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Breast cancer (BC) prevalence has reached an epidemic scale with half a million deaths annually. Current deficits in BC management include predictive and preventive approaches, optimized screening programs, individualized patient profiling, highly sensitive detection technologies for more precise diagnostics and therapy monitoring, individualized prediction and effective treatment of BC metastatic disease. To advance BC management, paradigm shift from delayed to predictive, preventive and personalized medical services is essential. Corresponding step forwards requires innovative multilevel diagnostics procuring specific panels of validated biomarkers. Here, we discuss current instrumental advancements including genomics, proteomics, epigenetics, miRNA, metabolomics, circulating tumor cells and cancer stem cells with a focus on biomarker discovery and multilevel diagnostic panels. A list of the recommended biomarker candidates is provided.

**Keywords:** breast cancer • cancer stem cells • circulating tumor cells • omics • personalized medicine • predictive diagnostics • prognosis • stratification • targeted therapy • validated biomarker

**Introduction: deficits in breast cancer management & expectations by multilevel diagnostics**

Breast cancer (BC) has been recognized as becoming epidemic in the 21st century [1] with around half a million deaths and about 1.7 million new cases annually [2]. Although BC mortality rates have slightly decreased over last decades, the incidence rates continue to escalate [3]. BC is a multifactorial disease with highly heterogeneous risk factors, origin, individual phenotypes and consequently, different molecular profiles, sensitivity to therapeutic approaches and frequently unpredictable clinical outcomes [4]. Current deficits in BC management include suboptimal screening programs exhibiting a number of cases with both false-positive and -negative diagnosis [5], an evident lack of screening programs focused specifically on BC predisposition and initiation in young subpopulations, individualized patient profiling for more precise diagnosis and treatments tailored to the person that altogether result in enormous socioeconomic burden [1,6]. In particular, the overall annual costs for BC management across Europe are reported as being at the level of €15.0 billion [7]. Furthermore, study by Meneses et al. has underlined that the BC-linked economic burden is in liaison with decreased motivation and productivity at work, days missed from work, change in partner’s income, financial adversity and an overall poorer quality of life [8].

Currently, there is an evident lack of articles providing evidence and summarizing biomarker panels for multilevel diagnostic approaches, which are particularly promising to optimize the overall BC management [1,6]. Potential complementarity and integration of...
several detection technologies at molecular and cellular levels in use or under development into innovative approaches by multilevel diagnostics is considered in this paper. Biomarker panels are overviewed as provided by individual technologies, namely genomics, transcriptomics, proteomics, metabolomics as well as detection and analysis of circulating tumor cells (CTCs) and cancer stem cells (CSCs). The crucial role of integrative bioinformatics in development and successful clinical application of innovative approaches by multilevel diagnostics is discussed.

**Crucial role of *omics in BC diagnostics & treatment**

*omics technologies encompass the utilization of detection methodology focused on complex molecular profiling. Most frequently considered complementary approaches are genomics, transcriptomics, proteomics, metabolomics and lipidomics. Specifically in the field of BC research, *omics technologies are currently applied to understand the molecular mechanisms, to analyze contributing molecular pathways [9], BC predisposition and phenotyping [10–12], heterogeneity of BC and BC metastatic disease (BCMD) [18], therapy targeting [14], drug resistance [15] and others [16–18].

**Genomic analysis in BC**

Cancer genomics has become a vital tool in cancer management. It employs different genomic approaches such as the next-generation sequencing platform, genome testing and genome wide association studies [19–25]. Clinical implementation of genome testing is well justified, for example, for adjuvant chemotherapy application [26]. Next-generation sequencing platform provides multitude of genomic data including probabilistic approach for cancer predisposition, discovery of cancer-related genes/pathways and structural DNA modification (mutations, changes of corresponding gene copy number and chromosomal rearrangements), analysis of intratumoral heterogeneity and drug resistance [19]. Engaging the currently available genomic tools manifests a potential for better diagnosis and prognosis of BC [20–24]. Table 1 provides information about powerful biomarker candidates detectable by genomics.

Complementary to this subchapter considering BC relevant DNA structure modifications such as polymorphisms, mutations, gene copy number, the follow-up subchapters are dedicated to the next levels of biomarker detection, namely the gene regulation-specific patterns.

### Table 1. Predictive and prognostic biomarkers detectable by genomics.

<table>
<thead>
<tr>
<th>Biological material</th>
<th>Biomarker (DNA template)</th>
<th>Comments</th>
<th>Useful for</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood lymphocytes</td>
<td><em>BRCA1</em> mutations (genomic DNA)</td>
<td>Higher in TNBC compared with non-TNBC; worsen OS in carriers compared with noncarriers</td>
<td>BC predictive diagnostics</td>
<td>[10,27–28]</td>
</tr>
<tr>
<td>Primary invasive breast carcinomas tumor samples (human)</td>
<td><em>TP53</em> mutations</td>
<td>Poor prognosis in ER+</td>
<td>BC therapy monitoring/prognostic</td>
<td>[29]</td>
</tr>
<tr>
<td>Peripheral blood samples</td>
<td><em>MTHFR</em> C677T polymorphism (genomic DNA)</td>
<td>Significantly associated with BC risk, BC development</td>
<td>BC predisposition/predictive diagnostics</td>
<td>[30–36]</td>
</tr>
<tr>
<td>Peripheral blood samples</td>
<td><em>hOGGT1</em> polymorphism c.977C&gt;G (genomic DNA)</td>
<td>Relevance for development of p53+ TNBC phenotype in premenopausal patients</td>
<td>BC therapy monitoring/prognostic</td>
<td>[37]</td>
</tr>
<tr>
<td>Whole blood samples</td>
<td>SNP rs17506395 in p63</td>
<td>TT genotype: poor prognosis in BC in Han Chinese population</td>
<td>BC therapy monitoring/prognostic</td>
<td>[38]</td>
</tr>
<tr>
<td>Blood samples</td>
<td>SNP rs28366003 in <em>MT2A</em> (genomic DNA)</td>
<td>Risk of BC in Polish population</td>
<td>BC predictive diagnostics</td>
<td>[39]</td>
</tr>
<tr>
<td>Tissue samples (human) and peripheral blood samples</td>
<td>SNP rs17849079 in <em>PIK3CA</em> (genomic DNA)</td>
<td>Prevalent in BC cases compared with controls in Arab population</td>
<td>BC predictive diagnostics</td>
<td>[40]</td>
</tr>
<tr>
<td>TNBC tissue samples (human)</td>
<td>High <em>EGFR</em> copy number</td>
<td>Associated with poor outcome in TNBC phenotype</td>
<td>BC therapy monitoring/prognostic</td>
<td>[41]</td>
</tr>
</tbody>
</table>

BC: Breast cancer; OS: Overall survival; rs: Reference SNP ID; TNBC: Triple-negative breast cancer.
Gene regulation level comprises a number of complementary approaches & allows for more precise diagnosis & targeted treatments in BC

Above-described BC-relevant alterations in DNA structure are the basic informational level considering genetic predisposition to pathology and pharmacogenetic characteristics relevant for the drug application.

The following paragraphs are focused on more comprehensive level of ‘post-genomic’ gene regulation to be essentially considered for individual patient profiles estimating synergistic effects of all risk factors including internal and external stress factors, lifestyle, diet, behavior-related biomarker patterns, among others.

Gene patterns differently regulated in BC

The data summarized below furnish information about biomarker-candidate elements in regard to molecular patterns linked to gene regulation (gene amplification/overexpression), which is specifically altered in BC. Table 2 outlines a particular importance of specific gene expression signature for BC and BCMD prognosis. Hence, a recurrence of triple-negative breast cancer (TNBC) can be predicted [42].

Epigenetic patterns specific for BC

In contrast to the basic DNA information analyzed by genomics, epigenomics analyses DNA patterns that are relevant for the specific gene activation such as DNA methylation status [48,49]. A role of epigenetic deregulation (specifically altered epigenetic patterns) has been suggested for BC pathology based on comprehensive analysis by ‘epigenome-wide maps’ [50]. An importance of DNA methylation in BC patient stratification, prediction and prognosis is evidence-based [51–53]. Furthermore, a biomarker panel is proposed specifically for BC progression, which comprises the following genes: BRCA1, DAPK1, MSH2, CDKN2A, PGR, PRKDCBP and RANKL [52]. The functional role of above-mentioned genes includes tumor suppression, regulation of gene transcription, proapoptotic activity, kinase inhibition and cell survival regulation. The other panel which is indicated to have prognostic value comprises BC-specific epigenetic patterns of the following 13 genes: CST6, DBC1, EGFR, GREM1, GSTP1, IGFBP3, PDGFRB, PPM1E, SFRP1, SFRP2, SOX17, TNFRSF10D and WRN [53]. Additionally, the same study demonstrated that the luminal type B tumors have higher average methylation status compared with basal-like tumors of the 48 selected genes [53] that are indicative for more precise BC phenotyping and stratified treatments. This gene panel was selected by previously performed genome-wide methylation studies, due to its relevance in BC. Epigenetic alterations in patterns of histones are shown to be involved in BC pathological mechanisms [54–56] being relevant for BCMD, patient stratification and related prognosis [57–60]. Table 3 provides summarizing information to the most potent biomarker candidates. Corresponding predictive and/or prognostic value is notified.

### Table 2. Predictive and prognostic biomarkers patterns differentially regulated in breast cancer.

<table>
<thead>
<tr>
<th>Biological material</th>
<th>Biomarker (DNA template)</th>
<th>Comments</th>
<th>Useful for</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue samples (human), BC cell line (human)</td>
<td>PAK1</td>
<td>Amplification is associated with TamR</td>
<td>BC therapy monitoring/prognostic and optimal BC therapy algorithms/predictive</td>
<td>[43,44]</td>
</tr>
<tr>
<td>Tissue samples (mice), cell line (human) and BC tissue samples (human)</td>
<td>Nanog</td>
<td>Altered expression contributes to BCMD, associated with poor prognosis</td>
<td>BC therapy monitoring/prognostic and BCMD therapy monitoring/prognostic</td>
<td>[45,46]</td>
</tr>
<tr>
<td>Tissue samples (human)</td>
<td>Oct-4 and Nanog</td>
<td>Overexpression promotes EMT process, contributing to BCMD</td>
<td>BC therapy monitoring/prognostic and BCMD therapy monitoring/prognostic</td>
<td>[46]</td>
</tr>
<tr>
<td>Normalized gene expression data and frozen-archived tumor material from early-stage BC patients</td>
<td>41-gene signature</td>
<td>Predict DMFS and OS in node-negative BC</td>
<td>BC therapy monitoring/prognostic, BCMD therapy monitoring/prognostic</td>
<td>[47]</td>
</tr>
<tr>
<td>Tissue samples (human)</td>
<td>7-gene signature</td>
<td>Predict distant recurrence in TNBC phenotype undergone adjuvant chemotherapy</td>
<td>BC therapy monitoring/prognostic, BCMD therapy monitoring/prognostic</td>
<td>[42]</td>
</tr>
</tbody>
</table>

BC: Breast cancer; BCMD: Breast cancer metastatic disease; DMFS: Distance metastasis-free survival; EMT: Epithelial mesenchymal transition; OS: Overall survival; TamR: Tamoxifen resistance; TNBC: Triple-negative breast cancer.
The role of specific miRNA patterns is reported for BC & BCMD

miRNAs are small (18–25 nucleotides), noncoding and evolutionary conserved RNAs discovered originally in *Caenorhabditis elegans* [77,78]. miRNAs function by negatively regulating expression of human genes affecting a number of biological processes such as cell development, differentiation, proliferation and apoptosis [78,79].

miRNA molecules have a crucial role in tumorigenesis, which is complex, yet not entirely understood. Several miRNA species are reported as associated with metastatic disease in a spectrum of cancer types, for example, lymph node metastasis at early stages of squamous cell carcinoma [80], bone metastases spread by prostate cancer [81], gastric cancer development [82], colorectal cancer development and progression [83].

The role of miRNAs in BC and metastatic disease is reported with estimated high diagnostic and prognostic values [84–87]. Hence, miRNA are shown to regulate signaling pathways implicated in BC development and progression [88]. Further supporting this, interplay between chemokine (C–C motif) ligand 18 (CCL18), a promoter of BC metastasis and miR progression has been illustrated [88]. This study indicates miR-98 and miR-27b as downregulated by CCL18, which in turn creates a feedback loop that renders the CCL18-activated-N-Ras/ERK/PI3K/NFκB/Lin28b signaling pathway responsible for epithelial mesenchymal transition characteristic for metastatic activity [88]. Considering technological progress behind the issue, next-generation sequencing is used for detecting miRNA patterns specific for BC and their profiling in this patient cohort now [89,90]. Advanced technologies that utilize a profiling of circulating miRNAs (ct-miR) in blood may lead to a sufficient progress in BC diagnostics, due to minimally invasive and simplified sampling of blood, serum/plasma and urine [91,92]. Contextually, biobanking plays an important role in the field of miRNA research and prospective studies, which may lead to novel biomarker panels [93]. In order to bring miRNAs ‘from bench to bedside’, following deficits should be resolved: creation of robust prospective clinical trials, standardization of the analytical approach, detection of the exact origin of circulating miRNAs (tumor vs by-products of normal or dead cells) and validation of the disease-specific signature/miRNA patterns [94].
### Table 4. Predictive and prognostic miRNAs in breast cancer and metastatic disease.

<table>
<thead>
<tr>
<th>Biological material</th>
<th>Biomarker</th>
<th>Comments</th>
<th>Useful for</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line (human)</td>
<td>miR-10b</td>
<td>Highly expressed in TNBC and BCMD</td>
<td>BC therapy monitoring/prognostic and BCMD therapy monitoring/prognostic</td>
<td>[84,95]</td>
</tr>
<tr>
<td>Cell line (human, mouse), tumor samples, serum samples</td>
<td>miR-181a</td>
<td>Upregulated in BCMD; biomarker for early-stage diagnosis</td>
<td>BC early stages; BC therapy monitoring/prognostic; BCMD therapy monitoring/prognostic</td>
<td>[86,96–97]</td>
</tr>
<tr>
<td>Cell line (human)</td>
<td>miR-221/222</td>
<td>Specific TamR patterns</td>
<td>BC therapy monitoring/prognostic</td>
<td>[98,99]</td>
</tr>
<tr>
<td>Cell line (human)</td>
<td>miR-205</td>
<td>Downregulated in HER2+</td>
<td>BC therapy monitoring/prognostic</td>
<td>[100]</td>
</tr>
<tr>
<td>Cell lines, tumor samples</td>
<td>miR-30c</td>
<td>Promotes invasive potential in BC cells</td>
<td>BC therapy monitoring/prognostic</td>
<td>[101,102]</td>
</tr>
<tr>
<td>Breast tumor samples</td>
<td>miR-190-b</td>
<td>Highly upregulated in ER+ phenotype</td>
<td>BC therapy monitoring/prognostic</td>
<td>[85]</td>
</tr>
<tr>
<td>Tissue samples, cell line (human)</td>
<td>miR-126</td>
<td>Lower circulating levels/downregulated in BC</td>
<td>BC therapy monitoring/prognostic</td>
<td>[103–105]</td>
</tr>
<tr>
<td>Tissue samples</td>
<td>miR-29b</td>
<td>Low expression in primary tumors</td>
<td>BC therapy monitoring/prognostic</td>
<td>[106]</td>
</tr>
<tr>
<td>Tissue samples, serum, cell lines (human)</td>
<td>miR-21</td>
<td>Upregulated in BC</td>
<td>BC therapy monitoring/prognostic</td>
<td>[107–112]</td>
</tr>
<tr>
<td>Tissue samples, serum, cell lines (human), urinary</td>
<td>miR-155</td>
<td>Upregulated in BC and BCMD (HER2+ and TNBC)</td>
<td>BC therapy monitoring/prognostic; BCMD therapy monitoring/prognostic</td>
<td>[112–120]</td>
</tr>
<tr>
<td>Plasma sample, tissue</td>
<td>miR-210</td>
<td>Upregulated in BC and BCMD</td>
<td>BC therapy monitoring/prognostic; BCMD therapy monitoring/prognostic</td>
<td>[121–124]</td>
</tr>
<tr>
<td>Serum</td>
<td>Circulating 9-miR signature</td>
<td>Differentiate between early-stage ER+ BC and healthy controls</td>
<td>BC early stages</td>
<td>[87]</td>
</tr>
<tr>
<td>FFPE tumors</td>
<td>5-miR signature</td>
<td>Downregulated in BC patients with early tumor recurrence</td>
<td>BC therapy monitoring/prognostic</td>
<td>[125]</td>
</tr>
</tbody>
</table>

miRNA patterns relevant for BC are summarized in Table 4. Corresponding diagnostic and prognostic value is notified.

Proteomic patterns specific for BC predisposition, development & progression
Clinical differential proteomics as the promising tool for BC risk assessment
A new integrative concept for better BC management has been recommended in context of molecular patient profiling utilizing clinical differential proteomics [6]. This approach emphasizes the crucial importance of multilevel diagnostics and application of technologies complementary to the protein level of detection. Consequently, the overall task includes multimodal diagnostic approaches, disease-specific biomarker-patterns, individual patient profiles, creation of medical records and treatments tailored to the person [6]. Creation of the patient-specific 'molecular portrait' is an essential part of the diagnostic strategy. Furthermore, noninvasive analytical tools utilizing blood samples for clinical proteomics and complementary analytical technologies are well justified in case of BC, due to a direct relevance of molecular patterns in blood for BC pre-

Table 5. Biomarker candidates nominated for specific diagnostic purposes.

<table>
<thead>
<tr>
<th>Biological material</th>
<th>Biomarker</th>
<th>Comments</th>
<th>Useful for</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC cell line (human)</td>
<td>PFN1</td>
<td>Downregulated in BCMD</td>
<td>BC therapy monitoring/prognostic; BCMD therapy monitoring/prognostic</td>
</tr>
<tr>
<td></td>
<td>Annexin A6</td>
<td>Increased expression in BC</td>
<td>BC therapy monitoring/prognostic</td>
</tr>
<tr>
<td>FFPE tumor samples</td>
<td>Vimentin</td>
<td>Upregulated in TNBC</td>
<td>BC therapy monitoring/prognostic</td>
</tr>
<tr>
<td>Surgical specimen, blood samples, FFPE samples</td>
<td>MMP-9</td>
<td>Overexpressed in BC and BCMD</td>
<td>BC therapy monitoring/prognostic; BCMD therapy monitoring/prognostic</td>
</tr>
<tr>
<td>Frozen primary tumor samples</td>
<td>11-protein signature</td>
<td>Predict metastasis in TNBC</td>
<td>BC therapy monitoring/prognostic; BCMD therapy monitoring/prognostic</td>
</tr>
<tr>
<td>FFPE samples</td>
<td>10-protein expression panel</td>
<td>Stratify aggressive phenotype in familial BC</td>
<td>BC predisposition; BC therapy monitoring/prognostic</td>
</tr>
</tbody>
</table>

disposition, development and progression. Therefore, analysis of molecular patterns in circulating leucocytes as the minimally invasive diagnostic tool and construction of diagnostic windows based on individual patient profiling for efficient BC risk assessment is highly recommended [6]; the approach that is further demonstrated as clinically useful [126]. Consequently, a panel of functional groups of proteins involved in BC-related molecular mechanisms is provided (see Box 1) [6].

