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Breast Cancer

Differential Chemotherapeutic Sensitivity for Breast Tumors With "*BRCA*ness": A Review

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. *BRCA*ness • Breast cancer • *BRCA* mutations • Differential chemosensitivity

Learning Objectives

Explain the concept of sporadic "*BRCA*ness" in breast tumors.

Describe the current status, obstacles, and future direction for utility of sporadic "*BRCA*ness" in breast tumors.

ABSTRACT

BRCA1 or *BRCA2* mutations predispose to cancer development, primarily through their loss of role in the repair of DNA double-strand breaks. They play a key role in homologous recombination repair, which is a conservative, error-free DNA repair mechanism. When mutated, other alternative, error-prone mechanisms for DNA repair take over, leading to genomic instability. Somatic mutations are rare in sporadic breast tumors, but expression of *BRCA1* and *BRCA2* genes can be downregulated in other mechanistic ways. These tumors have similar features in terms of their phenotypic and genotypic profiles, which are normally regulated by these genes, and mutations lead to defective DNA repair capacity, called "*BRCA*ness." Attempts have been made to exploit this differentially expressed feature between tumors and normal tissues by treatment with DNA-damaging chemotherapy agents. Cells with this functional *BRCA* deficiency should be selectively susceptible to DNA-damaging drugs. Preclinical and early clinical (primarily retrospective) evidence supports this approach. In contrast, there is emerging evidence of relative resistance of tumors containing *BRCA1* or *BRCA2* mutations (or *BRCA*ness) to taxanes. In this review, we summarize the data supporting differential chemotherapeutic sensitivity on the basis of defective DNA repair. If confirmed with available, clinically applicable techniques, this differential chemosensitivity could lead to treatment choices in breast cancer that have a more individualized biologic basis. ***The Oncologist*** 2013;18:909–916

Implications for Practice: Women with germline *BRCA* mutations are more prone to develop breast, ovarian, and other cancers because of the inability to repair DNA damage effectively. These mutations cause a small minority of breast cancers, but studies have shown that such tumors respond better when treated with DNA-damaging chemotherapy agents. Evidence shows that non-mutated tumors also have defective DNA repair or "*BRCA*ness" caused by other mechanisms and behave similarly to *BRCA*-mutated tumors. Some clinical data support that tumors with *BRCA*ness respond better to DNA-damaging chemotherapy. Preliminary data suggest that tumors with intact *BRCA1* respond better to treatment with antitubulin agents. In this review, we discuss *BRCA*ness and the clinical data supporting preferential responses to different chemotherapy agents. No standardized test to detect *BRCA*ness exists yet, and various techniques are being developed because this test could affect chemotherapy choice.

INTRODUCTION

Breast cancer is a heterogeneous disease with different clinicopathological features, responses to treatment, and prognoses. Progress in the development of targeted therapies (HER-2/neu targeting with monoclonal antibodies and small molecule inhibitors) has made a substantial difference in both response and survival. Despite significant clinical advances, there are still 40,000 women in the U.S. who die of breast cancer each year [1]. Consequently, there is a continuing need to search for other potential therapeutic strategies.

It is well known that women with germline mutations in *BRCA1* or *BRCA2* are at increased risk of developing breast and ovarian cancers [2]. In addition, there is a higher risk of pancreatic, prostate, and male breast cancer [2]. This risk is thought to be related to the roles of *BRCA1* and *BRCA2* genes in DNA repair. DNA damage activates cell-cycle check points and recruitment of DNA repair machinery. In cells deficient in *BRCA1* or *BRCA2*, there is defective DNA repair of double-strand DNA breaks (DSB) through homologous recombination (HR), which is a conservative DNA repair mechanism with a high degree of fidelity.

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Alternative error-prone, potentially mutagenic DNA repair mechanisms like non-homologous end joining and single-stranded annealing compensate but lead to genomic instability [3]. The relative roles of *BRCA1* and *BRCA2* in repair of DNA DSB have been explored and better defined over the past two decades. *BRCA1* is a critical organizing molecule that has been linked to a range of cellular processes beyond DNA repair, like transcriptional regulation and chromatin remodeling. *BRCA2* function in HR is primarily via regulation of *RAD51* activity [4]. *BRCA2* regulates RAD51 recombinase, which is a critical step in strand invasion and homology-directed repair [4].

