



HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2019 February 15.

Published in final edited form as:

Clin Cancer Res. 2018 February 15; 24(4): 777–783. doi:10.1158/1078-0432.CCR-17-1327.

Mutations in Homologous Recombination Genes and Outcomes in Ovarian Carcinoma Patients in GOG 218: an NRG Oncology/ Gynecologic Oncology Group Study

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Abstract

Purpose—We hypothesized that mutations in homologous recombination repair (HRR) genes beyond *BRCA1* and *BRCA2* improve outcomes for ovarian carcinoma (OC) patients treated with platinum therapy and would impact the relative benefit of adding prolonged bevacizumab.

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Experimental design—We sequenced DNA from blood and/or neoplasm from 1,195 women enrolled in GOG-0218, a randomized phase III trial in advanced OC of bevacizumab added to carboplatin and paclitaxel. Defects in HRR were defined as damaging mutations in 16 genes. Proportional hazards models were used to estimate relative hazards for progression-free survival (PFS) and overall survival (OS).

Results—Of 1,195 women with OC, HRR mutations were identified in 307 (25.7%). Adjusted hazards for progression and death compared to those without mutations were lower for women with non-*BRCA* HRR mutations (HR 0.73, 95% CI 0.57 – 0.94, p=0.01 for PFS; HR 0.67, 95% CI 0.50 – 0.90, p=0.007 for OS) and *BRCA1* mutations (hazard ratio (HR) 0.80, 95% CI 0.66 – 0.97, p=0.02 for PFS; HR 0.74, 95% CI 0.59 – 0.94, p=0.01 for OS) and were lowest for *BRCA2* mutations (HR 0.52, 95% CI 0.40 – 0.67, p<0.0001 for PFS; HR 0.36, 95% CI 0.25 – 0.53, p<0.0001 for OS). A test of interaction showed no difference in the effect of bevacizumab on PFS between cases with and without mutations.

Conclusions—HRR mutations, including non-*BRCA* genes, significantly prolong PFS and OS in OC and should be stratified for in clinical trials. The benefit of adding bevacizumab was not significantly modified by mutation status.

Keywords

Ovarian cancer; BRCA; homologous recombination; bevacizumab; somatic mutation

INTRODUCTION

BRCA1 and *BRCA2* are key components of the BRCA-Fanconi anemia DNA repair pathway that controls DNA repair via homologous recombination.(1–3) Germline and somatic mutations affecting homologous recombination repair (HRR) genes are relatively common, found in approximately one-third of ovarian carcinoma patients, with the majority of mutations occurring in *BRCA1* and *BRCA2*.(4,5) Ovarian carcinoma patients with inherited mutations in *BRCA1* and *BRCA2* have longer five-year survival, likely due to improved sensitivity to platinum chemotherapy.(6–8) This may also be true for somatic and germline mutations in other HRR genes, though the number of cases with non-*BRCA* HRR mutations studied to date is small.(5) In addition, *BRCA1* and *BRCA2* mutated ovarian carcinomas may have a lower angiogenic profile(9) and more favorable immunophenotype than those without mutations, which might also contribute to better outcomes.(10,11)

GOG-0218 was a randomized, phase III trial for patients with primary advanced ovarian, peritoneal, and fallopian tube carcinoma (OC), examining the addition of the anti-angiogenesis drug bevacizumab to standard chemotherapy with carboplatin and paclitaxel. Patients in this study who received extended bevacizumab had a significant prolongation in median progression-free survival (PFS) of 3.8 months.(12) In the parallel European ICON-7 trial, a non pre-specified subgroup analysis suggested that extended bevacizumab was of greatest benefit for patients with poor prognostic features such as stage IV disease, or unresectable or suboptimally resected tumors, demonstrating a benefit in overall survival (OS) in these “high-risk” patients.(13)

We hypothesized that OC patients with inherited mutations in HRR genes, including a select set of genes beyond *BRCA1* and *BRCA2*, have longer survival and improved platinum sensitivity and may derive less benefit from extended bevacizumab. We tested this hypothesis using tissues from patients in GOG-218 who had provided consent for translational research.