Furthermore, Table 5 summarizes protein biomarkers demonstrated as specific for BC at the level of predisposition to BCMD and diagnostically useful for individual BC phenotypes. Alterations specific for BC are notified and diagnostic/prognostic utility is described, respectively.

Metabolomics demonstrates a high potential of clinical utility in BC management

Well-detectable metabolic differences have been described as specific for BC and metastatic disease [138–141]. Consequently, metabolomics profiling is considered as being a powerful methodology for prediction and prognosis in BC development and progression [142,143]. Metabolomics is the technological tool analyzing health and disease stage-specific metabolite profiles in biological samples such as body fluids and tissue homogenates. The approach entails utilizing qualification and quantification of biomarkers in individual and comparative metabolic patterns. The most clinically useful approaches result in metabolite profiling and metabolic fingerprinting. Metabolite profiling is currently utilized for diagnostics of a whole spectrum of cancers [144]. Thereby, patient stratification is possible regarding cancer predisposition and cancer subtype followed by more precise prognosis and targeted therapy approaches [145–148]. Metabolomics employs highly sensitive technologies based on NMR spectroscopy and mass spectrometry. The methodology is well standardized with an excellent level of results' reproducibility that is decisive for its successful clinical application. Table 6 summarizes metabolic biomarkers specific for BC diagnosis and prognosis. According to currently published reports, following metabolic pathways are certainly involved in BC pathology: gluconeogenesis, tricarboxylic acid cycle, pentose phosphate pathway [149], serine/glycine pathway [140], glutamine and beta-alanine metabolism [146]. Many others are currently under consideration that further increases the potential of clinical utility for metabolomics in the overall BC management.

Table 6. Metabolites and metabolic pathways involved in breast cancer pathology.

<table>
<thead>
<tr>
<th>Biological material</th>
<th>Biomarker</th>
<th>Comments</th>
<th>Useful for</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Low levels of histidine, higher levels of glucose and lipids</td>
<td>Predict disease relapse in early stage of BC</td>
<td>BC therapy monitoring/prognostic</td>
<td>[138]</td>
</tr>
<tr>
<td>Urine, peripheral venous blood sample</td>
<td>PGE2</td>
<td>Increased levels in BC associated with increased BC risk</td>
<td>BC therapy monitoring/prognostic</td>
<td>[142–143,150]</td>
</tr>
<tr>
<td>Tissue samples, cell lines (human)</td>
<td>Glutamine metabolism</td>
<td>Enhanced in ER- and TNBC phenotypes and is associated with reduced patient survival in HER2+ phenotype</td>
<td>BC therapy monitoring/prognostic</td>
<td>[141,145–148]</td>
</tr>
<tr>
<td>Cell lines, tissue samples</td>
<td>Glycine/serine metabolism</td>
<td>Pathway highly activated in BC</td>
<td>BC therapy monitoring/prognostic</td>
<td>[139–140,151]</td>
</tr>
<tr>
<td>Tissue samples</td>
<td>Glycine</td>
<td>High levels in HER2+ phenotype</td>
<td>BC therapy monitoring/prognostic</td>
<td>[141]</td>
</tr>
</tbody>
</table>

BC: Breast cancer; TNBC: Triple-negative breast cancer.

Table 7. Circulating tumor cells in breast cancer prediction and prognosis.

<table>
<thead>
<tr>
<th>Biological material</th>
<th>Biomarker</th>
<th>Comments</th>
<th>Useful for</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral whole blood sample</td>
<td>CTC enumeration</td>
<td>Predict treatment outcome in BCMD</td>
<td>BC therapy monitoring/prognostic; BCMD therapy monitoring/prognostic</td>
<td>[168]</td>
</tr>
<tr>
<td>Cell lines (human) and peripheral blood sample</td>
<td>PLS3</td>
<td>High expression in CTC undergoing EMT</td>
<td>BC therapy monitoring/prognostic</td>
<td>[170]</td>
</tr>
<tr>
<td>Blood sample</td>
<td>CD133-expressing CTCs</td>
<td>More frequent in luminal tumors</td>
<td>BC therapy monitoring/prognostic</td>
<td>[172]</td>
</tr>
</tbody>
</table>

BC: Breast cancer; BCMD: Breast cancer metastatic disease; CTC: Circulating tumor cell; EMT: Epithelial mesenchymal transition.
Cellular level
Circulating tumor cells
CTCs, dislocated from a cancerous tissue via blood flow, demonstrate high metastatic potential nesting distanced organs and developing secondary tumors of particular aggressive phenotypes. Clinical interest in the CTCs research field is permanently increasing, due to two main reasons: first, CTC can be detected by minimally invasive approach in peripheral blood and second, a lot of diagnostic information can be received for more precise prediction and prognosis [152]. Information provided by CTCs has been recently demonstrated to have an independent prognostic value in metastatic disease [153], in monitoring treatment response [154,155], efficacy of chemotherapy [156] and prediction of recurrence [157]. Despite low density of CTC in circulating blood flow, innovative detection technologies allow for an accurate tracing of CTC and their representative isolation from patients’ blood samples [158,159]. Clinical application of CTC is considered for a spectrum of cancer types [160–166]. BC is demonstrated as spreading the highest number of CTC in blood flow [152] followed by high CTC level in prostate and other cancer types [167]. Contextually, this approach is well promising in diagnosis and prognosis of breast and prostate cancer metastatic disease. Hence, a prospective study on 393 metastatic BC patients revealed that serial CTC enumeration and related parameters such as changes in CTCs over a course of treatment are helpful for efficient monitoring of the disease progression and treatments tailored to the patient [168]. Furthermore, recent studies on Plastin3, a novel biomarker for CTCs detection [169], highlight that, first, it is of great clinical utility in BC regarding the risk of recurrence and second, it is a marker for poor prognosis especially relevant for TNBC cases [170]. CTCs could also play an important role in the detection of BC at early stages and associated pathology development [171]. Table 7 summarizes potential CTC-related biomarker candidates useful for BC and BCMD prognosis and therapy monitoring.

Cancer stem cells
CSCs are also known as tumor-initiating cells. CSC populations are highly heterogeneous with a particular potential for reproduction and generation of cells differentiated into cancer phenotypes [173,174]. It has been

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**Table 8. Cancer stem cells in breast cancer therapy monitoring and prognosis.**

<table>
<thead>
<tr>
<th>Biological material</th>
<th>Biomarker</th>
<th>Comments</th>
<th>Useful for</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue samples [171,172] and cell line (human [172])</td>
<td>CD44+/CD24−</td>
<td>Marker for basal-like tumors, associated with more aggressive phenotype</td>
<td>BC therapy monitoring/prognostic</td>
<td>[190,191]</td>
</tr>
<tr>
<td>Tissue samples [172,173] and cell line (human [172])</td>
<td>ALDH1+</td>
<td>Marker for basal-like and HER2+ overexpressing tumors</td>
<td>BC therapy monitoring/prognostic</td>
<td>[191,192]</td>
</tr>
</tbody>
</table>

BC: Breast cancer.

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**Figure 1. Potential biomarker panels in breast cancer management.**

BC: Breast cancer; BCMD: Breast cancer metastatic disease.
suggested that bulk of BC tumor consists of differentiated CSC [175–179]. Furthermore, CSCs strongly modulate (increase) tumor drug resistance [180–182]. Consequently, current CSC-related studies are dedicated to innovative therapy approaches targeted specifically to the CSC populations [183–185].

Breast cancer stem cells are known to be highly tumorigenic. They are characterized by increased chemoresistance compared with the differentiated BC cells [181,182]. They are involved in local invasion and migration being responsible for metastatic activity [186], aggressive phenotype in HER2-overexpressing breast cancer stem cells [187] and altered drug resistance [188]. The most common phenotypes used for identification of CSC in BC are CD44+/CD24-low/- and elevated ALDH [189]. Specifically in BC, well-characterized detail of CSCs may result in promising adjuvant therapies tailored to stratified BC subtypes and individual CSC populations. CSC biomarker patterns specific for BC are summarized in Table 8. Corresponding clinical utility is noted.

### Conclusion

Breast cancer is a multifactorial disease with highly heterogeneous risk factors, origin, individual phenotypes, and consequently, different molecular profiles, sensitivity to therapeutic approaches, and frequently unpredictable clinical outcomes. Breast cancer metastatic disease is the major cause of about a half of million deaths annually registered for the BC patient cohort worldwide. There are evident deficits in the overall BC management. Consequently, a paradigm change towards predictive, preventive, and personalized medicine (PPPM) in the overall breast cancer management is essential and urgent. For the most effective implementation of advanced PPPM services in the field of breast cancer, an innovative approach of multilevel diagnostics is recommended.

### Future perspective

BC is a multifactorial disease with highly heterogeneous risk factors, origin, individual phenotypes and consequently, different molecular profiles, sensitivity

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**Executive summary**

**Deficits in breast cancer management & expectations by multilevel diagnostics**

- Current deficits in breast cancer (BC) management include suboptimal screening programs demonstrating a number of cases with both false-positive and -negative diagnosis, an evident lack of screening programs focused specifically on BC predisposition and initiation in young subpopulations, individualized patient profiling for more precise diagnosis and treatments tailored to the person that altogether result in enormous socioeconomic burden.
- Furthermore, there is an evident lack of articles providing evidence and summarizing biomarker panels for multilevel diagnostic approaches, which are particularly promising to optimize the overall BC management.
- The crucial role of integrative bioinformatics in development and successful clinical application of innovative approaches by multilevel diagnostics is discussed.

**Crucial role of *omics in BC diagnostics & treatment**

- Next-generation sequencing platform provides multitude of genomic data including probabilistic approach for cancer predisposition, discovery of cancer-related genes/pathways and structural DNA modification (mutations, changes of corresponding gene copy number and chromosomal rearrangements), analysis of intratumoral heterogeneity and drug resistance.
- Gene regulation level comprises a number of complementary approaches and allows for more precise diagnosis and targeted treatments in BC.
- Epigenetic deregulation in BC pathology has been detected by ‘epigenome-wide maps’; an importance of DNA methylation in BC patient stratification, prediction and prognosis is evidence-based.
- The role of miRNAs in BC and metastatic disease is reported with estimated high diagnostic and prognostic values. However, in order to bring miRNAs ‘from bench to bedside’, following deficits should be resolved: creation of robust prospective clinical trials, standardization of the analytical approach, detection of the exact origin of circulating miRNAs (tumor vs by-products of normal or dead cells) and validation of the disease-specific signature/miRNA-patterns.
- Creation of the patient specific ‘molecular portrait’ is an essential part of the diagnostic strategy: differential proteomics is the promising tool for that.
- Metabolomics demonstrates a high potential of clinical utility in BC management.

**Cellular level**

- Clinical interest in the circulating tumor cells is permanently increasing due to two main reasons: first, circulating tumor cells can be detected by minimally invasive approach in peripheral blood, and second, a lot of diagnostic information can be received for more precise prediction and prognosis.
- Breast cancer stem cells (BCSCs) are highly tumorigenic being responsible for metastatic activity. Individual characterization of BCSCs may result in promising adjuvant therapies tailored to stratified BC subtypes and individual BCSC populations.
to therapeutic approaches and frequently unpredictable clinical outcomes. Currently, there are evident deficits in the overall BC management such as suboptimal screening programs differentiating early versus late adulthood and the elderly, lack of the targeted prevention specifically in teenager age and young subpopulations, patient stratification for more precise diagnosis and treatments tailored to the person. Over the next 5–10 years, the overall BC management will remarkably evolve utilizing the paradigm change from reactive to PPPM. The most effective implementation of advanced PPPM services includes:

- Innovative approaches of multilevel diagnostics considered in this paper – see Figure 1;
- Application of individualized patient profiles [6,126,193].

References
Papers of special note have been highlighted as:
• of interest; ** of considerable interest


** Demonstrating an urgent necessity for paradigm shift in the overall breast cancer management from reactive to predictive, preventive and personalized medical services, summarizing all-known breast cancer-related risk factors and providing recommendations for innovative multilevel diagnostic approaches.


** Summarizing protein biomarker panels specific for breast cancer development and progression and providing complete information about functional groups involved in BC pathology; the paper useful for creation of individualized patient profiles, targeted prediction and prevention of breast cancer.

- Creation of optimized screening programs [6,126];
- Development of novel biomarker panels for BC therapeutics [194];
- Application of multidrug treatments tailored to the person [195].

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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** Demonstrating high level of clinical utility for multidimensional (multomics) data to identify the cancer-related pathways and to improve the precision of diagnosis.


17 Pauling JK, Christensen AG, Batra R et al. Elucidation of epithelial–mesenchymal transition-related pathways in a...
• Summarizing the role of next-generation sequencing in breast cancer diagnostics, providing information for novel targets for treatments and disease monitoring tailored to the person.


33 Li K, Li W, Dong X. Association of 677 C>T (rs1801133) and 1298 A>C (rs1801131) polymorphisms in the MTHFR gene and breast cancer susceptibility: a meta-analysis based on 57 individual studies. PLoS ONE 9(6), e71290 (2014).


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**Summarizing epigenetic networks and demonstrating biological value and potential clinical utility of epigenome-wide maps for early breast carcinogenesis.**


77 Krishnan P, Ghosh S, Wang B et al. Next generation sequencing profiling identifies miR-574-3p and miR-660-5p


**Summarizing the role of miRNA panel in breast cancer diagnosis and treatment considering the mechanisms of tumor initiation, promotion of breast cancer metastatic disease and drug resistance as well as targeting new therapies.**


•• Demonstrating clinical utility of multilevel diagnostics and providing recommendations for implementation of biomarker panels by innovative minimal-invasive blood analysis.


136 Liu NQ, Stirling C, Look MP et al. Comparative proteome analysis revealing an 11-protein signature for aggressive triple-


** Presenting innovative technology for breast cancer risk assessment and providing recommendations for clinical application.


Management of women with a hereditary predisposition for breast cancer

Ismail Jatoi* & John R Benson2

Women with a hereditary breast cancer predisposition have three management options: screening, chemoprevention (risk-reducing medication) and risk-reducing surgery. However, no randomized trials have addressed the effect of these strategies in mutation carriers. In the general population, randomized trials failed to demonstrate a benefit for screening in premenopausal women. Moreover, although chemoprevention reduces breast cancer incidence in high-risk populations, this benefit is potentially confined to estrogen receptor-positive tumors. Finally, observational studies suggest that prophylactic mastectomy and even prophylactic salpingo-oophorectomy reduces breast cancer risk in BRCA mutation carriers, but there are systematic biases associated with such studies. Therefore, women with a hereditary predisposition for breast cancer should be informed of the three risk-reducing strategies, and that their benefits are not fully understood.

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Hereditary predisposition indicates innately increased susceptibility to development of a disease based on genetic makeup and in particular specific germline mutations passed from one generation to another [1]. These genetic changes are not necessarily causative and phenotypic manifestations of cancer are variable. In recent years there has been an upsurge of interest in identifying women with genetic predisposition and this has been facilitated by enhanced understanding of the genetic basis for breast cancer susceptibility derived from genome-wide linkage analysis and mutational screening [2]. The focus has shifted from high-penetrance genes to intermediate- and low-penetrance mutations that confer lower levels of risk individually but collectively are more significant [3]. There has been frenzied public interest in genetic testing and risk reduction strategies following revelation in the New York Times in May 2013 that the famed actress Angelina Jolie had chosen to undergo bilateral prophylactic mastectomy (BPM) due to carriage of a BRCA1 gene mutation [4]. Genetic counseling and testing for breast cancer predisposition has been widely implemented in many countries and the number of women seeking gene testing continues to rise. Nonetheless, despite these advances in genetics, approximately 30% of the familial breast cancer risk remains unaccounted for by mutations in currently known genes [5].

Risk factors for breast cancer
The three major risk factors for development of breast cancer are gender, age and family history/genetic susceptibility [6,7]. Gender is the single greatest risk factor with breast cancer being...
100-fold more common in women than men [7,8]. Thus approximately 12.7% of all women in the USA will be diagnosed with breast cancer (one in eight) compared with a figure of only 0.1% for men [7]. The second most important risk factor is age, with disease incidence rising dramatically during the fourth and fifth decades but less so after age 50 years (Clemmenson’s hook) and evidence of incidence leveling off after the age of 75–80 years [8]. This effect of ageing is illustrated by comparisons of estimated risk levels for younger versus older women. Thus for a 30-year-old woman, the risk of developing breast cancer during the next 10 years of life is 0.44% (one in 227) but the corresponding risk for a 70-year-old woman is 3.82% (one in 26) – a tenfold difference [8]. Finally, family history and genetic predisposition are associated with variable levels of risk depending on intensity of familial clustering (numbers of first-degree relatives with early onset/bilateral breast cancer) and identification of specific gene mutations [9].

The etiology of breast cancer is multifactorial and the vast majority of cases are nonhereditary (sporadic) with no identifiable predisposing gene mutation [10]. Nonetheless, environmental and lifestyle factors are important determinants of risk which can potentially be modified to reduce overall risk for development of breast cancer [11]. Thus, maintaining a healthy weight, undertaking regular exercise, limiting alcohol intake, avoiding/cessation of smoking and participation in regular screening may potentially help minimize risk in those individuals with a genetic predisposition [12]. There is evidence that environmental and lifestyle factors can modify risk in mutation carriers; for example, a recent population-based study in Israel found that breast cancer risk amongst BRCA1 or BRCA2 mutation carriers was much higher for women born after 1958 when compared with those born prior to this date [13]. Changes in lifestyle are could potentially be responsible for these differences between birth cohorts. It is tempting to speculate that contemporary levels of obesity have increased breast cancer among mutation carriers. However, it is also important to note that this study relied on hospital records to discern whether family members were diagnosed with breast or ovarian cancer in cohorts born before 1958 and after 1958. Thus, it is also possible that the higher incidence rates in the later cohort might be due to differences in screening and diagnostic methods between these two cohorts.

