*Review*

# BARD1 and breast cancer: the possibility of creating screening tests and new preventive and therapeutic pathways for predisposed women.

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**Abstract:** Current oncological developments are based on improved understanding of genetics, and especially the discovery of genes whose alterations affect cell functions with consequences for the whole body. Our work is focused on the most important of these genes, the BARD1 and its oncogenic role in breast cancer. Most importantly, the study points to new avenues in the treatment and prevention of the most frequent female cancer based on BARD1 research. The BARD1 and BRCA1 proteins have similar structures and functions, and they combine to form the new molecule BARD1-BRCA1 heterodimer. Through ubiquitination, this heterodimer has significant effects on individual proteins, enabling, among others, the destruction of damaged DNA fragments. Ubiquitination, as well as stabilizing chromatin, or regulating the number of centrosomes, confirms the protective cooperation of BARD1 and BRCA1 in the stabilization of the genome. The overexpression of the oncogenic isoforms BARD1β and BARD1δ permit cancer development.

The introduction of routine tests, for instance, to identify the presence of the BARD1β isoform, would make it possible to detect patients at high risk of developing cancer. On the other hand, introducing BARD1δ isoform blocking therapy, which would reduce estrogen sensitivity, may be a new line of cancer therapy with potential to modulate responses to existing treatments. It is possible that the BARD 1 gene offers new hope for improving breast cancer therapy.

**Keywords:** breast cancer, BARD1, surveillance, management, genetic testing, predisposition, susceptibility, neoadjuvant, chemotherapy

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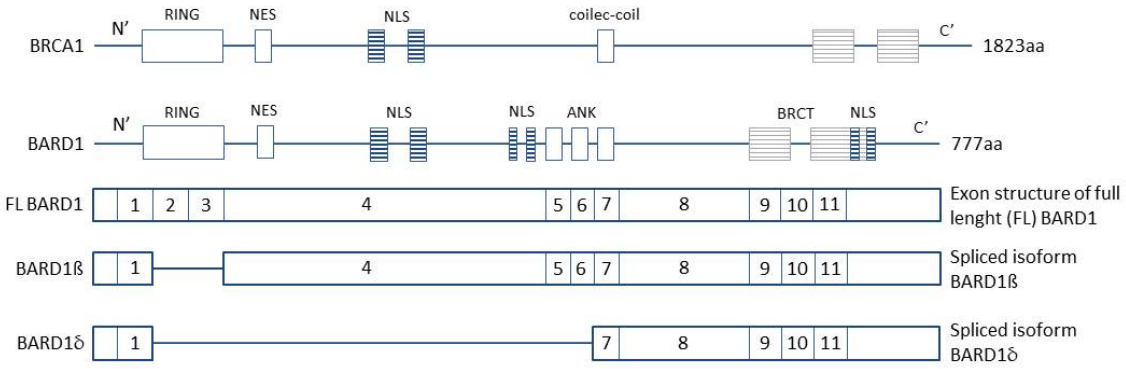
## 1. Introduction

In 1996, Wu et al. discovered a binding partner protein of BRCA1 (BReast CAncer type 1) which they named BRCA1-associated RING domain 1 (BARD1) [l]. The BARD1 gene is located on chromosome 2 and encoded by the sequence 2q34-q35. Its product is a 777 amino acid protein composed of an N-terminal RING-finger domain, three Ankyrin repeats (ANK) domains and two tandem BRCA1 C-terminal (BRCT) domains (Figure 1). The BARD1 protein structure is like that of the BRCA1, however it is different from that of the BRCA2 (BReast CAncer type 2), the second gene associated with breast cancer [2]. BARD1 and BRCA1 can form a heterodimer by their N-terminal RING finger domains which form a stable complex [3].

**Figure 1.** Schematic structures of BRCA1, BARD1 and isoforms: BARD1δ, BARD1β




**Legend:** RING finger domains enables to form stable complex between BRCA1 and BARD1. NES are nuclear export signals. NLS are the potential nuclear localization signals. BARD1 has six NLS, which together with NES are necessary for proper intracellular localization of BARD1. ANK (Ankyrin repeats) interacts with several proteins including p53 and NF-κB. BRCT (BRCA1 carboxy-terminal domain) motifs combine with a key lysine residue (K619) and bind to poly(ADP-ribose) (PAR) and interact with DNA strand breaks.