MATERIALS AND METHODS

GOG-0218 clinical trial details have been previously described.(12) DNA from tumor and blood from patients on GOG-0218 were sequenced using BROCA, a targeted capture, massively parallel sequencing test developed at the University of Washington.(14) All available germline DNA samples extracted from blood were sequenced (N = 788). These patients have been previously described.(8) In order to improve detection of HRR deficiency in this group by detection of somatic mutations in HRR genes, we also sequenced DNA from neoplastic tissue from a subset of these patients with negative germline testing (N = 324). Finally, we sequenced DNA from neoplastic tissue from 407 additional cases that did not have available DNA from blood, detecting both germline and somatic mutations (bringing the total number of sequenced patients to 1195). Distinguishing between germline and somatic mutations could not be done with certainty in the group with only tumor sequencing. As the effect on outcome was predicted to be similar, germline and somatic mutations were combined for analyses. All patients provided written informed consent as approved by an institutional review board (IRB), in accordance with ethical guidelines as described in the U.S. Common Rule. As patients were not specifically consented for open access genomic data, complete sequencing data such as BAM files cannot be shared publicly.

A subset of genes predicted to impact homologous recombination repair (HRR) when mutated was selected prior to analysis based on review of the available literature and expert opinion, including: *ATM*, *ATR*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*, *RBBP8*, *SLX4*, and *XRCC2*. Sequencing reads were aligned to the human reference genome (hg19). Variants were identified using GATK37 and Pindel after indel realignment and base quality calibration. Copy number variations (CNVs) were detected in germline samples as previously described.(14–16) Missense mutations were only included if proven to be damaging in functional assays or classified as likely pathogenic on ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>).(17)

Clinical information was collected as per the GOG-0218 protocol.(12) All pathology was centrally reviewed. PFS and OS were defined as the time between enrollment and progression or death, respectively. Proportional hazards models were used to provide estimates of the relative hazards for progression and death by genotype, adjusted by clinical characteristics. The effect of mutation status on the impact of bevacizumab on progression was assessed with a test of interaction. All P values are 2-sided.

RESULTS

Study Population

Of 1,873 women in GOG 218, 1,195 (63.8%) had DNA available for sequencing. Baseline characteristics of sequenced and not sequenced patients are described in Table 1. The distribution of race was significantly different between the groups, with more non-Hispanic Whites and fewer Asian/Pacific Islanders in the sequenced group ($p < 0.001$, chi-square). There were more optimally debulked stage III patients and less stage IV patients in the sequenced group ($p < 0.001$, chi-square). The distribution of histologic subtypes also differed, with fewer patients with clear cell carcinoma in the sequenced group ($p = 0.012$, chi-square). The median PFS duration for those who were not sequenced was 10.5 months compared to 13.5 months for those sequenced ($p < 0.001$) and the median overall survival for those not sequenced was 30.9 months compared to 46.2 months for those who were sequenced.

Sequencing results

In total, 307/1,195 (25.7%) sequenced patients had damaging mutations in one of the selected genes affecting HRR. There were 148 (12.4%) with mutations in *BRCA1*, 78 (6.5%) with mutations in *BRCA2*, and 81 (6.8%) with mutations in other HRR genes. Ten patients (0.8%) had more than one mutation. Of these 10, three were in the *BRCA1* mutation group, with additional mutations in *RBBP8* (one) and *NBN* (two), and four were in the *BRCA2* group (one with *CHEK2*, one with *RBBP8*, one with both *ATM* and *CHEK2*, and one with *BRIP1* and *CHEK2*). Of the 81 patients in the other HRR group, there were 84 mutations in the following genes: 12 in *ATM*, five in *ATR*, three in *BARD1*, five in *BLM*, 19 in *BRIP1*, two in *CHEK2*, two in *MRE11A*, three in *NBN*, six in *PALB2*, seven in *RAD51C*, seven in *RAD51D*, eight in *RBBP8*, two in *SLX4*, and three in *XRCC2*. Details of the patients with non-*BRCA* HRR mutations are provided in Supplemental Table 1.

In the subset of 324 patients with negative germline testing who had subsequent sequencing of neoplastic DNA, there were 32 (9.9%) somatic mutations in HRR genes (17 (5.2%) in *BRCA1*, seven (2.2%) in *BRCA2*, and eight (2.5%) in other HRR genes). In a small exploratory analysis, these 32 “definitely somatic” mutation carriers had significantly lower hazards of both progression and death when compared to 174 patients with “definitely germline” mutations (Supplemental Figure 1). Mutation rates by the source of sequenced DNA are described in more detail in Supplemental Table 2.

