Secondary Somatic Mutations Restoring *BRCA1/2* Predict Chemotherapy Resistance in Hereditary Ovarian Carcinomas

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ABSTRACT

Purpose

Secondary somatic *BRCA1/2* mutations may restore *BRCA1/2* protein in hereditary ovarian carcinomas. In cell lines, *BRCA2* restoration mediates resistance to platinum chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors. We assessed primary and recurrent *BRCA1/2*-mutated ovarian carcinomas to define the frequency of secondary mutations and correlate these changes with clinical outcomes.

Methods

Neoplastic cells were isolated with laser capture microdissection, and DNA was sequenced at the site of the known germline *BRCA1/2* mutation. When secondary mutations were found that restored wild-type sequence, haplotyping was performed using single nucleotide polymorphisms in tumor and paired lymphocyte DNA to rule out retention of the wild-type allele.

Results

There were 64 primary and 46 recurrent ovarian carcinomas assessed. Thirteen (28.3%) of 46 (95% CI, 17.3% to 42.6%) recurrent carcinomas had a secondary mutation compared with two (3.1%) of 64 (95% CI, 1.0% to 10.7%) primary carcinomas ($P = .0003$, Fisher's exact test). Twelve (46.2%) of 26 (95% CI, 28.7% to 64.7%) platinum-resistant recurrences had secondary mutations restoring *BRCA1/2*, compared with one (5.3%) of 19 (95% CI, 1.2% to 24.8%) platinum-sensitive recurrences ($P = .003$, Fisher's exact test). Six (66.7%) of nine (95% CI, 34.8% to 87.8%) women with prior breast carcinoma had a recurrent carcinoma with a secondary mutation, compared with six (17.1%) of 35 (95% CI, 8.2% to 32.8%) with no history of breast carcinoma ($P = .007$, Fisher's exact test).

Conclusion

Secondary somatic mutations that restore *BRCA1/2* in carcinomas from women with germline *BRCA1/2* mutations predict resistance to platinum chemotherapy and may also predict resistance to PARP inhibitors. These mutations were detectable only in ovarian carcinomas of women whom have had previous chemotherapy, either for ovarian or breast carcinoma.

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INTRODUCTION

Ten percent to 15% of ovarian carcinomas occur in women with heterozygous germline mutations in *BRCA1* and *BRCA2* (*BRCA1/2*).^{1,2} *BRCA1/2* are tumor suppressor genes, and *BRCA1/2* proteins are instrumental in repairing damaged DNA, through homologous recombination.^{3,4} The vast majority of primary carcinomas in patients with *BRCA1/2* mutations have deletions of the wild-type *BRCA1/2* allele,⁵⁻⁷ leading to a neoplasm deficient in *BRCA1/2* protein. *BRCA1/2*-deficient carcinomas have decreased capacity to repair DNA and are sensitive to chemotherapy with DNA cross-linking agents such as cisplatin and carboplatin (platinum agents)⁸⁻¹⁰ and are susceptible to synthetic lethality from poly

(ADP-ribose) polymerase (PARP) inhibitors.¹¹⁻¹³ Women with *BRCA1/2*-associated ovarian carcinoma have improved survival compared with those with sporadic ovarian carcinomas when treated with platinum agents.¹⁴⁻¹⁶ Despite this, the majority of women with *BRCA1/2*-mutated ovarian carcinomas ultimately develop recurrent disease that is resistant to platinum agents.

Our group and another have recently outlined a novel mechanism for resistance to platinum chemotherapy using *BRCA2*-deficient cancer cell lines.¹⁷⁻¹⁹ Secondary mutations that restore functional *BRCA2* protein can be induced by exposing *BRCA2*-deficient pancreatic or ovarian cancer cell lines to cisplatin or PARP inhibitors. The resulting clones with restored *BRCA2*

are resistant to both cisplatin and PARP inhibitors.¹⁷⁻¹⁹ Furthermore, secondary mutations restoring wild-type sequence or a functional reading frame have been seen in a small number of ovarian neoplasms from both *BRCA1* and *BRCA2* mutation carriers.¹⁷⁻²⁰

This study aimed to define the frequency of secondary mutations that restore *BRCA1/2* function within the ovarian carcinomas of *BRCA1/2* mutation carriers and correlate these changes with therapeutic outcomes.