Germline mutation in one *BRCA1* or *BRCA2* allele is sufficient to predispose for cancer development [5]. There is a loss of heterozygosity, with loss of the normal allele while retaining the mutant allele, in the tumor tissue of susceptible individuals, suggesting that the genes play a normal role as tumor suppressors [6, 7]. Somatic mutations in *BRCA1* or *BRCA2* do not occur frequently in sporadic (or nonfamilial) breast cancer [8, 9], but potentially any (somatic) inactivation of the genes could result in their phenotypic repression [10]. A phenomenon called "*BRCA*ness" (or, more properly, "*BRCA*lessness") has been reported in sporadic cancers that do not have the germline mutations in *BRCA1* or *BRCA2* but that display similar inactivation of the BRCA-related genes and consequently have defective HR [3].

Inactivation of the *BRCA1* gene leads to breast tumor histology that has a higher grade, that is more likely to be estrogen receptor (ER) negative, that has v-MYC avian myelocytomatosis viral oncogene homalog (CC-MYC) overexpression, and that lacks ERBB2 (HER-2/neu) amplification [11, 12]. *BRCA1* gene mutation-associated breast cancers typically display a basal-like molecular subtype [13, 14] similar to that composing the majority of triple-negative breast cancers (TNBCs). This finding suggests that therapeutic approaches targeted to *BRCA1* mutation-associated breast cancers might be applicable to sporadic TNBCs. Unlike the familial *BRCA1* mutation-associated tumors, which have a characteristic phenotype, familial *BRCA2* mutation-associated tumors do not have a consistent, distinct molecular phenotype. With currently available methods, it is hard to delineate histopathological characteristics that differentiate familial *BRCA2* mutation-associated tumors from sporadic cancer, but *BRCA2*-associated tumors tend to be ER positive and HER2 negative [15].

The therapeutic implications of *BRCA1* or *BRCA2* mutations or of a sporadic *BRCA*ness phenomenon remains unproven, but initial clinical evidence (see below in clinical data section) suggests that there could be higher activity of DNA damaging agents like alkylators, platinators, and anthracyclines in these groups. In addition, preliminary data suggest relative resistance of such tumors to agents that act by stabilizing microtubule polymers (e.g., the taxane class of drugs). This review addresses the possible relationship of the defective *BRCA1* and/or *BRCA2* function (in mutation carriers and sporadic breast cancers) in relation to sensitivity to different chemotherapeutic agents.

Etiology of Sporadic BRCAness

The *BRCA1* gene is transcriptionally regulated by a CpG island promoter that is unmethylated in all normal human cell types [16]. The *BRCA1* gene is inactivated by aberrant DNA methylation in approximately 15% of sporadic breast cancers overall, with a higher incidence of epigenetic inactivation in TNBC [17–19]. The frequency of epigenetic inactivation exceeds the frequency of genetic mutation of *BRCA1* in breast cancer and is likely to be a primary force behind *BRCA*ness. A second feature that can influence the *BRCA*ness of a cell is gene dosage. The *BRCA1* gene resides on chromosome 17 in a region frequently lost in breast cancer. Consequently, homozygous deletions of this region may lead to *BRCA1* haploinsufficiency, reflecting a lesser but potentially relevant degree of *BRCA*ness [20].

In contrast to *BRCA1*, hypermethylation of the BRCA2 gene promoter is not reported as a cause of its inactivation [21]. Instead, reports of chromosome 11 open reading frame 30 (C11orf30; also known as *EMSY*) gene amplification have been implicated in *BRCA2* inactivation [22]. *EMSY*, when overexpressed, inhibits *BRCA2* transcriptional activity by interacting with exon 3 of *BRCA2*. *EMSY* amplification has been reported in up to 13% of sporadic breast cancers with most of them having ER-positive tumor biology [3].

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Detecting BRCAness

Several efforts to identify this signature have been reported using different methodologies. These efforts include array comparative genomic hybridization (aCGH), quantitative real-time polymerase chain reaction (qPCR), multiplex ligation-dependent probe amplification (MPLA), and immunohistochemistry (IHC). Techniques may be based on DNA, RNA, or proteins and have been reported using frozen or formalin-fixed tumor tissue. Each technique has its unique advantages and disadvantages, and those details are beyond the scope of this review. Currently no standard way of defining or detecting *BRCA*ness has found its way to clinical application, but this is an area of intense investigation.

