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Personalized Medicine and Imaging

Clinical Cancer Research

# Location of Mutation in *BRCA2* Gene and Survival in Patients with Ovarian Cancer

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## Abstract

**Purpose:** BRCA2 plays a central role in homologous recombination by loading RAD51 on DNA breaks. The objective of this study is to determine whether the location of mutations in the RAD51-binding domain (RAD51-BD; exon 11) of *BRCA2* gene affects the clinical outcome of ovarian cancer patients.

**Experimental Design:** A study cohort of 353 women with ovarian cancer who underwent genetic germline testing for *BRCA1* and *BRCA2* genes was identified. Progression-free survival (PFS), platinum-free interval (PFI), and overall survival (OS) were analyzed. The Cancer Genome Atlas (TCGA) cohort of ovarian cancer ($n = 316$) was used as a validation cohort.

**Results:** In the study cohort, 78 patients were carriers of germline mutations of *BRCA2*. After adjustment for FIGO stage and macroscopic residual disease, *BRCA2* carriers with truncating mutations in the RAD51-BD have significantly prolonged 5-year PFS [58%; adjusted HR, 0.36; 95% confidence interval (CI), 0.20–0.64; $P = 0.001$] and prolonged PFI (29.7 vs. 15.5 months, $P = 0.011$), compared with noncarriers. *BRCA2* carriers with mutations located in other domains of the gene do not have prolonged 5-year PFS (28%, adjusted HR, 0.67; 95% CI, 0.42–1.07; $P = 0.094$) or PFI (19 vs. 15.5 months, $P = 0.146$). In the TCGA cohort, only *BRCA2* carriers harboring germline or somatic mutations in the RAD51-BD have prolonged 5-year PFS (46%; adjusted HR, 0.30; 95% CI, 0.13–0.68; $P = 0.004$) and 5-year OS (78%; adjusted HR, 0.09; 95% CI, 0.02–0.38; $P = 0.001$).

**Conclusions:** Among ovarian cancer patients, *BRCA2* carriers with mutations located in the RAD51-BD (exon 11) have prolonged PFS, PFI, and OS. *Clin Cancer Res; 24(2); 326–33. ©2017 AACR.*

## Introduction

Germline mutations in *BRCA1* and *BRCA2* genes have been identified as predisposing to hereditary breast and ovarian cancers (1). Up to 20% of high-grade serous ovarian carcinomas (HGSOC) show germline and/or somatic mutations of *BRCA1/BRCA2* genes (2–5). Ovarian cancer patients with such mutations have better survival than noncarriers (2, 6, 7). Importantly, almost all the ovarian cancer patients in those studies received platinum-based chemotherapy, inducing interstrand crosslinks (8). Interstrand crosslinks formed by platinum lead to severe distortion of the DNA double helix, and consequently double-strand breaks (DSB; refs. 9, 10). Homologous recombination (HR), a major mechanism for protecting genome integrity in proliferating cells, is pivotal in repairing DSBs that arise during the processing of interstrand crosslinks.

From a biological point of view, BRCA1 and BRCA2 are both key players of DNA damage repair but have different functions (11). BRCA1 is a pleiotropic DNA damage response protein that functions in both DNA damage checkpoint activation and repair, including HR (11, 12). In contrast, the primary function of BRCA2 is HR. BRCA2 interacts directly with RAD51 and promotes its specific recruitment to DSBs sites where recombination is initiated (11). RAD51 is essential for HR. The improved survival of *BRCA1/BRCA2* germline mutation carriers, especially *BRCA2* carriers, compared with noncarriers (13, 14) has been linked to the role of these proteins in the HR pathway. However, there is no clear explanation of why *BRCA2* carriers fare better than *BRCA1* carriers.

BRCA2 is one of the largest proteins in the human body. The central portion of the protein contains 8 repeat sequences (called BRC repeats), which bind to RAD51, named RAD51-binding domain (RAD51-BD; refs. 11, 15). A second

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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

BRCA2 plays a central role in homologous recombination by loading RAD51 on DNA double-strand breaks. Ovarian cancer patients who were carriers of *BRCA2* germline mutation and were treated with DNA damage agent platinum showed prolonged survival. We questioned whether the location of the mutation in various functional domains of BRCA2 has an impact on the clinical outcome for ovarian cancer patients. In two independent cohorts of ovarian cancer patients, we observed that only those patients whose germline or somatic mutations of *BRCA2* are located within the RAD51-binding domain of the protein have prolonged platinum-free interval and survival, compared with noncarriers. These results suggest that not all *BRCA2* carriers are highly sensitive to DNA damage agents, and the response depends on the location of the mutation in the various functional domains of the protein.

large segment of BRCA2 encompasses a DNA-binding domain (Fig. 1). Genetic and functional studies of BRCA2 revealed that mutations located in RAD51-BD impair the ability of BRCA2 to recruit RAD51, hampering HR (16, 17). In the current report, we investigated whether mutations in the RAD51-BD (exon 11) of the *BRCA2* gene impact progression-free survival (PFS), platinum-free interval (PFI), and overall survival (OS) in ovarian cancer patients.

