frontiers | Research Topics

Reviews in breast cancer 2023

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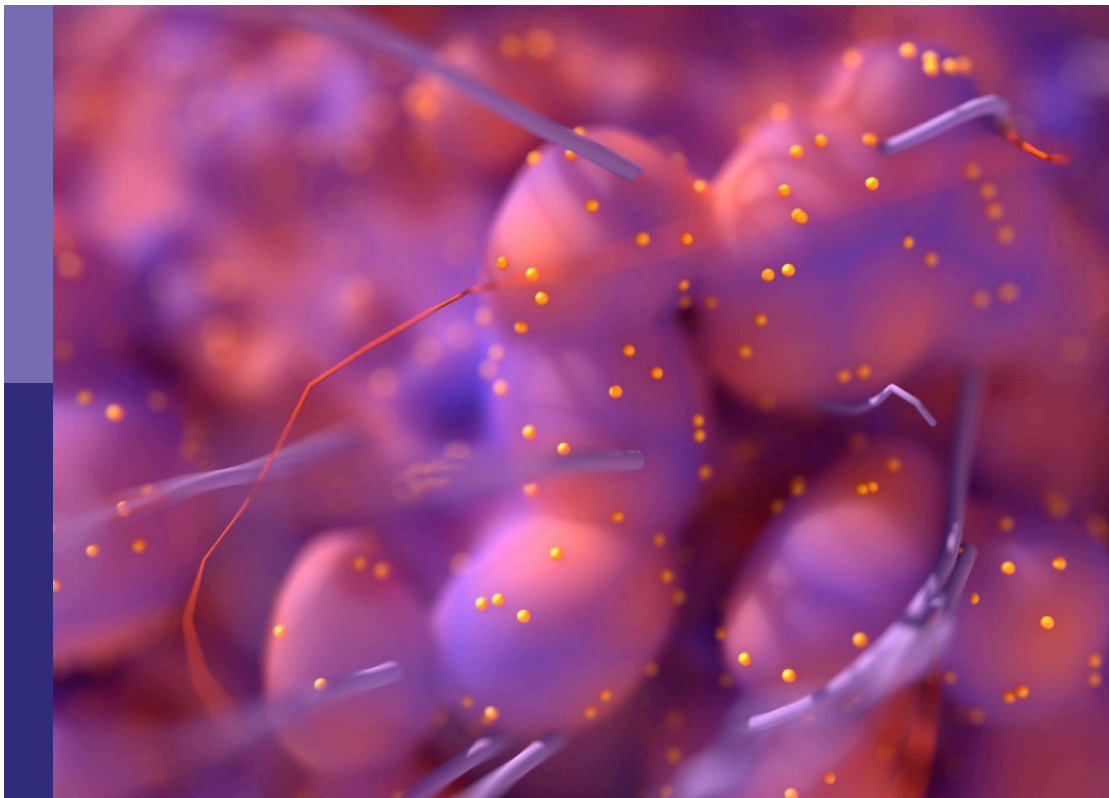
Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-8325-5609-2
DOI 10.3389/978-2-8325-5609-2

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Reviews in breast cancer: 2023

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Citation
De Miglio, M. R., Ni, K., Mohammed, S., Bezerra, D. P., eds. (2024). *Reviews in breast cancer: 2023*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5609-2

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frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 16 October 2024
DOI 10.3389/fonc.2024.1488263

OPEN ACCESS

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RECEIVED 29 August 2024
ACCEPTED 02 October 2024
PUBLISHED 16 October 2024

CITATION
Bezerra DP, Ni J, Mohammed SI and De Miglio MR (2024) Editorial: Reviews in breast cancer: 2023.
Front. Oncol. 14:1488263.
doi: 10.3389/fonc.2024.1488263

Editorial: Reviews in breast cancer: 2023

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KEYWORDS

breast cancer, epidemiology, review, tumor microenvironment, biomarkers

Editorial on the Research Topic
Reviews in breast cancer: 2023

Breast cancer is a heterogeneous disease characterized by several subgroups that can be identified through molecular biomarkers, which may serve as predictive indicators (1). Globally, female breast cancer ranks as the second most common cancer, with 2,308,897 new cases diagnosed, and the fourth leading cause of cancer-related deaths, with 665,684 fatalities in 2022 (2). Furthermore, the GLOBOCAN Cancer Tomorrow prediction tool estimates that the incidence of breast cancer will increase by more than 46% by 2040 (3).