The recognized steps to incorporation of a predictive biomarker into clinical care involve analytic validation, then clinical validation, and finally assessment of clinical utility [23]. It is important to recognize at the outset that analytic validation of a standardized technique that can be applied to clinical pathological material has not yet been accomplished, as reflected by the multiplicity of methods currently in use; clinical validation and ultimate assessment of clinical utility could then be formally addressed. Other predictive markers with proven utility, including HER2 and the ER, went through a lengthy process on the way to achieving these goals for adoption into clinical practice, and we hope the same may ultimately prove to be true of a test for HR deficiency (HRD).

Preferential Effect of Chemotherapy in Relation to *BRCA1* or *BRCA2* Expression

The key roles that the *BRCA1* and *BRCA2* genes play in DNA repair is through HR, and their dysfunction affects this least error-prone repair mechanism. Consequently, these cells could be more sensitive to chemotherapy agents that produce DNA damage by causing strand breaks through failure to reseal cleavable complexes in strand passage or by intercalation with base pairs (e.g., anthracyclines) or through DNA adduct formation (e.g., alkylators and platinating agents) with subsequent intra- or interstrand DNA crosslinks and resultant DSB [24].

Preclinical data suggest that low levels of BRCA1 in cell lines correlate with resistance to taxanes and vinca alkaloids [24, 25]. This response may be dependent on tumor type, with breast cancer cell lines preferentially showing this effect [25]. These breast cancer cell line data have been replicated in vivo in mice, where docetaxel resistance was noted in spontaneous breast tumors that have deletion of *BRCA1* [26]. The dominant mechanism by which *BRCA1* is involved in taxane response is unknown, but several have been proposed. One is a differential apoptotic response, which is mediated by *BRCA1* induction of Growth Arrest and DNA-Damage Inducible (GADD45A; also known as GADD45) transcription [27]. Another mechanistic hypothesis is a BRCA1-induced increase in c-Jun N-terminal kinase (JNK)/stress-activated protein kinase phosphorylation, with subsequent apoptosis in BRCA1-expressing cells treated with paclitaxel [28–30]. Taxanes disrupt mitotic spindle assembly by stabilizing microtubules and thereby triggering expression of a spindle check point. *BRCA1* is important for transcriptional upregulation of spindle assembly checkpoint proteins, and loss of its function inhibits their critical disruption by taxanes [30]. Despite published clinical reports suggesting an analogous direct relationship between loss of BRCA2 expression and taxane resistance [31], a direct pathophysiological link between the *BRCA2* gene and sensitivity to the taxane class of drugs has yet to be demonstrated.

Clinical Studies of *BRCA1* or *BRCA2* Mutation Carriers

The majority of the published clinical reports are retrospective (Table 1). Regimens used in those studies were reported as anthracycline-based or cyclophosphamide, methotrexate, and 5-flourouracil (CMF)-like regimens. Higher response rates were reported overall among patients with *BRCA1* or *BRCA2* mutations when treated with DNA-damaging regimens. The largest series reported responses in 121 *BRCA1* or *BRCA2* mutation carriers with metastatic breast cancer compared with matched sporadic breast cancer patients [32]. All patients in both groups received anthracycline-based or CMF or CMF-like regimens as treatment. Patients with *BRCA2* mutations had a higher overall response rate (ORR), higher progression-free survival (PFS), and higher overall survival (OS) after the start of such chemotherapy in first-line treatment compared with their matched controls. In the same series, a nonsignificant trend for increased ORR and PFS was noted in *BRCA1* mutation carriers. In this study, the cohorts were matched by age, year of primary cancer diagnosis, and year of metastatic breast cancer diagnosis but not for other predictive factors of known importance, such as ER and HER2, which could confound interpretation of the results.

