Germline mutations of the BRCA1 gene account for approximately 5% of breast and ovarian cancer cases, and lower than normal BRCA1 expression or function may be an important contributing factor in sporadic cancers. The major role of BRCA1 is to respond to DNA damage by participating in cellular pathways for DNA repair, mRNA transcription, cell cycle regulation, and protein ubiquitination. Because most chemotherapeutic agents function by directly or indirectly damaging DNA, the role of BRCA1 as a regulator of chemotherapy-induced DNA damage has been the subject of an increasing number of investigations. We review published preclinical and clinical evidence that the level of BRCA1 function in an individual patient’s tumor can guide the choice of chemotherapeutic agents for breast and ovarian cancer. We conclude that a loss of BRCA1 function is associated with sensitivity to DNA-damaging chemotherapy and may also be associated with resistance to spindle poisons. We recommend that prospective clinical studies investigating the role of BRCA1 in the response to chemotherapy be conducted. [J Natl Cancer Inst 2004;96:1659–68]

The BRCA1 gene was first localized to chromosome 17q by genetic linkage in 1994 (1). Loss-of-function mutations in BRCA1 have been reported to confer up to an 82% risk of developing breast cancer and up to a 54% risk of developing ovarian cancer by the age of 80 years (2). Germline mutations in BRCA1, however, account for approximately 5% of breast and ovarian cancer cases (Fig. 1), and lower than normal expression of BRCA1 may be an important contributing factor in sporadic tumors (3–8).

The BRCA1 gene encodes a 220-kDa nuclear protein that responds to DNA damage by participating in cellular pathways responsible for DNA repair, mRNA transcription, cell cycle regulation, and protein ubiquitination (Fig. 2) (9). Because most chemotherapeutic agents function by directly or indirectly damaging DNA, the role of BRCA1 after chemotherapy-induced DNA damage and as a predictive biomarker of response to these drugs has been the subject of several studies (Table 1).

The estrogen receptor is routinely used as a predictive biomarker for the response of breast cancer to hormonal treatment (10); however, estrogen receptor status does not independently predict response to cytotoxic chemotherapy (11). Other biomarkers, including HER-2, which predicts response to the antibody trastuzumab (12), are under investigation as predictive biomarkers of response to cytotoxic chemotherapy. Unfortunately, to date, incompatible or conflicting data preclude the use of estrogen receptor status and HER-2 status to guide chemotherapeutic treatment (13–17).

The DNA damage response gene p53 has been investigated as a predictive biomarker for response to DNA-damaging chemotherapy in breast and ovarian cancer, and contradictory results have been obtained. Some studies have indicated that p53 mutations increase sensitivity to DNA-damaging drugs, and other studies have indicated that they decrease sensitivity to such drugs (17,18). In addition, p53 has been investigated as a biomarker for response to paclitaxel, again with inconclusive results (19–23). Other potential predictive biomarkers, such as BCL2, cyclin D, cathepsin B, vascular endothelial growth factor, and Ki-67, are also under investigation, but clinical validation is required before these biomarkers can be applied (24).

Because no predictive biomarker for response is currently available to guide the choice of cytotoxic chemotherapy for breast or ovarian cancer, the choice of drugs is usually empiric, and 30%–70% of patients with measurable disease will fail to respond (25–29). The purpose of this article is to review the evidence that, in breast and ovarian cancers, BRCA1 can be used as a predictive biomarker of response to DNA-damaging chemotherapeutic agents and possibly also to mitotic spindle poisons.

**METHODOLOGY**

This review examines the preclinical and clinical evidence that BRCA1 plays a role in modulating response to chemotherapeutic agents and can be used as a predictive marker to guide the choice of chemotherapy for breast and ovarian cancer patients. We searched Entrez PubMed and Medline for papers published between January 1, 1994, and August 31, 2004, by use of the term BRCA1 and each of the following terms: treatment, chemotherapy, response, predictive marker, DNA damage, antimicrotubule agents, spindle poisons, paclitaxel, docetaxel, vinorelbine, vinca alkaloids, alkylating agents, cyclophosphamide, platinum, cisplatin, etoposide, doxorubicin, topoisomerase poisons, 5-fluorouracil, and methotrexate. If papers identified by these criteria cited reports not identified in the original search that were relevant to BRCA1 and the response to chemotherapy, we also included these reports in our review. We describe results...
from preclinical and clinical studies that apply to the interaction between BRCA1 and chemotherapy. Thus, we reviewed all published preclinical and clinical data identified regarding BRCA1 and the response to chemotherapy.

**BRCA1 Status and Response to Chemotherapy**

BRCA1 is posttranscriptionally modified in the response to DNA damage, as is p53, and both BRCA1 and p53 may be involved in cellular events that follow treatment with chemotherapeutic agents. Both BRCA1 and p53 are phosphorylated by the ataxia telangiectasia mutated (ATM) protein in response to double-stranded DNA breaks that lead to activation of DNA damage response pathways (30). Tumors that carry BRCA1 mutations frequently also carry p53 mutations (31). Moreover, mouse embryos with a conditional knockout of the Brca1 gene and with wild-type p53 genes die via a p53-dependent mechanism (32), suggesting that loss of p53 function is required for a cell to tolerate loss of BRCA1 function (33), an observation that may be important when considering the response of cancer cells to DNA-damaging drugs. BRCA1 also appears to be involved in modulating the cellular response to mitotic spindle poisons, such as taxanes and vinca alkaloids. However, the response to spindle poisons appears to be p53 independent, and loss of p53 function may actually improve response to these agents (34).