METHODS

Patients with primary and/or recurrent ovarian, peritoneal, or fallopian tube carcinomas and a confirmed *BRCA1/2* mutation were identified using the University of Washington and Cedars-Sinai Women's Cancer Institute Gynecologic Oncology Tissue Banks, as approved by the Human Subjects Committee of the institutional review board at each institution, as well as one patient from Edinburgh Cancer Research Centre with institutional review board approval through Fred Hutchinson Cancer Research Center. Neoplastic samples were most often formalin-fixed paraffin embedded blocks, with some samples frozen in optimal cutting-temperature compound. One neoplastic sample was isolated from a malignant pleural effusion, using the CELlection Epithelial Enrich protocol (Invitrogen, Carlsbad, CA).

Neoplastic cells in blocks were isolated from surrounding normal cells by laser capture microdissection using a Veritas system (Moelcular Devices, Sunnyvale, CA). Neoplastic DNA was extracted using the PicoPure DNA extraction kit (Arcturus; Applied Biosystems, Foster City, CA), and neoplastic and normal DNA (from lymphocytes) was amplified by polymerase chain reaction (PCR), using primers flanking the known mutation in *BRCA1* or *BRCA2*. PCR products were purified and sequenced with BigDye Terminator v3.1 (Applied Biosystems) using the ABI 3100 Genetic Analyzer (Applied Biosystems). Sequences at the mutation site were analyzed for relative ratios of wild-type and mutant sequences by comparing fluorescent peak heights. In cases with suspected secondary mutations, intragenic heterozygous single nucleotide polymorphisms (SNPs) were identified in lymphocyte DNA and compared with neoplastic DNA to rule out contamination with non-neoplastic cells or retention of the wild-type allele. If a heterozygous SNP was not identified, microsatellite testing was used.

Platinum sensitivity was defined by the response to platinum chemotherapy after the carcinoma was sampled (rather than the usual definition using the treatment-free interval before the recurrence). Carcinomas that responded to platinum chemotherapy and were in remission for at least 6 months after chemotherapy were defined as platinum sensitive; all others were labeled platinum resistant.

The number of patients with a secondary mutation was too small to adequately assess a survival difference between those with and without secondary mutations.