Among various classes of DNA-damaging chemotherapeutic agents, cisplatin has been consistently reported to produce a higher ORR among *BRCA1* mutation carriers. Two prospective clinical trials further assessed this hypothesis by administering cisplatin to patients with *BRCA1* mutations. In a neoadjuvant trial of 25 *BRCA1* mutation patients, there was a clinical complete response (cCR) and associated pathological complete response (pCR) (defined as no evidence of invasive cancer in breast and lymph nodes) in 18 patients after treatment with four cycles of cisplatin at 75 mg/m2 [33, 34]. In the metastatic *BRCA1*-associated breast cancer group, 20 patients were treated with cisplatin at similar doses for six cycles [35]. Response was observed in 16 patients, with 9 achieving complete response (CR) and 7 obtaining partial response (PR). These prospective clinical trials support the concept of exploiting defective HR in patients with *BRCA1* (and *BRCA2*) mutations by treatment with platinum chemotherapy agents; however, this observation needs to be confirmed in larger, prospective clinical trials. Another caveat to any generalization about "platinators" is that experience in other cancer types indicates that drugs in the same family are not always equivalent (e.g., cisplatin is superior to carboplatin in the curative intent setting in testicular cancer), and this possibility exists in breast cancer as well, especially when carboplatin is given at an area under the curve of 2 rather than 6.

Efficacy of taxanes in patients with *BRCA1* or *BRCA2* mutations has been evaluated in two retrospective studies. One study reported a lower response rate (RR) in patients with *BRCA1* mutations who received a neoadjuvant docetaxel-based combination regimen [36]. This study reported that 6 of 15 patients with *BRCA1* mutations responded compared with 29 of 29 matched control patients (without the mutation). The cases were matched for age and treatment center. In addition to different tumor biology in the two cohorts (*BRCA1* carriers were more likely to be triple negative), the regimen compared was a combination of docetaxel and doxorubicin and may confound the conclusions. Another study reported that taxanes were less effective (in terms of RR and PFS) in hormone receptor-negative *BRCA1* mutation-associated metastatic breast cancers when matched to their sporadic breast cancer controls [31]. Again, appropriate control for other predictive markers for response was not done between the cohorts, and that significantly confounds the results. This is noted in the same study where 11 patients with hormone receptor-positive *BRCA1* mutation-associated tumors have similar PFS as their sporadic counterparts. In addition, 10 patients with hormone receptor-positive *BRCA2* mutation-associated breast cancer had a higher RR and similar PFS compared with sporadic breast cancer patients when treated with taxanes [31].

Studies Correlating BRCAness in Sporadic Tumors and Clinical Response

Table 2 lists the studies that report clinical correlation of a BRCA1- or BRCA2-like signature with administration of different chemotherapeutic agents. BRCA1- or BRCA2-like signature was determined with a variety of nonstandardized techniques (aCGH, MPLA, qPCR, or IHC). There is a higher incidence of BRCA1-like signature in tumors with triple-negative histology compared with BRCA2-like signature, which is reported to be higher in hormone receptor-positive breast tu-

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Table 1. Studies reporting chemotherapy efficacy in patients with *BRCA1* or *BRCA2* mutations

First author	Mutations assessed	Type of study	No. of patients	Chemotherapy regimen	Results
Delalogue [53]	*BRCA1* and *BRCA2*	Retrospective	15 *BRCA1*, 5 *BRCA2*, and 57 matched controls	Neoadjuvant anthracycline-based regimen	A pCR was noted in 53% of *BRCA1*, 0% of *BRCA2*, and 14% of controls
Chappuis [54]	*BRCA1* and *BRCA2*	Retrospective	7 *BRCA1*, 4 *BRCA2*, and 27 noncarriers	Neoadjuvant anthracycline-based regimen	*BRCA1* and *BRCA2* carriers had higher cCR (93% vs. 30%) and pCR (36% vs. 4%) compared with noncarriers
Byrski [36]	*BRCA1*	Retrospective	44 *BRCA1* and 41 matched controls	Various neoadjuvant regimens: CMF, CMFP, AC, FAC, AT	80% of *BRCA1* carriers had PR or CR compared with 95% of noncarriers; *BRCA1* carriers responded poorly to AT (40%) compared with controls (100%)
Gronwald [33], Byrski [34]	*BRCA1*	Prospective	25 *BRCA1*	Neoadjuvant treatment with cisplatin	72% of *BRCA1* carriers had cCR and pCR
Kriege [32]	*BRCA1* and *BRCA2*	Retrospective	93 *BRCA1*, 28 *BRCA2*, 121 matched controls	Anthracycline-based or CMF/CMF-like regimen in metastatic breast cancer	Compared with sporadic patients, *BRCA2* carriers have significantly higher ORR and improved PFS and OS; for *BRCA1*, a nonsignificant trend for increased ORR and PFS was noted
Byrski [55]	*BRCA1*	Retrospective	102 *BRCA1*	Various neoadjuvant regimens: CMF, AT, FAC, AC, cisplatin	A pCR was noted in 23%, highest with cisplatin (83%), intermediate with AC (22%) and FAC (21%), lowest with AT (7%) and CMF (8%)
Byrski [35]	*BRCA1*	Prospective phase II	20 *BRCA1*	Metastatic breast cancer treated with cisplatin	ORR 80% (45% cCR, 35% PR); median TTP: 12 mo
Kriege [31]	*BRCA1* and *BRCA2*	Retrospective	35 *BRCA1*, 13 *BRCA2*, 95 matched controls	Taxanes (docetaxel or paclitaxel) in metastatic breast cancer patients	Compared with their matched controls, HR-negative *BRCA1* carriers had a lower ORR (42% vs. 20%), but HR-positive *BRCA2* carriers responded better to taxanes (89% vs. 38%)
Isakoff [56]	*BRCA1* and *BRCA2*	Prospective	86 TNBC patients: 9 *BRCA1*, 2 *BRCA2*	Platinum chemotherapy for metastatic TNBC	ORR in patients positive fpr *BRCA1* or *BRCA2* was 54.6% (5 PR, 1 CR)