**BRCA1 and Resistance to DNA-Damaging Chemotherapeutic Agents**

Therapeutic agents commonly used in breast and ovarian cancer treatment can damage DNA through various mechanisms. The agents can be broadly divided into four major groups by the type of damage they cause. The first group of drugs includes alkylating agents that cause DNA interstrand cross-links; these cross-links lead to the arrest of DNA replication forks and then to double-strand DNA breaks (35,36). The second group of agents inhibits topoisomerases I and II, and this group includes agents such as etoposide, mitoxantrone, irinotecan, and topotecan. Before replication, topoisomerases introduce temporary breaks in the DNA strands to allow the DNA to unwind. Inhibition of topoisomerases stabilizes the topoisomerase–DNA complex and causes the arrest of DNA replication forks and double-strand DNA breaks (37). The second group of agents also includes anthracyclines, such as doxorubicin and epirubicin, that inhibit topoisomerase II, but these drugs have additional modes of action, such as DNA interstrand cross-linking and the generation of oxygen free radicals (38). The third group of agents includes platinum-based compounds that form adducts with DNA, although some of these drugs can also cause double-stranded DNA breaks by introducing interstrand breaks (39) (Table 1). The fourth group of agents includes bleomycin, which directly damages DNA by causing double-strand breaks (40).

Both breast and ovarian cancers are treated with DNA-damaging drugs in the adjuvant and metastatic setting. In ovarian cancer, a platinum drug (either cisplatin or carboplatin) is used alone or in combination with a taxane (29). Until relatively recently, breast cancer was treated with a combination of DNA-damaging drugs, such as an anthracycline and cyclophosphamide, although taxanes are now frequently added to these regimens (41).

Effective DNA repair requires mechanisms that sense DNA damage, prevent replication of abnormal DNA, replace damaged nucleotides, and, depending on the severity of the damage, prevent or promote apoptosis. BRCA1 is involved in all of these processes, and these processes may be mediated in part through the association of BRCA1 with a large protein complex named the BRCA1-associated genome surveillance complex (BASC). Components of this complex include DNA damage detection molecules (such as ATM), DNA repair molecules (such as RAD50, MRE11, NBS1, and BLM), and mismatch repair molecules (such as MLH1, MSH2, and MSH6) (42).

After a DNA break occurs, the cell repairs the damage to allow survival or, if the break is not repaired, the cell undergoes apoptosis to prevent proliferation of abnormal DNA. BRCA1
### Table 1. Preclinical and clinical data by author and reference regarding the usefulness of BRCA1 as a predictive marker

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism*</th>
<th>Preclinical studies (refs) evaluating effect of loss of BRCA1 function</th>
<th>Clinical studies (refs) evaluating effect of loss of BRCA1 function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topotecan</td>
<td>Topo I poison</td>
<td>Fedier et al. (102)</td>
<td>Chappuis et al. (107)</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Topo I poison</td>
<td>Fedier et al. (102)</td>
<td>Delaloge et al. (108)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Topo II poison/free radical damage</td>
<td>Fedier et al. (102)</td>
<td>(BRCA1-mutant tumors; treatment included alkylating agent)</td>
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<tr>
<td></td>
<td></td>
<td>Sylvain et al. (103)</td>
<td></td>
</tr>
<tr>
<td>Epirubicin</td>
<td>Topo II poison/free radical damage</td>
<td></td>
<td>Egawa et al. (110) (Sporadic tumors; treatment included alkylating agent)</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Topo II poison</td>
<td>Lafarge et al. (61)</td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>Topo II poison</td>
<td>Quinn et al. (59)</td>
<td></td>
</tr>
<tr>
<td>Bleomycin</td>
<td>Direct double-strand DNA breakage</td>
<td>Fedier et al. (102)</td>
<td></td>
</tr>
<tr>
<td>Busulfan</td>
<td>Interstrand linkage</td>
<td>Fedier et al. (102)</td>
<td></td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>Interstrand linkage</td>
<td>Moynahan et al. (49)</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Adduct formation and interstrand linkage</td>
<td>Hussain et al. (58)</td>
<td>Cass et al. (105) (BRCA1-mutated tumors)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quinn et al. (59)</td>
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<td></td>
<td></td>
<td>Sylvain et al. (103)</td>
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<td>Tassone et al. (60)</td>
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<td></td>
<td></td>
<td>Bhattacharya et al. (101)</td>
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<tr>
<td>Carboplatin</td>
<td>Adduct formation and interstrand linkage</td>
<td>Fedier et al. (102)</td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>Mostly adduct formation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Enhanced microtubule polymerization</td>
<td>Sylvain et al. (103)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Zhou et al. (104) (ovarian models)</td>
<td></td>
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<tr>
<td>Docetaxel</td>
<td>Enhanced microtubule polymerization</td>
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<tr>
<td>Vinorelbine</td>
<td>Depolymerization of microtubules</td>
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<tr>
<td></td>
<td></td>
<td>Quinn et al. (59)</td>
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*Topo = topoisomerase.

appears to promote cell survival after DNA damage by preventing apoptosis and participating in repair pathways (43,44).  

