Mutations in Homologous Recombination Genes Predict Platinum Response and Survival in Ovarian, Fallopian Tube, and Peritoneal Carcinomas

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