- **High-risk predisposing genes**

  **BRCA1 & BRCA2**

Although many individuals may possess a relative with breast cancer and therefore by definition have a positive family history for the disease, only about 10% of all breast cancers are currently attributable to germline mutations [2]. Of particular relevance are the high-penetrance mutations, and these have an autosomal dominant pattern of inheritance consistent with vertical transmission of high-risk genetic predisposition from one generation to another [9]. The precise level of risk and the probability of harboring a mutation in a recognized high-penetrance gene is determined by family history of breast (and some other) cancers [2]. A method termed linkage analysis was previously used to calculate lifetime risk for development of breast cancer based on information from family cancer history such as the number of affected first and second degree relatives with breast/ovarian cancer, age of onset and bilateral breast cancer [14]. Hereditary predisposition occurs in only about 10% of all cases, and is conferred by cancer susceptibility genes that conform to Knudson’s model or ‘two-hit hypothesis’ [15,16]. Approximately half of mutations in hereditary breast cancer involve the breast cancer genes BRCA1 and BRCA2 [2]. These are large tumor suppressor genes located on chromosomes 17q21 and 13q12 respectively and mutated in approximately 1/300 individuals [17]. Among BRCA1 mutation carriers, the average cumulative risks for breast and ovarian cancer by 80 years of age are about 67 and 45% respectively, while for BRCA2 mutation carriers, the average cumulative risks for breast and ovarian cancer are 66 and 12%, respectively [2]. However, there is considerable variation in reported magnitude of risk for breast and ovarian cancer among BRCA1 and BRCA2 mutation carriers with risk partly dependent on precise location of the mutation within the gene [18]. Initial estimates of risk were based on studies of selected high-risk families whilst more recent estimates are derived from population-based studies and these are generally associated with lower levels of risk.

At the cellular level, the effects of BRCA1 and BRCA2 are recessive and both copies of an allele must be lost or mutated for cancer to develop. Individuals with a germline mutation in these genes have a dominantly inherited susceptibility and the second hit occurs in the somatic copy. Over 75% of breast cancers that develop
in BRCA1 mutation carriers are estrogen receptor (ER)-negative, and 69% are triple-negative (ER-negative, progesterone receptor-negative and EGFR2-negative) [2]. By contrast, 77% of breast cancers in BRCA2 mutation carriers are ER-positive, and only 16% are triple-negative [2]. Women with triple-negative breast cancer under the age of 50 years have a much greater likelihood of harboring a BRCA1 gene mutation, potentially justifying screening for BRCA mutations [19].

It should also be noted that after an initial diagnosis of unilateral breast cancer, BRCA1 and BRCA2 mutation carriers have an elevated risk of developing contralateral breast cancer and this has therapeutic implications [20]. For these patients, bilateral mastectomy should be considered as an option [20].

Other high-risk genes
Besides BRCA1 and BRCA2 mutations, there are several other gene mutations that dramatically increase the risk of developing breast cancer (high-penetrance mutations). These include mutations in the STK11 (Peutz-Jeghers syndrome), pTEN (Cowden’s syndrome), TP53 (Li-Fraumeni syndrome), CDH1 (diffuse hereditary gastric cancer syndrome) and PALB2 (partner and localizer of BRCA2) genes [9,21]. These mutations are generally associated with other malignancies and disease manifestations. Thus, the STK11 mutation is associated with gastrointestinal polyposis and breast cancer, and the pTEN mutation with thyroid cancer and breast cancer. The TP5 mutation is associated with numerous other cancers such as sarcomas, brain tumors and gastrointestinal tumors in addition to breast cancer. The CDH1 mutation is linked with gastric cancer and invasive lobular breast cancer and the PALB2 mutation with breast cancer. No further high-penetrance genes have been identified from genome-wide linkage studies and it seems unlikely that any other major genes in this class exist [22]. Mutations of TP53 are common in many cancers and promote tumor growth by removing a ‘brake’ on cell proliferation [23]. Germline TP53 mutations are associated with a lifetime risk for development of breast cancer in excess of 50% [24]. The PALB2 gene encodes for a protein that interacts with the BRCA2 gene product to repair damaged DNA and maintain fidelity of DNA replication [21]. Mutations of the PALB2 gene are associated with a breast cancer risk of 35–40% by age 70 years which is slightly lower than for BRCA2 mutations [21]. The pTEN gene is also involved in growth regulation and mutations confer a more variable level of risk ranging from 20 to 50% [25].

- Low/intermediate-risk predisposing genes
In addition to the BRCA1 and BRCA2 mutations, the high-penetrant gene mutations discussed above individually confer high lifetime risks (generally greater than 50%), but collectively these mutations are estimated to account for no more than 20–25% of the familial aggregation of breast cancer [26,27]. Much of the residual unexplained familial cancer risk is likely to be due to low- and moderate-penetrance mutations [27]. Technological advances and mapping of single nucleotide polymorphisms that are common genetic variants has permitted comprehensive analyses with identification of potential candidate genes such as CHEK2, ATM, FGFR and TNC9 [28]. CHEK2 and ATM are considered moderate-risk gene mutations whereas mutations in the FGFR and TNC9 genes confer low risk. Protein-truncating mutations in CHEK2 confer a lifetime breast cancer risk of about 20–30% but probably contribute no more than 3% to familial relative risk [29].

Genetic testing & risk prediction
Breast cancer risk assessments falls into two distinct categories: assessment of the risk of carrying a mutation that increases breast cancer risk, and assessment of breast cancer risk with or without such a mutation [30]. Genetic testing represents a great advance in provision of accurate risk quantitation. Once a mutation has been identified for a particular family, that individual has a very high chance of developing breast cancer without some form of intervention. Herein lies a potential dilemma; when women consent to genetic testing, they must be counseled appropriately and able to cope with the information gained from the test – whatever the result [31]. Women who wish to consider genetic testing should be referred to genetic counselors and a detailed family history obtained to determine whether genetic testing is warranted and which specific mutations should be sought. Counseling should take account of broader issues such as personal medical history as well as ethnicity and cultural influences. NICE has recently recommended that women undergo genetic testing when the chance of finding a high-penetrance mutation is 10% (reduced from the previous
mandate of 20%) [32]. Most women overestimate their risk and genetic testing allows accurate risk assessment that more confidently informs any proposed management decisions [33].

Three categories of mutation are recognized – positive (deleterious) pathogenic mutations, no mutations and variations of uncertain clinical significance. When a variation of uncertain clinical significance is found on genetic testing, the associated cancer risk is unknown and therefore default back to indirect risk assessment is necessary using models such as Claus, Boadicea and Tyrer-Cuzik [30]. Testing with low-penetrance gene panels can create potential clinical dilemmas. How will results of these tests lead to a change in management? Is the information useful? An individual may have a very strong family history of breast cancer, yet the panel is negative. Genetic testing should be offered to the person affected first; if a mutation, for example, in CHEK2 is found in the mother but not the individual, is their particular risk the same as the general population or a higher level of risk (20–30%) based on family history of breast/ovarian cancer (and determined by other as yet nonidentified gene mutations). There are no trials of low-penetrance genes and patients should be warned that there is much uncertainty regarding the significance of any result. It remains unclear whether newer techniques for genetic testing improve risk prediction as compared with already existing methods based on family history and empiric risk assessment tools. Ultimately, genetic testing must yield clinically actionable results. The challenge at present is how to maximize the yield of clinically actionable results whilst minimizing results that may confuse patients with little chance of benefit.

Management options for women with high-penetrance mutations
Once a gene mutation is identified in an asymptomatic woman, three management options should be discussed:

- Screening;
- Chemoprevention (risk-reducing medication);
- Risk-reducing surgery.

Management of these high-risk women can be challenging and sometimes a woman may choose a combination of more than one option depending on personal circumstances and estimated levels of risk. For instance, a BRCA1 mutation carrier may elect to undergo breast cancer screening until she has had her children and completed breastfeeding. She may subsequently opt for risk-reducing surgery targeted at both breasts and ovary (bilateral mastectomy with immediate reconstruction and bilateral salpingo-oophorectomy).

- Screening

Although no randomized trials have specifically addressed the efficacy of breast cancer screening in mutation carriers, there are trials that have examined mortality benefits of screening in the general population [34]. At total of nine trials have examined the effectiveness of mammography screening and there are two trials that have examined the efficacy of screening breast self-examination (BSE). The two trials on screening BSE were undertaken in Shanghai, China and St Petersburg, Russia, and showed that screening BSE had no benefit in reducing breast cancer mortality [35,36]. Additionally, two trials in India are currently assessing the potential value of screening clinical breast examination, and mortality data are eagerly awaited from these trials [37,38].

Meta-analyses of mammography screening trials demonstrate reductions in breast cancer-specific mortality of about 20% for the intervention group, with much of the benefit confined to women aged 50–69 years of age at trial entry [34]. It should be noted that most mutation carriers seek screening prior to age 50 years, and the efficacy of mammography screening in this age group has not been conclusively demonstrated. Indeed, two mammography screening trials (Canadian National Breast Screening Study 1 and the UK Age trial) were specifically designed to assess the efficacy of mammographic screening in women below aged 50 years and neither showed any benefit [39,40]. Moreover, improvements in therapy are likely diminishing both absolute and relative benefits of population-based mammography screening [41]. This is consistent with the observed trends over time in the mammography screening trials. The first randomized trial of breast cancer screening (Health Insurance Plan, NY, USA) was initiated in 1963 and demonstrated that mammography screening could reduce breast cancer mortality by about 25–30% [42]. Of note, adjuvant systemic therapy was not generally available to patients during the era of the Health Insurance Plan trial since which time there has been an incremental
Chemoprevention (risk-reducing medication)

This is a potential means of reducing breast cancer risk in mutation carriers, but once again there is no randomized trial data that has specifically addressed the efficacy of breast cancer risk-reducing medication in mutation carriers. Nonetheless, these trials of risk-reducing medication (Table 1) have addressed the benefits of these medications in women at high risk for breast cancer amongst whom there would be some BRCA mutation carriers. Placebo-controlled trials have evaluated both selective ER modulators in pre- and post-menopausal women and aromatase inhibitors in postmenopausal women. Trials of tamoxifen have revealed a reduction of about 50% (ranging from 31 to 67%) in the cumulative incidence of both invasive and noninvasive breast cancer at a median follow-up ranging from 96 to 158 months [46–49]. It has not been fully established if the benefit of risk-reducing medication is confined to lowering risk of only ER-positive breast cancers in mutation carriers [50]. Risk reductions in excess of 50% were noted for BRCA2 but not BRCA1 mutation carriers in the NSABP P-1 study which may relate to ER positivity of tumors in BRCA2 mutation carriers, but there were very few known BRCA mutation carriers in this study [51]. The International Breast Intervention Study (IBIS)-1 likewise randomized high-risk pre- and postmenopausal women to either tamoxifen or placebo with some women having used hormone replacement therapy and about one-third having undergone hysterectomy [52]. At 20 years follow-up breast cancer incidence was significantly lower in the intervention group (7.8%) compared with the control arm (12.3%) with a sustained benefit from tamoxifen beyond the initial 5-year pulse of treatment. An update of the head-to-head comparison of tamoxifen and another selective ER modulator (raloxifene) in the STAR trial confirms that there is some attrition of efficacy of raloxifene relative to tamoxifen with a significantly increased risk of invasive cancer in the raloxifene arm [53]. Aromatase inhibitors (alone or sequence before/after tamoxifen) have been shown to have marginally improved antitumor efficacy than tamoxifen alone and are associated with notably greater reductions in contralateral cancer – up to 70% compared with 50% for tamoxifen [54]. Although aromatase inhibitors are associated with a lower risk of thromboembolism compared with tamoxifen, there is an increased risk of fractures and arthralgias. The potential side effects of aromatase inhibitors has led to some caution in pursuance of risk-reducing strategies based on these agents [55]. Nonetheless, based on positive results from the MAP3 trial that examined the benefit of exemestane (25 mg daily for 5 years) versus placebo in a randomized comparison of 4560 high-risk postmenopausal women, current guidelines for use of risk-reducing medications incorporate an aromatase inhibitor option (see Table 1) [56]. Thus tamoxifen (20 mg per day orally for 5 years) should be discussed as an option for risk reduction in pre- and postmenopausal women, whilst a similar recommendation applies to raloxifene (60 mg per day orally for 5 years) for postmenopausal women only. Furthermore, American Society of Clinical Oncology (ASCO) guidelines now acknowledge the aromatase inhibitor exemestane (25 mg per day orally for 5 years)
as an additional option for breast cancer risk reduction in postmenopausal women.

Although clinical trials have shown these agents can reduce the risk of developing breast cancer, no mortality benefit has been conclusively demonstrated. Moreover, as previously indicated, there remains some concern that these agents might only be effective in the primary prevention of ER-positive breast cancer and are perhaps ineffective in prevention of ER-negative disease. Thus, in the context of BRCA mutation carriers, these agents might be recommended for patients with BRCA2 mutations, but not for those who carry a BRCA1 abnormality. As mentioned above, approximately 77% of breast cancers in the BRCA2 cohort are ER positive (comparable to the general population) while three quarters of breast cancers developing in BRCA1 mutation carriers are ER negative and 69% are triple-negative breast cancer.

**Surgery**

For women with a genetic predisposition to breast cancer, risk-reducing surgery has been associated with the greatest potential benefit in terms of decreasing the chance of developing breast cancer. Bilateral prophylactic surgery seems to reduce the expected risk of breast cancer by more than 90% among carriers of a gene mutation [44]. In a seminal retrospective study of more than 600 women at moderate to high risk who had undergone BPM, the incidence of breast cancer was reduced by 89.5% (p < 0.001) [57]. An established Gail model for risk estimation was used to predict the number of breast cancer cases expected in these two groups without surgical intervention. This together with several smaller studies have validated BPM as a management option for women at increased risk for development of breast cancer [44]. However, there are no randomized trials that have specifically examined the effectiveness of risk-reducing surgery in mutation carriers. Nonetheless, both prospective and retrospective cohort studies indicate that BPM is associated with reduced breast cancer risk of more than 90% in BRCA1 and BRCA2 mutation carriers [10]. Moreover, among those women with a BRCA gene predisposition, bilateral prophylactic salpingo-oophorectomy (BSO) was associated with ovarian and other BRCA-related adnexal cancer risk reductions of between 80 and 96% [58]. It should be noted that BSO (rather than bilateral oophorectomy alone) is the recommended surgical procedure for risk-reduction in BRCA1 and BRCA2 mutation carriers because these women are also at increased risk for development of fallopian tube cancers.

Systematic biases potentially threaten the validity of observational studies and several of these have relevance to studies on risk-reducing surgery. These include performance bias, detection bias and selection bias [10]. The first of these may result if the performance of a specific risk-reducing operation is not confirmed in an objective manner (i.e., from checking of medical or surgical records) and investigators are reliant instead on self reports. Attrition bias may result if follow-up of patients who undergo risk-reducing surgery is different from the control group [59]. For example, patients having risk-reducing surgery might be discharged from further follow-up while nonsurgical patients might continue with regular follow-up and monitoring. This could lead to under-reporting of malignancies in the risk-reducing surgery group. Detection bias may result if follow-up of patients who undergo risk-reducing surgery is different from the control group [59]. For example, patients having risk-reducing surgery might be discharged from further follow-up while nonsurgical patients might continue with regular follow-up and monitoring. This could lead to under-reporting of malignancies in the risk-reducing surgery group. Detection bias may result if outcomes are not assessed consensually for both groups in the study [60]. For example, women who undergo risk-reducing mastectomy are generally not referred for any subsequent breast imaging whilst controls continue to be screened. In consequence, detection of higher rates of occult cancers would be expected in the control group. Finally, the baseline characteristics of women undergoing risk-reducing surgery

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**Table 1. Trials of chemopreventive agents.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Women (n)</th>
<th>Median follow-up (months)</th>
<th>Relative risk (95% CI)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSABP P-1</td>
<td>13,338</td>
<td>84</td>
<td>0.38 (95% CI: 0.28–0.5)</td>
</tr>
<tr>
<td>Royal Marsden</td>
<td>2471</td>
<td>158</td>
<td>0.6 (95% CI: 0.43–0.86)</td>
</tr>
<tr>
<td>IBIS-I</td>
<td>7139</td>
<td>96</td>
<td>0.66 (95% CI: 0.5–0.87)</td>
</tr>
<tr>
<td>Italian</td>
<td>5408</td>
<td>132</td>
<td>0.77 (95% CI: 0.51–1.16)</td>
</tr>
<tr>
<td>STAR</td>
<td>19,747</td>
<td>81</td>
<td>1.24 (95% CI: 1:05–1.47)</td>
</tr>
<tr>
<td>IBIS-II²</td>
<td>3864</td>
<td>60</td>
<td>0.47 (95% CI: 0.5–0.87)</td>
</tr>
<tr>
<td>MAP3³</td>
<td>4560</td>
<td>35</td>
<td>0.35 (95% CI: 0.18–0.70)</td>
</tr>
</tbody>
</table>

¹Estrogen receptor-positive breast cancer.
²Postmenopausal women.
may differ from those who do not have surgical intervention, thus introducing an element of selection bias. For example, women having risk-reducing surgery might be of higher socio-economic status with better access to healthcare that can potentially influence measured outcomes. Collectively, these various sources of bias may lead to over-estimation of the benefit of risk-reducing surgery.

It is claimed that BSO not only reduces ovarian cancer risk, but also decreases breast cancer risk by 50% among mutation carriers when undertaken during the premenopausal years [61]. This effect is presumably due to estrogen deprivation and epidemiological studies have shown that for unselected patients, ovarian ablation reduces the risk of breast cancer in premenopausal women by up to two-thirds. When risk-reducing surgery is combined (BPM and BSO) the net reduction in risk of developing breast cancer is 95%. Once again, these estimates of benefit should be interpreted with caution and recent analyses have suggested that much of the apparent benefit of BSO on breast cancer risk might be accounted for by selection bias rather than being a true reduction in risk [62]. However, others continue to argue that BSO does indeed have benefit in reducing breast cancer risk in BRCA mutation carriers [63]. Nonetheless, oophorectomy should not be recommended specifically to reduce breast cancer risk (in conjunction with surveillance imaging) and risk-reducing mastectomy should remain the optimal approach for minimizing risk of developing breast cancer in BRCA1 and BRCA2 mutation carriers.

Notwithstanding these limitations of observational studies, it is highly unlikely that there will ever be randomized evidence available to assess the efficacy of risk-reducing surgery in mutation carriers. The decision to proceed with risk-reducing surgery is greatly influenced by an individual’s personal beliefs and values and a woman is unlikely to relinquish her right to self-destiny in this respect. Therefore future management strategies will be based on observational studies that have consistently shown huge benefits in terms of risk-reduction from surgical ablation.

**Contralateral prophylactic mastectomy in mutation carriers**

Several of these issues relating to BPM are pertinent to CPM in patients with a proven BRCA1/2 gene mutation. It is clear that the latter group of patients have an elevated risk of developing breast cancer due to an inherent genetic predisposition present within all cells of the contralateral breast tissue. If a BRCA1 or BRCA2 mutation carrier without any personal history of breast cancer is considered eligible for BPM, then a CPM in the same individual must be easier to justify on statistical probability for breast cancer development [10]. The risk of developing contralateral breast cancer in mutation carriers appears to be three- or four-times greater than that of average risk cohorts [64]. Moreover, a recent study from The Netherlands suggests that age at diagnosis of the first breast cancer is a significant predictor of contralateral breast cancer risk in BRCA1 and BRCA2 mutation carriers [65]. Those diagnosed with breast cancer before age 41 years had a 10-year contralateral breast cancer risk of 23.9% compared with 12.6% for those diagnosed 41–49 years of age. Thus mutation carriers who present with unilateral breast cancer should generally consider bilateral mastectomy with immediate breast reconstruction rather than breast conserving surgery or unilateral mastectomy, particularly if they are young.