## Materials and Methods

Clinical and genetic data were collected from a study cohort of ovarian cancer patients screened for germline mutations of *BRCA1* and *BRCA2* genes. We analyzed the curated dataset of HGSOC from The Cancer Genome Atlas (TCGA) as a validation cohort.

### Study cohort

Study participants were women with confirmed invasive epithelial ovarian or fallopian tube or peritoneal carcinoma, who had been tested for germline *BRCA1* or *BRCA2* pathogenic mutations through blood tests between January 1995 and December 2015 and who received platinum-based chemotherapy. Ovarian cancer patients referred to the clinical genetics Units' at Centre Leon Bérard (Lyon, France), Institut du Cancer Jean Mermoz (Lyon, France), and Hôpitaux Universitaires de Genève (Geneva, Switzerland) were included in the cohort. To increase the number of *BRCA2* carriers in the study cohort, only *BRCA2* carriers were included from Institut Curie (Paris, France). The study was conducted following ethical guidelines of the Declaration of Helsinki. The study was reviewed by the local Institutional Review Board in each hospital. Informed consent was obtained from all living patients in Geneva. All the French patients consented to the use of their data at the time of genetic analysis. Clinical and pathologic data were collected from medical records. These included patient demographics, tumor characteristics, surgical staging, macroscopic residual disease, platinum sensitivity, recurrence, and survival status. Surgical stage was classified according to the International Federation of Gynecology and Obstetrics (FIGO) at diagnosis. Information regarding residual disease following primary surgery was acquired from medical records. Pathology data, including histologic subtypes, tumor stages, and grades, were obtained from pathology reports.

### Genetic analysis

*BRCA1* and *BRCA2* mutations were classified as truncating according to the ENIGMA *BRCA1/2* Gene Variant Classification Criteria (http://www.enigmaconsortium.org/). Women with variants of uncertain significance were considered noncarriers. Blood samples for germline DNA testing were obtained when the patients were referred to clinical genetics Unit. All participants were screened for *BRCA1* and *BRCA2* mutations. The *BRCA2* gene comprises 27 exons and encodes a 3418 amino-acid (AA) protein (Fig. 1). RAD51-BD corresponds to the region covering AA 1003-2082 of BRCA2 (exon 11). DNA-BD corresponds to AA 2481-3186 (http://www.ncbi.nlm.nih.gov/protein/NP_000050.2). The BRCA2 protein has a second binding site to RAD51 located in the carboxy-terminus (named RAD51-binding site or TR2), which we excluded from the analysis because it is dispensable for HR (11, 12).

### TCGA cohort

We obtained the TCGA database of 316 HGSOC patients. Detailed patient information, including age at diagnosis, tumor stage and grade, and surgical outcome, has been described previously (13). Whole-exome sequencing of germline and somatic DNA was performed in all cases (2).

### Outcome measures

The primary endpoint was PFS. Secondary endpoints were PFI and OS. Date of first relapse was defined as the first instance of disease progression based on CT imaging by RECIST or clinical progression. PFS was defined as the interval between histologic diagnosis and first relapse, death, or the last follow-up (censored). OS was defined as the interval between histologic diagnosis and the date of death from any cause or last follow-up (censored). PFI was defined as the interval between the time of completion of platinum-based chemotherapy and the date of first relapse or death. Platinum-sensitive patients were defined as those having PFI >6 months.

### Statistical analysis

Patient characteristics were compared using Pearson $\chi^2$ or Fisher exact test for frequencies, and Kruskal–Wallis test for age distribution. Characteristics were compared pair by pair (*BRCA2* carriers' vs. noncarriers). The survival functions of the different subgroups were estimated using the Kaplan–Meier method and compared using a log-rank test. The relative hazards for each mutation group were estimated with a Cox proportional hazards model adjusted for tumor stage and macroscopic residual disease. The categorical covariates included in the model were FIGO stage (stage I–II vs. III–IV) and macroscopic residual disease (absent vs. present). $P \leq 0.05$ was considered statistically significant; all tests were two-sided. Statistical analysis was carried out using R software.