**Role of BRCA1 in DNA repair.** BRCA1 has been reported to be involved in both homologous recombination and nonhomologous end joining of double-stranded DNA breaks and in nucleotide excision repair of DNA adducts. Homologous recombination occurs primarily during the G2 and S phases of the cell cycle and is the least error-prone method of repairing DNA double-strand breaks. Homologous recombination begins with BRCA1, RAD50, MRE11, and NBS1 (45) forming a protein complex with 5′–3′ exonuclease activity that exposes the 3′ ends on either side of the break (46). The break is then repaired by one of two homologous repair systems: single-strand annealing, which uses regions of homology between the complementary strands to align the strands of DNA (47), and strand invasion, which uses the sister chromatid as a template to repair the break. BRCA1 appears to participate in strand invasion by forming a complex with BRCA2, RAD51, and proliferating cell nuclear antigen at regions of DNA damage (47,48). The participation of BRCA1 in homologous recombination in the response to DNA-damaging chemotherapeutic agents is supported by the observation that homologous recombination repair is deficient in embryonic murine stem cells carrying a BRCA1 gene with mutation in exon 11 and, consequently, these cells are sensitive to the alkylating agent mitomycin C (49). The alternative pathway used to repair double-strand DNA breaks is nonhomologous end joining repair. This repair pathway is less accurate than homologous end joining because it does not use the sister chromatid as a template to rejoin the broken DNA ends. This method of DNA repair is used for the majority of DNA breaks in mammalian cells and is active throughout the cell cycle (50). In the absence of BRCA1, DNA repair may be rerouted down this less precise and, therefore, potentially more harmful pathway (51). However, recent work has suggested that BRCA1 may also be required for accurate nonhomologous end joining repair (52) because of its ability to regulate microhomology in nonhomologous end joining repair. BRCA1 may be involved in this process by facilitating alignment of short areas of base homology on either side of the DNA break before ligation (53). Thus, in the absence of BRCA1, homologous recombination is deficient and nonhomologous recombination may become even less accurate, resulting in defective repair that may increase the toxicity of DNA damage. In addition to homologous and nonhomologous repair pathways, BRCA1 has been reported to be involved in nucleotide excision repair. This pathway is used in the repair of DNA adducts, such as those formed by platinum compounds, and may have a role in the repair of interstrand cross-links caused by alkylating agents (54). This process involves the excision of a 24- to 32-base fragment of single-strand DNA containing the adduct, followed by resynthesis and ligation.
that uses the complementary strand as a template (55,56). The nucleotide excision repair pathway can be further subdivided into the transcription-coupled repair and the global genomic repair pathways, and BRCA1 may be involved in both (57). In transcription-coupled repair, transcriptionally active DNA is preferentially repaired. BRCA1 has been indirectly linked to this repair pathway because it forms a complex with MSH2 and MSH6, both of which have been implicated in transcription-coupled repair (42), and because it is required for the transcription-coupled repair of oxidative 8-oxoguanine lesions in human cells (57). The requirement for BRCA1 in transcription-coupled repair may explain the increased cisplatin sensitivity that has been observed in BRCA1-deficient cells (58–61). Although the exact mechanism by which BRCA1 may participate in transcription-coupled repair of cisplatin-mediated DNA damage is unclear, the mechanism may involve BRCA1-mediated protein ubiquitination (62). Cisplatin-associated adducts have been shown to block the transcriptional enzyme RNA polymerase II, causing transcriptional arrest (63), which leads to the ubiquitination and degradation of the large subunit of RNA polymerase II, thereby allowing access to DNA repair proteins (64–67). BRCA1 binds to the BRCA1-associated ring domain protein 1 (BARD1) and forms a complex that has been reported to have ubiquitin E3 ligase activity and associates with RNA polymerase II (68). It is therefore possible that BRCA1 is involved in the ubiquitination and degradation of RNA polymerase II after transcriptional arrest. In this model, loss of BRCA1 function would prevent the removal of the transcriptional machinery from DNA, thereby preventing repair proteins from accessing the damaged region. BRCA1 has also been implicated in global genomic repair because BRCA1 can modulate the transcriptional activation of XPC (xeroderma pigmentosum C protein), DDB2 (damaged DNA binding protein), and GADD45 (growth arrest and DNA damage response protein) and because, in BRCA1 deficient cells, the repair of ultraviolet-induced adducts by the global genomic repair system is abnormal (69). However, it is unclear whether the global genomic repair pathway participates in the repair of chemotherapeutic agent-induced adducts, such as those induced by platinum-based drugs.