There is a paucity of data specifically addressing the efficacy of CPM in BRCA1 and BRCA2 mutation carriers, either in terms of risk reduction or improved survival. Van Sprundel and colleagues examined the incidence of invasive contralateral breast cancer among a group of 148 women who were mutation carriers for BRCA1 and BRCA2 and had received treatment for early stage unilateral invasive breast cancer [66]. Just over half of these women underwent CPM whilst the others remained under intensive surveillance. At a median follow-up of 3.5 years, one patient had developed an invasive contralateral cancer in the CPM group while six were observed in the surveillance group (p < 0.001) [66]. Therefore CPM reduced the risk of contralateral breast cancer by more than 90% and this is of similar magnitude to the risk reduction associated with BPM. There was no impact of CPM on breast cancer specific survival with this limited period of follow-up.

There would appear to be a compelling argument for offering CPM to mutation carriers who are diagnosed with unilateral breast cancer [20,65]. Additional factors such as dense breast parenchyma and/or florid benign changes would strengthen this decision-making process in favor of prophylactic surgery.
Surgical techniques

When contemplating risk-reducing mastectomy there are several technical points to consider. First, it is essential to ensure that breast imaging has been undertaken within the preceding 3 months to exclude any incidental breast cancer [67]. Bilateral diagnostic MRI is usually recommended for BRCA mutation carriers, but despite a normal MRI examination in these circumstances, definitive histo-pathological examination of the mastectomy specimen may reveal a small cancer and patients should be warned about this prior to surgery. Another issue pertains to surgical staging of the axilla; if pre-operative MRI shows normal axillary nodes with no parenchymal abnormality, the chance of finding tumor foci within the axillary nodes is very low and does not justify routine sentinel lymph node biopsy. The latter is not without risk and can increase the chance of infection post-operatively and lead to seroma formation and lymphedema in about 5% of patients [68].

Several types of mastectomy are utilized in the context of risk-reducing surgery for mutation carriers [10].

• Subcutaneous mastectomy
This procedure is historical to some extent and although previously employed in the context of surgical prophylaxis is no longer recommended as a risk-reducing strategy for mutation carriers [10,69]. Subcutaneous mastectomy aims to remove all breast parenchymal tissue whilst preserving a thin sliver of breast tissue deep to the nipple–areola complex (NAC) in order to ensure viability of the latter. This operation is often undertaken in younger women with dense breast tissue that can be difficult to dissect off the under-surface of the NAC without compromise of vascular supply. The procedure can be done through an incision along the inframammary fold although this can present problems with accessing the superior limit of the breast tissue.

• Skin-sparing mastectomy
This procedure refers to extirpation of the nipple–areola complex and breast tissue with preservation of the breast skin envelope [10]. It is feasible to dissect the skin and subcutaneous tissues from the breast parenchyma without risk of leaving remnant breast tissue and therefore skin-sparing mastectomy ensures almost complete removal of breast tissue while maintaining the benefits of skin preservation for purposes of immediate reconstruction. Some surgeons consider this to be the optimal breast cancer risk-reducing strategy for mutation carriers. It potentially achieves the combined goals of maximal risk-reduction whilst ensuring excellent cosmetic outcomes (nipple reconstruction can be undertaken at a later stage).

• Nipple-sparing mastectomy
This is the latest development in preservation of the breast skin envelope [10]. Unlike subcutaneous mastectomy where breast tissue is deliberately retained deep to the NAC, nipple-sparing mastectomy – or total skin-sparing mastectomy – aims to remove almost all breast tissue in the vicinity of the nipple–areola complex. The main lactiferous ducts converge upon the nipple and breast tissue and are inextricably linked with the tissues of the nipple itself. The areola can be readily dissected off the underlying breast tissue but in younger women with dense breasts this can be technically challenging and sometimes a thin layer of breast tissue must be retained to ensure viability of the NAC. With a nipple-sparing mastectomy, excision of the retro-areolar tissue is a particularly important maneuver and a balance must be achieved between complete excision of the duct system and preservation of blood supply to the NAC. The ducts are usually cored out of the nipple, although micro-anatomical studies suggest that breast tissue within the nipple contains no terminal duct lobular units. Nipple-sparing mastectomy is an appropriate option for risk-reduction but remains controversial for patients with established breast malignancy [70,71]. Patients should be offered the choice of nipple preservation and informed that there is no evidence of any significant attrition of risk-reduction consequent to retention of the NAC. However, patients should be warned about the possibility of nipple necrosis and need for any subsequent debridement. These risk-reducing procedures are not followed by adjuvant treatments such as radiotherapy or chemotherapy that may compromise the blood supply to the NAC or increase the chance of infection.

• Simple mastectomy
This procedure is appropriate for patients not seeking immediate breast reconstruction in order to avoid redundant flap tissue that would otherwise result from any skin-sparing mastectomy.
without restoration of the breast mound \[10\]. There is no evidence that more extensive extirpation reduces the risk of any subsequent cancer and simple mastectomy is only indicated for mutation carriers who decline immediate breast reconstruction.

Despite careful dissection within the oncological plane, some breast tissue will remain following either skin-sparing or nipple-sparing mastectomy and this is particularly likely at the periphery of the breast where access is more restricted with certain types of incision \[72\]. In theory, mutation carriers will continue to have a low risk of breast cancer development due to this residual tissue but there is no evidence at present that any form of radiological surveillance is indicated following risk-reducing surgery by a competent operator \[2\]. Many clinicians discharge mutation carriers from further follow-up after such surgery but others recommend screening clinical breast examination every 6 months. Breast cancer developing in mutation carriers following mastectomy is likely to be manifest as a superficial lesion which is readily detectable on clinical examination. These follow-up visits provide an opportunity for continued dialog with patients and their families (some of whom may be scheduled for risk-reducing surgery themselves at a future date).

**Conclusion**

The management of women with a hereditary predisposition for breast cancer will continue to be a focus of interest in the years ahead with increasing utilization of genetic testing services \[73\]. Women who harbor a mutation conferring elevated risk for breast cancer development have several options to potentially reduce that risk. Physicians and healthcare personnel should be appropriately trained and educated to discuss these options and guide the informed consent process. Surgeons must liaise appropriately with genetic counselors as well as medical oncologists and gynecologists to ensure patients receive comprehensive assessment and a coordinated plan for any risk reduction. Further advances in technology and bioinformatics may assist in more accurate risk prediction and defining which patients with low-penetrance genes may benefit from interventions aimed at risk reduction.

**Future perspective**

In the years ahead, wider use of genetic testing will result in the identification of large numbers of women who have a hereditary predisposition for breast cancer. In this article, we have emphasized the high-penetrance mutations (\textit{BRCA1}, \textit{BRCA2}, \textit{p53}, \textit{pTEN}, \textit{CDH1}, \textit{STK11}, \textit{PALB2}), for which prophylactic surgery, breast MRI

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**EXECUTIVE SUMMARY**

- Three major risk factors for development of breast cancer are gender, age and family history/genetic susceptibility.
- There has been an upsurge of interest in identifying women with a genetic predisposition for breast cancer
- Only about 10% of all breast cancers are currently attributable to germline mutations.
- High-penetrance mutations that increase breast cancer risk include those in the \textit{BRCA1}, \textit{BRCA2}, \textit{STK11} (Peutz-Jeghers syndrome), \textit{pTEN} (Cowden’s syndrome), \textit{p53} (Li-Fraumeni syndrome), \textit{CDH1} (diffuse hereditary gastric cancer syndrome) and \textit{PALB2} (partner and localizer of \textit{BRCA2}) genes.
- NICE has recently recommended that women undergo genetic testing when the chance of finding a high-penetrance mutation is 10% or greater.
- Once a high-penetrance gene mutation has been identified in an asymptomatic woman, three risk-reducing strategies should be discussed: screening (to include breast MRI screening), chemoprevention and risk-reducing surgery. However, there are no randomized trials that have addressed the effect of these risk-reducing strategies in mutation carriers.
- Observational studies suggest that bilateral prophylactic mastectomy reduces breast cancer risk by more than 90% in mutation carriers.
- If unilateral breast cancer is diagnosed in a mutation carrier, bilateral mastectomy should be considered, because these patients have a three- or four-times greater risk of developing contralateral breast cancer than average risk cohorts.
- Following bilateral prophylactic mastectomy, mutation carries will continue to have a low risk of breast cancer development due to the presence of some residual tissue, but there is no evidence that any form of radiological surveillance is beneficial.
screening and chemoprevention are potential risk-reducing strategies. However, little is known about the impact of these risk-reducing strategies on quality of life, and more research is needed to address this issue. Moreover, potential strategies to lower breast cancer risk in women who carry low- or moderate-penetrance mutations have received little attention. For these patients, life-style changes will likely play an important role, and additional research is needed to better understand how alterations in diet, maintaining a healthy weight, exercise and reduction in alcohol intake may potentially serve to lower breast cancer risk in women who harbor low- or moderate-penetrance mutations.

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References
Papers of special note have been highlighted as:

• of interest; •• of considerable interest


• Excellent review of the characteristics of breast and ovarian cancers associated with the BRCA1 and BRCA2 mutations, and also an excellent overview of the role of prophylactic surgery in the prevention of those cancers.

• Provides an overview of the role of surgery for the prevention of breast, ovary, colon, thyroid and gastric cancers, for individuals with a hereditary predisposition for those cancers.

• Overview of important concepts pertaining to epidemiology, local therapy therapy and adjuvant systemic therapy of early breast cancer.

• Interesting editorial, calling for population-based screening for BRCA1 and BRCA2 mutation carriers.
Management of women with a hereditary predisposition for breast cancer

REVIEW


52 • There were very few BRCA mutation carriers in this study, but the study suggests a benefit of tamoxifen in preventing breast cancers in BRCA2 mutation carriers.


59 • One of the first studies to demonstrate a benefit of bilateral prophylactic mastectomy in reducing breast cancer risk in high-risk individuals.

60 Kauff ND, Satagopan JM, Robson ME et al. Risk-reducing salpingo-oophorectomy in...


Intratumor and circulating clonal heterogeneity shape the basis of precision breast cancer therapy

“...rapid developments in technological genome systems and computational network methods open new avenues in understanding etiopathogenesis on the basis of large-scale translational research studies.”

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The recent evidence of spatiotemporal genomes and tumor evolution has led to the development of breakthrough next-generation sequencing (NGS) technologies. Intratumor heterogeneity (ITH) and circulating clonal diversity represent two of the most possible explanations of primary and secondary resistance. In this editorial, we discuss how extensive biobanking for each individual patient with subsequent genome sequencing can open novel horizons for precision medicine in breast cancer.

Approximately half a century following the war against cancer and the discovery of DNA double helix, millions of patients still die from the disease. The initial enthusiasm on personalized medicine after the completion of the first human genome sequence draft at the beginning of this millennium, was followed by skepticism on the basis of complexity of noncoding genome functionality and nonlinear transcription.

Although the ENCODE project [1,2] has revolutionized biomedical research highlighting the necessity for a long-term basic research efforts, at the same time it has shaped innovative horizons in improving NGS technologies and developing breakthrough methods for understanding cancer evolution and resistance to current therapies [3,4].

Progress in personalized prevention & treatment

Progress in basic, translational and clinical research and implementation of new discoveries into routine clinical practice is faster in breast cancer than in any other cancer type. Advances in single gene testing have led to the development of a personalized approach in the prevention setting and targeted therapy. Breast cancer is still a major health problem for women taking into consideration that 1.67 million new cases are diagnosed each year worldwide [5].

KEYWORDS
• breast cancer • clonal diversity
• intratumor heterogeneity
• next-generation sequencing
• therapy

“The recent evidence of spatiotemporal genomes and tumor evolution has led to the development of breakthrough next-generation sequencing technologies.”

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20 years after the discovery of germline mutations \textit{BRCA1} and \textit{BRCA2}, which are associated with life-time high risk of breast and ovarian cancer, effective primary prevention, has developed as a result of personalized therapies based on both family history and \textit{BRCA1/2} testing \cite{6}. Moreover, more aggressive risk-reducing surgery including bilateral mastectomy and bilateral salpingo-oophorectomy has been suggested for \textit{BRCA1} than \textit{BRCA2} mutation carriers \cite{6}.

In the multimodal treatment setting, established clinical models on the basis of both traditional and genetic criteria provide better selection of patients improving oncological and quality-of-life outcomes. For example, age, tumor size (T), node status (N), histological grade (G) and molecular features including estrogen receptor and progesterone receptor (ER/PR) status as well as HER2 status represent the modern algorithm for decision-making in adjuvant treatment of early breast cancer (M0).

Trastuzumab (Herceptin\textsuperscript{®}, Genentech, Inc., CA, USA) added to chemotherapy for HER2-positive patients has improved overall survival (OS) rates in the adjuvant \cite{7} and metastatic setting (M1). More recently, trastuzumab–emtansine conjugate (T-DM1, KADCYLA\textsuperscript{®}, Genentech, Inc., CA, USA) prolongs mean OS \cite{8}. Moreover, Palbociclib has recently received regulatory fast-track approval opening a new therapeutic horizon for postmenopausal women with \textit{ER}/HER2-negative metastatic breast cancer \cite{9}.

From interpatient to intrapatient heterogeneity
Tumor heterogeneity has long been considered as genetic variation for most solid malignancies. In recent years, the development of high-throughput technologies has enhanced the ability of many studies to access the impact of tumor heterogeneity in the clinic. Most of these studies have suggested different genetic characteristics not only among patients with the same cancer type (interpatient heterogeneity) \cite{10}, but also between primary and metastatic tumor(s) in the same individual patient \cite{3}. However, a few other studies have shown high similarities between primary and metastatic tumor, suggesting the pre-existence of a small cell population within the primary tumor responsible for metastasis (reviewed also in \cite{3}).

Next-generation sequencing systems
This relative uncertainty of individual patient’s tumor heterogeneity could be overcome in recent years by the implementation of NGS systems. NGS systems have revolutionized biomedical research because of clinical validity and continuing lowering cost of these technologies. Numerous genomic studies using tumor-normal pairs for each individual patient including whole-exome sequencing (WES) \cite{11} and whole-genome sequencing (WGS) \cite{12,13} have dramatically been increased over the last 6 years. Even in this NGS era, multiple challenges are emerging, regarding their potential for improving clinical treatment and patients’ outcomes. Two NGS strategies including a conventional and a breakthrough approach have been developed providing promise for clinical implications.

Following single biopsy-based modern oncology, most NGS studies available are based on the same concept. In the largest WES study reported by Lawrence \textit{et al}. \cite{11} on 4742 tumor-normal pairs, 892 breast cancers were analyzed. In this study, the \textit{SETBD1} gene has been discovered for

“\textbf{The unprecedented potential of advancing next-generation sequencing systems and methods to explore spatiotemporal genomes and tumor evolution, raises for the first time rational hope for deep understanding of mechanisms underlying therapeutic resistance and metastasis in response to therapy.}”
Intratumor & circulating heterogeneity in breast cancer

Intratumor & circulating clonal heterogeneity

Exploiting these new data from basic and translational research, landmark studies open new predictive and therapeutic horizons for breast cancer. Developing new methods, dynamics of genomics clones’ evolution [16], ITH and circulating genomic clones diversity can now be explored. These techniques and methods provide the potential not only to explain, but also to overcome therapeutic resistance and prevent metastasis.

Identification of ITH, which represents genetic characteristics of different cell subpopulations within the primary tumor, could provide important clinical implications in overcoming resistance to current therapeutics. Multiregional NGS analysis has been suggested as the optimal method to reveal ITH. In the largest ITH study available, Yates et al. [16] have performed WGS and targeted sequencing of the primary tumor in 50 patients with breast cancer including 303 solid tumor samples. Sequencing data were compared in 18 patients who had undergone neoadjuvant treatment followed by surgical resection. This intelligent method could reveal not only ITH but also clonal evolution in response to neoadjuvant treatment. In 13 patients, targetable mutations were resulted from clonal evolution following neoadjuvant treatment.

Noninvasive methods with major potential clinical implications have recently been developed such as the circulating tumor DNA followed by NGS analysis (ctDNA-NGS). The aim from a clinical perspective is to use this method as a biomarker to predict therapeutic resistance and relapse before it clinically occurs. Murtaza et al. [17] have performed a serial ctDNA-NGS analysis in 19 plasma samples obtained from six patients with breast, ovarian and lung cancer. Quantification of allele fractions in plasma identified the emergence of mutations associated with acquired therapeutic resistance. This method not only can potentially predict resistance-based metastatic relapse, but also opens new avenue for drug-development design. In breast cancer, one patient that was treated with chemotherapy, was found to have an activating mutation in PIK3CA. These data establish a proof-of-principle that ctDNA exome sequencing can be used as biomarker to predict acquired resistance but additional larger studies are required.

Future perspective & conclusion

The unprecedented potential of advancing NGS systems and methods to explore spatiotemporal genomes and tumor evolution, raises for the first time rational hope for deep understanding of mechanisms underlying therapeutic resistance and metastasis in response to therapy.

Indeed, once the vast majority of women with nonmetastatic breast cancer receive systemic chemotherapy and targeted therapy and a substantial proportion among them develop relapse, it is crucial to understand the reasons why. Exploiting recent evidence on dynamic clonal evolution [15,16] we could explain therapeutic resistance-based metastatic relapse. Preliminary data suggest that dynamic evolution depends on the kind of mutation. On one hand most data support dynamic evolution of point mutations only, while on the other large structural genome changes are pre-existing and remain stable during tumor growth and progression [18,19]. However, these finding require validation by large studies.

Clinical evidence that almost 25% of women with HER2-positive breast cancer treated with guidelines-based recommendation therapy develop relapse, clonal evolution identification
following systemic therapy could reduce this resistance-based treatment failure. The potential of ITH and subclonal emergence identification in the neoadjuvant treatment and postsurgical setting [16] along with repeated ctDNA-NGS can reveal the emergence of resistant genome-wide alterations responsible for clinical relapse several months before it clinically occurs.

In summary, rapid developments in technological genome systems and computational network methods open new avenues in understanding etiopathogenesis on the basis of large-scale translational research studies. This understanding of molecular mechanisms landscape underlying therapeutic resistance shapes new horizons in robust biomarkers and drug-development strategy to reach precision cancer medicine.

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References
Restoring anti-oncodriver Th1 responses with dendritic cell vaccines in HER2/neu-positive breast cancer: progress and potential

HER2/neu is expressed in the majority of in situ breast cancers, but maintained in 20–30% of invasive breast cancer (IBC). During breast tumorigenesis, there is a progressive loss of anti-HER2 CD4\textsuperscript{pos} Th1 (anti-HER2Th1) from benign to ductal carcinoma in situ, with almost complete loss in IBC. This anti-HER2Th1 response can predict response to neoadjuvant therapy, risk of recurrence and disease-free survival. Vaccines consisting of HER2-pulsed type I polarized dendritic cells (DC1) administered during ductal carcinoma in situ and early IBC can efficiently correct anti-HER2Th1 response and have clinical impact on the disease. In this review, we will discuss the role of anti-HER2Th1 response in the three phases of immunoediting during HER2 breast cancer development and opportunities for reversing these processes using DC1 vaccines alone or in combination with standard therapies. Correcting the anti-HER2Th1 response may represent an opportunity for improving outcomes and providing a path to eliminate escape variants.