## Results

### Characteristics of the study cohort

Of the 353 women included in the cohort, 87 were excluded from the analysis: 74 women were found to carry *BRCA1* mutation

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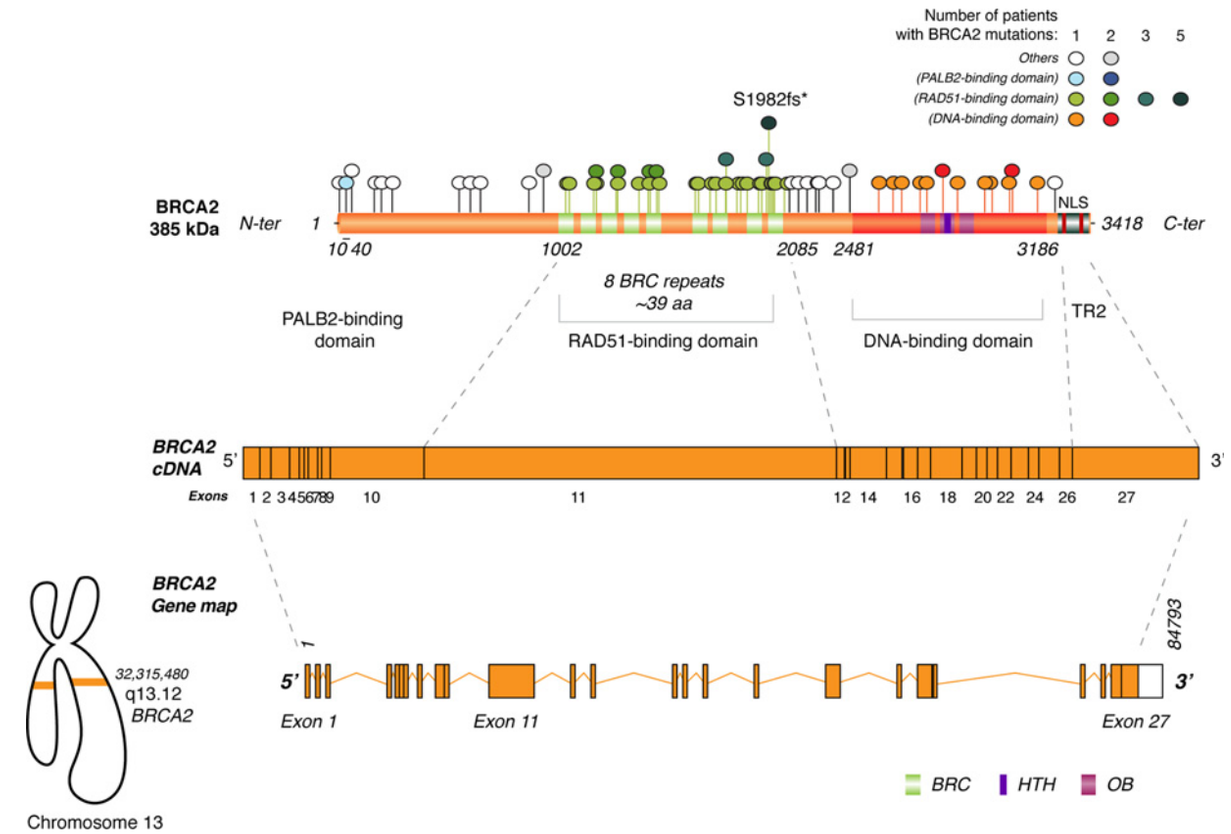


**Figure 1.**

Germline mutations of *BRCA2* gene in the study cohort of ovarian cancer patients. The human *BRCA2* gene is located on the long arm of chromosome 13 (13q12.3) and is composed of 27 exons that encode for a protein of 3,418 amino acids. The N-terminal domain of BRCA2 is involved in interaction with PALB2. BRCA2 contains 8 BRC repeats located in the central portion of the protein; they are primarily involved in binding to monomeric RAD51 (RAD51 binding domain: RAD51-BD). The BRCA2 DNA-binding domain (DNA-BD) promotes BRCA2 binding to single-stranded DNA (ssDNA) and poly(ADP-ribose). The C-terminus of BRCA2 contains the TR2 domain, which interacts with RAD51 nucleofilaments.

(they will be analyzed in another study), 2 patients harbored mutations in both *BRCA1* and *BRCA2* genes, 9 patients did not receive adjuvant chemotherapy (5 noncarriers, 1 *BRCA1*, and 3 *BRCA2* carriers), and follow-up data were not available for 2 noncarriers (Supplementary Fig. S1). In total, 266 women (152 from Centre Leon Bérard, 40 from Hôpitaux Universitaires de Genève, 26 from Institut du Cancer Jean Mermoz, and 48 from Institut Curie) were analyzed for outcome: 188 were tested negative for germline mutations in *BRCA1* and *BRCA2* genes (hereafter "noncarriers") and 78 were found to carry *BRCA2* mutations (hereafter "*BRCA2* carriers"). All mutations were germline. Patient demographics and clinical and treatment characteristics are summarized in Table 1.