Role of BRCA1 in cell cycle regulation. In addition to playing a role in DNA repair pathways, BRCA1 has been implicated in the regulation of cell cycle checkpoints (70–73). Our group has reported that, after exposure to chemotherapeutic agents that cause double-strand DNA breaks, the G2/M-phase checkpoint is defective in the BRCA1-mutant HCC1937 breast cancer cell line. Reconstituting BRCA1 function in these cells, through transfection of a wild-type gene, restores the function of this checkpoint (59). The mechanisms through which BRCA1 regulates various cell cycle checkpoints may be related, at least in part, to the finding that BRCA1 is a substrate for the so-called DNA damage response kinases, including ATM, ATM-related kinase (ATR), and cell cycle checkpoint kinase 2 (CHK2) (Fig. 3). ATM is activated by the type of double-strand DNA breaks that are caused by ionizing radiation, whereas ATR is activated by stalled DNA replication forks (74). Therefore, the ATR-mediated phosphorylation of BRCA1 may be particularly important in mediating the response to drugs, such as alkylating agents or topoisomerase II poisons, which result in stalled DNA replication forks. DNA damage response kinases, such as ATM, ATR, and CHK2, appear to have both overlapping and distinct phosphorylation sites on BRCA1, but only some of these sites have been characterized. BRCA1 is phosphorylated by the ATM kinase on serines 1387, 1423, 1457, and 1524 in response to ionizing radiation (75,76), by CHK2 on serine 988 in response to ionizing radiation (77), and by ATR on serine 1423 in response to UV radiation (78). Phosphorylation of BRCA1 on serine 1423 is required to activate the G2/M-phase checkpoint, whereas activation of the intra–S-phase checkpoint requires phosphorylation on serine 1387 (71,72). The mechanism used by phosphorylated BRCA1 to modify the activity of the various checkpoints is still unclear, but it may be partly associated with the transcriptional activation of key checkpoint proteins. BRCA1 apparently stimulates the transcription of the p21 and p27 genes, which arrest cells at the G1/S-phase boundary and in S phase, respectively, by inhibiting cyclin-dependent kinase 2 (79–81). BRCA1 also stimulates the transcription of GADD45, which can activate the G2/M-phase checkpoint by binding to the cyclin B–cdc2 complex and preventing its localization to the nucleus, and BRCA1 represses the expression of cyclin B, also thereby activating the G2/M-phase checkpoint (73,82,83). In addition, BRCA1 induces the expression of 14–3–3σ, which binds to and sequesters cdc25C in the cytoplasm, thus preventing it from activating the cyclin B–cdc2 complex (70,84). BRCA1 also stimulates the transcription of the Weel kinase, which phosphorylates and thus inhibits cdc2 to arrest cells at the G2/M-phase checkpoint (70). Finally, BRCA1 transcriptionally represses the polo-like kinase (PLK) gene, which encodes a protein required for progression into mitosis (85). Therefore, BRCA1 can function at multiple levels to regulate the G2/M-phase checkpoint. Although phosphorylation of BRCA1 has been associated with cell cycle checkpoint regulation, a recent study has demonstrated that the phosphorylation of serine 988 by CHK2 is associated with DNA damage repair. Mutation of this residue results in defective DNA damage repair but does not activate cell cycle checkpoints (86). Thus, phosphorylation of BRCA1 appears to promote DNA repair and prevent replication of damaged DNA by activating cell cycle checkpoints.

Association of BRCA1 with Fanconi anemia proteins. Additional evidence for the importance of BRCA1 in the response to DNA-damaging chemotherapy comes from studies of Fanconi anemia, a rare autosomal recessive condition that results in progressive bone marrow failure and a predisposition to cancer. Cells cultured from these patients are highly sensitive to the DNA-damaging drugs mitomycin C or cisplatin. Eight Fanconi anemia proteins have been identified, six of which (A, C, E,
F, G, and L) form a core complex that is involved in the monoubiquitination and subsequent activation of Fanconi anemia protein D2 (87). After DNA damage, BRCA1 interacts with the ubiquitinated form of Fanconi anemia protein D2, but the purpose of this interaction is unclear. BRCA1 also specifically interacts with the Fanconi anemia core complex through an association with the amino terminus of Fanconi anemia protein A (88). This complex may be involved in detecting the arrest of the DNA replication fork after chemotherapy and the subsequent ATR-mediated activation of Fanconi anemia protein D2 (89). BRCA1 also associates with Fanconi anemia protein D1, which has been identified as BRCA2 (the product of the second breast cancer susceptibility gene), and both proteins have been implicated in the repair of chemotherapy-induced double-strand DNA cross-links through homologous recombination (49,90,91). Finally, decreased expression of Fanconi anemia F protein as a result of promoter methylation has been associated with cisplatin sensitivity in ovarian cancer (92), demonstrating that the association of Fanconi anemia proteins with BRCA1 may be important in the repair of chemotherapy-induced DNA damage. BRCA1 thus may function at multiple levels in the response to chemotherapy-induced DNA damage. BRCA1 has been reported to be involved in several DNA repair pathways, such as homologous recombination, nonhomologous end-joining, and nucleotide excision repair, and it has also been implicated in cell cycle control after the detection of DNA damage. In this manner, BRCA1 may prevent the propagation of abnormal DNA.

BRCA1 and Response to Spindle Poisons

Although the BRCA1 gene is a DNA damage response gene, it also appears to play a role in the regulation of mitosis and may be involved in modulating the response to spindle poisons, such as taxanes or vinca alkaloids. Taxanes, such as paclitaxel and docetaxel, bind to β-tubulin and stabilize microtubules, thereby blocking their depolymerization, whereas vinca alkaloids, such as vincristine or vinorelbine, also bind to β-tubulin but promote depolymerization of microtubules. Treatment with either group of drugs arrests cells in mitosis and results in apoptotic cell death (34,93).