Clinical Characteristics by Mutation Status

The proportion of patients in each mutation group by clinical characteristics is shown in Table 2. Mutation status did not differ by disease stage or primary site. Mutations in HRR genes were found in all histologic subtypes. The overall mutation rate in the high-grade serous carcinomas was 27.0% (262/971), which was not significantly different from the rate in unspecified carcinoma (22/101, 21.8%, $p = 0.29$), endometrioid (10/42, 23.8%, $p = 0.73$), clear cell (6/28, 21.4%, $p = 0.67$), or mucinous carcinoma (2/7, 28.6%, $p = 1.0$). The low-grade serous carcinomas had a lower HRR mutation rate of 10.9% (5/46, 95% confidence interval 4.8–23.1%, $p = 0.02$, Fisher’s exact) when compared to high-grade serous carcinomas.

Effect of Mutation Status on Survival

Damaging mutations in *BRCA1*, *BRCA2*, or other non-*BRCA* HRR genes were all associated with longer PFS and OS relative to cases without mutations (Figure 1). After adjusting for study treatment, stage of disease, size of residual disease, and initial performance status, adjusted hazard ratios for both progression and death were significantly lower in cases with HRR mutations when compared to those with no mutations (Figure 1). This effect was strongest for *BRCA2* mutations and was similar for *BRCA1* and non-*BRCA* HRR mutations.

Interaction Between Mutation Status and the Effect of Bevacizumab on Progression

For this analysis, only patients from arm 1 (carboplatin/paclitaxel with placebo) and arm 3 (carboplatin/paclitaxel with bevacizumab and bevacizumab maintenance) were included (N = 809). In patients with no mutations (N = 581), extended bevacizumab significantly prolonged PFS (15.7 months vs. 10.6 months, HR 0.71, 95% CI 0.60 – 0.85, p=0.0001), Figure 2A. In those with mutations (N = 228), extended bevacizumab conferred a median PFS of 19.6 months vs. 15.4 months, (HR 0.95, 95% CI 0.71 – 1.26, Figure 2C). Using a test of interaction, mutation status did not significantly modify the effect of extended bevacizumab on progression (0.95/0.71) = 1.33, 95% CI 0.95 – 1.85, p = 0.10.

DISCUSSION

GOG-0218 was a large randomized, placebo-controlled phase III clinical trial with standardized treatment, central pathology review, and follow up, providing a unique opportunity to assess the impact of HRR deficiency on clinical outcomes. In this trial, patients with OC with defective HRR, defined by damaging somatic or germline mutations in one of 16 genes, had significantly prolonged PFS and OS when compared to those without mutations (Figure 1). This is the first study in OC large enough to separately assess the impact on outcomes of non-*BRCA* HRR mutations, which are less common than mutations in *BRCA1* and *BRCA2*, though our smaller single institution study showed a trend towards improved overall survival with mutations in a subset of non-*BRCA* genes.(5) We carefully selected a list of 14 non-*BRCA* genes a priori that we thought would be most likely to impact outcomes based on *in vitro* data. OCs with mutations in other HRR pathway genes such as *FANCM*, *FANCA*, and *FANCI* were not classified as HRR deficient. Interestingly, the non-*BRCA* HRR mutations were associated with PFS and OS curves that were almost identical to *BRCA1* mutations, supporting our a priori selection of HRR genes as meaningful.

As expected, *BRCA1* and *BRCA2* mutations were associated with longer survival, with the most profound effect seen for *BRCA2* mutations with a median survival advantage of 33 months compared to those without mutations. These patients were treated years before poly (ADP-ribose) polymerase (PARP) inhibitors became available, and these outcome differences would likely be even greater with current treatments. Previous studies have also demonstrated a better outcome for *BRCA2* compared to *BRCA1* mutations (5–8), but ours is the first large study to include somatic mutations and to assess outcomes relative to mutation status for patients treated on a phase III clinical trial with standardized treatment and follow-

up. Given the meaningful impact of HRR deficiency on both PFS and OS, the presence of HRR mutations, including *BRCA1* and *BRCA2*, should be carefully considered in the design and analyses of OC clinical trials.