Abbreviations: AC, doxorubicin, cyclophosphamide; AT, doxorubicin, docetaxel; cCR, clinical complete response; CMF, cyclophosphamide methotrexate, 5-flourouracil; CMFP, cyclophosphamide methotrexate, 5-flourouracil, prednisone; CR, complete response; FAC, flourouracil, doxorubicin, cyclophosphamide; HR, hormone receptor; ORR, overall response rate; OS, overall survival; pCR, pathological complete response; PFS, progression-free survival; PR, partial response; TNBC, triple-negative breast cancer; TTP, time to progression.

mors [18]. In addition to the different methodologies to identify *BRCA*ness, information regarding other predictive markers for treatment outcome is not uniformly reported in all studies, a serious known shortcoming. Currently, there are no standardized ways to compare these techniques, making cross-study comparisons difficult.

Tumors with BRCA1-like signature determined by aCGH are reported to be more sensitive to anthracycline-based or high-dose-platinum-based chemotherapy in two large retrospective studies [18, 37]. One of the studies reported that a BRCA2-like signature was surprisingly common among ER-positive patients, and such patients had a much higher rate of pathological response to neoadjuvant treatment with an anthracycline-based regimen [18]. The second large series found that 18% of all breast tumors had a BRCA1-like aCGH. A strength of this study was that specimens were assessed retrospectively from a randomized clinical trial comparing conventional chemotherapy with high-dose chemotherapy in stage II/III breast cancer patients. Among those patients with a BRCA1-like profile, significantly better recurrence-free survival (RFS) and OS was noted after treatment with the high-dose regimen, which contained platinator and alkylators at doses requiring autologous stem cell support for hematological recovery, versus the control arm [37].

Smaller studies have shown less consistent results. One study reported significantly higher BRCA1 messenger RNA (mRNA) levels in patients responding to treatment with cyclophosphamide and epirubicin [38]. The authors hypothesized that *BRCA1* is needed for apoptosis, and an intact BRCA1 protein pushes the cell into apoptosis when DNA damage occurs. Preclinical and other clinical data reviewed suggest otherwise, and a further technical issue may involve intracellular localization of the RNA message being measured. Similarly, another report assessed BRCA1 promoter methylation in sporadic patients with TNBC and found decreased RFS at 5 years in patients whose tumors had BRCA1 promoter methylation, implying impaired, rather than augmented, effectiveness of an anthracycline-based approach in the setting of defective HR [39]. One possible difficulty with this observation is that it makes an assumption that BRCA1 promoter methylation accounts entirely for a *BRCA*ness profile, whereas other causes (e.g., gene dosage) are known to exist. Moreover, the regi-

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Table 2. Studies reporting chemotherapy efficacy in sporadic *BRCA*ness

