Clinical Cancer Research

Tumor *BRCA1* Reversion Mutation Arising during Neoadjuvant Platinum-Based Chemotherapy in Triple-Negative Breast Cancer Is Associated with Therapy Resistance

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Abstract

Purpose: In germline *BRCA1* or *BRCA2* (*BRCA1/2*) mutation carriers, restoration of tumor *BRCA1/2* function by a secondary mutation is recognized as a mechanism of resistance to platinum and PARP inhibitors, primarily in ovarian cancer. We evaluated this mechanism of resistance in newly diagnosed patients with *BRCA1/2*-mutant breast cancer with poor response to neoadjuvant platinum-based therapy.

Experimental Design: PrECOG 0105 was a phase II neoadjuvant study of gemcitabine, carboplatin, and iniparib in patients with stage I–IIIA triple-negative or *BRCA1/2* mutation–associated breast cancer ($n = 80$). All patients underwent comprehensive *BRCA1/2* genotyping. For mutation carriers with moderate or extensive residual disease after neoadjuvant therapy, *BRCA1/2* status was resequenced in the residual surgical breast tumor tissue.

Results: Nineteen patients had a deleterious germline *BRCA1/2* mutation, and four had moderate residual disease at surgery. *BRCA1/2* sequencing of residual tissue was performed on three patients. These patients had *BRCA1* 1479delAG, 3374insGA, and W1712X mutations, respectively, with LOH at these loci in the pretreatment tumors. In the first case, a new *BRCA1* mutation was detected in the residual disease. This resulted in a 14–amino acid deletion and restoration of the *BRCA1* reading frame. A local relapse biopsy 4 months later revealed the identical reversion mutation, and the patient subsequently died from metastatic breast cancer.

Conclusions: We report a *BRCA1* reversion mutation in a patient newly diagnosed with triple-negative breast cancer that developed over 18 weeks of platinum-based neoadjuvant therapy. This was associated with poor therapy response, early relapse, and death. *Clin Cancer Res; 23(13); 3365–70. ©2017 AACR.*

Introduction

Individuals with a heterozygous germline mutation in the *BRCA1* or *BRCA2* (*BRCA1/2*) genes are predisposed to a range of familial cancers, including breast and ovarian carcinomas (1). The *BRCA1/2* genes are tumor suppressors that encode proteins critical for the accurate repair of DNA double-strand breaks by homologous recombination. The interplay between the BRCA1/2 proteins and RAD51, an essential DNA repair protein, preserves genomic stability by avoiding more error-prone DNA repair pathways (2–4). Tumors from patients with deleterious *BRCA1/2* mutations usually have loss of the wild-type allele and are thus *BRCA1/2*-deficient, whereas *BRCA1/2* heterozygosity is retained by normal cells (5, 6). Somatic inactivation of the wild-type *BRCA1/2* allele and methylation of the *BRCA1* promoter domain with subsequent epigenetic silencing are other reported mechanisms (7).

Approximately 10% to 20% of invasive triple-negative breast cancers arise in the setting of *BRCA1/2* deficiency (8–10). *BRCA1/2*-deficient breast cancers have decreased capacity to repair DNA, resulting in enhanced sensitivity to chemotherapy with cross-linking agents, such as cisplatin and carboplatin, and susceptibility to PARP inhibitors due to a synthetic lethal interaction (11–13). In a small proof-of-concept trial, the PARP inhibitor olaparib led to objective tumor response in 41% of patients with advanced breast cancer with a deleterious germline *BRCA1/2* mutation (14). Clinical trials are now underway to assess the efficacy of PARP inhibitors as single agents and in combination with chemotherapy in the advanced as well as curative settings for triple-negative and *BRCA1/2*-associated breast cancers. Likewise, platinum agents have shown efficacy for *BRCA1/2*-associated breast cancers (15, 16), with a recent phase III study demonstrating substantial improvement in the objective response rate and progression-free survival with carboplatin compared with docetaxel in patients with a deleterious *BRCA1/2* mutation and advanced disease (17).