Table 1. Characteristics of Women With Secondary Mutations

Patient	Inherited Mutation	Carcinoma	Stage of Primary	Secondary Mutation	Response of Primary Carcinoma to Platinum	Response of Recurrent Carcinoma to Platinum	History of Previous Breast Cancer	Progression-Free Survival (months)	Interval Since Last Chemotherapy* (months)	Overall Survival (months)
Primary carcinomas										
UWF35	B1.120A>G	Primary fallopian tube	IA	Reversion to wild-type	Nonevaluable		Yes, with chemotherapy	27 (censored)		27 (censored)
UW40	B1.2594delC	Primary ovarian	IV	Reversion to wild-type	Resistant		Yes, with chemotherapy	8		23
Recurrent carcinomas										
CS1	B2.5193delC	Recurrent ovarian	IIIC	B2.5177insA	Sensitive	Resistant	No	24	19	125 (censored)
UWF52	B1.2800delAA	Recurrent ovarian	IIIC	Reversion to wild-type	Resistant	Resistant	Yes, chemotherapy unknown	11	5	27
UWF51	B1.300T>G	Recurrent primary peritoneal	IIIC	Reversion to wild-type	Resistant	Resistant	No	6	0	69
UW400	B2.4075delGT	Recurrent ovarian	IIIC	Reversion to wild-type	Sensitive	Resistant	Yes, with chemotherapy	12	7	16 (censored)
UW91	B1.185delAG	Recurrent ovarian	IIIC	Reversion to wild-type	Sensitive	Resistant	Yes, chemotherapy unknown	52	47	89
UW40	B1.2594delC	Recurrent ovarian	IV	B1.2606_2628del23	Resistant	Resistant	Yes, with chemotherapy	8	2	23
UW304	B2.6174delT	Recurrent ovarian	IIIB	Reversion to wild-type	Sensitive	Resistant	Yes, tamoxifen	24	7	98
UW200	B2.4689delAC	Recurrent ovarian	IIIB	Reversion to wild-type	Sensitive	Sensitive	No	28	23	74 (censored)
UW200	B2.4689delAC	2nd recurrent ovarian†	IIIB	Reversion to wild-type	Sensitive	Resistant	No	28	13	74 (censored)
UWF27	B1.185delAG	Recurrent ovarian	IIC	Reversion to wild-type	Sensitive	Resistant	Yes, with chemotherapy	17	12	53
UW80	B1.185delAG	Recurrent ovarian	IIIC	Reversion to wild-type	Sensitive	Resistant	No	25	11	109
CS2	B1.1912T>A B2.8765delAG	Recurrent ovarian	IIC	Loss of mutant <i>BRCA2</i> allele	Sensitive	Resistant	No	38	33	90
PEO1	B2.5193C>G	Recurrent ovarian	IIIC	B2.5193C>T	Sensitive	Resistant	No	22	16	34

*Defined as the time off chemotherapy before the sampled recurrence.

†This is the second recurrent carcinoma for patient UW200, which occurred 13 months after completing chemotherapy for the first recurrent carcinoma. It was ultimately platinum resistant because of disease progression within 2 months of completing another course of platinum chemotherapy.

Statistical analysis was performed using Instat or Prism software (Graphpad, San Diego, CA). Contingency tables were made for comparison of categorical variables, and *P* values were derived using Fisher's exact test.

RESULTS

Description of Samples

We analyzed 64 primary (48 *BRCA1*, 16 *BRCA2*) and 46 recurrent (32 *BRCA1*, 14 *BRCA2*) ovarian, fallopian tube, and primary peritoneal carcinomas (subsequently collectively referred to as ovarian carcinomas). The 46 recurrent carcinomas were from 44 women, as two women had two separate recurrences analyzed. Seven primary and 15 recurrent carcinomas were previously published.^{17,18,20} All carcinomas had deletion of the wild-type *BRCA1/2* allele, as determined by no wild-type sequence being detected or by confirmation with SNPs or microsatellite testing.

Secondary Mutations in Primary and Recurrent Ovarian Carcinomas

Secondary mutations restoring *BRCA1/2* were more common in recurrent ovarian carcinomas (13 [28.3%] of 46; 95% CI, 17.3% to 42.6%) than in primary carcinomas (two [3.1%] of 64; 95% CI, 1.0%

to 10.7%; *P* = .0003, Fisher's exact test). Secondary mutations were more common in recurrent ovarian carcinomas of *BRCA2* mutation carriers (six [46.2%] 13; 95% CI, 23.0% to 71.1%), compared with those of *BRCA1* mutation carriers (six [19.4%] 31; 95% CI, 9.2% to 36.4%), but this did not achieve statistical significance (*P* = .13; Appendix Fig A1, online only).

Of the 46 recurrences, previous chemotherapy regimen information was complete on 42. Twenty-five recurrences were sampled after only one previous chemotherapy regimen, and four had secondary mutations (16%; 95% CI, 6.5% to 34.9%). In contrast, 17 recurrences were sampled after two or more chemotherapy regimens, and eight contained secondary mutations restoring *BRCA1/2* (47%; 95% CI, 26.0% to 69.2%; *P* = .04, Fisher's exact test). Of the four recurrent cases with secondary mutations sampled after only one chemotherapy regimen for ovarian carcinoma, three had received chemotherapy previous to their ovarian cancer diagnosis for breast carcinoma.