Using an inducible BRCA1 expression system in the MDA-MB-435 breast cancer cell line, we demonstrated that induction of BRCA1 expression after paclitaxel treatment led to activation of the mitotic checkpoint and subsequent apoptotic cell death (59,73). The mitotic checkpoint occurs at the metaphase–anaphase boundary and ensures that chromosomes segregate between the daughter cells during mitosis. Failure of spindles to attach to one or more kinetochores (the spindle attachment regions of each chromatid) activates the checkpoint, and this activation persists until attachment is achieved (94). The mechanism by which BRCA1 functions in this checkpoint is unclear but may involve its interaction with the spindle microtubule proteins α-, β-, and γ-tubulins during mitosis (95). In particular, BRCA1 appears to interact with γ-tubulin through a region in BRCA1 exon 11 (96); mutation of this exon leads to chromosomal instability, further supporting the hypothesis that binding of BRCA1 to γ-tubulin is involved in the correct segregation of chromosomes during mitosis (97). These studies, therefore, support a mechanism in which BRCA1 participates in the detection of abnormal mitosis and the induction of apoptosis to prevent the replication of aneuploid cells.

The mechanism by which BRCA1 participates in sensitivity to spindle poisons may involve the stress-activated c-jun N-terminal kinase (JNK) pathway. This pathway is specifically activated after treatment with spindle poisons, and spindle poison–induced apoptotic cell death is dependent on its activation (34,98). In addition, BRCA1 is apparently a potent activator of the JNK pathway (99). Evidence for an interaction between BRCA1 and spindle poisons through the JNK pathway comes from the observation that loss of BRCA1 expression results in decreased JNK activation after paclitaxel treatment (61). The BRCA1-mediated cell death pathway appears to include the sequential activation of the kinases H-Ras, MEKK4, and JNK and an interaction between Fas ligand and the Fas receptor protein, followed by apoptosis mediated through caspases 9 and 3 (100).

Thus, the evidence suggests that BRCA1 modulates an apoptotic pathway in response to spindle damage; this role is in direct contrast to its role in promoting DNA repair and cell survival after treatment with DNA-damaging drugs. The preclinical models that follow suggest that this differential affect on cell survival may influence the response of breast and ovarian cancer cells to treatment (Fig. 4).

BRCA1 and Chemotherapy in Preclinical Models

Preclinical studies addressing the interaction between BRCA1 and chemotherapeutic agents are outlined in Table 1. The initial studies in this area were carried out in mouse embryonic stem cells. In keeping with the role of BRCA1 as a modulator of DNA damage response pathways, Brca1-disrupted mouse embryonic stem cells were more sensitive to the alkylating agent mitomycin C and the platinum compound cisplatin than cells expressing wild-type Brca1 (49,101). Murine embryonic fibroblasts with a disrupted Brca1 gene were also more sensitive to the platinum compounds carboplatin and oxaliplatin, the topoisomerase I poisons irinotecan and topotecan, and the topoisomerase II poisons doxorubicin, etoposide, and mitoxantrone than cells with a normal Brca1 gene (102). However,
sensitivity to antimetabolite drugs, such as gemcitabine or 5-fluorouracil, was not increased in Brca1-disrupted mouse embryonic fibroblasts (102).

In related studies, we investigated the role of BRCA1 in chemotherapeutic response by use of the BRCA1-mutant HCC1937 breast cancer cell line and an isogenic control cell line expressing wild-type BRCA1 (59). We reported that reconstitution of BRCA1 in the HCC1937 cell line via transfection resulted in a reduced level of apoptotic death after treatment with cisplatin, bleomycin, or etoposide, compared with the level of apoptotic cell death in an unmodified HCC1937 cell line. In agreement with the mouse embryonic fibroblast models, which found no interaction between Brca1 and antimetabolites (102), the reconstitution of functional BRCA1 in HCC1937 cells did not affect their sensitivity to 5-fluorouracil (59). In their study, Tassone et al. (60) found that HCC1937 cells carrying wild-type BRCA1 were more resistant to cisplatin and were more sensitive to doxorubicin than BRCA1-mutant HCC1937 cells. These results are surprising because etoposide and doxorubicin both inhibit topoisomerase II activity and because the expression of a functional BRCA1 in mouse embryonic fibroblasts confers increased resistance to doxorubicin (102). The reason behind the conflicting results for etoposide and doxorubicin in the HCC1937 cell model is unclear but may be related to yet-to-be-discovered BRCA1-independent effects of doxorubicin.

In contrast to BRCA1-associated hereditary breast cancer, sporadic breast and ovarian cancers typically have lower than normal levels of BRCA1 expression rather than the absence of function caused by the BRCA1 mutation. Approximately 30% of invasive breast cancers and 70% of ovarian cancers have reduced BRCA1 expression that is, at least in part, caused by methylation of the BRCA1 promoter (3,5,8). Model systems that appear to replicate the in vivo situation generally use either an antisense-, a small-interfering RNA (siRNA)–, or an RNA-specific enzyme–based approach to inhibit expression of endogenous BRCA1. Reduction of BRCA1 expression results in increased sensitivity to etoposide (61), indicating that the reduced BRCA1 expression observed in sporadic cancers may also be involved in the response of these tumors to DNA damage–based chemotherapy.

Further evidence documenting the importance of BRCA1 in the response to DNA-damaging drugs comes from cisplatin-resistant breast and ovarian cancer cell lines. These cell lines overexpress BRCA1, and their resistance to cisplatin has been attributed to increased BRCA1-dependent DNA repair. Accordingly, inhibition of BRCA1 by antisense RNAs increased cisplatin sensitivity (58), whereas overexpression of BRCA1 in murine ovarian cancer cells increased resistance to cisplatin, etoposide, and doxorubicin (103).