The majority of patients had serous carcinomas and advanced stages (III/IV; 88%). Two thirds (67%) of the patients had no macroscopic residual disease and 85% were platinum sensitive. All patients received a platinum agent, and 95% of the patients received a combination with taxane.

There was no difference between the two groups regarding age of diagnosis ($P = 0.814$), histologic subtype ($P = 0.451$), FIGO staging ($P = 0.655$), and the presence of macroscopic residual disease ($P = 0.15$). Platinum sensitivity rates were not statistically different in the two groups of patients: 85% of noncarriers and 86% of *BRCA2* carriers were sensitive ($P = 0.722$). However, *BRCA2* carriers had longer PFI ($P = 0.008$).

### BRCA mutation and survival in the study cohort

Median follow-up of the cohort was 4 years. After adjustment for major prognostic factors (stage and macroscopic residual disease), PFS at 5 years was significantly higher in *BRCA2* compared with noncarriers ($P < 0.001$; Fig. 2A; Supplementary Table S1). Regarding OS, *BRCA2* carriers had significantly higher 5-year OS compared with noncarriers ($P < 0.001$; Supplementary Table S2).

### Location of mutations in BRCA2 and clinical outcome in the study cohort

Among the 78 *BRCA2* carriers of the study cohort, 42 had mutations located within the RAD51-BD (Fig. 1; Supplementary Table S3), including 5 carriers of the Ashkenazi founder mutation c.5946del/p.Ser1982Argfs*22. They had no macroscopic residual disease ($P = 0.009$) more frequently and had significantly prolonged PFI ($P = 0.011$), compared with noncarriers. There was no significant difference between the 36 other *BRCA2* carriers and noncarriers, regarding any of the clinical, pathology, and treatment characteristics (Table 1).

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**Table 1.** Clinical and pathology characteristics of the study cohort

| | | | All cases | | | *BRCA2* carriers | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Characteristics** | | **All cases** ***n*** **= 266** | **Noncarriers** ***n*** **= 188** | ***BRCA2*** **carriers** ***n*** **= 78** | ***P*** | **RAD51-BD** ***n*** **= 42** | ***P*** | **Other domains** ***n*** **= 36** | ***P*** |
| Age (in years) | Median (min-max) | 59 (25–88) | 59 (25–88) | 59 (37–81) | 0.814 | 61 (37–81) | 0.278 | 57 (44–70) | 0.094 |
| Histologic subtype | Serous | 220 (83%) | 150 (80%) | 70 (91%) | 0.451 | 38 (90%) | 0.663 | 32 (91%) | 0.308 |
| | Low grade | 12 | 12 | — | | — | | — | |
| | High grade | 180 | 119 | 61 | | 34 | | 27 | |
| | Missing | 28 | 19 | 9 | | 4 | | 5 | |
| | Endometrioid | 13 (5%) | 11 (6%) | 2 (3%) | | 2 (5%) | | — | |
| | Low grade | 4 | 4 | — | | — | | — | |
| | High grade | 7 | 6 | 1 | | 1 | | — | |
| | Missing | 2 | 1 | 1 | | 1 | | — | |
| | Carcinoma, NOS | 15 (6%) | 12 (6%) | 3 (4%) | | 2 (5%) | | 1 (3%) | |
| | Clear cell | 9 (3%) | 8 (4%) | 1 (1%) | | — | | 1 (3%) | |
| | Carcinosarcoma | 4 (2%) | 4 (2%) | — | | — | | — | |
| | Transitional | 4 (2%) | 3 (2%) | 1 (1%) | | — | | 1 (3%) | |
| | Missing | 1 | | 1 | | — | | 1 | |
| FIGO stage | 1 | 26 (10%) | 18 (10%) | 8 (11%) | 0.655 | 6 (14%) | 0.274 | 2 (6%) | 0.909 |
| | 2 | 10 (4%) | 7 (4%) | 3 (4%) | | 2 (5%) | | 1 (3%) | |
| | 3 | 165 (64%) | 121 (66%) | 44 (59%) | | 21 (50%) | | 23 (70%) | |
| | 4 | 57 (22%) | 37 (20%) | 20 (27%) | | 13 (31%) | | 7 (21%) | |
| | Missing | 8 | 5 | 3 | | | | 3 | |
| Macroscopic residual disease | Absent | 172 (67%) | 116 (64%) | 56 (75%) | 0.150 | 35 (85%) | 0.009 | 21 (62%) | 0.846 |
| | Present | 83 (33%) | 64 (36%) | 19 (25%) | | 6 (15%) | | 13 (38%) | |
| | Missing | 11 | 8 | 3 | | 1 | | 2 | |
| Platinum sensitive | | 226 (85%) | 159 (85%) | 67 (86%) | 0.722 | 35 (83%) | 0.588 | 32 (89%) | 0.704 |
| PFI (months) | Median range (min–max) | 17 (0–281) | 15.5 (0–228) | 20.9 (1–281) | 0.008 | 29.7 (1–254) | 0.011 | 19 (1–281) | 0.146 |