The BARD1-BRCA1 heterodimer, as an E3 ubiquitin ligase, is essential in numerous cell regulations [4]. Generally, it enables ubiquitin to be attached, which marks proteins for further degradation. Due to this ability, the BARD1-BRCA1 heterodimer is engaged in the DNA damage response pathway. BRCT motifs combine with a key lysine residue (K619) and bind to poly(ADP-ribose) (PAR), which targets the BARD1-BRCA1 heterodimer to DNA damage sites. Afterward, the BARD1-BRCA1 heterodimer ubiquitinates RNA polymerase II, preventing the transcription of the damaged DNA and maintaining its genetic stability.

The BARD1-BRCA1 heterodimer also functions in the modulation of the chromatin structure. It conducts ubiquitination of H2A/H2AX, H2A and H2B that creates the conditions for transcriptional activity [5].

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The BARD1-BRCA1 association also has a function in the regulation of the centrosome number during mitosis. BRCA1 locates the centrosomes and then the BRCA1-BARD1 ubiquitin ligase regulates centrosome numbers by targeting gamma-tubulin [6].

Estrogen and progesterone levels have a significant role in the pathogenesis of breast cancer. They affect cells via estrogen receptors alpha (ER$\alpha$) and beta (ER$\beta$). This results in the activation of a gene responsible for cell proliferation. The BARD1-BRCA1 heterodimer is responsible for the ubiquitination and subsequent degradation of ER$\alpha$, resulting in reduction of cellular ER$\alpha$ levels.

BARD1 is not only an important factor in the ubiquitinating complex but also functions as a protein in the BRCA1-independent pathways. BARD1 has a crucial role during the induction of apoptosis by the stabilization of p53 [7]. It also inhibits mRNA maturation during genotoxic stress through having an impact on CstF-50 (cleavage stimulation factor) [8].

All these functions prove that full length-BARD1 (FL-BARD1) has an important tumor suppressor role. However, in neoplastic pathogenesis, BARD1 isoforms antagonize FL-BARD1 and enable uncontrolled proliferation. The main cancerous isoforms are BARD1$\beta$ and BARD1$\delta$.

The first of these, BARD1$\beta$, has an adverse effect on the Aurora kinases A and B. It stabilizes Aurora B and forms a complex with BRCA2 and Aurora B during telophase and cytokinesis that results in overriding the mitotic checkpoint. Thus, Aurora and BARD1$\beta$ expression levels might be predictive biomarkers for responses to Aurora inhibitors. The second important isoform is BARD1$\delta$. It interacts with ER$\alpha$ and antagonizes FL-BARD1 that results in a higher response rate to estrogens. Inhibiting BARD1 isoform expression might be a novel pathway for therapeutic intervention or prevention for predisposed women.

Breast cancer is the second most common neoplasia in the female population. Despite this fact, no more than 40% of familial breast cancers have been identified as having causative gene mutations [9]. Most of these are mutations in either the BRCA1 or the BRCA2 genes. A better understanding of the pathogenesis of breast cancer may enable the development of new therapeutic methods. It would also enable new screening tests to be implemented that are based on the presence of mutations that might be discovered soon. The latest reports show that deleterious BARD1 variants may be the reason for hereditary breast cancer in BRCA1 and BRCA2 negative families [10].

The BARD1 protein also seems to be an interesting starting point in analyzing the causes of drug resistance in breast cancer cases. About 70% of breast cancers are ER positive. Despite using multiple drugs that are ER antagonists (e.g., tamoxifen) we still observe numerous relapses, even during 15 years of post-treatment follow-up. The main limitation in solving this problem is that the mechanisms of chemoresistance are still too-little understood. However, it seems that the BARD1 protein, that is associated with so many cellular mechanisms, can play a valid role here. It has been proved that patients following breast cancer relapse have significantly higher expressions of the BARD1 and BRCA1 proteins resulting from the activated PI3K/AKT pathway [11].

The aim of our review is to investigate the role of the BARD1 gene in the assessment of predisposition to breast cancer. We would also like to probe the utility of BARD1 in surveillance programs or as a potential target of new anticancer therapies, including sensitivity to chemotherapy.