Preclinical results thus indicate that the presence of BRCA1 mutation or the reduction of BRCA1 expression increases sensitivity to several DNA-damaging agents, in agreement with the role of BRCA1 in DNA damage repair. Studies on the role played by BRCA1 in modulating cellular response to chemotherapy, however, have not focused exclusively on DNA damage–based chemotherapy. Two groups (59,60) using the HCC1937 cell model reported statistically significantly increased sensitivity to spindle poisons (paclitaxel and vincristine) when functional BRCA1 was reconstituted into the cell line, in contrast to the increased resistance to DNA-damaging agents mentioned above. Several other model systems and experimental approaches have been used to validate these findings in vitro. Induced expression of BRCA1 in the MD-MB-435 breast cancer cell line increased the sensitivity of the cells to paclitaxel and vincristine by increasing apoptosis (59). Thus, a dose–effect relationship appears to exist between BRCA1 expression and spindle poison toxicity. In keeping with the other models described, inhibition of BRCA1 expression with siRNA technology in the T47D breast cancer cell line resulted in paclitaxel resistance (59). Similarly, inhibition of endogenous BRCA1 expression in the HBL100 breast cancer cell line, using mRNA-specific RNA enzymes, caused resistance to the spindle poisoning drugs paclitaxel and vincristine (61).

Although all results described so far suggest that BRCA1 is required for paclitaxel sensitivity in breast cancer cell lines, two studies have reported conflicting results for human and mouse ovarian cancer cell lines. Decreased paclitaxel sensitivity was reported when BRCA1 was expressed in the BRCA1-mutant SNU-251 human ovarian cell line (104), and expression of a dominant negative BRCA1 in the ID-8 murine ovarian cell line increased the sensitivity of the cell line to paclitaxel (103). Why BRCA1 should behave differently in ovarian and breast cancer cells treated with spindle poisons is unclear. If confirmed, however, these results may provide important information on the function of BRCA1.

Overall, preclinical results have indicated that BRCA1 inhibits apoptosis after treatment with DNA-damaging chemotherapeutic agents but that BRCA1 is required for the induction of apoptosis in response to spindle poisons, particularly in breast cancer cell lines. BRCA1, therefore, appears to act as a differential mediator of apoptosis in breast cancer cells that is dependent on the nature of the chemotherapeutic agent. The situation with BRCA1 and spindle poisons in ovarian cancer cell models is less well defined and clearly requires further investigation.

**BRCA1 and Chemotherapy in Clinical Studies**

A summary of clinical studies reviewed that address the role of BRCA1 gene in response to chemotherapy is in Table 1. Unfortunately, all trials to date have been retrospective in nature, and no trial was designed specifically to study the role of BRCA1 in response to chemotherapy. Some studies have grouped both BRCA1 and BRCA2 mutant carriers together, which is not desirable because the genes are not homologous and have separate, although often complementary, functions with response to DNA damage (105). Indeed, the genetic profiles of BRCA1 and BRCA2 mutant tumors are distinct, as shown by microarray analysis (106). Consequently, grouping patients with BRCA1 and BRCA2 mutations may result in the loss of important data regarding the distinct effects of these genes.

Evidence for the sensitivity of BRCA1-mutated tumors to DNA damage comes from a small retrospective study by Chappuis et al. (107). They identified seven BRCA1 mutation carriers, four BRCA2 mutation carriers, and 27 noncarriers, all of whom had received neoadjuvant chemotherapy for breast cancer. Most patients received four cycles of an anthracycline and cyclophosphamide-based regimen. Ten of the eleven patients who were BRCA1 and/or BRCA2 mutation carriers had a complete clinical response, compared with two of the 11 matched noncarrier patients with sporadic breast cancer; and so the authors concluded that BRCA1 or BRCA2 mutations might increase the response to DNA-damaging chemotherapy (107).
Further evidence for chemosensitivity in BRCA1 mutation carriers comes from a study by Delaloge et al. (108). They retrospectively compared 15 patients carrying a BRCA1 mutation who had locally advanced breast cancer and five patients carrying a BRCA2 mutation who had locally advanced breast cancer, with 57 matched control patients with locally advanced sporadic breast cancer. All patients were treated with anthracycline- and cyclophosphamide-based chemotherapy. One hundred percent of the patients with a BRCA1 mutation, 80% of patients with a BRCA2 mutation, and 63% of the patients with sporadic breast cancer had a clinical response. When tumors were examined microscopically after surgery, 53% of the patients with a BRCA1 mutation, none of the patients with a BRCA2 mutation, and 14% of the patients with sporadic breast cancers had responded completely. The authors concluded that tumors with a BRCA1 mutation were more sensitive to DNA-damaging chemotherapy than tumors with a BRCA2 mutation or sporadic breast cancers (108).

Goffin et al. (109) retrospectively studied 278 Ashkenazi Jewish patients with early breast cancer and found that, compared with noncarriers, BRCA1 mutation carriers had a worse overall survival if they did not receive adjuvant chemotherapy (relative risk [RR] = 3.3; \( P = .01 \)). They commented that BRCA1 mutation carriers also seemed to have a greater benefit from DNA-damaging adjuvant chemotherapy than patients with sporadic breast cancer, in keeping with an increased sensitivity to DNA-damaging drugs among mutation carriers (109).