Mutations in HRR genes were found in all histologic subtypes of OC, and rates were only significantly lower in low-grade serous carcinomas, 5/46 (10.9%) vs. 262/971 (27%) in high-grade serous carcinomas, $P = 0.02$, (Table 2). All tumors in GOG-0218 underwent centralized review by gynecologic pathologists, minimizing pathologic misclassification. These results are similar to our recent observation that germline HRR mutations are distributed across many histological sub-types without a clear predilection for high-grade serous carcinomas.(8) Therefore, HRR status cannot be assumed by histologic type. Some clinical trials targeting HRR deficiency, such as those using PARP inhibitors, are restricted to patients with high-grade serous carcinomas,(18) or to those with either high-grade serous or high-grade endometrioid histology.(19) Our data do not support restricting access to these clinical trials, or to germline genetic testing or tumor sequencing for HRR defects based on histologic subtype alone.

The role of anti-angiogenesis targeted therapy in ovarian carcinomas with and without HRR deficiency has not been previously defined. In the subset of patients with HRR mutations in GOG-0218, extended bevacizumab did not confer a statistically significant prolongation in PFS (Figure 2C). However, there was insufficient evidence that bevacizumab had a different effect on PFS or OS in HRR-mutated versus non-mutated cases, using a test of interaction (Figure 2). The power of this analysis was limited by not having translational samples from all participants in GOG-0218, therefore the number of mutation carriers was relatively small. Sequenced patients were less likely to be stage IV and had better PFS and OS, which also may have reduced our ability to detect a difference. Within the similar ICON7 trial, a subset of 359 cases subjected to expression profiling, proliferative and mesenchymal subtypes may have derived greater benefit from bevacizumab therapy, however tests of interaction were not done to formally test that hypothesis. The mutation status of the ICON7 cancers was not determined.(20) Further studies are needed to identify ovarian carcinomas best treated with bevacizumab, but our data do not currently support *BRCA* mutation status as a determinant.

This study has several limitations. While germline sequencing data is available on all patients (by sequencing DNA from blood or tumor), around 40% of patients did not have tumor sequenced. This likely underestimated the true mutation frequency by missing some somatic mutations. In addition, as germline and somatic HRR mutations are rarely present in the same patient, utilizing a “germline negative” group ($N = 324/1195$, 27.1%) for additional tumor sequencing likely overestimated the somatic mutation rate by excluding those with germline mutations who were unlikely to have somatic mutations (Supplemental Table 2). Exome sequencing data from The Cancer Genome Atlas (TCGA) demonstrated somatic or germline mutations in DNA-repair genes in around 40% of patients with high-grade serous histology ovarian carcinomas,(4) compared to 27% in patients with high-grade serous carcinomas in this trial. This difference is likely due to a combination of 1) our pre-selected shorter HRR gene list chosen to maximize likelihood of therapeutic impact, 2) our incomplete tumor sequencing data in 40% of cases, and 3) our more stringent definition of damaging mutations compared to TCGA. Finally, mutations in non-*BRCA* “other” HRR

genes were too few to allow a survival analysis by individual gene, necessitating combining these patients into one group for analysis.

In conclusion, HRR deficiency is a strong predictor of both PFS and OS in ovarian carcinoma, including mutations in 14 non-*BRCA* HRR genes (*ATM*, *ATR*, *BARD1*, *BLM*, *BRIP1*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*, *RBBP8*, *SLX4*, and *XRCC2*). Histologic subtype does not provide sufficient information to predict either inherited or somatic HRR mutations. There is insufficient evidence that HRR mutations should be a criterion, either positive or negative, in the decision to use bevacizumab maintenance therapy for advanced ovarian carcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Conflict of Interest Statements: Dr. Backes has received research grants from Eisai Incorporated, ImmunoGen, and Clovis. Dr. Burger has received honoraria for leading web based tumor board discussions for Roche. Dr. Tewari reports participation in advisory boards for Genentech and was a member of Roche's speaker's bureau for 2 years. Dr. Mannel is on an advisory board for Genentech.