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Keywords: adoptive cell therapy • checkpoint inhibitor • chemotherapy • dendritic cell • immunotherapy • multimodality • radiotherapy • targeted therapy

The rationale for targeting HER2

Although HER2 (ERBB2) is transiently expressed during fetal and normal breast development, as well as during breast growth in pregnancy, overexpression of HER2/neu can contribute to breast tumorigenesis. HER2/neu has become the most studied tumor-associated antigen (TAA), as it is a molecular oncodriver overexpressed in 20–30% of invasive breast cancers (IBC) and in about 13–56% of ductal carcinoma in situ (DCIS) lesions [1].

HER2/neu is an immunogenic protein that elicits both humoral and cellular immune responses in patients with HER2\textsuperscript{pos} tumors, but patients with HER2/neu overexpressing tumors often demonstrate diminished existing immunity directed against the protein [2]. In IBC, HER2/neu is associated with a heightened risk for invasion, suggesting a crucial role for HER2/neu in stimulating a tumorigenic microenvironment. Whether by ‘immunoediting’ or other immune evasion mechanisms, a diminished cellular immune response to HER2/neu in the tumor microenvironment of HER2\textsuperscript{pos} breast cancer (HER2\textsuperscript{pos}BC) is associated with poorer prognosis [2]. On the contrary, an increased cellular and humoral response against HER2/neu has been associated with decreased tumor development and improved outcomes [3]. Clinical implications of HER2/neu expression in DCIS (HER2\textsuperscript{pos}DCIS) are not as clear, but present data suggest that it predicts the presence of invasive foci [4], and increases risk of disease recurrence, although only approximately 20% of HER2\textsuperscript{pos}DCIS recurs as HER2\textsuperscript{pos}BC [5]. This low rate of HER2\textsuperscript{pos}DCIS conversion to HER2\textsuperscript{pos}IBC may be due to...
pathway and phenotype instability in HER2<sup>pos</sup> cancer cells, with natural or induced immunity possibly shaping the resulting tumor phenotype [6].

With the exception of antibody-based HER2 targeting, HER2-directed immunotherapy in BC has not been as successful as initially expected, there has only been limited success in the setting of advanced disease where tumor cells have already escaped immunosurveillance. The future of HER2-targeted vaccination trials should be geared toward early-stage HER2<sup>pos</sup>BC, potentially halting progression of HER2<sup>pos</sup>-DCIS to IBC (Figure 1) or situations to prevent recurrence. HER2 vaccines must be created based on immunologic principles of circumventing tolerance, a primary mechanism of escape, by strengthening the weak pre-existing anti-HER2 CD4<sup>pos</sup> Th1 (anti-HER2Th1) immune response [6].

**Loss of anti-HER2 CD4<sup>pos</sup> Th1 during breast tumorigenesis**

Recently, we have identified a progressive loss of anti-HER2Th1 immune response in HER2<sup>pos</sup>-BC patients relative to healthy controls, with an early and progressive decrease in immune competence in patients with HER2<sup>pos</sup>-BC [7]. Specifically, there is a loss of anti-HER2Th1 response during breast tumorigenesis, where healthy patients have a strong anti-HER2Th1 immune response that is diminished in patients with DCIS and nearly absent in patients with IBC [7]. This supports the hypothesis that the level of circulating anti-HER2Th1 response correlates to immune response in patients with HER2<sup>pos</sup>-BC after treatment [7]. It is likely that this immune deficit begins in DCIS and continues to decline with disease progression. This depressed anti-HER2Th1 response is driven predominantly by Th1 phenotypes. In patients who had undergone neoadjuvant therapies, preserved anti-HER2Th1 response was associated with pCR and improved disease-free survival (DFS), while a depressed response was found in patients without pathologic complete response (pCR) and recurrent disease after treatment [8]. These findings may potentially lead to the use of anti-HER2Th1 monitoring for patients receiving HER2-targeted therapies to identify those at risk of clinicopathologic failure.

**Role of immune response & immunoediting during breast tumorigenesis**

The breast parenchyma normally harbors immune cells such as cytolytic CD8<sup>pos</sup> T cells, Th1 cells and NK cells.
that aid in development, lactation, involution and may participate in immunosurveillance of the breast [9]. The presence of high-density CD8$^{\text{pos}}$ T cells in tumors and nearby stroma is associated with improved prognosis in BC, suggesting that immune effector cells have identified the malignant cells and are actively mounting an appropriate immune response. Potent immune effectors act by eliminating tumors directly via exocytosis of cytotoxic granules, or indirectly, by producing INF- $\gamma$ to activate Th1 immune response. It is thought that under immunosurveillance, the host eliminates evolving tumor cells continuously during elimination, until some malignant cells manage to outnumber and stress the immune response, evading immune recognition through a process described as 'immunoediting' [9]. Initially malignant clones are effectively eliminated by the immune response but as the tumor grows and expands a state of tumor equilibrium is reached (Figure 1). If this process becomes ineffective or fails, malignant cells may escape from immunological control to proliferate and manifest clinically as invasive and metastatic cancer [10].

The immunoediting phenomenon is evident during breast tumorigenesis mediated by HER2. HER2/neu is present in nonmalignant cells at basal levels, but overexpression leads to malignant transformation. We hypothesize that in the initial stage of malignant transformation, the mounted immune response is robust enough against this HER2/neu overexpression and may eliminate HER2$^{\text{pos}}$DCIS from becoming clinically apparent. If elimination is not achieved, HER2/neu overexpressing tumor cells and the immune response develop a state of equilibrium where the anti-HER2Th1 immune response is unable to completely eliminate malignant cells, but is able to prevent them from becoming invasive. This equilibrium state may be clinically manifested as HER2$^{\text{pos}}$DCIS presenting with microcalcifications on mammography [11]. These calcifications may be a reflection of necrosis caused by immune effectors cells (anti-HER2Th1 response) attempting to eliminate tumor cells during this period of homeostasis (Figure 1). The anti-HER2Th1 response although diminished remains somewhat active in DCIS [7]. This phase of tumor equilibrium in DCIS may be boosted using DC vaccines, which, by stimulating both CD4$^{\text{pos}}$ and CD8$^{\text{pos}}$ T cells and IL-12, can maximize INF- $\gamma$ production and the functionality of Th1 cells thereby tip the equilibrium state toward elimination again (Figure 1).

If the process of equilibrium continues, the tumor may remain stagnant and suspended as DCIS with no disease progression to invasion. The alternative to equilibrium in DCIS would be tumor progression and developing invasion (IBC). If progression occurs, there are likely two scenarios where the immune response would be ineffective at halting disease progression. First, the anti-HER2Th1 immune response further erodes over time [7] and becomes virtually absent allowing tumor escape. This hyporesponsive state may be due to a chronic exhaustion of the immune system leading to increased inhibitory receptors [11], decreased effector cytokines or impaired cytotoxicity, allowing for tumor escape. The result is continued expression of HER2 on the IBC. The second scenario is one where anti-HER2Th1 response is sustained, but tumor cells manage to evade immunosurveillance via phenotypic shifting. In this scenario, the immune system remains responsive, but tumor cells lose or down-regulate HER2/neu expression resulting in a new phenotype, such as triple-negative breast cancer (TNBC) or estrogen receptor positive BC (ER$^{\text{pos}}$BC) (Figure 1).

There are numerous examples of ER$^{\text{pos}}$ non-HER2 primary tumors developing systemic metastasis that are HER2$^{\text{pos}}$ and also examples of HER2$^{\text{pos}}$ primary tumors that lose expression of HER2 [12]. Using our understanding of immunoediting and immune escape, there are numerous opportunities to use DC vaccines to boost anti-HER2 immunity when needed and boost immunity against oncodrivers involved with escape to prevent recurrence.

**Activation of type I polarized DC drive strong anticancer Th1 responses**

Dendritic cells (DCs) are a heterogeneous group of specialized APCs [13], found in lymphoid and nonlymphoid tissue. They are classified according to their ability to elicit a specific immune response based on polarization of a T-cell response. DCs are typically found in immature form, and maturation is triggered by proinflammatory cytokines such as IL-1, IL-6, IFN- $\gamma$ and TNF-$\alpha$ [14]. Additional signals such as bacterial lipopolysaccharide and IFN-$\gamma$ drive IL-12 production leading to pulsed type I polarized DC1 [15]. Once maturation and activation occurs, migration to nearby lymph nodes is facilitated [16], where activated DCs deliver costimulatory signals essential for T-cell activation such as CD40, CD80, CD86, CD46 ligands and Toll-like receptors [17]. DCs signal activation and polarization of T cells is crucial for the differentiation of CD8$^{\text{pos}}$ T cells into cytotoxic T lymphocytes (CTLs) [18] and for the polarization of CD4$^{\text{pos}}$ T cells into their different effectors (Th1, Th2 and Th17) [19].

During BC development and progression, CD4$^{\text{pos}}$ T cells may be a crucial element in the tumor ‘immunoediting’ process. Therefore, there is increasing interest in activating CD4$^{\text{pos}}$ T cells because of their role as helpers in maintaining CD8$^{\text{pos}}$ cells as functionally active [20] and indirect effects on other innate cells such as...
as NK, macrophages and DCs [21]. CD4\(^{\text{pos}}\) are significantly increased during breast cancer and the subsets dynamically change with disease progression. In early-tumor stages, Th1 cells are the dominant population of CD4\(^{\text{pos}}\) T cells, while in the advanced tumor stages, FoxP3\(^{\text{pos}}\) Treg and Th17 cells become the dominant populations [22]. Th1 cells secrete high-level cytokines such as IFN-\(\gamma\) and TNF-\(\alpha\), both key components of initiating Th1-polarized response and to the antitumor function of DC1 vaccines [19]. DC1s are very efficient in the presentation of antigens and production of IL-12 [23] that polarizes T cells toward the IFN-\(\gamma\) Th1 phenotype [24]. IL12 has multiple roles with inherent antitumor effect, antiangiogenic capabilities, activation of NK cells as well as enhancing adaptive immunity and improving sensitization to tumor antigens [25].

Despite their ability to prime an immune response, results have been disappointing when testing DC vaccines in late-stage BC. One reason may be because, in advanced disease, DCs are unable to mount a strong enough immune response to overcome the overwhelming immunosuppressive tumor microenvironment present in tumors that have escaped immunosurveillance. This may be one reason why, in early stages of BC, DC vaccines have enjoyed some clinical successes [3]. Another potential reason for the ineffectiveness of DC vaccines in late-stage disease is that inflammatory type DC vaccines have been used in most studies that do not necessarily drive strong Th1 response [18]. Increasing understanding of the dynamics of DCs activation, treatment of early-stage BC and different adjuvant settings will allow for improvements that warrant further investigation.

**Effectiveness of DC1 vaccines administered in the equilibrium phase of DCIS**

We suggest that HER2\(^{\text{pos}}\)/DCIS represents the equilibrium phase of immunoediting, where Th1 immune response is in homeostasis with cancer cells in the ducts. Supporting this argument, data suggest that a strong Th1 proinflammatory signature in tumor microenvironment is associated with improved outcomes [13]. Therefore, if we can stimulate a strong Th1 immune response during the equilibrium phase of disease, it may be possible to prevent recurrence of the preinvasive lesions or halt disease progression to IBC.

A group of 27 patients with HER2\(^{\text{pos}}\)/DCIS received HER2-pulsed DC1 vaccines in the neoadjuvant setting [2]. These patients all required surgical resection of their HER2\(^{\text{pos}}\)/DCIS. In vaccinated subjects pCR was achieved in 18.5% of all patients (ER\(^{\text{neg}}\) = 40 vs ER\(^{\text{pos}}\) 5.9%) suggesting shift from equilibrium to elimination. Among those patients without a pCR in about 50% of HER2 expression was eradicated in residual DCIS (sustained HER-2/neu expression ER\(^{\text{neg}}\)=10% vs ER\(^{\text{pos}}\) = 47.1% \(p = 0.04\)). Postvaccination phenotypes were significantly different between ER\(^{\text{pos}}\) and ER\(^{\text{neg}}\) subjects (\(p = 0.01\)). The conversion of HER2\(^{\text{pos}}\)/DCIS to HER2\(^{\text{neg}}\) phenotypes after vaccination demonstrated the potential process of tumor escape in that a single target may not be sufficient to completely treat or eradicate disease. Interestingly, most of the DCIS lesions that changed were ER\(^{\text{pos}}\) and subsequent trials have demonstrated this group can have similar complete response rate by combining antiestrogen therapy with DC1 vaccination (Lowenfeld L, Press Oncoimmunology 2016, Manuscript submitted). This suggests that future experimental BC vaccines may be more effective by either targeting multiple oncodrivers or combining with other therapies that block additional pathways to prevent the escape phase of immunoediting [26].

Postimmunization, sensitization of T cells to at least one class II peptide was observed in 22 of 25 evaluable subjects, while 11 of 13 subjects were successfully sensitized to class I peptides. Perhaps most importantly, anti-HER2 peptide responses were observed up to 52 months postimmunization. These data show even in the presence of early-stage BC, such DC1 are potent inducers of durable Th1-polarized immunity, suggesting potential clinical value for development of cancer immunotherapy [27]. There is no significant difference in immune response detected systemically after vaccination in patients with HER2\(^{\text{pos}}\) or ER\(^{\text{neg}}\) and ER\(^{\text{pos}}\) tumors, but complete tumor regression was significantly more common in patients with ER\(^{\text{neg}}\) compared with ER\(^{\text{pos}}\)/DCIS [28]. This proposed the concept of ER signaling as an escape pathway in HER\(^{\text{neg}}\)/BC resistance to anti-HER2-targeted therapies. When looking at the effect of antiestrogen therapy in combination with DC1 vaccination, there is an increase rate of pCR and decreases recurrence in patients with ER\(^{\text{pos}}\)/HER2\(^{\text{neg}}\)/BC. Interestingly, the addition of antiestrogen therapy increased the anti-HER2Th1 response in regional sentinel nodes (Lowenfeld L, Press Oncoimmunology 2016, Manuscript submitted).

The increased interest in targeted vaccination against HER\(^{\text{pos}}\)/BC has shown promising results. This data provide rationale for developing vaccinations to reduce recurrence in patients with ER\(^{\text{neg}}\) and HER2\(^{\text{pos}}\)/DCIS in whom there personalized therapies other than standard surgery and radiation do not exist. There is a large push in the community to develop novel, rationale-targeted therapies for DCIS that also provide protection against new breast events.

The other area where DC1 vaccines may have clinical benefit is in patients with HER2\(^{\text{pos}}\) IBC where the anti-HER2Th1 response is severely compromised (Figure 1) [7]. These patients have substantial risk of
recurrence and include those patients with residual disease following neoadjuvant therapy, and advanced stage III and IV patients that are no evidence of disease (NED). Ongoing research with DC and non-DC vaccines in early-stage trials are currently opened and results are pending. (Table 1). We have demonstrated that the anti-HER2 Th1 response can be restored using DC1 vaccines in these patients [29]. Whether or not restoration of Th1 response translates to diminished recurrence must await larger trials.

Use of DC1 vaccines during tumor escape in combination with anti-HER2 therapies

Loss of anti-HER2Th1 immune response is associated with lack of pCR to adjuvant chemotherapy and HER2-targeted therapies [7] as well as predicts increased of recurrence and diminished DFS [8]. Though DC vaccination has not yet proven to be an effective treatment of BC, other adjuvant treatment modalities have been shown to aid in promoting the immune response to tumor cells when given in combination with DC vaccines. These treatments often work synergistically with vaccination by aiding T-cell recognition of tumor cells. Some therapies, such as chemotherapy or radiation, may induce changes in the tumor microenvironment, allowing a more complete response to DC vaccination. In combination with other therapies, such as HER2-targeted therapies, DC vaccination can aid in overcoming resistance [29,30]. Hence, pCR may be best achieved using combination therapy with DC vaccination (Figure 2).

Adjuvant chemotherapy

The tumor microenvironment may play a role in the recognition and response of the immune system to tumor cells. It has been observed that a high level of lymphocytic infiltration in breast tumors predicts a better prognosis and a better response to chemotherapy [31]. Chemotherapy has the potential to augment the tumor microenvironment by increasing CD4 T-cell infiltration (Figure 2), demonstrating a positive correlation to pCR [32]. Because vaccination, especially with DCs, can stimulate activation of T cells and potentially improve lymphocytic infiltration of breast tumors, concomitant or sequential administration of vaccines with low-dose chemotherapy may improve treatment outcomes. Sequential administration of a low dose of the chemotherapeutic drug, cyclo-

<table>
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<th>Phase</th>
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DCIS: Ductal carcinoma in situ; MUC-1: Mucin-1.
phosphamide, along with vaccination against HER2 has been shown to induce de novo HER2-specific T helper-dependent immunity [33]. Low-dose doxorubicin, paclitaxel and methotrexate have also been shown to enhance HER2-specific immunity with vaccination as well as stimulate DC maturation and differentiation [33–35]. Therefore, chemotherapy may be better utilized in order to maximize the effectiveness of the antitumor immune response.

**Combinations of vaccines with HER2-directed therapy**

Trastuzumab is a monoclonal antibody that targets the HER2/neo protein by binding to the extracellular domain, triggering HER2 internalization and degradation. The use of trastuzumab induces passive immunity to HER2, although a major limitation of this therapy after continued use is the development of drug resistance. About 70% of patients who initially respond to therapy experience progression of the disease within approximately a year, suggesting the development of an acquired resistance to the antibody [36]. Patients undergoing neoadjuvant trastuzumab therapy plus chemotherapy may not show pCR due to depressed anti-HER2Th1 cell response [29]. Conversely, combination use of trastuzumab with vaccination against HER2 can significantly sensitize CTLs to tumor cells expressing the peptide and result in increased tumor cell lysis specific to HER2 [30,37]. In addition, it has recently been demonstrated that the anti-HER2Th1 response is positively correlated with pCR in patients given neoadjuvant chemotherapy plus trastuzumab. Those patients with a depressed anti-HER2Th1 response did not show pCR, but it was shown that this could be augmented with use of HER2-targeted DC vaccination [29]. Additionally, there is a strong association between immune gene expression and DFS following treatment with adjuvant trastuzumab, suggesting that there is a subset of HER2+ tumors with a high level of immunologic activity [38]. Thus, DC vaccination may prove to be a useful adjunct to trastuzumab therapy by restoring immune response to HER2.