Cases with secondary mutations are summarized in Table 1. Two secondary mutations were insertions or deletions that restored a functional reading frame, 11 were reversions to wild-type sequence, one was a synonymous mutation restoring the wild-type amino acid sequence, and one was loss of the mutant *BRCA2* allele. An example of

Fig 1. Samples from individual with germline *BRCA2* 5193delC mutation demonstrating a secondary somatic mutation restoring *BRCA2* in her ovarian carcinoma. (A) Lymphocyte DNA sequence of the heterozygous germline *BRCA2* 5193delC mutation. (B) DNA sequence from her primary ovarian carcinoma. Only mutant 5193delC sequence is seen, consistent with loss of the wild-type *BRCA2* allele in neoplastic cells. (C) DNA sequence from recurrent ovarian carcinoma obtained 10 years after initial diagnosis. This patient had multiple chemotherapeutic regimens for recurrent disease, including a poly (ADP-ribose) polymerase (PARP) inhibitor before surgical resection of this recurrence (CS1 in Table 1). An insertion of an A base is seen at nucleotide 5177, which restores the open reading frame disrupted by the 5193delC mutation and restoring full-length *BRCA2* protein. Both the original *BRCA2* 5193delC sequence and the 5177insA/5193delC combination sequence are seen, suggesting that the secondary mutation occurs in just one of a duplicated mutant allele or in just a proportion of the neoplastic cells analyzed. Postoperatively, the recurrent carcinoma was resistant to platinum chemotherapy and subsequently to a trial of a PARP inhibitor. (D) Single nucleotide polymorphism (SNP) haplotyping differentiates the germline mutant and wild-type alleles. Lymphocyte DNA demonstrates an A/G SNP within *BRCA2*. The primary carcinoma has only G, indicating that the G is on the mutant allele. The recurrent carcinoma also has predominantly G, indicating that the secondary mutation occurred on the mutant allele. (E) Allele diagrams show the relative positions of the SNP, the original mutation, and the secondary mutation.

sequences from a patient with a secondary mutation is shown in Figure 1.

Secondary Mutations and Platinum Resistance

Of 64 primary ovarian carcinomas, four (6.3%) were primarily platinum resistant, 56 were platinum sensitive, three were of unknown platinum sensitivity, and one was nonevaluable for response. One of four platinum-resistant primary ovarian carcinomas had a secondary mutation restoring *BRCA1* (25%; 95% CI,

5.3% to 71.4%), as compared with 0 of 56 platinum-sensitive primary carcinomas (95% CI, 0% to 6.3%; $P = .07$, Fisher's exact test; Fig 2A).

In contrast, of 46 recurrent ovarian carcinomas, 26 (56.5%) were platinum resistant (defined as recurrence within 6 months of stopping secondary chemotherapy after the recurrent carcinoma was sampled), 19 were platinum sensitive, and one was unknown. Twelve of 26 platinum-resistant recurrences had secondary mutations restoring *BRCA1/2* (46.2%; 95% CI, 28.7% to 64.7%), as compared with one of