Increased chemosensitivity associated with BRCA1 mutations has also been observed in ovarian cancer. In a retrospective study of 71 Jewish patients with ovarian cancer, Cass et al. (105) demonstrated that patients with BRCA1 or BRCA2 mutations had a better response to platinum-based chemotherapy than patients with sporadic disease. This group also had a better prognosis that was possibly associated with their tumor sensitivity to platinum (105).

The clinical and preclinical results indicate that tumors with BRCA1 mutations have increased sensitivity to DNA-damaging drugs. However, reduced BRCA1 expression observed in sporadic breast cancer has yielded contradictory results. Egawa et al. (110) reported that, in breast cancer patients, decreased BRCA1 mRNA expression (defined as a BRCA1 mRNA expression level less than 55% of the \( \beta \)-glucuronidase mRNA expression level) was associated with a less favorable response to DNA-damaging chemotherapy. They studied 51 patients with locally advanced breast cancer who were treated with cyclophosphamide and epirubicin. Sixty-five percent of the patients expressing high levels of BRCA1 mRNA responded to this treatment, compared with 32% of patients expressing low levels of BRCA1 mRNA (110). The conflicting results between Egawa’s study and those investigating chemotherapeutic sensitivity in BRCA1-mutant breast cancers (60,111) may reflect the difference between low levels of BRCA1 expression in sporadic disease compared with complete loss of BRCA1 function observed in BRCA1 mutation–associated disease. Alternatively, these conflicting results may indicate that mRNA levels do not always reflect the presence of functional BRCA1 protein.

From the available preclinical and clinical studies to date, it appears that BRCA1 inactivation through mutation confers sensitivity to DNA-damaging drugs. Decreased expression of BRCA1 also results in sensitivity to DNA-damaging agents in preclinical models, although loss of BRCA1 expression in sporadic breast or ovarian cancer has not been associated with chemosensitivity in clinical studies to date.

The role of the BRCA1 gene in the response to spindle poisons has been less well characterized in clinical studies because the participation of this gene in the response to abnormal mitosis has only recently been reported (59–61,73). This activity of BRCA1 may be important because spindle poisons, in particular the taxanes, are commonly used as treatments for breast and ovarian cancer. In ovarian cancer, the taxanes are usually given in combination with a DNA-damaging platinum-based drug, such as cisplatin or carboplatin (112,113). In breast cancer, the taxanes are used to treat metastatic disease and are increasingly used as adjuvant therapy in combination or sequentially with DNA-damaging drugs, such as anthracyclines and cyclophosphamide (41). The vinca alkaloids, such as vinorelbine, are used mostly as single agents in the treatment of metastatic breast cancer (93).

Egawa et al. (114) demonstrated, in a small study of 25 patients with locally advanced or locally recurrent breast cancer, a non–statistically significant trend of lower expression of BRCA1 mRNA being associated with increased sensitivity to docetaxel. This finding is in disagreement with preclinical studies that indicated that an increased level of BRCA1 protein results in increased taxane sensitivity. An explanation for the difference in these results may be that Egawa et al. measured BRCA1 mRNA expression, whereas the preclinical models measured BRCA1 protein expression. The preclinical studies also used paclitaxel, rather than docetaxel, which has a slightly different mechanism of action (115). Another possibility is that BRCA1 levels immediately after chemotherapy may increase in patients who are sensitive to therapy but remain low in patients who are resistant to therapy. Further prospective studies investigating BRCA1 protein expression before and after taxane chemotherapy might clarify whether BRCA1 expression is related to sensitivity to taxanes.

**Conclusions**

Results of preclinical and clinical studies indicate that loss of BRCA1 function through mutation confers sensitivity to the DNA-damaging chemotherapy commonly used in breast and ovarian cancer. Because normal cells should have normal BRCA1 function and, therefore, normal levels of DNA repair, the loss of BRCA1 function may prove to be the factor that selects for cancer cell death after DNA-damaging treatments. Results of preclinical models also predict that loss of BRCA1 expression in sporadic breast cancers should result in increased sensitivity to DNA-damaging treatments. However, the only clinical study to specifically address this issue has reported a contradictory result—an increase in sensitivity (110). Therefore, the response to DNA-damaging chemotherapy may differ between tumors that have reduced BRCA1 function through epigenetic silencing and tumors that have lost all BRCA1 function through mutation.

Results of preclinical studies have also suggested that BRCA1 may be required for the response to spindle poisons. This observation may be particularly important for patients carrying BRCA1 mutations or for patients with sporadic tumors that have lost BRCA1 expression and are thus resistant to spindle poisons. In contrast, cancer cell lines that have become resistant to DNA-damaging chemotherapy through repeated ex-
posure overexpress BRCA1 (58). This observation raises the possibility that human breast or ovarian tumors that have developed overexpression of BRCA1 as a mechanism of resistance to DNA damaging drugs may be particularly sensitive to spindle poisons.