After adjustment for stage and macroscopic residual disease, only *BRCA2* carriers with mutations located in the RAD51-BD had significantly higher 5-year PFS than noncarriers ($P = 0.001$), whereas the other *BRCA2* carriers did not have significant prolonged 5-year PFS ($P = 0.094$, Fig. 2B; Table 2). Regarding OS, events (deaths) occurred in 9 (11.5%) of *BRCA2* carriers, insufficient for survival subgroup analyses.

We investigated whether mutations located in other domains of *BRCA2* than the RAD51-BD impacted the outcomes of carriers. In our study cohort, 14 *BRCA2* carriers harbored mutations located in the DNA-BD (AA 2481-3186; Fig. 1; Supplementary Table S3). Their 5-year PFS was not significantly longer than noncarriers [27%; HR = 0.73; 95% confidence interval (CI), 0.40–1.41; $P = 0.38$]. *BRCA2* carriers with mutations located in exons 1–10 (19%; HR = 0.72; 95% CI, 0.36–1.42; $P = 0.338$) or exons 12–27 (32%; HR = 0.64; 95% CI, 0.35–1.16; $P = 0.143$) did not show higher 5-year PFS, compared with noncarriers.

### Location of mutations in BRCA2 and survival in the TCGA cohort

To validate our observations, we explored the correlation between location of mutation within *BRCA2* gene and survival in the TCGA cohort of HGSOC ($n = 316$). All patients received platinum-based chemotherapy. Among the 34 patients who had germline or somatic mutations of the *BRCA2* gene (hereafter "*BRCA2* carriers"), two of them harbored mutations in both *BRCA1* and *BRCA2* genes and were excluded from analysis. Three additional patients with missense mutations were excluded, resulting in 29 *BRCA2* carriers being analyzed. We excluded patients with somatic or germline mutations of *BRCA1*. A total of 247 patients had no somatic or germline mutations of *BRCA1* or *BRCA2* (hereafter "noncarriers").

Among the 243 patients for whom the information on residual disease was available, 20% had no macroscopic residual disease. After adjustment for stage and macroscopic residual disease, 5-year PFS was significantly higher in *BRCA2* carriers with mutations within the RAD51-BD ($P = 0.004$), while *BRCA2* carriers with mutations in other domains did not show better 5-year PFS than noncarriers ($P = 0.639$; Table 3; Fig. 3A). Five-year OS was significantly higher only in *BRCA2* carriers with mutations within the RAD51-BD compared with noncarriers ($P = 0.001$). *BRCA2* carriers whose mutations were not located in the RAD51-BD did not show any difference in OS compared with noncarriers ($P = 0.594$; Table 3; Fig. 3B).

## Discussion

In this study, we addressed the correlation between *BRCA2* genotype and ovarian cancer patients' survival, according to the functional domains of the protein. Data from two independent cohorts showed that ovarian cancer patients treated with platinum and harboring germline and/or somatic mutations located at the RAD51-BD (exon 11) of the *BRCA2* gene have prolonged survival compared with noncarriers of *BRCA1*/*BRCA2* mutations. Mutations occurring in the *BRCA2* gene at other locations than the RAD51-BD did not impact the outcome, compared with noncarriers. Previous work investigated the correlation between type of mutation (base change, simple deletion, etc.) or location of the mutation across *BRCA2* gene and patient outcome, and the authors did not report a correlation with outcome (3, 18). This can be explained by the absence of analysis of the location based on the different functional domains of the protein (3).