With the increased recognition of DNA-damaging therapies as the forefront agents for *BRCA1/2*-associated tumors, an unexpected

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Translational Relevance

Restoration of tumor *BRCA1* or *BRCA2* (*BRCA1/2*) function by a secondary mutation in these genes is recognized as an acquired mechanism of resistance to platinum and PARP inhibitors and has been reported primarily in ovarian cancer. How frequently this occurs in clinical practice is not well-known but may have significant implications for rational treatment selection in *BRCA1/2* mutation–associated cancers. We report a case of a *BRCA1* reversion mutation that developed over 18 weeks of neoadjuvant therapy in a newly diagnosed patient with triple-negative breast cancer. This was associated with rapid relapse after platinum-based therapy and death from metastatic breast cancer. Given the growing number of DNA repair–targeted therapeutics currently under development for *BRCA1/2* mutation–associated cancers, further characterization of the prevalence, time course, and risk factors for reversion mutation development are warranted, as these events are likely to have greater relevance for clinical practice and drug development.

mechanism of drug resistance has garnered clinical attention: the restoration of *BRCA1/2* function due to secondary mutations of *BRCA1/2* in tumors. Given that defects in homologous recombination secondary to an underlying *BRCA1/2* mutation lead to cellular sensitivity to DNA-damaging agents, the restoration of DNA repair proficiency results in acquired resistance to these drugs. To date, this drug resistance mechanism has been reported primarily in patients with advanced ovarian cancer who had disease progression after platinum or PARP inhibitor therapy (7, 18–24). In this study, we evaluated this novel mechanism of drug resistance in newly diagnosed patients with early-stage *BRCA1/2*-mutant breast cancer who had a poor response to platinum-based neoadjuvant chemotherapy.

Materials and Methods

Clinical trial study design and treatment regimen

PrECOG 0105 was a single-arm phase II neoadjuvant study in patients with newly diagnosed clinical stage I–IIIA triple-negative [estrogen receptor (ER) $\leq$ 5%, progesterone receptor (PR) $\leq$ 5%, and human epidermal growth factor receptor 2 (HER2)-negative (0 or 1+ by immunohistochemistry or FISH unamplified)] or *BRCA1/2* mutation–associated breast cancer. Eighty patients were treated with carboplatin, gemcitabine, and iniparib, intravenously, for 6 cycles before definitive surgery. A total of 80 patients were enrolled at multiple cancer centers through PrECOG. Details of the clinical trial methods and results of the study endpoints have been published previously (16).

Study endpoints

The primary endpoint of the clinical study was complete pathologic response rate, defined as no invasive carcinoma in the breast or axillary lymph nodes. The extent of residual disease was assessed using the residual cancer burden (RCB) index (25). The RCB index is a validated prognostic marker of distant relapse-free survival in patients who have been treated with neoadjuvant chemotherapy [RCB 0: complete pathologic response (pCR); RCB I: minimal residual disease; RCB II: moderate residual disease; and, RCB III: extensive residual disease); ref. 25].

Molecular analyses

In addition to comprehensive germline *BRCA1/2* testing using serum samples, all patients underwent research core biopsies of the primary breast tumor before therapy initiation; five to ten 5-μm tissue sections of fixed or frozen tumor tissue were collected and processed at Myriad Genetics, Inc. DNA extraction from formalin-fixed, paraffin-embedded or frozen tumors was conducted, and next-generation DNA sequencing using a liquid hybridization assay (Agilent SureSelect) followed by Illumina sequencing was performed. Residual disease tissue was collected in patients who went to surgery and did not achieve pCR and processed using the same assay methodology (26).

To determine *BRCA1/2* mutation status, variant and large rearrangement detection was performed on sequence from *BRCA1* and *BRCA2*. Complete descriptions of the mutation detection methods have been previously described (27). Only mutations classified as deleterious or suspected deleterious on the basis of previously described criteria were included in the analysis (28).

Baseline tumor gene expression profiling, copy number alterations and measures of genomic scars (homologous recombination deficiency assay) were assessed for any correlation with treatment response to neoadjuvant therapy. For *BRCA1/2* mutation carriers with unfavorable response to neoadjuvant therapy, e.g., RCB II, tumor *BRCA1/2* status was re-sequenced in the residual surgical tissue.

DNA extractions from two independent samples of the pretreatment tumor specimen were also performed for patient 15 to assess for a reversion mutation. In both samples, there were between 800 and 1,800 unique clonal reads through the region where the reversion mutation was located. On the basis of simulations, we would have 95% confidence to detect the reversion mutation in this number of clonal reads, if the mutation occurred in at least 1 in 250 cells.