First author	Genes assessed	Type of study	No. of patients	Chemotherapy regimen	Results
Egawa [43]	*BRCA1* and *BRCA2*; mRNA levels by qPCR	Retrospective	25	Docetaxel in patients with locally advanced/recurrent tumors	40% RR in tumors with high BRCA2 mRNA levels compared with 100% in tumors with low BRCA2 levels; a nonsignificant trend toward lower BRCA1 mRNA expression among responders was noted
Egawa [38]	*BRCA1* and *BRCA2*; mRNA levels by qPCR	Retrospective	51	Cyclophosphamide, epirubicin in patients with locally advanced/ recurrent tumors	65% RR in patients with tumors with high BRCA1 mRNA levels compared with 32% in tumors with low BRCA1 levels; no difference in response was noted in relation to BRCA2 levels
Kurebayashi [41]	*BRCA1* expression by IHC	Retrospective	50	Taxane-based ($n = 19$) or anthracycline-based ($n = 25$) regimens in patients with metastatic breast cancer	No *BRCA1* expression in 58%; in the taxane subgroup, absent *BRCA1* expression was associated with shorter TTP (6.5 mo vs. 14.7 mo); no difference noted in RR or TTP with *BRCA1* expression when treated with anthracycline-based regimen
Silver [57]	*BRCA1* mutations, BRCA1 mRNA levels, BRCA1-PM,	Prospective	28	Cisplatin given neoadjuvantly	22% had pCR; low BRCA1 mRNA expression and BRCA1 PM associated with good response to cisplatin
Rodriguez [42]	*BRCA1*-like signature (69-gene LDA by qPCR)	Retrospective	105	FEC ($n = 50$), AC ($n = 16$), and TET ($n = 39$) were given to TNBC patients neoadjuvantly	Tumors with defective DNA repair gene expression are more sensitive to anthracyclines and are resistant to taxane-based chemotherapy
Lips [18]	*BRCA1* and *BRCA2*; aCGH, BRCA1-PM, BRCA1 mRNA levels, *EMSY* amplifications	Retrospective	163	Dose-dense AC given neoadjuvantly	BRCA1-like aCGH found to be higher in TNBC (57% vs. 6% in ER positive); BRCA2-like aCGH associated with higher pCR and near-pCR
Vollebergh [37]	BRCA1 aCGH	Retrospective	230[a]	HD-PB vs. FEC X6	18% had BRCA1-like aCGH, and it was associated with better outcomes after HD-PB chemotherapy (RFS and OS)
Sharma [39]	BRCA1-PM	Retrospective	39	Anthracycline-based regimen administered neoadjuvantly or adjuvantly to TNBC	30% had BRCA1 PM; 5-yr RFS was 27% in those with PM, compared with 61% without
Oonk [58]	*BRCA1*-like status by MLPA	Retrospective	101	Adjuvant cyclophosphamide based, AC, FEC, TAC, CMF administered to TNBC	65% have BRCA1-like profiles; no difference in prognosis when treated with conventional chemotherapy
Sharma [40]	*BRCA1* mutation, BRCA1-PM, *BRCA1* expression	Retrospective	30	Neoadjuvant caroboplatin, docetaxel, and erlotinib to TNBC	20% had *BRCA1* mutations, 30% had BRCA1 PM, 15% had low *BRCA1* expression; patients with *BRCA1* insufficiency had a better RFS (81% vs. 54%) and OS (83% vs. 46%) compared with those without BRCA1 insufficiency
Telli [50]	HRD assay by genomewide SNP analysis by Affymetric MIP arrays or DNA sequencing	Prospective	77	Neoadjuvant carboplatin, gemcitabine, and iniparib to TNBC	Germline mutations in *BRCA1* and *BRCA2* were detected in 16% and 5%, respectively; somatic mutations of *BRCA1* and *BRCA2* were detected in 1%; responders had a significantly higher HRD score (in all patients and *BRCA1* and *BRCA2* mutation carriers)

[a]Initial analysis started on 320 patients, but results are reported for 230 patients.