Our hypothesis is biology driven. Through HR, BRCA2 plays a central role in repairing DSBs induced by interstrand crosslinks (9). It coordinates the formation of RAD51 filaments at DSBs. BRCA2 interacts with monomeric RAD51 primarily via the highly conserved BRC repeats encoded by exon 11 (RAD51-BD). DNA DSBs induced by platinum can be considered as acting as a "targeted chemotherapy" in *BRCA2*-mutated tumors. We hypothesized that mutations at sites crucial for the association between

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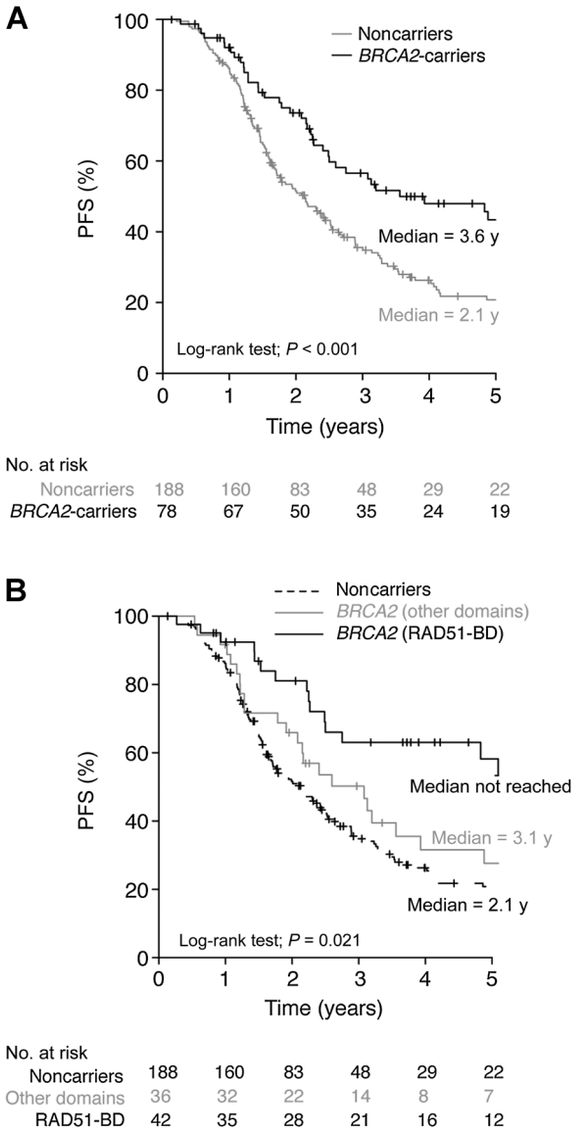


**Figure 2.**

*BRCA2* genotype correlates with PFS in the study cohort. **A,** PFS for *BRCA2* carriers and noncarriers. **B,** PFS for *BRCA2* carriers having mutations located in RAD51-BD (exon 11), other *BRCA2* carriers, and noncarriers.

RAD51 and BRC repeats could impair the ability of BRCA2 to recruit RAD51 to DNA breaks, hampering HR (Supplementary Fig. S2; refs. 16, 17) and impacting patients' survival.

Of course, this study has several limitations. First, the cohort is derived from a retrospective study that only included patients screened for germline mutations. The criteria for genetic analysis of *BRCA1/BRCA2* genes have evolved over time. Systematic screening for all ovarian cancer patients has recently been implemented. Thus, our cohort study included patients selected for their young age at diagnosis and/or significant family history of cancer and does not reflect the general population of ovarian cancer patients. Second, our noncarrier group probably included some patients with somatic mutations of *BRCA1*, *BRCA2*, and other HR genes (2). The enrichment in *BRCA2* carriers, who were mainly recruited at Institut Curie could also be considered as a limitation. On the other hand, compared with the literature, our study cohort is representative of *BRCA2* carriers in terms of clinical characteristics (age of diagnosis, histologic subtype, stage, etc.) and clinical behavior with *BRCA2* carriers showing significantly prolonged survival compared with noncarriers (4, 13).

Two thirds of the patients included in the study cohort had no macroscopic residual disease, explaining high survival rates, as described previously (19). The low number of events in the *BRCA2* carriers group allowed subgroup investigation only for PFS, not OS. OS data for *BRCA2* patients will require longer follow-up before subgroup analysis. It should be noted that PFS has been shown to be a surrogate marker for OS in *BRCA* carriers in the TCGA cohort (13) and the GOG clinical trials 218 and 262 (4). Moreover, we observed in the TCGA cohort that OS was dramatically prolonged in these patients. One major difference between our study cohort and the TCGA cohort is the percentage of patients with no macroscopic residual disease, which was lower in the TCGA cohort (66% vs. 20%, respectively).

Our study and the TCGA cohort showed that only mutations in the RAD51-BD of *BRCA2* gene lead to prolonged PFS, PFI, and OS in ovarian cancer patients, compared with noncarriers. As BRCA2 is a very large protein, it is possible that mutations in other domains of the protein could also impact clinical outcome of ovarian cancer patients. In our study cohort, we did not observe that mutations located in the DNA-BD impact PFS.