## 2. Materials and Methods

The article reviews the literature using the Pubmed, Google Scholar and Elsevier Clinical Key databases. Articles were screened for relevance, those with the most up-to-date information were

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selected for inclusion. In addition, a manual search of the reference lists of previously captured articles was carried out to increase the likelihood of choosing essential studies.

## 3. Results

### *3.1. BARD1 gene and predisposition to breast cancer*

Genetic predisposition to breast cancer can be divided into three different levels [12,13], depending on the risk of breast cancer and the degree of penetrance. The first level is comprised of high-risk heterozygous, and highly penetrant gene mutations. The second level is associated with genes of intermediate penetrance and a moderate risk of breast cancer. The third level consists of low-penetrance breast cancer susceptibility alleles, and common polymorphisms (SNPs - single nucleotide polymorphisms) [10,13] (Table 1).

Since its discovery in 1996, the BARD1 gene and its various mutations have been extensively studied for breast cancer susceptibility. In a study of over 65,000 American non-BRCA1 and non-BRCA2 patients (mean age at diagnosis 48.5) with breast cancer, pathogenic variants in BARD1 in white women were associated with a significant moderately increased risk of breast cancer. The pathogenic variant (PV) in this population, proved to be quite rare (<1 out of 500 breast cancer cases) [17].

**Table 1.** Levels and characteristic of genetic predisposition to breast cancer

| Level of predisposition | Gene penetration | Risk of breast cancer | Examples of affected genes | Characteristics | Reference |
|---|---|---|---|---|---|
| **I** | High | High | BRCA1 and BRCA2, TP53, CDH1, STK11, PTEN | Mutations in BRCA1 and BRCA2 are responsible for 16-40% of hereditary breast and ovarian cancers and site-specific breast cancer; inTP53 is associated with up to 85% risk of developing breast cancer by age 60; germline mutations in CDH1 and STK11 are associated with 39-52% and 32-54% risk of developing breast cancer, respectively; germline mutations in the PTEN gene promoter are associated with an 85% lifetime risk of breast cancer | [9,14,15,16] |
| **II** | Intermediate | Moderate | ATM, CHEK2, BRIP1, BARD1, PALB2 | Mutations in these genes are responsible for a 2- to 4-fold increase in the risk of breast cancer in comparison to population-based risk | [13] |

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|---|---|---|---|---|---|
| **III** | Low | Low | FGFR2, RAD51 | FGFR2 SNPs increase the risk of breast cancer by increasing the response to estrogen; RAD51 SNP2 i.e. are considered as BRCA1/2 mutations carriers risk modifiers | [10,13] |

BARD1 is not only thought to be a breast cancer susceptibility gene, but also a gene predisposing to triple negative breast cancer (TNBC) [18]. Furthermore, in a study of 10,901 TNBC patients, it was established that BARD1 was one of the most common non-BRCA1/2 genes to mutate. Among other genes [18], BARD1 was proven to be statistically significantly associated with a moderate to high risk of TNBC with an incidence of 0.5-0.7% [18]. The same study established that the PVs in BARD1 were associated with a lifetime risk of TNBC in 7% of cases; and a 21% risk of breast cancer for Caucasian patients and 39% risk of TNBC for African American patients [18]. In a different study of 289 African American patients, 144 of whom were cases of familial breast cancer, only 1 incidence of PV in the BARD1 gene was found [19]. In another study of 1,824 female American patients with TNBC, 97% of which were white, 1.9% African, 0.6% Asian and 0.6% Hispanic, deleterious mutations in BARD1 were detected 9 times, with an incidence of 0.3-0.5% [20].

Outside the United States, there has also been researched on BARD1 in Europe, Korea, and Australia. Out of 120 Korean breast cancer patients negative for BRCA1/2 mutations, PVs in the BARD1 gene were identified in two patients [21]. A Finnish study of 94 BRCA1/2 negative breast cancer families, established an incidence of 7.4% of Cys557Ser allele in the BARD1 gene in comparison with an incidence of 1.4% in the healthy controls [22]. Moreover, the BARD1 Cys557Ser allele was also reported in an Italian study with an incidence of 2.5% [23]. These studies may indicate that the BARD1 Cys557Ser allele is of European origin.