Fig 2. Secondary mutations in primary and recurrent carcinomas by platinum sensitivity and a history of previous breast cancer. (A) and (C) refer to primary ovarian carcinomas. (B) and (D) refer to recurrent ovarian carcinomas. (A) One (25%) of four platinum-resistant primary ovarian carcinomas had secondary mutations restoring *BRCA1/2*, compared with 0 of 56 platinum-sensitive primary ovarian carcinomas ($P = .07$, Fisher's exact test). Three carcinomas had unknown platinum status and one individual was nonevaluable. (B) Twelve (46.2%) of 26 platinum-resistant recurrent ovarian carcinomas had secondary mutations restoring *BRCA1/2*, compared with one (5%) of 19 platinum-sensitive recurrent ovarian carcinomas ($P = .003$). One carcinoma had unknown platinum status. Two carcinomas with secondary mutations are from the same patient (one sensitive and one resistant), and two carcinomas without secondary mutations are from the same patient (one sensitive and one resistant). (C) Two (10.5%) of 19 primary ovarian carcinomas obtained from women with a history of breast carcinoma had secondary mutations restoring *BRCA1*, compared with 0 of 45 obtained from women with *BRCA1/2* mutations and no history of breast carcinoma ($P = .08$, Fisher's exact test). (D) Six (66.7%) of nine recurrent ovarian carcinomas from women with a history of breast carcinoma before their diagnosis of ovarian carcinoma had secondary mutations restoring *BRCA1/2*, compared with six (17.1%) of 35 recurrent ovarian carcinomas from women with no prior history of breast carcinoma ($P = .007$, Fisher's exact test).

19 platinum-sensitive recurrences (5.3%; 95% CI, 1.2% to 24.8%; $P = .003$, Fisher's exact test, Fig 2B).

One primary carcinoma with a secondary mutation occurred in a 0.8-mm stage IA fallopian tube carcinoma diagnosed at the time of risk-reducing salpingo-oophorectomy, in a carrier of a germline *BRCA1* 120A>G mutation who was previously treated with chemotherapy for breast cancer (UWF35, Table 1). She received three cycles of docetaxel and carboplatin for the tubal carcinoma and has no evidence of recurrence 2 years later. She was considered nonevaluable for response given her lack of residual disease after initial surgery. The other patient with a secondary mutation in a primary carcinoma had a germline *BRCA1* 2594delC mutation and stage IV primary platinum-resistant disease (UW40, Table 1).²⁰ She also was treated previously with chemotherapy for breast cancer (unknown agents). She subsequently experienced recurrence within 3 months of completing primary platinum-containing chemotherapy.

The patient with a secondary mutation in a platinum-sensitive recurrent carcinoma (UW200) initially presented with stage IIIB ovarian carcinoma, treated with primary surgery and six cycles of carboplatin and paclitaxel. She then experienced recurrence 3 years later, when the recurrent sample with a secondary mutation was obtained. This was a small-volume recurrence that was removed with no visible residual disease. She was treated with intraperitoneal cisplatin and intravenous paclitaxel for six cycles and did not experience recurrence again for 13 months after completing that regimen. Her recurrence at that time was also found to have a secondary mutation and was platinum resistant with disease progression within 2 months of completing platinum-based therapy. Interestingly, her recurrent carcinoma has subsequently had a partial response on a clinical trial combining carboplatin, gemcitabine, and a PARP inhibitor. However, the lack of a complete response to platinum is consistent with platinum resistance.

Secondary Mutations and Response to PARP Inhibition

Six patients with platinum-resistant recurrent ovarian carcinomas were treated with PARP inhibitors (Table 2). Three carcinomas had secondary mutations restoring *BRCA1/2* (including the one just described), two did not respond, and one had a partial response. Two patients who did not have secondary mutations were noted to have a complete response to PARP inhibition, and one had a partial response.

Secondary Mutations and History of Previous Breast Carcinoma and Chemotherapy

A history of breast carcinoma occurring before the primary ovarian carcinoma was present in 19 (29.7%) of 64 patients who had primary carcinomas sequenced. Two (10.5%) of 19 (95% CI, 3.2% to 31.7%) had secondary mutations in their primary ovarian carcinoma after a previous breast carcinoma, compared with 0 of 45 with no history of breast carcinoma (95% CI, 0 to 7.7%; $P = .08$, Fisher's exact test; Fig 2C). Data regarding prior treatment with chemotherapy was incomplete; however, at least 11 of 19 had chemotherapy for breast carcinoma before diagnosis with ovarian carcinoma. Interestingly, the two primary carcinomas with secondary mutations that restored *BRCA1* had received their chemotherapy for breast carcinoma 10 and 11 years before their diagnosis of fallopian tube or ovarian carcinoma, respectively.