Financial Support: This work was supported by National Cancer Institute grants to the Gynecologic Oncology Group Administrative Office (CA 27469), the Gynecologic Oncology Group Statistical and Data Center (CA 37517), the Gynecologic Oncology Group Tissue Bank (U10 CA27469, U24 CA114793, and U10 CA180868), NRG Oncology Grant (1 U10 CA180822), R01CA157744 (M. King), R01CA175716 (T. Walsh and M. King), and by the Ovarian Cancer Research Foundation Alliance (E. Swisher and B. Norquist), The Breast Cancer Research Foundation (M. King), the Department of Defense Ovarian Cancer Research Program OC120312 (E. Swisher), the V Foundation Translational Research Award (E. Swisher), The Women's Reproductive Health Research Career Development Award 5K12HD001264-13 (B. Norquist), The Nancy Kelley Jensen Faculty Fellowship for Gynecologic Oncology Research (B. Norquist), and the Wendy Feuer Research Fund for the Prevention and Treatment of Ovarian Cancer (E. Swisher).

Role of Funding Sources: The funders/sponsors 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.

The following Gynecologic Oncology Group member institutions participated in the primary treatment studies: Cancer Trials Support Unit, University of Oklahoma Health Sciences Center, University of California Medical Center at Irvine-Orange Campus, Fred Hutchinson Cancer Research Center, Ohio State University Comprehensive Cancer Center, Gynecologic Oncology Network/Brody School of Medicine, Mayo Clinic, Metro-Minnesota CCOP, Abramson Cancer Center of the University of Pennsylvania, University of North Carolina at Chapel Hill, Abington Memorial Hospital, University of Alabama at Birmingham, Rush University Medical Center, Walter Reed National Military Medical Center, Washington University School of Medicine, University of Kentucky, University of Iowa Hospitals and Clinics, Roswell Park Cancer Institute, Duke University Medical Center, University of Colorado Cancer Center – Anschutz Cancer Pavilion, University of Chicago, Cleveland Clinic Foundation, Women's Cancer Center of Nevada, University of Hawaii, Mount Sinai School of Medicine, Northwestern University, Fox Chase Cancer Center, Yale University, Women and Infants Hospital, University of California at Los Angeles Health System, Memorial Sloan Kettering Cancer Center, Indiana University Hospital/Melvin and Bren Simon Cancer Center, University of Minnesota Medical Center-Fairview, University of Mississippi Medical Center, University of Pittsburgh Cancer Institute, University of New Mexico, Case Western Reserve University, The Hospital of Central Connecticut, University of Texas-Galveston, Cancer Research for the Ozarks NCORP, Cooper Hospital University Medical Center, University of Virginia, Moffitt Cancer Center and Research Institute, State University of New York Downstate Medical Center, University of Wisconsin Hospital and Clinics, Carle Cancer Center, Northern Indiana Cancer Research Consortium, Penn State Milton S. Hershey Medical Center, Wake Forest University Health Sciences, Stony Brook University Medical Center, University of Massachusetts Memorial Health Care, Fletcher Allen Health Care, Michigan Cancer Research Consortium Community Clinical Oncology Program, MD Anderson Cancer Center, Gynecologic Oncology of West Michigan PLLC, Central Illinois CCP, Delaware Christiana Care CCOP, Virginia Commonwealth University, University of Cincinnati, Tufts-New England Medical Center, Scot and White Memorial Hospital, Tacoma General Hospital, Northern New Jersey CCOP, Virginia Mason CCOP, New

York University Medical Center, Aurora Women's Pavilion of Aurora West Allis Medical Center, Wisconsin NCI Community Oncology Research Program, Evanston CCOP-North Shore University Health System, Cancer Research Consortium of West Michigan NCORP, Missouri Valley Cancer Consortium CCOP, William Beaumont Hospital, Colorado Cancer Research Program NCORP, Saint Louis-Cape Girardeau CCOP, University of Texas Southwestern Medical Center, University of Illinois, Kalamazoo CCOP, Upstate Carolina CCOP, Heartland Cancer Research CCOP and Wichita CCOP.