Abbreviations: AC, doxorubicin, cyclophosphamide; aCGH, array comparative genomic hybridization; CMF, cyclophosphamide methotrexate, 5-flourouracil; ER, estrogen receptor; FEC, fluorouracil, epirubicin, cyclophosphamide; HD-PB, high dose platinum-based; HRD, homologous recombination deficiency; IHC, immunohistochemistry; LDA, low-density array; MIP, molecular inversion probes; MLPA, multiplex ligation-dependent probe amplification; OS, overall survival; pCR, partial complete response; PM, promoter methylation; qPCR, quantitative real-time polymerase chain reaction; RFS, recurrence-free survival; RR, response rate; SNP, single nucleotide polymorphism; TAC, docetaxel, doxorubicin, cyclophosphamide; TET, docetaxel, epirubicin plus docetaxel; TNBC, triple-negative breast cancer; TTP, time to progression.

mens used were reported as "anthracycline-based" adjuvant therapy, but frequently the standard approach involves administering an anthracycline and taxane, which may confound the drawn conclusion, especially if taxanes are less effective in tumors with defective repair. The same group recently reported clinical correlation of BRCA1 insufficiency (defined by either *BRCA1* mutation or BRCA1 promoter methylation or decreased BRCA1 mRNA levels) with outcome in 30 TNBC patients who were treated with neoadjuvant combination of carboplatin, docetaxel, and erlotinib [40]. Patients with BRCA1 insufficiency had better RFS and OS when compared with those without BRCA1 insufficiency, but data for the mutation patients versus those with *BRCA*ness were not provided.

Data are relatively scant with regard to taxanes and sporadic *BRCA*ness. In a study of 50 patients with metastatic breast cancer, time to progression was shorter in patients with absent BRCA1 expression (as determined by IHC) when treated with taxanes [41]. This result is in concordance with preclinical data suggesting that intact BRCA1 is required for optimal taxane activity. In addition, it is supported by another small study of TNBC tumor patients in which a BRCA1-like signature was associated with relative resistance to taxane-based therapy [42]. In contrast, a study in 25 patients with locally advanced/recurrent breast tumors reported that a significantly lower BRCA2 mRNA level was noted among docetaxel responders compared with nonresponders [43]. Interpretation of this observation is complicated by the lack of

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Less well known are data that intact *BRCA1* function may be important for an optimal response to taxane-based therapy. Particularly among the patient subgroup with triple-negative disease, in which 30% to 50% may have *BRCA*ness with loss of *BRCA1* function, the presence of this feature may predict a better therapeutic index for DNA-damaging therapy (platinator, alkylator, anthracycline); it may predict for less benefit from antitubulin treatment with taxanes.

a known mechanistic basis for specific effects of BRCA2 loss on taxane effectiveness. In the same study, there was a nonsignificant trend toward low BRCA1 mRNA levels in responders, whereas the preclinical data reviewed would support a relationship between intact BRCA1 function and taxane efficacy but in the opposite direction. All of these studies are retrospective, and the numbers of patients evaluated are small, precluding any definitive conclusions.

Discussion

BRCA1 and *BRCA2* genes play a critical role in HR repair of DNA damage. Mutations in those genes or sporadic inactivation leading to *BRCA*ness have been shown to have therapeutic implications, likely with an increased sensitivity to DNA-damaging agents. Less well known are data that intact *BRCA1* function may be important for an optimal response to taxane-based therapy. Particularly among the patient subgroup with triple-negative disease, in which 30% to 50% may have *BRCA*ness with loss of *BRCA1* function [18], the presence of this feature may predict a better therapeutic index for DNA-damaging therapy (platinator, alkylator, anthracycline); it may predict for less benefit from antitubulin treatment with taxanes. Conversely, for the majority with intact *BRCA1* function, taxane therapy may have the better therapeutic index. Because current standard of care chemotherapy for the triple-negative subset typically involves the combination of anthracycline/alkylator and taxane, it is important to determine the validity of these hypotheses; findings could result in superior outcomes based on more individualized choices. Currently, there are various techniques and methodologies to determine *BRCA*ness, each with its own limitations. To mount appropriate prospective trials, there is a pressing need to develop reproducible, standardized techniques for the determination of *BRCA*ness on formalin-fixed pathologic specimens.

Although *BRCA2* is known to play an important role in HR repair, correlation of *"BRCA2*ness" with outcome to DNA damaging therapy is less well investigated, as is the frequency of this phenomenon. If the correlation is similar to that for *BRCA1* downregulation and chemotherapy class response [18], and if the frequency is not rare, ER-positive patients may also benefit differentially from a choice of agent based on mechanism of action in the context of defective DNA repair. Again, the developmental of clinically applicable techniques to detect *BRCA2*ness will be critical to testing this hypothesis.