The landscape of breast cancer research is continually evolving, with new insights and innovations emerging rapidly. This series of article collections on the Research Topic "Reviews in Breast Cancer 2023" aimed to showcase cutting-edge research, highlighting recent advances in the field and emphasizing key directions and new possibilities for future investigations.

In this Research Topic, Roheel et al. conducted a systematic review of the global epidemiology of breast cancer, focusing on risk factors. The authors found that lifestyle factors, such as nutrition and exercise, as well as genetic variables, including DNA repair gene polymorphisms and mutations in breast cancer genes (BRCAs), are associated with breast cancer risk. Notably, most of the genetic variability was linked to Asian populations, whereas lifestyle factors were more commonly associated with breast cancer risk in the United States and the United Kingdom. This highlights the differences in demographic, genetic, and lifestyle risk factors across various countries.

These findings are corroborated by Nicolis et al., who emphasized the complex interplay of genetic, lifestyle, and environmental risk factors for breast cancer, noting significant differences between populations. The authors also highlighted the potential of artificial intelligence (AI) to revolutionize personalized breast cancer prevention and detection by tailoring procedures to individual risk factor profiles. Additionally, Chen et al. reported that hepatitis C virus infection is associated with an increased risk of breast cancer.

Abdel-Razeq conducted a systematic review focusing on the oncological safety of less aggressive surgical techniques, including skin-sparing and nipple-sparing mastectomies, for breast cancer patients with mutations in high-penetrance cancer-predisposing genes such as *BRCA1* and *BRCA2*, as well as for unaffected carriers. Additionally, Li et al. provided a

systematic review and meta-analysis of axillary treatment in patients with clinically node-negative and sentinel node-positive early breast cancer.

Xu et al. reviewed the role of matrix stiffness in breast cancer progression. Matrix stiffness, which refers to the progressive elastic force exerted by the extracellular matrix on cells, plays a crucial role in regulating various aspects of breast cancer, including tumorigenesis, proliferation, invasion, metastasis, drug resistance, immune evasion, and the growth of breast cancer stem cells. Owing to its significant impact, matrix stiffness has emerged as a potential target for breast cancer treatment. The authors concluded that a deeper understanding of matrix stiffness could pave the way for the development of new therapeutic options for breast cancer.

Several reviews (Ansari et al., Tollens et al., Zhang et al., and Zhang et al.) have concentrated on imaging technologies for breast cancer detection. The authors assessed current modalities, including magnetic resonance imaging (MRI), and examined emerging technologies, such as contrast-enhanced and elastography ultrasound combined with deep learning. Collectively, these reviews highlight the ongoing evolution of imaging technologies and emphasize a clear trend toward integrating traditional imaging techniques with advanced technologies such as contrast enhancement, elastography, and AI-driven analysis. This convergence is expected to enable earlier detection, improve diagnostic accuracy, and ultimately contribute to more favorable patient outcomes.

Beyond imaging, this Research Topic also includes reviews that address other critical aspects of breast cancer, such as novel diagnostic and therapeutic biomarkers (Long et al.), the tumor microenvironment (Akinsipe et al.), surgical interventions (Li et al.), endocrine and immunotherapy (Lan et al., Alaluf et al., and Sharaf et al.), and complementary therapies (Deng et al., and Li et al.). Additionally, Ali-Thompson et al. conducted a bibliometric analysis of HER2-positive breast cancer from 1987 to 2024, offering valuable insights into the research trends and developments in this specific subtype over the past decades.

Early cancer detection is critical for improving overall survival rates, as it enables the initiation of appropriate treatments before metastasis occurs (4). The identification of biomarkers, such as miRNAs, is emerging as a promising strategy for the early diagnosis of breast cancer. Wang et al. conducted a meta-analysis on the association between circulating miR-155 and breast cancer diagnosis and suggested that further large-scale clinical studies on this miRNA are warranted.

The primary cause of death in patients with breast cancer is disease progression due to metastasis and drug resistance. To address this challenge, there is a critical need for reliable molecular biomarkers that can predict disease response. In a meta-analysis and systematic review, Sang et al. found that low absolute lymphocyte counts and elevated neutrophil–lymphocyte ratios were associated with poor outcomes in metastatic breast cancer (mBC) patients. These findings underscore the significant prognostic value of these biomarkers in this patient population.