In three independent studies of the Polish population, a deleterious nonsense pathogenic BARD1 mutation, namely p.Q564X, was identified [10,24,25]. A study among 12,476 Polish and 1,459 Belarusian breast cancer patients, identified a 0.27% incidence of the PV in both study groups, assessing it as a low/moderate breast cancer predisposition gene. The p.Q564X BARD1 mutation is possibly a founder mutation, present at least in Central Europe. However, its presence in the Polish control subgroup (0.15%) might indicate its low penetrance. It is also important to point out, that a higher incidence of the mutation was found in (PR-)breast cancer patients than in the group of receptor-positive breast cancer patients (0.55% and 0.24%, respectively) [26]. An analysis of large mutations of the BARD1 gene in 504 breast cancer/ovarian cancer Polish patients was conducted and indicated that such mutations do not contribute to breast cancer predisposition [10]. This, however, does not contradict the role of BARD1 as a breast cancer susceptibility gene.

A study in Germany, inspecting germline loss of function (LoF) variants in the BARD1 gene, was conducted in 4,469 breast cancer patients, 23 (0.51%) of whom had LoF variants. Those patients showed a significantly younger mean age at first diagnosis than in the overall population sample (42.3 vs 48.6 years). LoF BARD1 variants were not significantly associated with patients with age at

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first diagnosis of >=50. This might suggest a need to intensify breast cancer surveillance programs and include testing for BARD1 PV [27].

However, controversy remains as to whether the BARD1 variant, in its rarity, can be clinically associated with increased breast cancer risk [21]. There have also been studies disputing that BARD1 is a moderate/high-risk breast cancer susceptibility gene [28]. In a study of 684 Australian non-BRCA1/2 patients with familial breast cancer, 4 cases of PVs in BARD1 were identified, and the study concluded there is no clinical value in testing for the BARD1 PV mutation in breast cancer families.

### 3.2. *Utility of BARD1 in surveillance programs*

Currently, BRCA1/BRCA2 is the best-known gene relating to breast cancer. Depending on their age, those carriers at high, or very high-risk need: regular breast self-examination, imaging such as mammography or breast magnetic resonance imaging (MRI) every 6 to 12 months, transvaginal ultrasound every six months, and CA-125 blood testing due to the risk of ovarian cancer [29,30]. Monitoring can also include prophylactic mastectomy and bilateral salpingo-oophorectomy; though these procedures severely affect the patient's quality of life and can hamper her psychosocial well-being as a result of infertility [31,32]. Bearing this in mind, it seems justified that stricter monitoring should be undertaken, including risk stratification based on genetic testing. We studied the BARD1 gene, which appears beneficial for patient observation. Based on 2019 study of a group of 4469 women, it was concluded that the BARD1 gene correlates with early onset of breast cancer and a worse prognosis [27,33]. The gene carriers should be screened at a younger age, especially because the gene has also been shown to be related to other cancers, including ovarian cancer, colorectal cancer, non-small-cell lung carcinoma and hepatocellular carcinoma [34,35]. However, the use of a multidirectional diagnostic pathway as a standard approach requires further cohort trials and could be of interest for future research. Breast cancer cells produce isoforms of the BARD1 gene which can be detected using specific antibodies. Interestingly, the isoforms can also be produced by spontaneous breast cancer that is not associated with BRCA group genes [36].

In addition to the most obvious role of a screening test, the antibody testing can also be used for monitoring treatment because the increased expression of BARD1 isoforms is associated with disease progression. Immunohistochemical testing of breast cancer samples shows more intense staining of the cytoplasm due to the overexpression of BARD1 isoforms. It is worth mentioning that the degree of staining was proportional to the degree of malignancy and size of the tumor. Comparing those observations with the Tumor-Nodes-Metastasis staging system, it can be hypothesized that overexpression of BARD1 isoforms is proportional to the size of the tumor and its malignancy grade, which in turn heralds a worse prognosis [37-39]. Recently, there has been a growing body of research suggesting that the BARD1 gene is only associated with low to intermediate risks of breast cancer [26]. Other genes, such as PALB2, BRIP1, ATM, CHEK2, RAD51C, RAD51D, NBN, NF1, and MMR should also be considered, as these can have a cumulative effect on the risk of breast cancer when in combination with the BARD1 gene [16,40]. Our research advocates strongly for patient observations based on multigenetic panel testing, or even for an individualized approach and monitoring based on genetic profiling [41].