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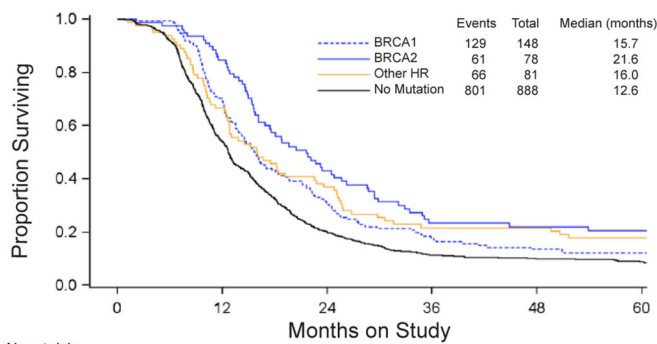
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Translational Significance

In the setting of a large clinical trial in primary ovarian cancer patients, we have demonstrated that damaging mutations in a selected set of genes affecting homologous recombination repair beyond *BRCA1* and *BRCA2* are associated with improved progression-free and overall survival, controlling for known prognostic features, with an outcome similar to that seen for *BRCA1* mutations. Cases with *BRCA2* mutations had even better outcomes. Given the magnitude of these effects, consideration should be given to assessing homologous recombination repair in the design and analyses of ovarian carcinoma clinical trials. We found mutations in homologous recombination repair genes including *BRCA1* and *BRCA2* in all histologic subtypes, with only low-grade serous having a significantly lower mutation frequency. Our data do not support restricting access to clinical trials, or to germline genetic testing, on the sole basis of histology. We did not see a differential effect of the impact of bevacizumab on progression-free survival by mutation status. Therefore, mutation status should not be used to decide who is a candidate for bevacizumab.

A. Progression Free Survival by Mutation Group

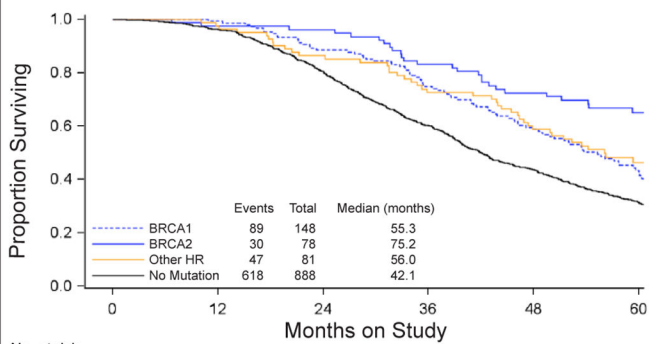


No. at risk	0	12	24	36	48	60
BRCA1	148	102	45	25	19	14
BRCA2	78	66	33	18	15	11
Other HR	81	54	29	17	17	4
No Mutation	888	475	175	98	83	47

Adjusted Hazards for Progression by Mutation Category

Gene Category	Total N	Events (%)	aHR (95% CI)	P-value
BRCA1	148	129 (87.2)	0.80 (0.66 - 0.97)	0.02
BRCA2	78	61 (78.2)	0.52 (0.40 - 0.67)	<0.0001
Other HRR	81	66 (81.5)	0.73 (0.57 - 0.94)	0.01
No Mutation	888	801 (90.2)	1.0 (referent)	Referent

B. Overall Survival by Mutation Group



No. at risk	0	12	24	36	48	60
BRCA1	148	147	130	108	84	43
BRCA2	78	76	74	64	53	32
Other HR	81	79	69	58	47	22
No Mutation	888	844	701	517	362	187

Adjusted Hazards for Death by Mutation Category

Gene Category	Total N	Events (%)	aHR (95% CI)	P-value
BRCA1	148	89 (60.1)	0.74 (0.59 - 0.94)	0.01
BRCA2	78	30 (38.5)	0.36 (0.25 - 0.53)	<0.0001
Other HRR	81	47 (58.0)	0.67 (0.49 - 0.90)	0.007
No Mutation	888	618 (69.6)	1.0 (referent)	Referent

Figure 1. Progression-free and overall survival in OC patients by mutation category
A. – B. Kaplan-Meier curves for progression-free and overall survival by mutation category, with adjusted hazard ratios for progression or death. Hazard ratios were adjusted for study treatment, stage of disease, size of residual disease, and initial performance status. The no mutation group was the referent group. Events were progression or death in 60 months. Abbreviations: aHR (adjusted hazard ratio).