Furthermore, SNP data from the homologous recombination deficiency assay was used to calculate the percent tumor content in each sample and to reconstruct the genome over the locus. This information allowed for calculation of predicted read frequency of both germline and somatic alleles.

All germline and tumor *BRCA1/2* mutation testing was performed by Myriad Genetics, Inc.

Results

Patients and tumor characteristics

Eighty patients received six cycles of gemcitabine, carboplatin, and iniparib in PrECOG 0105. Nineteen of these 80 patients (24%) carried a deleterious germline mutation in *BRCA1*, *BRCA2*, or both genes (Table 1). Four of these 19 patients had ER/PR-positive breast cancer (two *BRCA1* carriers and two *BRCA2* carriers), and the remaining 15 patients (78.9%) had triple-negative breast cancer. Deletion mutations leading to nonfunctional BRCA1/2 proteins were the primary mechanism (13 patients, 65%), including one patient with bilateral breast cancer with deletion of the entire *BRCA1* gene, as well as a patient with deletions in both *BRCA1* and *BRCA2* genes (*BRCA1*: 1048delA; *BRCA2*: 1366delA); both patients had excellent responses to neoadjuvant therapy. Insertions and missense mutations were

Table 1. Breast cancer subtype and *BRCA1/2* mutation status in PrECOG 0105

Characteristics	All patients (N = 80) *n* (%)
Breast cancer subtype	
Triple-negative	77 (96)
ER- and/or PR-positive/HER2-negative	3 (4)
BRCA1/2 mutation status	
BRCA1 mutation	14 (18)
BRCA2 mutation	4 (5)
BRCA1 and BRCA2 mutation	1 (1)

also observed (three and four patients, respectively). The affected gene and specific location of the germline mutations are shown in Table 2.

Response data

Among all study patients, 29 [36.3%; 90% confidence interval (CI), 27%–46%] achieved a pCR. The pCR rate was 33.0% among wild-type *BRCA1/2* patients (CI, 23%–44%), 47% among *BRCA1/2* mutation carriers (CI, 27%–68%), and 56% among *BRCA1/2* mutation carriers with triple-negative breast cancer (CI, 33%–77%). Among patients with germline *BRCA1/2* mutations, RCB class was as follows: RCB 0 = 10 (53%); RCB I = 6 (32%); RCB II = 4 (21%); and RCB III = 0 (0%). One *BRCA1* mutation carrier was lost to follow-up and never had surgery.

BRCA1 sequencing of residual tissue was available in three of four patients with RCB II response (no consent in one case, patient 1). These three patients had *BRCA1* 3374insGA (patient 2), 1479delAG (patient 15), and W1721X (patient 18) mutations with LOH at these loci in the pretreatment specimens (Table 3). Patients 2 and 15 had triple-negative breast cancer, whereas patient 18 had ER/PR-positive, HER2-negative breast cancer. No reversion mutations were detected in the residual tumor specimen at the time of surgery in patients 2 and 18. However, a new *BRCA1* mutation was detected in the residual surgical tumor specimen in patient 15. This reversion mutation comprised a 42-base pair deletion that overlapped with the original 2-base pair deletion. It resulted in a 14–amino acid deletion and restoration of the *BRCA1* reading frame. A relapse biopsy of an ipsilateral axillary lymph node 4 months later revealed an identical reversion mutation (Table 3, Figs. 1 and 2).

Because of the presence of nontumor DNA in the sample, it was not possible to determine whether there were residual tumor cells after neoadjuvant therapy that retained the original 1479delAG germline mutation and lacked the reversion mutation. However, upon analysis of two samples of extracted DNA in the pretreatment tumor specimen, no reversion mutation was detected. On the basis of our statistical constraints, we conclude that whether the reversion mutation was present in the pretreatment tumor specimen, it was in less than 1 in 250 tumor cells. DNA sequencing of the contralateral breast cancer specimen did not reveal evidence of a somatic reversion mutation. Specifically, 324 unique DNA sequence reads were present, spanning both the germline deletion mutation (1479delAG) and the somatic reversion, with 245 of the DNA sequence reads showing the germline mutation and zero reads showing the somatic reversion mutation.

Predicted and observed allele frequencies were consistent with the original mutation being germline and the subsequent reversion mutation being somatic using SNP data. The presence of the original mutation in the post-reversion mutation samples at levels consistent with residual germline DNA was also confirmation that the original mutation was germline (Appendix 1).