Pertuzumab another anti-HER2 mAb, similar to trastuzumab, targets the extracellular dimerization domain of the HER2 receptor. Developed in attempts to combat resistance to trastuzumab, pertuzumab prevents HER2/HER3 heterodimerization [39,40]. Coadministration of the two therapies has been shown to enhance clinical effectiveness compared with either one administered singly [40,41]. Pertuzumab has not yet been studied in combination with vaccination against cancer, but, considering its similar mechanism of action and synergistic effects with trastuzumab, DC vaccination could prove to be a clinically effective adjunct.

Similarly, lapatinib, a tyrosine kinase inhibitor (TKI) that disrupts the HER2/neu and EGFR, is used to treat HER2+BC. Like trastuzumab, patients may develop resistance fairly early. In attempts to combat this, vaccination against HER2 was given concomitantly with lapatinib in a clinical trial, which demonstrated safety of the combined treatment, but due to small sample size, no additional immunologic benefits were observed [42]. However, another TKI, axitinib, showed improved therapeutic efficacy when combined with DC vaccination in a preclinical model of murine melanoma [43]. Although DC vaccination combined with TKIs has not yet shown improved outcomes in breast cancer, improved anti-HER2 immunity acquired by vaccination may improve pCR and overcome resistance seen with continued use of TKIs [29,37].

**Radiation**

Radiation is an important part of anticancer therapy, with nearly two-thirds of patients with cancer receiving it at some point during the course of their treatment [44]. Radiation is known for its ability to damage DNA of tumor cells, eventually causing enough injury to prohibit cellular proliferation and/or cause tumor cell death. Along with the antitumor properties that radiation has on its own, it may also have the ability to amplify the effects induction of apoptosis and necrosis; so APCs are ultimately attracted to this environment. This radiation-induced tumor microenvironment increases expression of Fas, MHC class I molecules and several other cell surface proteins [45], which provoke a DC-mediated antigen-specific immune response [46], therefore stimulating a CTL response. Correspondingly, the goal of vaccination is to induce an antigen-specific immune response to tumor cells, so addition of radiotherapy to vaccination should have a synergistic effect. This effect has been shown in a preclinical model of subcutaneous murine colorectal carcinoma where, after combination therapy of local radiation and vaccination, there was a massive infiltration of T cells that was not seen in either modality alone [47]. Prostate cancer patients treated with radiotherapy in combination of anti-PSA DC vaccines were able to increase immune response against PSA, but there was no clear synergistic effect of the combination of radiation with vaccination [45]. At this point, there have been no studies examining the effect of radiation therapy in combination with DC vaccines in the treatment of BC, however the ability of radiation to enhance MHC class I expression and consequently CTL infiltration of tumor has been shown in other forms of cancer immunotherapy using monoclonal antibodies against molecules such as cytotoxic T-lymphocyte associated
antigen 4 (CTLA-4) and PD-1 \([48-50]\). Ultimately, radiotherapy may provide a useful adjunct to DC vaccination in the treatment of BC to affect the tumor microenvironment.

**Checkpoint inhibitors**

Tumors may utilize immune checkpoint pathways by expressing ligands that, under normal circumstances, would prevent aberrantly activated T cells from causing autoimmunity \([51,52]\). Tumors may effectively hide from the immune system with expression of these ligands. By blocking the interaction of ligands with immune effector cells using monoclonal antibodies (mAb), the tumor cells may now be easily ‘seen’ by the immune system. Hence, two of these checkpoint pathways that are currently areas of major investigation are the PD-1/PD-L1 and CTLA-4/B7 pathways, which have demonstrated significant success in melanoma, lung cancer and colon cancer \([53-55]\) – all tumors with significant production of neoantigens.

PD-1 is expressed on effector immune cells – including T cells, NK cells, activated monocytes and DCs \(\text{Figure 2}\) \([51]\). Contact of PD-1 with its ligand, PDL-1, results in T-cell inactivation and apoptosis \([56]\) and inhibits the activation of tumor-antigen-specific T cells \([57]\). Expression of PDL-1 on tumor cells may render them immunologically invisible. Like the PD-1 pathway, the CTLA-4 is a significant immune checkpoint receptor exploited in cancer. CTLA-4 is a CD28 homolog, which is upregulated upon T-cell activation and competes with CD28 for binding to APC ligands \([58]\). Tumor cells can express the CTLA-4 receptor on their surface \([59]\) and counteract the activity of the T-cell costimulatory receptor, CD28 \([60]\) impairing tumor-reactive T cells.

mAb against PD-1 and CTLA4 may restore the function of disabled CD8\(^{\text{TM}}\) T cells in cancer as well as preventing the depletion of activated memory B cells, resulting in a more robust immune response \([61]\). PD-1/PD-L1 blockade can neutralize the inhibi-
tation of DC–T-cell interactions, restore APC function, repair T-cell function by increasing IFN-γ production, promoting T-cell proliferation [52] and T-cell targeting of tumors (Figure 2).

The restoration of T-cell function with the use of mAb against these checkpoint inhibitors presents a synergistic opportunity for administration of DC vaccines. Using PD-1 mAb in conjunction with DC vaccination has shown an increase in CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses [56]. Along with restoring function of disabled immune cells, blocking the PD-1/PD-L1 checkpoint may further enhance the effectiveness of DC vaccination by preventing suppression of DC maturation [52]. In a murine breast cancer model, administration of a monoclonal antibody against PD-L1 in combination with DC vaccination induced more potent protective immunity than just DC vaccination alone [52]. Anti-HER2 DC vaccination combined with anti-CTLA4 therapy in a murine mammary carcinoma model also exhibited a significant increase in the frequency of tumor infiltrating CD4<sup>+</sup> and CD8<sup>+</sup> T cells [62]. A probable mechanism for the success in this therapeutic combination is the restoration and promotion of a robust Th1 response, encouraging cytotoxic CD8<sup>+</sup> T-cell response and infiltration into the tumor [62]. Checkpoint inhibitors and DC vaccination seem to be an advantageous synergistic combination for activation of the immune system in cancer therapy for which there are ongoing trials [58]. Ultimately, the immune response needs help targeting oncodrivers, and the combination of checkpoint inhibitors with DC vaccines may prove to be the most successful combination.

DC vaccines for breast cancer escape with loss of HER2 oncodriver expression

Genetic profiling has allowed us to identify different oncodrivers associated with IBC as well as the four principal subtypes. HER2<sup>+</sup> expressing tumor cells, as previously mentioned, may undergo phenotypic shifting or loss of immune response under anti-HER2 pressure (Figure 1) which may allow for disease progression to HER2<sup>−</sup>IBC with subtypes such as basal like (TNBC) or Luminal A (ER<sup>+</sup>BC). With this in mind, modulating the immune responses using DC pulsed with antigens against other oncodrivers may aid in eliminating residual BC cells and prevent recurrence of escape variants that lose HER2/neu expression. We will discuss some of the potential oncodriver targets in the different subtypes of BC.

ER-positive breast cancer oncodrivers

MUC1

Epithelial Mucin-1 (MUC1) is a large transmembrane protein and is the most widely expressed of the mucins, located on the apical surface of human epithelial cells lining glands or ducts. It is an overexpressed antigen found in about 90% of BC and is a cancer-specific class of vaccine target [63]. Elevated levels of MUC1 on the tumor have been associated with invasiveness, tumor growth, metastatic properties leading to poor prognosis in colon, pancreas, breast and bladder cancer [64,65]. MUC1 has been found to stimulate ER-mediated transcription, antagonize tamoxifen and contribute to ER-induced growth and survival of BC cells [66]. These findings support the idea that MUC1 is crucial to ER function in BC. It contributes to tumorigenesis by reducing degradation of EGFR, increasing cell proliferation and inhibiting cell death by protecting against oxidative and genotoxic stress [67-69]. MUC1 has been studied as a target in cancer immunotherapy due to the ability to induce humoral immune responses in healthy subjects and in cancer patients [70]. Patients vaccinated with MUC1 core peptide-pulsed DC vaccines with MUC1-positive cancer were also found to have a reduction in tumor size or tumor marker levels as well as prolonged survival, suggesting that MUC1 is sufficiently immunogenic to be used as a target for DC vaccination [71]. More recently, MUC1 peptide vaccine study was able to induce INF gamma CD4<sup>+</sup> and CD8<sup>+</sup> T cells that recognized tumor-associated MUC1. Although only T-cell preactivation outside the tumor bed, either in culture or by repetitive vaccination, showed to overcome escape phase of tumor ‘immunoediting’ [72].

HER2-positive breast cancer – other oncodrivers

hTERT

Human telomerase reverse transcriptase (hTERT) expression has been found to be increased in radioresistant HER2<sup>+</sup>-BC, while HER2 reduction has been shown to cause hTERT/telomerase activity (TA) downregulation. A widely expressed tumor antigen, hTERT, is expressed in more than 85% of all human cancers [73] and absent in most normal human cells [73], making it a favorable target for immunotherapy. hTERT is not a cellular growth receptor or signal transducer, but rather overexpression, prolongs tumor cellular survival by maintaining chromosomal integrity and protecting telomeric DNA [74]. TA has been found in up to 75% of breast carcinoma in situ lesions. Proto-oncogenes and growth factors, such as p53 and HER2, have been associated with the regulation of TA. Studies have shown that HER2 mediates hTERT expression through activation of NF-κB and c-myc [75]. In the setting of immunotherapy, it has been observed that the hTERT peptide, I540, binds with high affinity to HLA-A2, and can be used to generate specific CTLs in vitro that lyse a wide range of hTERT-positive tumor cell lines [73]. Overall, hTERT-specific
immunity has been achieved with induction of CD8<sup>+</sup> tumor infiltrating lymphocytes [76, 77]. Although there was some immunity to the TAA peptide hTERT, trials have not yet proven DC vaccination against hTERT to be an effective therapeutic strategy.

**HER3**

HER3 is another member of the EGFR family. Some studies have suggested that combination of HER2 and HER3 are crucial in cell growth, tumorigenesis and directly contribute in acquired resistance to therapies in HER2<sup>+</sup>BC and ER<sup>+</sup>BC. Although HER3 overexpression and activation is seen in invasive BC, a few studies have looked at it in the setting of DCIS, in which HER2<sup>2nd</sup> status is frequently seen [78]. HER3 can channel ErbB signaling to PI3K pathway leading to its aptness to favor tumor growth. Murine models have shown that overexpression of HER3 is seen in conjunction with high levels of HER2 [79]. This is thought to occur via gene amplification, transcription or translation. In cells with high HER2 and HER3, downregulation of HER3 affects both receptors, implying association of the two for signal maintenance [79]. This may have impact in HER2-targeted therapies and provides resistance against pertuzumab, mTOR inhibitors and tamoxifen. In the case of tamoxifen, downregulation of ERBB3 in MCF-7 cells rendered resistant by HER2 overexpression promotes Tamoxifen-induced apoptosis. This relationship allows for a chance at improving efficacy of HER2-targeted therapies, by hindering linking of HER2 to HER3 potentially using DC vaccines. HER-3 expression is also a poor prognostic factor in TNBC [80] as it can heterodimerize with EGFR as well as transduce signals with c-MET.

**Triple-negative breast cancer oncdrivers**

**c-MET & EGFR**

c-MET and EGFR amplification and overexpression have been associated with TNBC and basal-like subgroups. EGFR is found in 50% of BC and c-MET is overexpressed in 20–30% of cases. c-MET is a tyrosine kinase receptor that binds to ligand EGFR or HGF for organ development, but when an anomalous activation occurs it can lead to tumor development [81]. Overexpression has been associated with increasing tumor size, increased nodal involvement [82], decreased DFS, decreased overall survival and poor response to chemotherapy [83].

c-MET amplification is associated with treatment failure to trastuzumab and shorter time to disease progression [84] in HER2<sup>+</sup>BC, and resistance to anti-EGFR therapies in TNBC [85]. Currently, Phase II trials of anti-c-MET therapies are underway in the setting of advanced BC using targeted therapies such as tyrosine kinase inhibitors and monoclonal antibodies.

Combined c-MET/EGFR therapies have been studied in head and neck, lung and colon cancer and more recently for TNBC. EGFR/MET inhibition is synergic and therefore targeting both may provide for insight to establishing an effective therapeutic strategy in the setting on TNBC were limited therapies are available [85]. This role of c-MET and EGFR in BC merits further investigation and potentially using them as a DC vaccine target to block both pathways.

**P53**

p53 is a tumor suppressor gene, regulator of the cell cycle, cellular proliferation and apoptosis, which is found to be mutated in 25% of BC. It is most commonly seen in TNBC in about 50–80% [86]. Overexpression is associated with poor response to chemotherapy [87], DFS and overall survival [88]. Tumors cells are able to bypass the G1 checkpoint and complete the cell cycle, even in the presence of DNA damage in the setting of a p53 mutation [86]. Th1 response is significantly more prevalent in patients with tumors exhibiting high expression of p53. With this in mind, future immunotherapeutic approaches may potentially be geared toward Th1 polarization to allow a more effective immunotherapy in patients with advanced disease with p53 mutation [89].

**Conclusion**

Cancer immunotherapy is an important feature in the rapidly changing landscape of cancer treatment. Because breast cancer may represent a classic example of immunoediting, DC-based cancer vaccines can harness the potential to reinvigorate the immune system, moving toward elimination of tumor cells and shifting away from the equilibrium and escape stages. However, a one-size-fits-all theory will not likely be possible with DC vaccines. The use of vaccines in early stages of breast cancer, such as in DCIS, has claimed more success than in late-stage use, likely because, in DCIS, the tumor cells and the immune response have achieved a state of equilibrium. Vaccines may be able to more readily tip the scales in favor of the immune system, thereby eliminating equilibrium and moving toward elimination of the tumor. Although DC vaccines have enjoyed some success in early stages of breast cancer, they still have been limited success when used in later stages. This limited success has shifted focus toward using DC vaccines in the adjuvant setting to prevent recurrence. Vaccination in combination with targeted molecular and immune therapies that provide an additive or synergistic response, as well as using DC with cytotoxic therapies such as chemotherapy and radiation.
remain underexplored. Combinations with checkpoint inhibitors are rapidly moving forward. DC vaccination therapy may also be used to target numerous TAAs, giving it the potential to be a personalized tumor treatment of tumor immune escape for essentially any phenotypic subtype of breast cancers. The versatility of DC vaccines lends itself to being a powerful tool in the future of cancer therapy.

**Future perspective**
In the past, monotherapy with DC vaccines did not meet the expectations as a standalone therapy in advanced breast cancer. As we move forward, the focus of DC vaccines is being shifted to early disease and use in adjuvant settings. In addition, DC vaccines are being incorporated in combination with conventional therapies such as radiation, chemotherapy, and antiestrogen therapy as well as in combination with other immune adjuvants in the treatment of early-stage breast cancer. It is presumed that such uses of DC vaccines will reduce recurrence and could potentially be developed for primary prevention. The synergistic effects will yield improved outcomes as the era of personalized medicine continues.

**Executive summary**

**Targeting HER2 & loss of anti-HER2**
- HER2/neu is associated with a heightened risk for invasion and metastasis, suggesting a crucial role for HER2/neu during breast tumorigenesis. We have observed a progressive loss of anti-HER2 CD4pos Th1 (anti-HER2Th1) immune response during tumorigenesis in patients with HER-2 expressing breast cancer. HER2 vaccines can restore anti-HER2Th1 immune responses.

**Role of immune response & immunoediting during breast tumorigenesis**
- Since healthy women have robust anti-HER-2 CD4 Th1 responses, we hypothesize that in the initial stage of malignant transformation, the mounted immune response is robust enough against this HER2/neu overexpression and may eliminate HER2posDCIS or invasive breast cancer (IBC) from becoming clinically apparent. If elimination is not achieved, HER2/neu overexpressing tumor cells and the immune response develop into a stalemate of equilibrium where the anti-HER2Th1 immune response is unable to completely eliminate malignant cells, but is able to prevent them from becoming invasive. This equilibrium state may be clinically manifested as HER2posDCIS and may remain in this state of equilibrium and the anti-HER-2 Th1 response further erodes allowing tumor escape as HER-2negIBC or anti-HER-2Th1 response is sustained but ineffectual and tumor cells manage to evade immunosurveillance via phenotypic shifting.

**Activation of type 1 polarized DC drive strong anticancer Th1 responses**
- CD4pos T cells may be a crucial element in the antitumor immune response because of their role as helpers in keeping CD8pos cells functionally active. Th1 cells secrete high-level cytokines such as IFN-γ and TNF-α, both key components in antitumor function. These cytokines can induce apoptosis of HER-2 expressing breast cancer cells and upregulate MHC class I expression making the cells more susceptible to CD8-mediated cytolysis. Dendritic cell (DC) polarization toward high levels of IL-12 production specifically drive anti-HER-2 CD4 Th1.

**Effectiveness of DC1 vaccines administered in the equilibrium phase of DCIS**
- We suggest that HER2posDCIS represents the equilibrium phase of immunoediting where Th1 immune response is in homeostasis with cancer cells in the ducts. Stimulating a strong Th1 immune response may lead to eradication or return to the elimination phase. Results from ductal carcinoma in situ patients treated with HER-2-pulsed DC1 demonstrated that some patients who received HER2-pulsed DC1 vaccines alone achieved pCR. In addition, others have remaining tumor with loss of HER-2 suggesting tumors can escape. Combining vaccines targeting multiple oncdrivers or combination therapies may prevent immunoediting.

**Use of DC1 vaccines during tumor escape in combination with anti-HER2 therapies**
- Persistent diminished anti-HER2Th1 immune response is associated with lack of pCR to neoadjuvant chemotherapy and HER2-targeted therapies. Ongoing clinical trials as being performed to assess whether restoring anti-HER-2 CD4 Th1 can prevent recurrence or combined with initial therapies to cause more complete responses to standard therapy. Combining the anti-HER-2 therapies and other immune modulating agents such as checkpoint inhibitors are subjects of future trials to augment effective anti-HER-2 CD4 Th1 responses.

**DC vaccines for breast cancer escape with loss of HER2 oncdriver expression**
- Ineffectual anti-HER-2 Th1 responses may lead HER2pos expressing tumor cells to undergo phenotypic shifting or loss of immune response under anti-HER2 pressure which may allow for disease progression to HER2negIBC with subtypes such as basal like (triple-negative breast cancer) or Luminal A (ERposBC). Modulating immune responses using DC pulsed with antigens against other oncdrivers such as Mucin-1, c-MET, human telomerase reverse transcriptase, HER3 may aid in eliminating residual BC cells and prevent recurrence of escape variants that lose HER2/neu expression.
Financial & competing interests disclosure

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Work from our laboratory cited in this review was funded by National Institutes of Health RO1 CA096997, Henle Fund Research Fellowship (*) and Pennies in Action® Foundation. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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Papers of special note have been highlighted as:

- of considerable interest


** First description of the progressive and specific loss of CD4+ T cell immunity to a molecular oncodriver during breast tumorigenesis. Points into the negative clinical and pathologic outcomes associated with depressed anti-HER2 T cell immunity and implies that immune restoration with vaccination or other immune modulating strategies may be an option to diminish tumor progression or prevent recurrence.