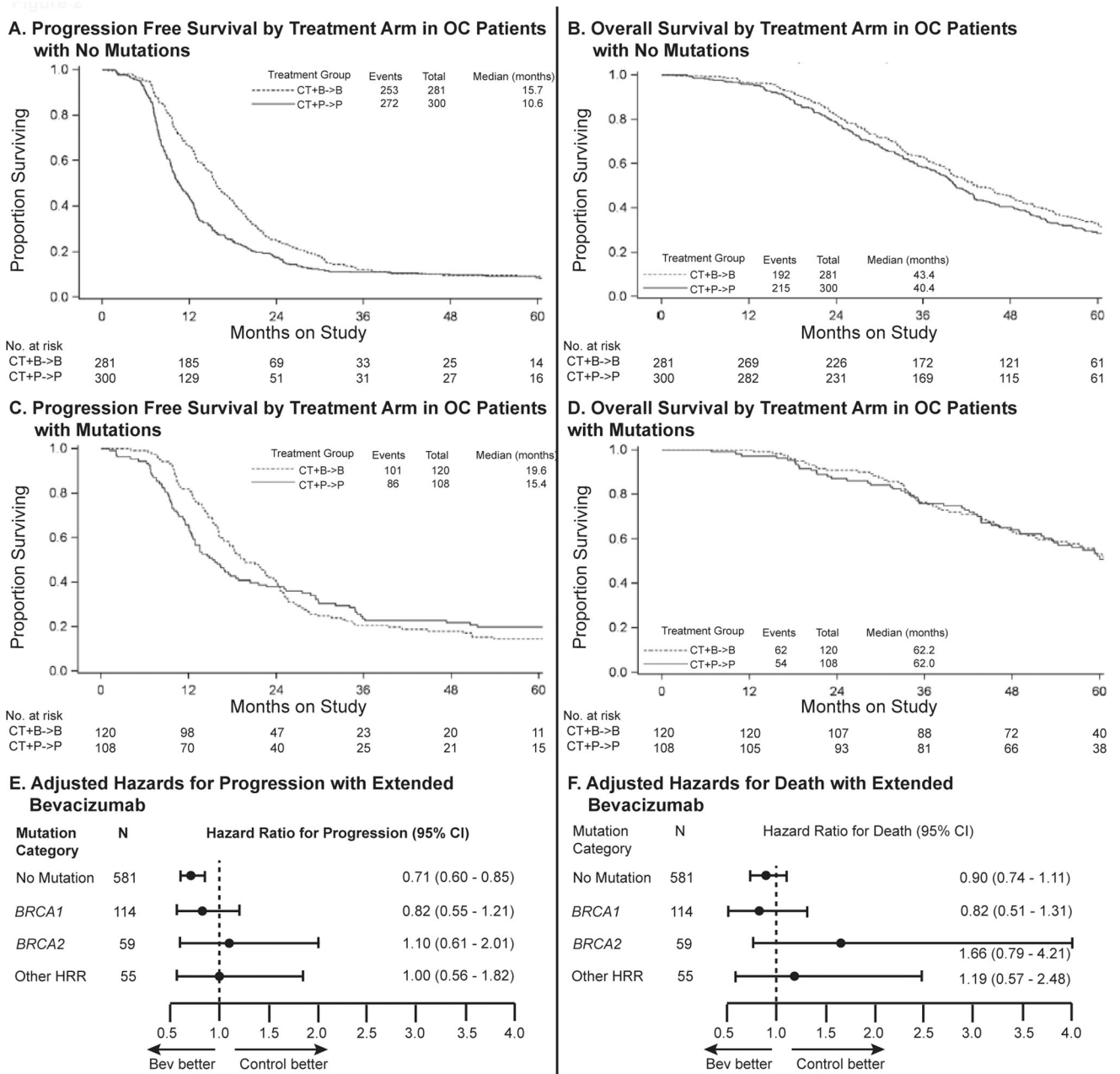


Figure 2. Effect of extended bevacizumab on survival in OC patients with and without mutations
A. – D. Kaplan-Meier curves for progression-free and overall survival in OC patients with and without mutations in genes affecting HRR. **E. – F.** Adjusted hazard ratios for progression or death by individual mutation categories. Using a test of interaction, mutation status did not significantly modify the effect of extended bevacizumab on progression (0.95/0.71) = 1.33, 95% CI 0.95 – 1.85, p = 0.10.