The number of patients studied, inappropriate grouping of BRCA1 and BRCA2 mutation carriers, the method of BRCA1 quantification, and retrospective design have limited the interpretation of studies designed to address the role of BRCA1 as a predictive marker of response to chemotherapy. We recommend that future studies be conducted that are prospective, adequately powered, and measure BRCA1 expression at both mRNA and protein levels. In future studies of sporadic breast cancer that investigate the association between BRCA1 expression and chemotherapeutic response, we recommend that patients be screened for BRCA1 mutations to help clarify some of the conflicting data because the expression level of mutant BRCA1 may be similar to that of functional BRCA1. We also recommend that, in future studies investigating the association between BRCA1 mutations and chemotherapeutic response, the BRCA1 gene be sequenced so that associations between the different mutations and chemotherapeutic response can be assessed. Finally, we suggest that the use of single-agent treatments rather than combination regimens would allow the association between BRCA1 expression or mutation and response to particular drug classes to be assessed more accurately. By these approaches, it may be possible to determine whether the BRCA1 gene is a useful predictive marker for chemotherapeutic choice in breast or ovarian cancer. These studies may also point the way to new chemotherapeutic strategies targeting BRCA1-mediated pathways that participate in cancer cell death or an improved response to conventional treatments.

REFERENCES


Lafarge S, Sylvain V, Ferrara M, Bignon YJ. Inhibition of BRCA1 leads to increased chemoresistance to microtubule-interfering agents, an effect that involves the JNK pathway. Oncogene 2001;20:6597–606.


Mingo-Sion AM, Marietta PM, Koller E, Wolf DM, Van Den Berg CL.
(98)
Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG,
Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders
Pichierri P, Rosselli F. The DNA crosslink-induced S-phase checkpoint
Ree AH, Bratland A, Nome RV, Stokke T, Fodstad O. Repression of
is an essential kinase that is regulated by Atr and required for the G(2)/M
(89)
phosphorylation of BRCA1 regulates DNA double-strand break repair.
(88)
BRCA1 interacts directly with the Fanconi anemia protein FANCA. Hum
Pichirrelli P, Rosselli F. The DNA crosslink-induced S-phase checkpoint
depends on ATR-CHK1 and ATR-NBS1-FANCD2 pathways. EMBO J
(87)
Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders
(86)
(85)
Mutation in BRCA2 stimulates error-prone homology-directed repair of
DNA double-strand breaks occurring between repeated sequences. EMBO J
2001;20:4704–16.
(84)
Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG,
Joenej H, et al. Disruption of the Fanconi anemia-BRCA pathway in
(83)
Vinorelbine is an active antiproiferative agent in pretreated advanced
(82)
Wassmann K, Benezza R. Mitotic checkpoints: from yeast to cancer. Curr
(81)
Subcellular localization of the BRCA1 gene product in mitotic cells.
(80)
Hsu LC, Doan TP, White RL. Identification of a gamma-tubulin-binding
(79)
amplification and a defective G2-M cell cycle checkpoint induce genetic
instability in BRCA1 exon 11 isoform-deficient cells. Mol Cell 1999;3:
389–95.
(78)
Mingo-Sion AM, Marietta PM, Koller E, Wolf DM. Van Den Berg CL.
Inhibition of JNK reduces G2/M transit independent of p53, leading to
endoreduplication, decreased proliferation, and apoptosis in breast cancer
(77)
Induction of GADD45 and JNK/ASK-dependent apoptosis following
(76)
Thangaraju M, Kaufmann SH, Couch FJ. BRCA1 facilitates stress-
induced apoptosis in breast and ovarian cancer cell lines. J Biol Chem
(75)
Bhattacharyya A, Ear US, Koller BH, Weichelbaum RR, Bishop DK.
The breast cancer susceptibility gene BRCA1 is required for subnuclear
assembly of Rad51 and survival following treatment with the DNA
(74)
Fedier A, Steiner RA, Schwarz VA, Lenherr L, Haller U, Fink D. The effect
of loss of Brca1 on the sensitivity to anticancer agents in p53-
(73)
Sylvain V, Lafarge S, Bignon YJ. Dominant-negative activity of a Brca1
truncation mutant: effects on proliferation, tumorigenicity in vivo, and
chemosensitivity in a mouse ovarian cancer cell line. Int J Oncol 2002;
20:848–53.
(72)
Zhou C, Smith JL, Liu J. Role of BRCA1 in cellular resistance to
doxorubicin and ionizing radiation in an ovarian cancer cell line carrying a
(71)
Case I, Baldwin RL, Varkey T, Moslehi R, Narod SA, Karlan BY.
Improved survival in women with BRCA-associated ovarian carcinoma.
Cancer 2003;97:2187–95.
(70)
Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, et
(69)
A significant response to neoadjuvant chemotherapy in BRCA1/2 related
(68)
Delaloge S, Klooij L, et al. BRCA1-linked breast cancer (BC) is highly
more chemosensitive than its BRCA2-linked or sporadic counterparts
[abstract 120], Nice (France): Program and abstracts of the 27th Congress
of the European Society for Medical Oncology 2002.
(67)
Impact of germline BRCA1 mutations and overexpression of p53 on
diagnosis and response to treatment following breast carcinoma: 10-year
(66)
Egawa C, Motomura K, Miyoshi Y, Takamura Y, Taguchi T, Tamaki Y,
et al. Increased expression of BRCA1 mRNA predicts favorable response
to anthracycline-containing chemotherapy in breast cancers. Breast Cancer
(65)
Quinn JE, Kennedy RD, Mullan PB, Gilmore PG, Johnston PG, Harkin
(64)
The breast cancer susceptibility gene BRCA1 transactivates the
endoreduplication, decreased proliferation, and apoptosis in breast cancer