Currently, a randomized phase II/III trial is ongoing in Europe comparing responses of TNBC patients with defective HR, treated in the neoadjuvant setting with intensified alkylating chemotherapy (doxorubicin, cyclophosphamide followed by carboplatin, thiotepa, and cyclophosphamide) versus standard chemotherapy with dose-dense doxorubicin/cyclophosphamide or docetaxel/capecitabine (ClinicalTrials.gov identifier NCT01057069). In addition, another randomized phase II trial (ClinicalTrials.gov identifier NCT00861705) is evaluating the addition of carboplatin with and without bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense adiramycin-cyclophosphamide in TNBC. Blood and fresh frozen and fixed tumor tissue are being collected in this study for future biomarker analysis.

To date, published clinical data regarding differential chemosensitivity based on *BRCA1* and *BRCA2* mutation status seem strongest supporting platinum agents. The level of evidence is still preliminary, given the number of trials and patients evaluated. Data regarding taxanes in more preliminary and current evidence would not support withholding treatment with these agents in mutation carriers outside of a clinical trial. The concept of *BRCA*ness in some sporadic tumors is provocative and, in our view, warrants further investigation. Studies have shown that sporadic *BRCA*ness occurs in a reasonable proportion of patients, especially among those with TNBC. What needs to be developed is a standardized methodology to identify the signature, and larger trials are needed to evaluate chemosensitivity of such tumors to DNA-damaging agents. Only then can the assessment of *BRCA*ness become part of clinical decision making outside of a clinical trial.

PARP1 and the Concept of "Synthetic Lethality"

Although related, it is important to distinguish between *BRCA*ness in the context of DNA-damaging therapy and "synthetic lethality" in the context of concurrent inhibition of the *PARP1* molecule. Synthetic lethality involved targeting of *PARP1* in the setting of defective HR repair, which results in reciprocal increased dependence on upregulated PARP1 as a component of alternative repair pathways, such as base excision repair. When there was combined inhibition of both pathways in such a setting, it resulted in synthetic lethality and cell death in preclinical systems [44, 45]. The value of this approach has been demonstrated in breast and ovarian cancer patients with *BRCA1* and *BRCA2* mutations [46] but has not been demonstrated conclusively in the setting of sporadic *BRCA*ness. Results of an initial promising phase II trial of the putative PARP inhibitor iniparib among sporadic metastatic breast cancer patients with TNBC have not been replicated in a larger phase III trial [47, 48]. Another phase II trial has failed to demonstrate any objective responses to the single-agent PARP inhibitor olaparib in TNBC patients [49]. In this study, however, no objective responses were noted, even among breast cancer patients with *BRCA1* or *BRCA2* mutations, which have been reported in other trials. A recent study of TNBC and *BRCA1* and *BRCA2* mutation-associated breast cancer reported that a higher HRD score predicts for pathological response after neoadjuvant platinum-based therapy in combination with iniparib [50]. Conflicting clinical data may be the result

of the small sample size, of tumors from the patient populations under study that were variably enriched for *BRCA*ness (not tested in the trials), and of the PARP inhibitor (iniparib) studied in some trials that has little actual activity against this target [51]. Several ongoing clinical trials of PARP inhibitors alone or in combination with chemotherapy (primarily platinum agents) [52] could provide more insight into the concept of synthetic lethality in sporadic breast tumors.

Conclusion

BRCA1 and BRCA2 germline mutations have an important role in DSB repair of DNA. Germline mutations or sporadic "BRCAness" cause defective BRCA1 or BRCA2 functions and subsequently impair DNA repair capacity. This feature makes these cells differentially more sensitive to DNA-damaging chemotherapeutic agents. There is emerging preclinical and some clinical evidence that such cells might be resistant to taxanes. Clinical studies (primarily retrospective and few prospective) have shown that this feature can be exploited for selecting chemotherapy agents, however, there is no standardized method to detect "BRCAness." The concept of "BRCAness" is provocative and may bear fruit as a guide in future treatment selection. However, the test of its value awaits the validation of a simple, reproducible laboratory assay which can be applied to clinical material. Until then, we recommend further pursuit of this concept only in the context of clinical trials.

Author Contributions

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Collection and/or assembly of data: Pavani Chalasani, Robert Livingston
Data analysis and interpretation: Pavani Chalasani, Robert Livingston
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