A very large proportion of pathogenic *BRCA2* mutations are truncating deletions, meaning that cells containing early mutations (exons 1–10) are likely to produce no BRCA2 protein. In an exploratory analysis, we compared *BRCA2* carriers with mutations located in either exons 1–10 or exons 12–27, and we did not observe a significant difference in the outcome of both groups. Our analysis is limited by the number of cases in each subgroup

**Table 2.** Multivariate model of PFS in the study cohort of patients with ovarian cancer according to location of mutations in *BRCA2* gene

| Variable | 3-year PFS rate (%) (95%CI) | 5-year PFS rate (%) (95% CI) | HR (95% CI) | *P* |
|---|---|---|---|---|
| *BRCA* | | | | |
| Noncarriers | 36 (29–44) | 21 (15–29) | 1 | |
| *BRCA2*- carriers other domains | 50 (36–70) | 28 (15–50) | 0.67 (0.42–1.07) | 0.094 |
| *BRCA2*- carriers RAD51-BD | 63 (49–81) | 58 (43–79) | 0.36 (0.20–0.64) | 0.001 |
| FIGO stage | | | | |
| 1–2 | 73 (59–91) | 65 (49–86) | 1 | |
| 3–4 | 37 (31–45) | 22 (16–29) | 2.68 (1.34–5.36) | 0.005 |
| Macroscopic residual disease | | | | |
| Absent | 55 (47–63) | 38 (31–48) | 1 | |
| Present | 15 (83–25) | 6 (23–15) | 3.20 (2.29–4.47) | <0.001 |

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**Table 3.** Multivariate model of PFS and OS in the TCGA cohort according to location of mutations in *BRCA2* gene

| Variables | 3-year OS rate (%) (95% CI) | 5-year OS rate (%) (95% CI) | HR (95% CI) | *P* | 3-year PFS rate (%) (95% CI) | 5-year PFS rate (%) (95% CI) | HR (95% CI) | *P* |
|---|---|---|---|---|---|---|---|---|
| *BRCA* | | | | | | | | |
| Noncarriers | 59 (53–67) | 25 (19–33) | 1 | | 15 (10–22) | 10 (6–17) | 1 | |
| *BRCA2*- carriers other domains | 60 (37–95) | 43 (22–82) | 0.83 (0.42–1.64) | 0.594 | 31 (14–70) | 23 (9–63) | 0.85 (0.43–1.68) | 0.639 |
| *BRCA2*- carriers RAD51-BD | 91 (75–100) | 78 (55–100) | 0.09 (0.02–0.38) | 0.001 | 46 (26–83) | 46 (26–83) | 0.30 (0.13–0.68) | 0.004 |
| FIGO stage | | | | | | | | |
| 1–2 | 91 (75–100) | 62 (35–100) | 1 | | 70 (47–100) | 47 (19–100) | 1 | |
| 3–4 | 60 (53–67) | 28 (22–36) | 3.69 (1.17–11.68) | 0.026 | 16 (11–22) | 12 (8–18) | 3.90 (1.23–12.36) | 0.021 |
| Macroscopic residual disease | | | | | | | | |
| Absent | 67 (52–85) | 38 (22–65) | 1 | | 38 (24–60) | 24 (11–54) | 1 | |
| Present | 57 (50–65) | 23 (17–32) | 1.67 (1.03–2.72) | 0.039 | 10 (6–17) | 9 (5–15) | 1.92 (1.22–3.04) | 0.005 |