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**Results from this study suggest that targeting the HER-2/neu pathway in early breast cancer and ductal carcinoma in situ using DC1 pulsed with HER-2/neu peptides boosted anti-HER-2 CD4 T-helper type 1 responses and caused regression in some of these lesions, especially those with estrogen-independent HER-2/neu-positive ductal carcinoma in situ.**

**Reviewed safety and tolerability of combination therapy of trastuzumab and a HER2/neu-specific vaccine, as well as demonstrated the potential use of vaccines to boost and maintain pre-existing immunity to HER2/neu with immunization. Moreover, they showed epitepope spread, which could be involved in modulating systemic mediators of tumor-induced immune suppression.**

**Discussed the importance of identifying patients who are likely to benefit from trastuzumab on the basis of evaluation of an immune response gene signature. Patients with low immune response signature did not obtain significant benefit from trastuzumab as those with high immune response signature. This opens the possibility for clinical trials designed to evaluate therapeutic approaches that might enhance the immune signature within HER2-positive tumors and thereby sensitize the tumors to biologic therapies. HER2-pulsed DC1 vaccines may be one way to boost the tumor immune signature.**

**Results from this study suggest that targeting the HER-2/neu pathway in early breast cancer and ductal carcinoma in situ using DC1 pulsed with HER-2/neu peptides boosted anti-HER-2 CD4 T1 helper responses and caused regression in some of these lesions, especially those with estrogen-independent HER-2/neu-positive ductal carcinoma in situ.**

**Reported safety and tolerability of combination therapy**
Dendritic cell vaccines in breast cancer

Review


72 Lakshmiraayanan V, Supekar NT, Wei J et al. MUC1 vaccines, comprised of glycosylated or non-glycosylated peptides or tumor-derived MUC1, can circumvent immunoeediting to control tumor growth in MUC1 transgenic mice. *PLoS One* 11(1), e0145920 (2016).


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Use of complementary therapies for side effect management in breast cancer: evidence and rationale

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Women diagnosed with breast cancer can experience chronic side effects after curative treatment concludes, negatively impacting survivorship. The most prevalent side effects addressed in the medical and nursing literature include symptoms such as hot flashes, fatigue, myalgias/arthralgias and cognitive impairment. Complementary therapies, particularly natural products including herbs, dietary supplements, vitamins, minerals, and probiotics, and mind–body techniques that include such modalities as yoga, meditation, massage, acupuncture, relaxation, tai chi and hypnosis show promise for treatment of some of these symptoms associated with cancer care. However, the research in this area is nascent and much more work is needed to understand symptom physiology and mechanisms of action of complementary therapies. The purpose of this paper was to summarize key evidence from Phase II and III randomized clinical trials in order to provide guidance to distinguish promising versus nonpromising interventions for symptom management.

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For approximately 3.1 million breast cancer survivors in the USA [1], side effects from treatment may lead to symptoms that persist long after active treatment [2]. These symptoms, such as cancer-related fatigue (CRF), hot flashes, myalgias/arthralgias and cognitive impairment, affect many women even a decade into their recovery thus diminishing overall health-related quality of life [3]. For difficult to manage symptoms, the use of complementary therapy modalities has increased within the past three decades, particularly within the cancer population [4]. Complementary

Practice points

- Complementary therapy use is common among women with a history of breast cancer and too often, this use is not reported to the medical care team. Therefore, it is important to ask patients if they are using any over-the-counter products or mind–body therapies and to ask with in an open, accepting manner.
- Women with breast cancer who use complementary therapy are typically younger, well-educated, of higher socioeconomic status and not currently receiving chemotherapy or hormone therapy.
- Some of the most prevalent physical symptoms experienced by women having been treated for breast cancer include hot flashes, fatigue, myalgias/arthralgias and cognitive impairment.
- Few natural products, also called dietary supplements, have been shown to be effective for symptoms in well-designed, placebo-controlled trials, and mechanisms of action are often not known.
- There is evidence that hypnosis can reduce hot flashes and may reduce cancer-related fatigue.
- Yoga has evidence for improving fatigue and cognitive impairment.
- Arthralgias/myalgias from aromatase inhibitors may be improved with acupuncture.

KEYWORDS
- AIMSS
- arthralgias/myalgias
- cognitive impairment
- complementary therapies
- fatigue
- ginseng
- hot flashes
- mind and body techniques
- yoga
- Ginkgo biloba
Prevalence of use of complementary therapies in women with breast cancer

Women with breast cancer express interest in complementary therapies, but historically underreport usage due to fear of dismissiveness from their providers [6]. Overall, patients report improved satisfaction when a provider can educate regarding options, integrate care and encourage patients to actively participate in their treatment course [7]. This facilitates a healthy patient-provider relationship while promoting hopefulness and empowerment toward self-care [6]. Integrating complementary therapies with conventional care has long existed in other countries around the world. Currently in European countries, complementary therapy use ranges from 14.9 to 73.1% of women with breast cancer [6]. Within the USA, the NCCIH [5] estimates that up to 90% of women with breast cancer have utilized some form of complementary therapy during their treatment course.

Women with breast cancer who use complementary therapy are typically younger, well-educated, of higher socioeconomic status (SES) and not currently receiving chemotherapy or hormone therapy [8,9]. Women with breast cancer tend to favor natural products (21%) and mind–body therapies (13%) for relief of long-term symptoms, improved overall health or for increased health-related quality of life [8,10–11].

Common side effects/toxicities women with breast cancer experience

Patients undergo a variety of treatments during their breast cancer experience that elicit a wide range of side effects and symptoms specific to that particular stage of care. During the surgical phase (mastectomy, lumpectomy or reconstruction), patients are at increased risk of postoperative symptoms such as infection, pain, swelling or impaired sleep. During chemotherapy, patients suffer from nausea and vomiting, neuropathy, fatigue and ‘chemo brain’ or cognitive impairment [1]. For patients undergoing radiation therapy, common side effects include skin irritation, soreness or localized swelling [1,8]. Patients continuing with long-term adjuvant endocrine therapy continue to experience fatigue (15–90%), vaginal changes (21–48%), hot flashes (50–70%) and musculoskeletal changes, arthralgias and myalgias [2,12]. Hence, survivors of breast cancer can face many side effects indefinitely into their survivorship [2].

Summary

Given the growing population of breast cancer survivors, and the prevalence of ongoing cancer-related symptoms [4], further knowledge regarding effective treatment of these symptoms is warranted. While complementary therapies are commonly used, the majority have not been rigorously tested for overall safety or efficacy [13]. There is preliminary research that attempts to elucidate the benefit, or lack of effect, of several complementary therapies. This paper will review the evidence for complementary therapies, specifically focusing on natural products and mind–body therapies, for some of the most common symptoms women with breast cancer experience, namely, hot flashes, fatigue, arthralgias/myalgias and cognitive impairment [4]. The literature search for this paper was performed from December 2015 to February 2016 and included PubMed, CINAHL, EMBASE and MEDLINE databases. First, the literature was searched for manuscripts that described the prevalence of symptoms in breast cancer survivors. The most prevalent symptoms formed the basis for the next level of the literature search. Search terms included combinations of the terms ‘breast cancer’, ‘complementary therapy’, ‘alternative therapy’ and ‘natural products’ with the following symptom terms: hot flashes, fatigue, menopausal symptoms, joint pain, arthralgias, myalgias, AIMSS and cognitive function. Studies included in this paper were published in English and included randomized, controlled clinical trials or pilot studies with pre–post intervention testing conducted in ambulatory settings in which the primary outcome of the study was the symptom of interest. Populations included breast cancer only and mixed populations in which the majority of participants had a breast cancer diagnosis. Manuscripts in which the symptom of interest was not the primary outcome or included pharmacologic therapy that
was not a dietary supplement were excluded. The reference lists for manuscripts selected for inclusion were scanned for relevant studies.

**Hot flashes**

Hot flashes are a prevalent symptom after menopause (natural and surgical), and also after treatment for breast cancer. They manifest as a sudden feeling of warmth, with or without sweating, that involves much of the body and may be associated with feelings of panic and anxiety [14]. It is estimated that 96% of women with breast cancer report hot flashes soon after beginning anticancer therapy [15] and up to 73% reporting hot flashes 6 years after diagnosis, even after estrogen deprivation therapy has been completed [16]. Hot flashes in women with breast cancer are particularly distressing since they can occur much earlier than their peer group. Estrogen deprivation therapies such as tamoxifen or aromatase inhibitors (AIs) can cause hot flashes and these therapies are taken for at least 5 or more years. Hot flash physiology is not definitively known, however, the current hypothesis suggests that dysregulation of the autonomic nervous system may contribute to hot flash symptomatology, both in terms of objective rises in temperature, and in subjective discomfort [17,18]. Management of hot flashes is critical for the maintenance of a high quality of life as they can interrupt sleep and thereby cause mood and cognitive alterations. There are some medications that are effective for reducing hot flashes, but these are mostly antidepressants that are associated with side effects and for some women, negative stigma. Therefore, women often seek complementary and alternative therapies for the relief of hot flashes, hoping for effectiveness without unwanted side effects.

**Natural products**

Studies of dietary supplements have been informed by the broad evidence base that serotonergic antidepressants have been effective in reducing hot flashes. Physiologic hypotheses for the etiology of hot flashes include low levels of serotonin as well as over activity of the sympathetic nervous system [17,19]. Natural products that have been evaluated for hot flashes include soy [20], black cohosh [21], vitamin E [22], magnesium oxide [23] and flaxseed (Table 1) [24]. Despite encouraging preliminary data, mostly in single arm trials, numerous randomized controlled trials (RCTs) evaluating various dietary supplements for hot flash reduction have been

<table>
<thead>
<tr>
<th>Study (year), study size</th>
<th>Natural product</th>
<th>Dose and duration</th>
<th>Primary outcome measure</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barton et al. (1998), n = 120</td>
<td>Vitamin E</td>
<td>Vitamin E 800 IU Placebo 4-week crossover design</td>
<td>HF score; severity × frequency per prospective HF diary, self-report</td>
<td>Statistically significant favoring vitamin E on crossover analysis but not clinically meaningful</td>
<td>[22]</td>
</tr>
<tr>
<td>Quella et al. (2000), n = 177</td>
<td>Phytoestrogens</td>
<td>Phytoestrogens 600 mg with 50 mg soy isoflavones: genistein, diadzein, glycitein Placebo 4-week crossover design</td>
<td>HF score; severity × frequency per prospective HF diary, self-report</td>
<td>No statistically significant difference, placebo looked a little better than the soy</td>
<td>[20]</td>
</tr>
<tr>
<td>Pruthi et al. (2012), n = 188</td>
<td>Flaxseed</td>
<td>Flaxseed bars 40 g flaxseed; 410 mg lignans Placebo bars 1 bar daily for 6 weeks 4-week crossover design</td>
<td>HF score; severity × frequency per prospective HF diary, self-report</td>
<td>HF reduction flaxseed: 29% HF reduction placebo: 28% Not statistically significant</td>
<td>[24]</td>
</tr>
<tr>
<td>Pockaj et al. (2006), n = 132</td>
<td>Black cohosh</td>
<td>Black cohosh 20 mg Placebo 1 pill twice per day 4-week crossover design</td>
<td>HF score; severity × frequency per prospective HF diary, self-report</td>
<td>HF reduction black cohosh: 20% HF reduction placebo: 27% Not statistically significant</td>
<td>[21]</td>
</tr>
<tr>
<td>Park et al. (2015), n = 289</td>
<td>Mg oxide</td>
<td>Mg oxide 800 mg Mg oxide 1200 mg Placebo Daily for 8 weeks 4-week crossover design</td>
<td>HF score; severity × frequency per prospective HF diary, self-report</td>
<td>HF reduction Mg oxide: 25% (800)–41% (1200) HF reduction placebo: 34% Not statistically significantly different</td>
<td>[23]</td>
</tr>
</tbody>
</table>

HF: Hot flash; Mg: Magnesium.
negative. More recently, a dietary supplement believed to increase concentrations of a serotonin metabolite and thus exhibiting an antidepressant effect, S-adenosylmethionine or SAMe, was evaluated for hot flash relief in a single arm pre-post-design study to determine if there was any activity supporting a larger Phase III trial. The study population included women with and without a history of breast cancer. The results were not supportive of any ability to reduce hot flashes, providing an effect equivalent to what is expected from a placebo [28].

• Mind–body techniques

Mind–body approaches have also been studied for hot flashes, namely, paced breathing, cognitive–behavioral therapy (CBT) and hypnosis. These modalities target the relaxation response that can potentially decrease sympathetic activation, negative cognitions and stress that can be triggers for hot flashes [17,19,26–27]. One CBT strategy has been studied in women with and without a history of breast cancer and evaluated in both a group delivered therapy and individually self-help delivered therapy [28,29]. The intervention included several components, namely, relaxation, paced breathing, cognitive restructuring, behavioral strategies (such as planning activities when energy levels are the highest) and stress/anxiety management. Sessions lasted 90 min for 6 weeks [28] or 120 min for 4 weeks [29] when delivered in a group. The self-help delivery utilized a workbook over 4 weeks and included two contacts with a clinical psychologist. Results of these studies demonstrated reductions in rating hot flashes and night sweats as a problem in the groups that received the intervention by group or through the self-help delivery, but actual frequency of hot flashes was not reduced. A four-arm RCT including over 400 women with a history of breast cancer compared physical exercise to the same CBT intervention described above (relaxation, paced breathing, cognitive restructuring), compared with both interventions (exercise and CBT) together versus a waitlist (WL) control group [30]. Similar results were shown with those receiving CBT reporting a decreased problem rating with hot flashes but actual frequency not decreasing. Hypnosis, on the other hand, has demonstrated improvements in the frequency and severity of hot flashes as well as a decrease in interference with activities and improvements in emotional parameters of anxiety and depressed mood [31,32]. Hypnosis for hot flashes is a mind–body therapy that uses relaxation and imagery to induce a deep relaxation where suggestions for cooling and stress reduction can be delivered to the subconscious. Studies have been completed in women with a history of breast cancer [31] as well as in women with natural or surgical menopause without breast cancer [32]. The study in breast cancer survivors used a WL control group, but in the study with a nonbreast cancer population, an attention control group was used as the control. Both studies demonstrated approximately 70% reductions in the frequency and severity of hot flashes, with additional benefits in reducing anxiety, depressed mood and improving sleep.

• Summary

In summary, there are no natural products, dietary supplements or herbs that have shown a benefit in hot flash reduction to date in rigorously designed trials. Compelling evidence exists for behavioral interventions that incorporate cognitive restructuring, relaxation and cooling suggestions as strategies to reduce hot flashes; hypnosis, in particular, has evidence leading to its recent inclusion as a treatment in a recent position statement by the North American Menopause Society [33].

Fatigue

Fatigue is a common side effect of cancer treatment. The National Comprehensive Cancer Network (2003) [34] defines CRF as “an unusual, persistent, subjective sense of tiredness related to cancer or cancer treatment that interferes with usual functioning.” CRF is multifactorial with cancer pathology and treatment toxicity to be the two key factors. Other contributing factors may include anemia, depression, infection and sleep disorders [35]. It is proposed that in response to cancer and its treatments, inflammatory cytokines signal the CNS and generate fatigue by altering neural processes [36]. CRF generally improves following treatment completion, but some level of fatigue may persist for years after that [37]. Studies in women with breast cancer indicate that fatigue is reported in up to 94% of patients undergoing cancer treatment [38–40]. Additionally, fatigue may be a persistent problem long into breast cancer survivorship. Numerous studies have reported fatigue prevalence of 20–66%, occurring up to 5–10 years after completion of adjuvant chemotherapy [41–44].
Use of complementary therapies for side effect management in breast cancer

**Perspective**

- **Natural products**
  There are no US FDA-approved pharmacologic agents for treating or preventing CRF and little evidence of benefit for commonly used drugs such as psychostimulants. Thus, studying natural products for fatigue is common. Preliminary evidence supports the use of Wisconsin ginseng for fatigue treatment as reported by two studies completed by the same investigators and evaluated American ginseng for the reduction of fatigue in a heterogeneous group of cancer survivors, both in those getting chemotherapy and/or radiation and in those who have completed all treatments [45,46]. Among these studies, a Phase III randomized, double-blind trial comparing 2000 mg of American ginseng to placebo for 8 weeks in 364 cancer patients, predominantly breast cancer, found a trend toward mean change in fatigue scores at 4 weeks and a statistically significant between-group mean change at 8 weeks, favoring ginseng over placebo [46]. Another plant product, this one from Brazil, is believed to have stimulant and aphrodisiac properties. It is called guarana and it is being studied for fatigue, although thus far the data are mixed. In one randomized, double-blind, placebo-controlled crossover trial including 36 breast cancer patients, 75 mg of guarana twice a day was not able to significantly improve CRF during radiation therapy [47]. However, in a more recent randomized, double-blind, placebo-controlled crossover study evaluating 50 mg of guarana twice daily in 75 women with breast cancer and receiving chemotherapy, guarana was shown to have a positive effect on CRF [48]. So, while data are not yet definitive on these two plant products, more research, particularly relating to their mechanisms of action, is needed to better understand their role in treating CRF.

- **Mind–body approaches**
  Mind–body techniques are among the most prevalent interventions studied for fatigue and some have shown promising results. Among these therapies, yoga has been the most studied in the breast cancer population. Two systematic reviews summarized findings from studies evaluating various types of yoga and provide support that yoga, from Iyengar to restorative, may be beneficial for reducing fatigue in women with breast cancer [49,50], with one of the reviews reporting effect sizes from individual trials ranging from 0.3 to 1.5 [49]. Another type of mind–body therapy that has been tested to improve CRF is mindfulness meditation, a program-based intervention that involves bringing attention to an individual’s present moment experiences with openness and acceptance. In breast cancer, three clinical trials testing mindfulness, all including fatigue as a secondary outcome, found significant improvements in fatigue scores [51–53], suggesting promise for mindfulness as an effective intervention for reducing fatigue in this population. In a study that tested yet another therapy, acupuncture, in 302 breast cancer patients with at least moderate fatigue, the study showed significant improvement in fatigue scores [54], warranting further research to establish the effectiveness of acupuncture for reducing CRF. Acupuncture has had mixed effects due to the lack of a standardized sham treatment, which has made this area of research challenging.

  Furthermore, mind and body techniques such as CBT showed some evidence in reducing fatigue among women with breast cancer. In a meta-analysis on the impact of behavioral techniques, including CBT and psychosocial support, in breast cancer patients, the authors showed a very small, yet statistically significant, effect on fatigue (effect size: -0.158) [55]. More recently, a clinical trial of 200 breast cancer patients comparing attention control to a group receiving a combined intervention (CBT and hypnosis) suggested that this combination has promise in treating CRF [56].

- **Summary**
  Research in breast cancer indicates that yoga appears to show the most efficacy for reducing fatigue as well as the dietary supplement, American ginseng, but more research is needed. Other mind and body techniques such as mindfulness and hypnosis with CBT, need more research before definitive conclusions about their effectiveness can be made.