Clinical narrative of patient with *BRCA1* reversion mutation

At the age of 25 years, this patient was diagnosed with stage IIIC right breast invasive ductal carcinoma that was ER/PR-negative and HER2-unamplified (i.e., triple-negative). A deleterious 1479delAG *BRCA1* mutation was detected at breast cancer diagnosis. She received neoadjuvant chemotherapy with adriamycin and cyclophosphamide, followed by a right breast lumpectomy

Table 2. PrECOG 0105 patients with *BRCA1/2* mutation–associated breast cancer

Patient ID	Gene mutated	Site of deleterious mutation	ER/PR status	RCB score
1	BRCA1	2392insT	TN	II
2	BRCA1	3374insGA	TN	II
3	BRCA1	917delTT	ER 0%, PR 1%	0
4	BRCA1	5385insC	TN	0
5	BRCA1	3607C>T	TN	N/A
6	BRCA1	del exons 23–24	TN	I
7	BRCA2	7297delCT	TN	0
8	BRCA2	8803delC	ER 90%, PR 5%	I
9	BRCA1	del exons 5–8	TN	0
10	BRCA1	187delAG	TN	0
11-L breast	BRCA1	Deletion of entire BRCA1 gene	TN	0
11-R breast	BRCA1	Deletion of entire BRCA1 gene	TN	0
12	BRCA1	Q284X969CTdel	TN	0
13	BRCA1	C61G (300T>G)	TN	I
14	BRCA2	G819delTG	ER 30%, PR 20%	I
15	BRCA1	1479delAG	TN	II
16	BRCA1 and BRCA2 (one breast lesion)	BRCA1: 1048delA	TN	I
		BRCA2: 1366delA	TN	I
17	BRCA2	5104delAA	TN	0
18	BRCA1	W1712X (5255G>A)	ER 50%, PR 30%	II
19	BRCA1	IVS16+6T>C	TN	0

Abbreviation: TN, triple-negative.

Table 3. Study patients with *BRCA1* mutation–associated breast cancer and RCB score of II

Patient No.	Deleterious mutation in original breast tumor	BRCA1 status in residual surgical tissue	BRCA1 status in recurrence (metastatic biopsy)
1	2392insT	N/A; patient did not provide consent	N/A
2	3374insGA	No secondary mutation	N/A
15	1479delAG	42-base pair deletion (Ser454_Lys467del)	42-base pair deletion (Ser454_Lys467del)
18	W1712X (5255G>A)	No secondary mutation	N/A

Abbreviation: N/A, not applicable.

and axillary lymph node dissection. She was found to have achieved a pCR at the time of surgery with no disease in the lymph nodes. This was followed by adjuvant paclitaxel and whole breast radiation (50.4 Gy).

The patient had an in-breast recurrence 1 year later; this was also high-grade, triple-negative carcinoma for which she proceeded with a right mastectomy, followed by additional cycles of anthracycline- and taxane-based chemotherapy.

Approximately 10 years later, she was diagnosed with a contralateral high-grade, triple-negative breast cancer. She enrolled in PrECOG 0105 and received six cycles of gemcitabine, carboplatin, and iniparib. She proceeded with a left mastectomy upon completion of experimental therapy, and this revealed a 2-cm residual invasive carcinoma of mixed lobular and ductal histology (RCB II) with a negative sentinel lymph node dissection.

Within months of completing neoadjuvant therapy, a recurrent left axillary mass was discovered, prompting additional locoregional and systemic treatment. One year later, she was found to have distant disease spread. Her disease progressed rapidly, and she died less than 2 years after her second primary breast cancer diagnosis.

Discussion

We report the case of a young woman with a history of bilateral triple-negative breast cancer and a germline *BRCA1* 1479delAG mutation who, over the course of 18 weeks of neoadjuvant platinum-based therapy, developed a 42-base pair somatic deletion in the *BRCA1* gene, leading to a reversion of the underlying 1479delAG deleterious germline *BRCA1* mutation. The same somatic *BRCA1* reversion mutation was identified in a metastatic focus in an axillary lymph node at the time of locoregional relapse, 4 months after the patient's definitive surgery. To our knowledge, there has been no previous report of a *BRCA1* somatic reversion mutation developing over the short span of neoadjuvant chemotherapy in a patient with newly diagnosed, early-stage breast cancer.