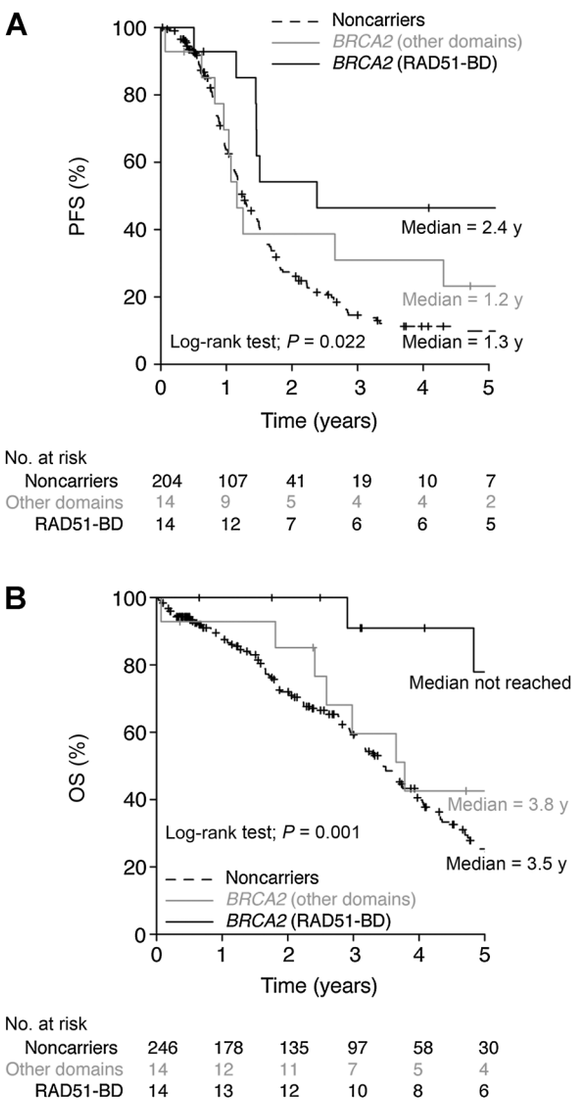


**Figure 3.**
*BRCA2* genotype correlates with survival in the TCGA cohort. **A,** PFS for patients with *BRCA2* mutations located in RAD51-BD (exon 11), other *BRCA2* carriers, and noncarriers. **B,** OS for patients with *BRCA2* mutations in RAD51-BD (exon 11), other *BRCA2* carriers, and noncarriers.

and needs to be confirmed on larger cohorts before deriving any conclusion. From a biological point of view, little is known about the functions of the large part of BRCA2 protein corresponding to exons 1–10 (AA 1-1002), except PALB2-BD (AA 10-40). This is an important question that needs to be addressed through preclinical functional studies.

Our work highlights the importance of RAD51 in the context of ovarian cancer. Germline mutations in *RAD51* paralogs (20, 21), in particular *RAD51C* and *RAD51D* genes, increase the risk for ovarian cancer (21) and lead to high genomic instability and sensitivity to PARP inhibitors (22). Secondary gene reversion mutations restoring the open reading frame of *RAD51C* or *RAD51D* is a mechanism of acquired resistance to PARP inhibitors (23), as it was previously shown for *BRCA1* and *BRCA2* genes (24). Edwards and colleagues studied 12 independently derived PARP inhibitor–resistant CAPAN1 tumor cell lines (*BRCA2* mutation p.Ser1982Argfs*22 located in the RAD51-BD) and found that none of them showed a secondary mutation event restoring the wild-type allele, but all exhibited *BRCA2* DNA deletion events that restored the open reading frame and encoded the RAD51-BD (24, 25). It is perhaps ironic that the secondary mutations in *BRCA2* or *RAD51* leading to resistance may also arise because of this same HR deficiency (25).

Although mutations of RAD51-BD concerns a minority of ovarian cancer, this has to be put in the perspective of other cancers, such as breast (26), pancreatic (27), or castration-resistant prostate cancers (28) where *BRCA2* mutations occur in 5% to 7% of the patients. This work, which needs further confirmation in larger cohorts (29), could have consequences for the clinical management of ovarian cancer patients. On the basis of the location of mutation in the *BRCA2* gene, we can predict whether there will be a survival advantage and prolonged PFI in *BRCA2* carriers compared with noncarriers. These patients may benefit more than other patients from reintroduction of platinum. This observation also questioned the impact of such alteration on selecting patients for PARP inhibitors. PARP inhibitors showed promising results in preclinical studies (30), but the response rate varies from one study to another (31, 32). PARP inhibitors lead to longer PFS especially in patients selected on the basis of their platinum sensitivity (31–35). Thus, platinum sensitivity probably acts as an "*in vivo* functional test" for HR defects (36), selecting *BRCA* carriers who will be more likely to benefit from PARP inhibitors. A recent report, showing an enrichment of *BRCA2* carriers with mutations located in the RAD51-BD among patients who are long responding (>2 years) to olaparib as a maintenance therapy, reinforces our observations (37). We are currently

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investigating the PFS and OS of *BRCA2* carriers treated with PARP inhibitors based on their genotype.

In conclusion, our data suggest that PFI and survival in *BRCA2* carriers are prolonged specifically when the mutations occur in the RAD-51 BD (exon 11) of the gene. These results confirmed the new paradigm of personalized therapy in *BRCA* carriers.

## Disclosure of Potential Conflicts of Interest

S.I. Labidi-Galy, M. Rodrigues, and A. Bodmer are consultant/advisory board members for AstraZeneca. O. Tredan is a consultant/advisory board member for AstraZeneca, Lilly, Novartis, Pfizer, and Roche. M-H. Stern has ownership interests (including patents) at Myriad Genetics. No potential conflicts of interest were disclosed by the other authors.

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# Location of Mutation in *BRCA2* Gene and Survival in Patients with Ovarian Cancer

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