Of 44 women with recurrent ovarian carcinomas (two women had two recurrences), nine (20.5%) had been previously treated for breast carcinoma. Six of nine recurrent ovarian carcinomas in women with a history of breast carcinoma had secondary mutations (66.7%; 95% CI, 34.8% to 87.8%), compared with six of 35 recurrent ovarian carcinomas in women with no history of breast carcinoma (17.1%; 95% CI, 8.2% to 32.8%; $P = .007$, Fisher's exact test; Fig 2D). Information on chemotherapy for breast carcinoma was incomplete; however, at least four of nine women had chemotherapy for breast carcinoma before their primary ovarian carcinoma, and three of four had secondary mutations restoring *BRCA1/2*. To date, no secondary mutation has been identified in a primary or recurrent ovarian carcinoma in an individual without any history of prior chemotherapy. Of women with breast carcinoma and a secondary mutation, the range of time from breast cancer diagnosis to ovarian cancer diagnosis was 60 to 156 months (median, 115.5 months).

Five women received neoadjuvant chemotherapy immediately before surgery for their primary ovarian carcinoma (range, three to four cycles), and none had detectable secondary mutations in their primary carcinomas.

Timing of Secondary Mutations

Patient UW40 had a 23 base-pair (bp) deletion downstream from her *BRCA1* 2594delC mutation that restores a functional reading frame (Fig 3A), as previously described.²⁰ Primers were designed to PCR amplify only the sequence containing the secondary 23-bp deletion. Using Sanger sequencing, this 23-bp deletion was detectable only

Table 2. Summary of Platinum-Resistant Recurrent Ovarian Carcinomas Treated With PARP Inhibition

Patient	Inherited Mutation	Carcinoma	Stage of Primary Carcinoma	Secondary Genetic Change in Carcinoma	PARP-Inhibitor Containing Regimen	Best Response to PARP-Inhibitor Containing Regimen
CS1	B2.5193delC	Recurrent ovarian	IIIC	5177insA	AZD-2281	Progressive disease
UW200	B2.4689delAC	Recurrent ovarian	IIIB	Reversion to wild-type	ABT-888 with carboplatin and gemcitabine	Partial response
UW80	B1.185delAG	Recurrent ovarian	IIIC	Reversion to wild-type	AZD-2281	Progressive disease
CS25	B1.185delAG	Recurrent ovarian	IIC	No secondary mutation	AZD-2281 and carboplatin	Complete response
CS15	B1.5382insC	Recurrent ovarian	IIIC	No secondary mutation	AZD-2281	Complete response
UW336	B1.5083del19	Recurrent ovarian	IIIC	No secondary mutation	AZD-2281	Partial response

Abbreviation: PARP, poly (ADP-ribose) polymerase.

Fig 3. A secondary mutation in the recurrent ovarian carcinoma identified at low levels in the primary carcinoma. (A) Sequences from UW40. Lymphocyte DNA demonstrates the *BRCA1* heterozygous germline mutation 2594delC and two heterozygous single nucleotide polymorphisms (SNPs). The primary ovarian carcinoma sequence shows both the 2594delC mutation and wild-type sequence, consistent with a secondary mutation restoring wild-type sequence. SNPs within the primary carcinoma confirm that only one allele is present, indicating that the secondary mutation occurred on the mutant allele, and the wild-type sequence present was not from retained wild-type allele. The recurrent ovarian carcinoma sequence has a 23-bp deletion that, when combined with the 2594delC mutation, restores the open reading frame. The 23-bp deletion is not detectable in the primary carcinoma using conventional sequencing. Adapted with permission from Swisher et al.²⁰ (B) Mutation-specific primers were designed to amplify sequences with the 23-bp deletion only using polymerase chain reaction. As expected, the 23-bp deletion amplifies well in the recurrent carcinoma (Rec Ca). This 23-bp deletion sequence can also be detected in two separate samples of the primary ovarian carcinoma, from the ovary (Prim Ca Ovary) and from a metastatic lesion in the omentum (Prim Ca Om). Control samples of an unrelated individual's carcinoma (Control DNA), the UW40's lymphocyte DNA (Lymph), and water (H₂O) do not have detectable sequence.