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Mammography and breast MRI remain the fundamental imaging modalities for the high and very high-risk patients. Decreasing mortality rates are thought to have resulted from more effective treatment [42]. Therefore, other diagnostic tools should be sought, not only for screening but also for risk management. An interesting approach might be radiogenomics, which brings together clinical assessment, imaging results and genetic background [43]. This approach would be of interest in relation to the immunohistochemical staining of the BARD1 gene, which in turn can be imaged in magnetic resonance scans. The precise diagnosis may play a role in decisions about whether to perform or postpone prophylactic surgical interventions due to breast cancer risk.

### *3.3. BARD1 gene as a potential target of new anticancer therapies including sensitivity to chemotherapy with a focus on breast cancer*

Several studies have shown that BARD1 may become a potential target for new therapies in the treatment of breast cancer patients. Zhu Y et al. [11] reported that tamoxifen-resistant breast cancer cells express significantly more BARD1 and BRCA1, leading to a resistance to DNA-damaging chemotherapy including cisplatin and adriamycin, but not to paclitaxel. The mutation of BRCA1 or BARD1 results in defective DNA damage repair which also leads to increased sensitivity to platinum-based chemotherapy. They suggested that microtubule-targeting drugs such as taxane may be superior to DNA-damaging drugs such as anthracyclines and cisplatin/carboplatin when planning chemotherapy for tamoxifen-resistant breast cancer patients. Zhu Y et al. proved that silencing BARD1 or BRCA1 expression using siRNAs or the inhibition of BRCA1 phosphorylation by Dinaciclib (inhibitor of CDKs) restores sensitivity to cisplatin in tamoxifen-resistant cells. Silencing both BARD1 and BRCA1 did not have an addictive effect, indicating that these two proteins work through the same pathway. The same authors also showed that the activated PI3K/Akt/mTOR pathway is responsible for the upregulation of BARD1 and BRCA1. PI3K inhibitors decreased the expression of BARD1 and BRCA1 in tamoxifen-resistant cells and resensitized them to cisplatin, both *in vitro* and *in vivo*. The PI3K/Akt/mTOR pathway is an intracellular signaling pathway that controls several pivotal processes, including proliferation, apoptosis, angiogenesis, and survival of cells. Mutations of the PI3K pathway are the most common genetic alterations in ER-positive early breast cancer, as well as in recurrent or metastatic cancers [44]. These mutations often lead to the hyperactivation of the PI3K pathway and play an important role in resistance to endocrine therapy. The PI3K/Akt/mTOR pathway can be blocked by US Food and Drug Administration (FDA)-approved mTOR inhibitor everolimus and several PI3K inhibitors currently in clinical trials [11]. The addition of CDK4 / 6 kinase inhibitors (Palbocyclib, Ribocyclib) to hormone therapy in the luminal breast cancer subtype reduces the risk of resistance resulting from the activation of these cyclins [45].

Li M et al. [46] reported the interaction of the BARD1 BRCT domain with poly(ADP-ribose) (PAR) and the consequent recruitment of the BARD1-BRCA1 complex to DNA repair after damage. The PAR pathway is of particular interest because the promising drugs that act on inhibiting the PAR polymerizing enzyme (PARP) are more efficient in cells BRCA1-mutated with a saved BARD1 tumor suppressor function.

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The protein poly ADP-ribosylation (PARylation acts as a signal to recruit DNA damage repair proteins like the BARD1-BRCA1 complex to repair Double Strand Breaks (DSBs). BARD1 BRCTs bind ADP-ribose, the basic unit of PAR, at DNA damage sites that mediate the rapid recruitment of BRCA1. PARP inhibition suppresses the recruitment of the BARD1-BRCA1 heterodimer to DNA damage sites and impairs DNA repair. PARP inhibitors (PARPi) selectively eliminate BRCA1-deficient cells. Recently, several PARPi have been approved by the FDA to treat various cancers, including metastatic TNBC and estrogen receptor-negative (ER-)/HER2+ breast cancer with BRCA mutations [47].

Ovarian and breast cancer patients who harbor BRCA1 mutations initially respond well to platinum and PARPi therapy but later develop resistance to both PARPi and platinum compounds [48,49,50]. Secondary mutations in BRCA genes as well as the gene methylation status of BRCA1, BRCA2 and other genes that control homologous recombination have been examined in patients' biopsies as potential resistance mechanisms. One way to overcome clinical resistance is to investigate whether the expression of FL or isoform of BARD1 could contribute to the success or failure of PARPi therapy [48].