Two types of somatic reversion mutations have been described. In the case of our patient, a genetic reversion event leading to deletion of a frameshift mutation resulted in correction of the original frameshift, thereby producing an allele that contains a new mutation, but which now encodes a DNA repair–proficient BRCA1 protein. A second class of mutations involves direct reversion to wild-type of the original mutation. Studies have shown that these types of somatic reversions account for the majority of *BRCA1/2* mutation–associated ovarian carcinomas with secondary restoration of the *BRCA1* or *BRCA2* reading frame (7). These ovarian tumors develop platinum resistance, and may also be resistant to PARP inhibitor therapy (15, 17, 21). Reversion mutations in this setting have been associated with a prior history of breast cancer or with exposure to more than one line of chemotherapy for the treatment of ovarian cancer. This has led to the hypothesis that such somatic mutations may have been present at the time of the ovarian cancer diagnosis in a small number of neoplastic cells that were subsequently selected by additional DNA-damaging therapy (17). Furthermore, somatic reversion mutations

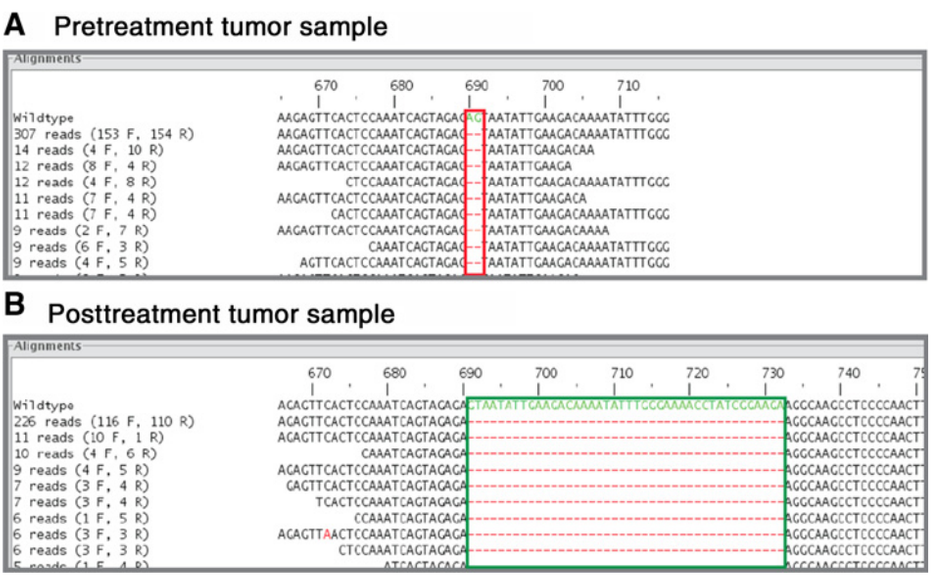

Figure 1.
Sequence data from tumor samples of patient 15.

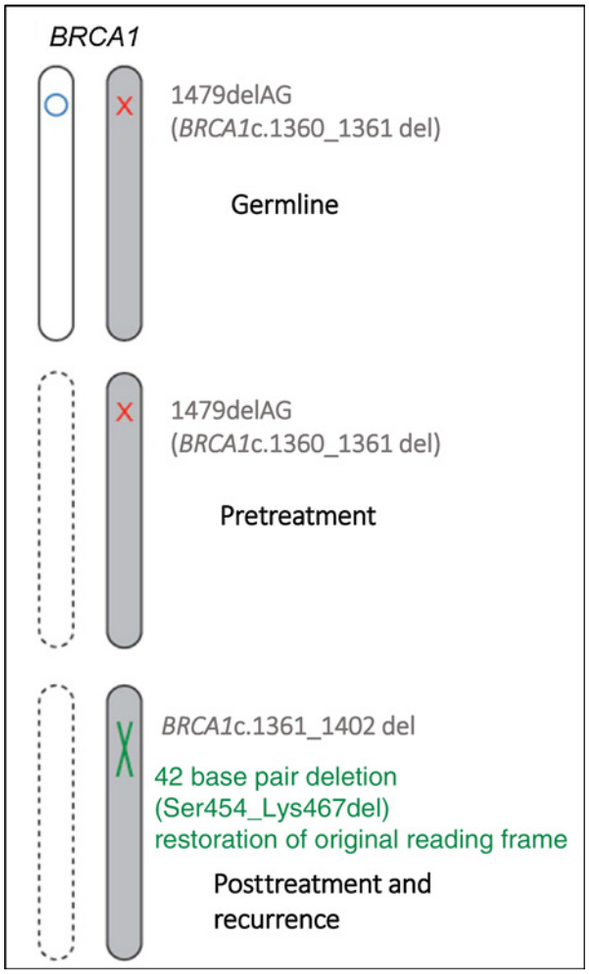

Figure 2.
Schematic of BRCA1 reversion mutation in pretreatment tumor compared with posttreatment and recurrence samples.