in the recurrent carcinoma and not in the paired primary carcinoma from the same individual. However, PCR amplification using the mutation-specific primers detected the secondary mutation in the patient's primary carcinoma (Fig 3B), indicating that the secondary mutation in the recurrent carcinoma was present in a minority of neoplastic cells at the time of diagnosis.

DISCUSSION

In this large series of *BRCA1/2*-mutated ovarian, tubal, and peritoneal carcinomas, the presence of secondary somatic mutations that restore *BRCA1/2* in recurrent carcinomas was significantly correlated with

response to platinum-based chemotherapy, with 12 (92%) of 13 carcinomas with restored *BRCA1/2* proving to be platinum-resistant (95% CI, 66.1% to 98.2%). When treating ovarian carcinomas, oncologists traditionally have used the interval from completion of platinum-based chemotherapy to disease progression to predict platinum sensitivity or resistance. When the treatment-free interval is greater than 6 months before progression, the patient is thought to have a platinum-sensitive recurrence and is often re-treated with platinum-based chemotherapy. In our series, the presence of a secondary mutation restoring *BRCA1/2* was a better predictor of platinum resistance than interval since last treatment (Table 1). Of 26 recurrent carcinomas that proved to be platinum resistant, 12 (46.2%)

of 26 (95% CI, 28.7% to 64.7%) could have been predicted by detection of a secondary mutation restoring BRCA1/2, as opposed to only four (15.4%) of 26 by treatment-free interval (95% CI, 6.3% to 33.7%; $P = .03$, Fisher's exact test, two-tailed). Additionally, secondary mutations were significantly more common in recurrent carcinomas sampled after more than one chemotherapy regimen than those sampled after a single chemotherapy regimen. The ability to predict response to platinum chemotherapy is clinically valuable, as it would allow the avoidance of unnecessary toxicity and earlier use of alternate agents. Although our data support the clinical utility of testing for secondary mutations in *BRCA1/2*-mutated ovarian carcinomas to predict platinum resistance, the clinical implementation may be challenging because this is a technically demanding test, requiring careful microdissection to obtain a pure neoplastic cell population. Additionally, the differentiation of a secondary reversion mutation from contamination with wild-type sequence from non-neoplastic cells requires careful allelic identification with SNP haplotyping or microsatellite testing.

There was a marked association between prior history of breast carcinoma and secondary mutations in either the primary or recurrent ovarian carcinomas, with the breast carcinoma often preceding the ovarian carcinoma by many years. Cumulative chemotherapy exposure is the most likely contributor to these secondary genetic changes. Cyclophosphamide, commonly used in patients with breast carcinoma, is a DNA cross-linking agent and theoretically would induce or select for BRCA1/2 restoration in similar fashion to platinum compounds.²¹ The occurrence of secondary mutations in ovarian carcinomas more than a decade after chemotherapy for breast cancer is not easily explainable. If breast cancer chemotherapy induced a secondary mutation that restored wild-type sequence in a non-neoplastic ovarian or tubal epithelial cell, such a cell would be homozygous for wild-type *BRCA1/2* and presumably be subsequently protected from neoplastic transformation. Alternatively, hypothesizing that the secondary mutation occurs in a cell that is already BRCA1/2 deficient (secondary to deletion of the wild-type allele) implies that the earliest neoplastic cell exists many years before clinical diagnosis. A better understanding of the timing of secondary mutations will shed light on the natural history and molecular progression

of hereditary ovarian carcinomas. The association of breast carcinoma with secondary mutations in subsequent ovarian carcinomas could also be unrelated to chemotherapy and perhaps reflects a more severe phenotype in those patients.