Table 1

Clinical Characteristics of OC Patients, Sequenced Vs. Not Sequenced

Characteristic		Sequenced	Not Sequenced
N		1195	678
Age	<40	40 (3.3%)	23 (3.4%)
	40 – 49	176 (14.7%)	90 (13.3%)
	50 – 59	385 (32.2%)	221 (32.6%)
	60 – 69	374 (31.3%)	236 (34.8%)
	70 – 79	205 (17.2%)	91 (13.4%)
	80	15 (1.3%)	17 (2.5%)
Race/Ethnicity *	Non-Hispanic White	1048 (87.7%)	518 (76.4%)
	Hispanic	51 (4.3%)	23 (3.4%)
	Non-Hispanic Black	46 (3.8%)	34 (5.0%)
	Asian/Pacific Islander	31 (2.6%)	86 (12.7%)
	Other or Unknown	19 (1.6%)	17 (2.5%)
Disease Site	Ovary	998 (83.5%)	565 (83.3%)
	Peritoneal	171 (14.3%)	103 (15.2%)
	Fallopian Tube	26 (2.2%)	10 (1.5%)
Stage *	III/Optimal	465 (38.9%)	175 (25.8%)
	III/Suboptimal	453 (37.9%)	299 (44.1%)
	IV	277 (23.2%)	204 (30.1%)
Histology **	HG Serous	971 (81.3%)	526 (77.6%)
	LG Serous	46 (3.8%)	25 (3.7%)
	Carcinoma, NS	84 (6.2%)	81 (14.2%)
	LG Endometrioid	4 (0.3%)	2 (0.3%)
	HG Endometrioid	38 (3.2%)	15 (2.2%)
	Clear Cell	28 (2.3%)	27 (4.0%)
	Mucinous	7 (0.6%)	12 (1.8%)
Treatment	CT + P -> P	408 (34.1%)	217 (32.0%)
	CT + B -> P	386 (32.3%)	239 (35.3%)
	CT + B -> B	401 (33.6%)	222 (32.7%)

Abbreviations: HG (high-grade, grades 2 and 3), LG (low-grade, grade 1), NS (not specified), CT (chemotherapy), P (placebo), B (Bevacizumab).

* P-value <0.001, chi-square,

** P-value 0.012, chi-square.

Table 2

Clinical Characteristics and Mutation Category in OC Patients

Characteristic	BRCA1	BRCA2	Other HRR	WT
N	148	78	81	888
Disease Site				
Ovary	131 (88.5%)	65 (83.3%)	68 (85.2%)	734 (82.7%)
Peritoneal	16 (10.8%)	12 (15.4%)	12 (14.8%)	131 (14.8%)
Fallopian Tube	1 (0.7%)	1 (1.3%)	1 (1.2%)	23 (2.6%)
Stage				
III/Optimal	59 (39.9%)	26 (33.3%)	37 (45.7%)	343 (38.6%)
III/Suboptimal	58 (39.2%)	29 (37.2%)	25 (30.9%)	341 (38.4%)
IV	31 (20.9%)	23 (29.5%)	19 (23.5%)	204 (23.0%)
Histology				
HG Serous	127 (85.8%)	70 (89.7%)	65 (80.2%)	709 (79.8%)
LG Serous*	4 (2.7%)	0	1 (1.2%)	41 (4.6%)
Carcinoma, NS	12 (8.1%)	6 (7.7%)	4 (12.6%)	79 (8.9%)
Endometrioid	3 (2.0%)	1 (1.3%)	6 (7.4%)	32 (3.6%)
Clear Cell	2 (1.4%)	1 (1.3%)	3 (3.7%)	22 (2.5%)
Mucinous	0	0	2 (2.5%)	5 (0.6%)
Treatment				
CT + P -> P	53 (35.8%)	28 (35.9%)	27 (33.3%)	300 (33.8%)
CT + B -> P	34 (23.0%)	19 (24.4%)	26 (32.1%)	307 (34.6%)
CT + B -> B	61 (41.2%)	31 (38.3%)	28 (34.6%)	281 (31.6%)

Abbreviations: HG (high-grade, grades 2 and 3), LG (low-grade, grade 1), NS (not specified), CT (chemotherapy), P (placebo), B (Bevacizumab).

Other HRR (other homologous recombination repair) refers to those with mutations in non-*BRCA* genes including *ATM*, *ATR*, *BARD1*, *BLM*, *BRIPI*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*, *RBBP8*, *SLX4*, and *XRCC2*.

* Low-grade serous carcinoma had a significantly lower total mutation rate when compared to high-grade serous carcinoma, $p=0.02$, Fisher's exact.