**Arthralgias/myalgias**

Treatment for hormone receptor-positive breast cancer has improved with the use of third-generation AIs, as estrogen-deprivation therapies. Compared with tamoxifen (a selective estrogen receptor modulator), AIs are associated with disease-free survival [57] and forgoes serious side effects of tamoxifen such as increased risk for thromboembolic events and endometrial cancer [58]. However, AIs present with their own disruptive side effects, and because a patient may
be prescribed an AI for over 5 years, they present a major concern for patients and providers alike in terms of adherence and quality of life. One of the most common and debilitating side effects is the occurrence of AI-induced musculoskeletal symptoms (AIMSS). AIMSS encompasses a variety of symptoms but are generally characterized as aches and pains in the joints and muscles after beginning AI use; these symptoms are commonly noted in the hands, knees and back, among others. There also appears to be changes in tenosynovial structures of affected areas and possible development of carpal tunnel syndrome; moreover, patients may describe stiffness or a feeling of sudden aging that is difficult to manage. Notably, musculoskeletal symptoms are defined and reported differently from study to study depending on the primary outcome and the measurements used.

Phase III clinical trials indicate that musculoskeletal symptoms occur between 11 and 36% during AI use; using patient-reported outcomes, though, this percentage may increase to over half. There is some evidence to indicate that the presence of AIMSS is actually associated with greater rates of disease-free survival than those who do not experience these symptoms. However, the discontinuation rate due to AIMSS is clinically significant at approximately 30% and symptoms will not necessarily disappear over time while remaining on an AI. Therefore, there is a compelling reason to treat and manage AIMSS to improve overall rates of adherence and, ultimately, survival.

One factor limiting treatment for AIMSS is that its etiology is unknown. Many hypotheses point to the severe estrogen depletion that occurs during AI use, although there are likely other significant etiologic factors. Furthermore, patients that have pre-existing joint pain, are younger or more recently experienced menopause, were treated with systemic taxanes and have insufficient levels of vitamin D, are potentially more at risk for AIMSS. There does also appear to be a genetic component to AIMSS; Mao et al. observed that polymorphisms in \textit{CYP19A1}, a gene known for its role in encoding aromatase, is correlated with the incidence of AIMSS. These risk factors may lead to a shorter mean time to discontinuation particularly if a patient is younger, has received chemotherapy with taxanes, or pain is severe. Primary conventional treatment for AIMSS includes the use of NSAIDS or switching to another AI or tamoxifen. In spite of relatively similar levels of estrogen depletion between AIs, the act of switching does appear to improve adherence rates and reduced severity of AIMSS. However, more effective treatments are needed.

- **Natural products**
  - One notable dietary supplement linked with AIMSS outcomes is vitamin D. Descriptive data suggest that those with inadequate or deficient vitamin D levels below 30 ng/ml are potentially more likely to be symptomatic. Khan \textit{et al.} propose that vitamin D3 supplementation at 50,000 IU per week is safe and may reduce functional disability resulting from AIMSS. It is unclear, however, how strong the relationship between vitamin D and AIMSS; in spite of many postmenopausal women having inadequate serum levels of vitamin D, Singh \textit{et al.} did not find a significant correlation between AIMSS and vitamin D levels. From these studies, it is clear that vitamin D is only one potential component contributing to the cause of AIMSS and that there are other underlying factors.

  Popular supplements such as glucosamine with chondroitin and omega-3-fatty acids (O3-FAs) have also been investigated based on their role in arthritis pain. In a single-arm, Phase II study by Greenlee \textit{et al.}, participants (n = 39) took 1500 mg/day of glucosamine-sulfate and 1200 mg/day of chondroitin-sulfate over the course of 24 weeks; at follow-up, there were significant, moderate improvements in joint pain severity, interference and functioning. However, these results cannot necessarily be generalized without a larger, placebo-controlled trial.

  Hershman \textit{et al.} conducted a randomized, placebo-controlled trial (n = 249) of 3.3 g/day of O3-FA compared with a soybean and corn oil placebo taken over 24 weeks. At 12 and 24 weeks, there were statistically and clinically significant changes in worst pain/stiffness scores (over 50%) for both groups, but no significant difference between the two groups was found. While O3-FAs may be beneficial for lowering abnormally high triglyceride levels in this group; with the current evidence, it is unlikely that O3-FAs provide a viable option for AIMSS treatment above placebo.

- **Mind–body techniques**
  - Evidence for mind and body therapies for AIMSS is limited even though patients often
express interest in complementary therapies that can provide relief from pain and improve quality of life [79,80]. Acupuncture is one option that has been associated with little to no known side effects. Crew et al. [81] provided promising results in a randomized, sham-controlled acupuncture intervention of 38 women for AIMSS that consisted of twice weekly treatments over 6 weeks; there was approximately a 50% improvement in worst pain score for the manual acupuncture versus a sham acupuncture versus WL control for ten sessions over 8 weeks. Both the electro-acupuncture and sham groups reported significant reductions in pain severity and interference at 8 and 12 weeks compared with the WL control group, yet there were no significant differences between the two groups themselves. Though the results suggest that acupuncture is no better than placebo, there is an ongoing debate as to whether or not sham acupuncture provides unintended physiologic benefits beyond placebo effects [82]. Nevertheless, a recent review and meta-analysis of acupuncture randomized, controlled trials by Chien et al. [83] found that there is no standardized intervention for AIMSS and that results trend toward reduced symptoms but conclusive evidence does not exist. Beyond acupuncture, there exist few other mind–body interventions that have been evaluated for AIMSS; feasibility studies conducted by Galantino et al. [79,84] provide promising evidence for Yoga and Tai-Chi in reducing pain severity and improving quality of life, respectively. Taken together, it is clear that much of the research on mind and body techniques remains in its preliminary stages.

### Cognitive impairment

Individuals with breast cancer can experience changes in cognitive function across the disease trajectory beginning after diagnosis and before any treatment to 20-year post-treatment [85,86]. Estimates of the prevalence of cognitive problems have been reported to be as high as 33% before treatment (range: 10–33%) and up to 61% after surgery, radiation therapy and/or chemotherapy (range: 10–61%) [87–97]. Variation among these estimates may be related to a number of study-related factors including differences in sample sizes, patient characteristics, treatment regimens, timing of assessments and definitions of impairment. Patient characteristics that may affect the expression of cognitive symptoms include age, cognitive reserve, presence of co-morbid conditions and genetic variations (APOE and COMT genotypes) [95–99]. Cognitive processes affected include attention, memory, processing speed and executive function [100]. These processes are necessary for everyday functioning including planning and achieving personal goals, learning and interacting with others [101,102]. Possible mechanisms underlying cognitive changes in breast cancer include central neurotoxicity from chemotherapy, immune and other physiologic responses to the cancer and cancer treatments (surgery, radiation therapy, chemotherapy, hormone therapy), mental or attentional fatigue and psychological and symptom distress [103–105]. Currently, there are no empirically validated interventions to optimize cognitive function in individuals with breast cancer. Complementary therapies are a promising group of interventions that may reduce cognitive symptoms and improve quality of life through a variety of mechanisms including neurologic modification, immune regulation, restoring attentional function, cognitive training and stress reduction. At least 13 research reports examined the benefits of complementary therapies in preventing cognitive decline or improving cognitive function (primary outcomes) in community dwelling women with breast cancer. These studies will be discussed below; Table 2 provides a detailed description of the therapies evaluated.

### Natural products

Only one study examined the effects of a natural product on cognitive function in women with breast cancer. The study by Barton et al. [106] used a randomized, placebo-controlled, double-blind study design to assess the efficacy of
<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Complementary therapy</th>
<th>Dose</th>
<th>Program</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barton et al. (2013)</td>
<td>Ginkgo biloba</td>
<td>60-mg capsules p.o. b.i.d</td>
<td>Ginkgo biloba administered orally before second cycle of adjuvant chemotherapy and continued until 1-month postadjuvant chemotherapy</td>
<td>[106]</td>
</tr>
<tr>
<td>Milbury et al. (2014)</td>
<td>Tibetan sound meditation</td>
<td>Two 60-min classes a week for 6 weeks. Home practice encouraged</td>
<td>Each session included focus on concentration and mindfulness. Practices included breathing exercises, visualizations and vocalization of meditation sounds</td>
<td>[107]</td>
</tr>
<tr>
<td>Lesiuk (2015)</td>
<td>Mindfulness-based music therapy</td>
<td>One 60-min class a week for 4 weeks plus 15–20 min daily home practice</td>
<td>Each session included focusing on a sound, focusing on music and a discussion of focus. Home practice included music exercises and written reflections</td>
<td>[108]</td>
</tr>
<tr>
<td>Cimprich (1993)</td>
<td>Restorative therapy</td>
<td>Three 20–30-min sessions per week for 3 months</td>
<td>Sessions included participating in restorative activities including spending time with nature (watch sunset), exploring things of interest (reading) and engaging in enjoying activities (making things)</td>
<td>[109]</td>
</tr>
<tr>
<td>Cimprich and Ronis (2003)</td>
<td>Restorative therapy – nature</td>
<td>Exposure to the natural environment for 120-min per week</td>
<td>Sessions included participating in restorative activities that focus on nature including visiting a scenic spot, bird watching, listening to sounds of nature, watching sunset and tending or plants/gardens</td>
<td>[110]</td>
</tr>
<tr>
<td>Alvarez et al. (2013)</td>
<td>Neurofeedback</td>
<td>Two 33-min sessions a week for 10 weeks</td>
<td>NeurOptimal System (Zengar Institute). Session includes placing sensors on head to analyze an EEG and identify phase state changes. Participants listen to music and experience brief interruptions to signal feedback</td>
<td>[111]</td>
</tr>
<tr>
<td>Komatsu et al. (2015)</td>
<td>Yoga</td>
<td>One 30-min orientation and 90-min yoga class. Home practice 15-min session using DVD encouraged</td>
<td>Hatha Yoga. Participants selected DVD(s) with differing themes (warm-up, low-intensity, high-intensity) for home practice after orientation and first yoga class. Yoga logs kept to record dose (no recommended dose)</td>
<td>[112]</td>
</tr>
<tr>
<td>Ferguson et al. (2007 and 2012)</td>
<td>Memory and Attention Adaptation Training</td>
<td>One 30–50-min session per month for 4 months, three phone contacts between monthly visits and daily home practice</td>
<td>Monthly sessions included education on chemotherapy-associated problems, self-awareness and identifying situations where cognitive problems occur and providing tailored self-regulation and compensatory strategies</td>
<td>[113,114]</td>
</tr>
<tr>
<td>Kesler et al. (2013)</td>
<td>Executive Function Training Program</td>
<td>Four 20–30-min online sessions a week for 12 weeks</td>
<td>Program included 13 different exercises from Lumos Labs. Exercises included switching games, mental rotation games, n-back memory games, spatial sequencing games, route planning and rule-based problem solving. Exercises tailored to individual’s ability and increased in difficulty over time</td>
<td>[115]</td>
</tr>
<tr>
<td>Von Ah et al. (2012)</td>
<td>Memory and speed processing program</td>
<td>Ten 60-min classes over 6–8 weeks</td>
<td>Memory training program included education on strategies for remembering word lists, sequences and text and practice (adapted from ACTIVE trial [Jobe et al., 2001]) Information Processing training program used the Insight program by Posit Science that was derived from the ACTIVE trial</td>
<td>[116]</td>
</tr>
<tr>
<td>Ercoli et al. (2015)</td>
<td>Cognitive training</td>
<td>One 120-min class a week for 5 weeks plus 20 min a week of daily home practice</td>
<td>Program focused on attention, memory and executive function. Program included education (in-class), practice with three levels of difficulty (in class and home practice using a training manual, CD and/or stopwatch), and goal setting</td>
<td>[117]</td>
</tr>
<tr>
<td>Damholdt et al. (2016)</td>
<td>Cognitive training</td>
<td>Five 30-min sessions a week for 6 weeks plus two phone contacts after randomization</td>
<td>Program included 12 tasks from HAPPYneuron Pro™ in Danish. Tasks included training in focusing attention, vigilance, verbal and visual memory and problem solving, learning strategies and maintaining and processing perceptions simultaneously. Difficulty levels increase after two error-free trials. Feedback about speed and accuracy given</td>
<td>[118]</td>
</tr>
</tbody>
</table>

b.i.d.: Twice daily; CD: Compact disc; DVD: Digital video disc; p.o.: Per orem (orally).
Ginkgo biloba in preventing cognitive changes in 166 women with breast cancer receiving adjuvant chemotherapy [106]. Researchers found no differences between individuals taking Ginkgo versus placebo in cognitive performance or self-report from baseline to 24-month postchemotherapy [106].

- Mind–body techniques

Two studies examined the benefits of meditation on cognitive function in women with breast cancer [107,108]. Lesiuk [108] used a pre–post-study design to assess the efficacy of a mindfulness-based music therapy intervention in preventing cognitive changes in 15 women with stage I–III breast cancer currently receiving chemotherapy and found significant improvement in attentional performance and perceived function.

The second study by Milbury et al. [107] used a RCT with a WL control group to assess the feasibility and preliminary efficacy of Tibetan Sound Meditation in optimizing cognitive function in 47 women with early-stage breast cancer (stage I–III) previously treated with chemotherapy (6–60 months prior). Researchers found Tibetan Sound Meditation to be feasible and enjoyable for participants and observed trends in cognitive improvement.

Two studies assessed the use of activities that restore attention in mentally fatigued individuals newly diagnosed with stage 0–III breast cancer [109,110]. Specifically, Cimprich [109] used a RCT to assess a restorative therapy intervention in 32 women after primary breast cancer surgery and found that the intervention group had a significantly greater mean gain in attentional function over four time points from 3- to 90-day postsurgery. A subsequent RCT by Cimprich and Ronis [110] used a pre–post design to assess a restorative therapy intervention focused on the natural environment in 157 women before and after breast cancer surgery. Researchers found that the intervention group had a significantly greater gain in attentional function after controlling for key covariates.

Alvarez et al. [111] used a repeated measure design with WL control period before the active intervention period to assess the efficacy of a neurofeedback intervention on optimizing cognitive function in 23 women with breast cancer previously treated with chemotherapy (6–60 months). Researchers found a significant improvement in cognitive function by self-report from before to 1-month after the neurofeedback intervention [111].

Komatsu et al. [112] used a pre–post-study design to assess the feasibility and preliminary efficacy of a home yoga program on perceived cognitive function in 18 women with breast cancer receiving adjuvant and neoadjuvant chemotherapies. Researchers found the yoga program to be safe and feasible but did not observe any improvement self-report cognitive functioning [112].

Six studies examined the effects of CBT on improving cognitive function in women with breast cancer. Three studies used investigator developed cognitive behavioral programs. In particular, two studies by Ferguson et al. [113,114] evaluated the efficacy of a brief CBT intervention, Memory and Attention Adaptation Training or MAAT, in women reporting cognitive problems postchemotherapy for stage I–II breast cancer. In a pilot study, these investigators used a repeated measures design to assess MAAT in 29 women and found it to be satisfactory for participants and found MAAT significantly improved objectively measured cognitive performance and self-reported cognitive symptoms [113]. A follow-up RCT with a WL control group assessed MAAT in 40 women and found that the intervention group had a significant mean gain in verbal memory performance [114].

The third study by Ercoli et al. [117] used a repeated measures design to assess a cognitive training program in 27 women reporting cognitive post-treatment for stage 0–III breast cancer. Researchers found a significant improvement in attention, memory and processing performance and self-report of cognitive functioning.

The remaining three studies examined the benefit of commercially available cognitive training programs [115–116,118]. Damholdt et al. [118] used a RCT with WL controls to assess a customized online cognitive training program from HAPPYneuron Pro in 41 women with a history of breast cancer reporting cognitive problems. Researchers found improvement in objectively measured verbal and working memory performance but not cognitive self-report.

Kesler et al. [115] used a RCT study design with WL controls in 41 women to assess a customized online cognitive training program from Lumos Labs in 41 women postchemotherapy for stage I–III breast cancer and found a significant improvement in attention and information processing function and a trend to improved cognitive self-report. Von Ah et al. [116] used a three-group RCT to assess the efficacy of a memory...
or commercially available information processing speed training program (Insight) versus WL controls in 82 women reporting cognitive problems postchemotherapy for stage I–II breast cancer. Researchers found a significant improvement in cognitive performance and self-report postintervention in the memory training group versus controls and the information processing speed group versus controls.

● Summary

Studies in the area of cognition and complementary therapy are many, but most can be considered preliminary due to the lack of active control groups, no blinding of the study hypothesis, small sample sizes and a failure to control for important confounding variables. No natural products appeared to benefit individuals with breast cancer; however, a variety of mind and body techniques appeared to have some promise.

Conclusion

Despite the prevalence of complementary therapy use, there continues to be limited understanding on overall safety, efficacy and long-term effects [9]. While vitamins, dietary supplements and herbs are described as ‘natural products’, if effective, these agents have biologic properties that can pose a risk of toxicity, drug interactions and often, unknown dose effects [9,11]. Further research is needed that matches proposed mechanisms of action with symptom biology in well-designed trials to move this area of science forward.

Preliminary data from this review suggest positive evidence especially surrounding mind and body interventions for hot flashes and fatigue, specifically hypnosis yoga and mindfulness [31,49–50]. Regarding other common side effects, such as AI-induced myalgias/arthralgias and cognitive dysfunction related to cancer treatment, potential benefit has been found in early studies surrounding acupuncture, restorative therapy and cognitive training, but these studies lack conclusive evidence due to various limitations. Studies with mind and body techniques need to control for the nonspecific effects of group and provider interaction at the very least, and to match strategies with mechanisms specific to the symptom under study.

Use of complementary therapies continues to be under-reported with only approximately 50% of women openly discussing complementary therapy with their clinician [11]. Particularly concerning are patients self-treating with biologic-based supplements, agents not screened by the FDA, with little clinical oversight. This presents a safety concern for clinicians, who may not be aware of potential risks due to lack of disclosure from the patient. Providers who are knowledgeable about the current evidence, as well as limitations of existing studies, have more options in their arsenal to improve therapeutic outcomes for their patients suffering from cancer-related symptoms [9,11].

While some mind–body therapies have the potential for efficacy, they are not without the investment of time, resources and commitment. The most well-studied behavioral interventions, such as hypnosis, CBT, yoga or mindfulness, require trained professionals to instruct, lead and conduct a series of sessions. Further well-designed studies are still needed to optimize active components, to elucidate adequate dosing and to inform the integration into clinical practice. The ability to match mechanisms of interventions to the etiology of symptoms will help to develop mind–body techniques that can more efficiently improve outcomes with a reasonable amount of time and resources.

Future perspective

Complementary therapies, such as mind–body techniques and natural products, have specific physiologic effects that need to be elucidated through rigorous clinical trials with translational objectives. Understanding mechanisms will facilitate the evaluation of interventions that match symptom physiology. Advancing the science in this way will have tremendous implications for the integration of complementary therapies into clinical practice.

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• of interest

www.cancer.org/acs/groups


• Overview of survivorship issues and evidence-based treatment.


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• An important overview about the physiologic changes known to be related to cognition.