The timing of when secondary mutations develop during the treatment of ovarian carcinoma is clinically important. Secondary mutations may be induced by DNA-damaging chemotherapy either for breast carcinoma or primary ovarian carcinoma. Alternatively, secondary mutations could be present in the primary carcinoma due to genomic instability and then selected by chemotherapy. Data from patient UW40 (Fig 3) reveals that secondary mutations can be present in rare cells in the primary carcinoma. Her secondary mutation may have been induced by chemotherapy for breast carcinoma. Whether secondary mutations restoring BRCA1/2 exist in primary ovarian carcinomas in women with no previous chemotherapy exposure is not known. In chronic myelogenous leukemia, *BCR-ABL* mutations that confer resistance to imatinib therapy in some cases are detectable by PCR in rare neoplastic cells before imatinib exposure.²² If secondary mutations that restore BRCA1/2 are already present in a small percentage of cells in a primary ovarian carcinoma similarly, this could have therapeutic implications. It is possible that the use of PARP inhibitors for prolonged consolidation after primary therapy could accelerate the selection of BRCA1/2-restored neoplastic cells and consequently accelerate the development of platinum resistance after recurrence.

These data add to the current understanding of *BRCA1/2* carcinogenesis. Loss of heterozygosity in *BRCA1/2* leads to a carcinoma deficient in BRCA1/2 protein. This carcinoma with decreased ability to repair DNA then becomes highly sensitive to DNA cross-linking agents such as platinum drugs. Secondary mutations restoring DNA repair function of BRCA1/2 can occur within the carcinoma, likely as a consequence of prior chemotherapy or perhaps from genomic instability and spontaneous mutation. This restoration of DNA repair can then lead to acquired resistance to platinum and likely resistance to PARP inhibition (Fig 4).

Not all platinum-resistant recurrent carcinomas had detectable secondary somatic mutations, indicating that other mechanisms of

Fig 4. Model for acquired resistance to platinum chemotherapy by secondary mutations restoring BRCA1/2. Secondary mutation is hypothesized to occur as a result of DNA-damaging chemotherapy, either from primary adjuvant therapy of the ovarian carcinoma or from previous chemotherapy for a separate carcinoma such as breast. This population of cells with restored BRCA1/2 and improved DNA repair is either induced or selected by adjuvant platinum therapy. Adapted with permission from Sakai et al.¹⁸

acquired resistance occur. Possibly, we missed some larger insertions or deletions that restore the *BRCA1/2* reading frame,¹⁹ as these alterations would be missed with the relatively short amplicons generated by our PCR of formalin-fixed tissues. Additionally, our retrospective series does not represent all women with *BRCA1/2*-associated ovarian carcinoma as we selected those cases who had known mutations as opposed to prospectively testing all cases. *BRCA1/2* mutation carriers selected in this fashion are likely to include a higher proportion of women with two primary cancers as well as women with better survival who are more likely to undergo clinical genetic testing.

Secondary mutations in *BRCA1/2* could also confer resistance to PARP inhibitors. Neoplastic cells with restored *BRCA1/2* are resistant to PARP inhibitors,¹⁷⁻¹⁹ and platinum-resistant *BRCA2*-mutated pancreatic cancer clones without secondary *BRCA2* mutations remain sensitive to PARP inhibition in vitro.¹⁸ Response data from our small number of cases treated with PARP inhibitors support the in vitro data that secondary *BRCA1/2* mutations predict PARP inhibitor resistance. However, larger studies are needed to determine whether testing for secondary *BRCA1/2* mutations could be used to determine which patients will benefit from PARP inhibition.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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