### *3.4. BARD1 gene mutations and neoadjuvant setting in breast cancer*

Current data on this subject mainly refer to TNBC which has higher incidence of pathogenic variants of the BARD1 gene [51]. Watanabe et al. analyzed thirty TNBC core biopsy specimens of patients with pathologic complete response (non-invasive cancer) and non-complete response following neoadjuvant chemotherapy (NACT), with regard to the aberrant DNA methylation status of the BARD1 gene (from a total number of 16 DNA repair genes) using bisulfite-pyrosequencing. Although hypermethylation of BRCA1 gene is associated with TNBC subtype and may impact chemosensitivity and progression under NACT, BARD1 gene hypermethylation revealed only a low-to-moderate influence on these processes [52]. Some other studies underline the low incidence and uncertain clinical impact of gene mutations other than BRCA1/2 (including BARD1), and the associated unfavorable outcomes for patients with breast cancer undergoing NACT [53]. Yet another study reported that BRCA1 and its associated protein BARD1 are upregulated in tamoxifen-resistant breast cancer cells, rendering the cells resistant to DNA-damaging chemotherapy [11,54].

## 4. Discussion

The US National Comprehensive Cancer Network (NCCN) guidelines do not routinely recommend BARD1 positive-patients undergo additional breast cancer screening (early breast MRI, mammography), which might need to be implemented [18]. This screening is usually only performed if the cases with family history indicating that the patient has an increased risk of breast cancer. For now, the risks of breast cancer connected with BARD1 remain poorly defined and of varying prevalence across different populations. Nonetheless, there is multiple instances, listed in the evidence above, that the PVs of BARD1 not only increase the risk of breast cancer, but also of TNBC and can be associated with age at first diagnosis of <=50.

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The BARD1 gene can significantly extend the monitoring options in patients at risk of breast cancer and during post-treatment follow-up. However, due to the low incidence of BARD1 mutations, such assumptions require further long-term population-based trials. At present, and considering potential benefits and costs, it seems that the possible uses of the BARD1 gene that we discussed can set a direction for further research rather than provide real options for widespread use.

Over the last few decades, molecular research has been intensified to further individualize the treatment of breast cancer patients. Personalization of systemic treatment is aimed at identifying a group of patients with unfavorable prognostic factors and at identifying patients who can benefit most from therapy [41]. The assessment of efficacy of PARPi in breast cancer patients with the relatively frequent LoF mutations of BARD1 would be of necessity to improve patients' outcomes. Ozden et al. [55] proved that BARD1β sensitizes colon cancer cells to poly PARP-1 inhibition even in an FL BARD1 background, thus suggesting that BARD1β may serve as a future biomarker for assessing the suitability of colon cancers for homologous recombination targeting with PARPi in the treatment of advanced colon cancer. Clinical trials of PARPi in neoadjuvant, mono- and combination therapy settings in breast cancer are ongoing.

Neoadjuvant chemotherapy offers opportunity to assess the molecular changes of heterogenic breast cancer tissue before and after chemotherapy, especially in the case of TNBC, in which BARD1 deleterious gene alterations are the most prevalent and NACT seem to have the greatest value.

## 5. Conclusions

Analyzing structure and functions of the BARD1 gene, we believe that BARD1 gene can play an important role in the pathogenesis of breast cancer and in the mechanisms of chemo-resistance of cancer cells as well.

It is reasonable to screen BARD1 gene isoforms in certain populations, especially in those with evidence of higher prevalence of mutations in the BARD1 gene. This approach would also have to be researched for its relevance to general breast cancer patient outcomes, survival rates, quality of life, influence on treatment decisions and cost-effectiveness.

Radiogenomics is a promising field of science as a bridge between molecular and imaging medicine. Broader prospective studies and standardization (i.e. immunohistochemistry studies with BARD1-directed antibodies) will provide determination of appropriate imaging biomarkers enabling "cancer cell visibility" before they can be introduced in to a clinical investigation.

Further research on the BARD 1 gene expression may contribute to the effective reversal of PARPi resistance and the wider introduction of new targeted therapies for the treatment of breast cancer patients.

Data on patients with BARD1 gene polymorphism undergoing NACT for breast cancer are limited. However, gene expression alterations after NACT can shed light on the pathogenesis of this multifactorial disease.

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