have also been reported with cisplatin treatment in breast and pancreatic cancer cell lines that had mutations in *BRCA2* (19) and with the PARP inhibitor olaparib for a male *BRCA2* mutation carrier with metastatic breast cancer (14).

BRCA1 is a large multidomain protein that includes the "really interesting new gene" (RING) finger, nuclear localization signal (NLS), coiled-coil, and BRCA1 C-terminus (BRCT) domains. Prior *BRCA1* somatic reversion mutations have been reported in the RING finger domain and in downstream nucleotide sites (Fig. 3). In ovarian cancer, *BRCA1* reversion mutations have been reported at several sites, including six patients with a 185delAG mutation and two patients with a 2594delC mutation (21, 24); however, reversion of the germline *BRCA1* 1479delAG mutation has not previously been reported in either breast or ovarian cancer.

Our study has limitations. Here, we report an association between a somatic *BRCA1* reversion mutation and therapeutic failure after neoadjuvant platinum-based therapy for breast cancer. However, we cannot establish a causal relationship, in particular, as our patient's breast cancer history was complex, given she had been treated for a contralateral high-grade breast cancer 10 years prior to her new triple-negative breast cancer diagnosis, and, as such, she was exposed to chemotherapy in the past. Her clinical response to gemcitabine, carboplatin, and iniparib may not be generalizable given her past oncologic history, and, as a result of past therapeutic exposures, she may have had other molecular triggers, besides the *BRCA1* reversion, leading to chemoresistant tumor cells. Furthermore, this is a relatively small study, and corroboration in a larger cohort of *BRCA1/2* carriers treated with neoadjuvant therapy for breast cancer is warranted.

We report a novel, acquired somatic frameshift mutation in *BRCA1* leading to reversion of a 1479delAG germline *BRCA1* mutation, which developed during 18 weeks of neoadjuvant platinum-based chemotherapy for triple-negative breast cancer. We conclude that this reversion mutation led to reduced treatment sensitivity, manifested as significant disease burden at definitive surgery and rapid metastatic recurrence shortly after completion of therapy. The timing and etiology of such a reversion mutation are unclear and may have stemmed from genomic instability of the primary tumor given an underlying deficiency in homologous recombination DNA repair. How frequently somatic reversion mutations occur in clinical practice is not well known but may have significant implications for rational treatment selection in *BRCA1/2* mutation–associated cancers. Given the growing number of DNA repair–targeted therapies, characterization of the prevalence, time course, and correlates of reversion mutations in *BRCA1/2* and other DNA repair genes is essential to guide patient care and drug development.

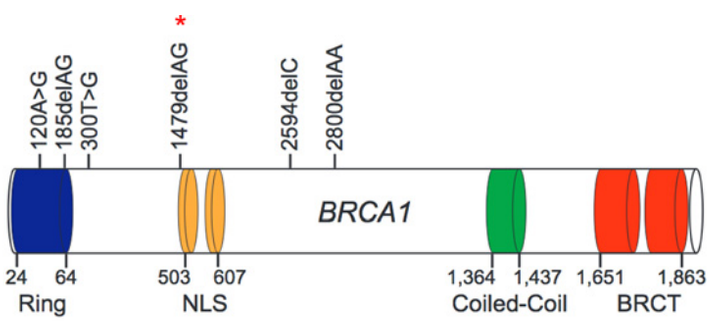

Figure 3.
Known *BRCA1* site–specific mutations with reported somatic reversion mutations.

★ Location of the reversion mutation described in patient 15, NLS = Nuclear Localization Sequence, BRCT = *BRCA1* C-Terminus

Disclosure of Potential Conflicts of Interest

S. Vinayak reports receiving company-sponsored meals from Tesaro during a clinical trial investigator's meeting. M.L. Telli is a consultant/advisory board member for Myriad Genetics. No potential conflicts of interest were disclosed by the other authors.

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Study supervision: K.M. Timms, M.L. Telli

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