Trophoblast cell-surface antigen 2 (TROP2) overexpression is associated with aggressive subtypes of breast cancer and drug resistance (5), and its silencing has been shown to reduce tumor growth, underscoring its oncogenic relevance (6). Yao et al. examined the variability of TROP2 expression across different breast cancer subtypes, its correlation with clinicopathological features, and its prognostic and predictive roles. These findings highlight the critical role of TROP2 in tumor dynamics, suggesting that TROP2 represents a compelling therapeutic target.

Wang et al. reviewed the roles and mechanisms of long noncoding RNAs (lncRNAs) in breast cancer progression, metastasis, and drug resistance. They explored lncRNA-based strategies and lncRNA-targeted therapies, emphasizing their potential to enhance the management of breast cancer patients in clinical practice.

In recent decades, significant advancements have been made in the treatment of hormone receptor-positive breast cancer, leading to notable improvements in survival and quality of life. As first-line treatments for hormone receptor-positive mBC patients, cyclin-dependent kinase 4/6 (CDK4/6) inhibitors markedly improve progression-free survival and overall survival. Horani et al. reviewed the literature on the role of CDK4/6 inhibitors in mBC progression. Additionally, Zhang et al. suggested that CDK4/6 inhibitors might also offer therapeutic benefits for HER2-positive breast cancer subtypes, presenting new possibilities for treatment development.

Myeloid differentiation factor 88 (MyD88) overexpression is closely associated with aggressive tumor characteristics, positioning it as a potential prognostic biomarker and therapeutic target. MyD88 plays a crucial role in modulating inflammatory and immune responses, highlighting its impact on the interaction between tumors and the immune system. Zheng et al. analyzed the mechanisms underlying the diverse roles of MyD88 in breast cancer, suggesting that translating these findings into clinical applications holds significant promise for precision medicine approaches, potentially enhancing patient prognosis and therapeutic strategies. Immunotherapy, often utilized in personalized cancer care, strengthens the ability of the immune system to recognize and eliminate cancerous cells.

Alqathama et al. reviewed key immune response-related pathways in breast cancer and discussed how natural compounds can function as immunomodulatory agents that target biomolecular pathways. Some natural compounds have been shown to inhibit immune checkpoints, as well as PD-L1, offering new avenues for therapeutic intervention.

Conclusions

Overall, the articles compiled in this Research Topic not only consolidate the latest advancements but also provide new insights into breast cancer research. This Research Topic serves as a valuable resource for researchers, clinicians, and policymakers dedicated to enhancing the diagnosis and treatment outcomes for patients with breast cancer.

Author contributions

DB: Writing – original draft, Writing – review & editing. JN: Writing – original draft, Writing – review & editing. SM: Writing – original draft, Writing – review & editing. MD: Writing – original draft, Writing – review & editing.

Acknowledgments

We are grateful to the Frontiers in Oncology Editorial Team members for their continuous, valuable and helpful support given to us for this Research Topic.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 14 September 2023
DOI 10.3389/fonc.2023.1265197

OPEN ACCESS

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RECEIVED 22 July 2023
ACCEPTED 29 August 2023
PUBLISHED 14 September 2023

CITATION
Abdel-Razeq H (2023) Surgical options for patients with early-stage breast cancer and pathogenic germline variants: an oncologist perspectives.
Front. Oncol. 13:1265197.
doi: 10.3389/fonc.2023.1265197

Surgical options for patients with early-stage breast cancer and pathogenic germline variants: an oncologist perspectives

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Breast cancer continues to be the most common cancer diagnosed among women worldwide. Family history of breast cancer is frequently encountered, and 5-15% of patients may carry inherited pathogenic germline variants, identification of which can be helpful for both; patients themselves and their unaffected close relatives. The availability and affordability of molecular diagnostics, like next generation sequencing (NGS), had resulted in wider adoption of such technologies to detect pathogenic variants of cancer-predisposing genes. International guidelines had recently broadened the indications for germline genetic testing to include much more patients, and also expanded the testing to include multi-gene panels, while some professional societies are calling for universal testing of all newly diagnosed patients with breast cancer, regardless of their age, personal or family history. The risk of experiencing a contralateral breast cancer (CBC) or ipsilateral recurrence, is well known. Such risk is highest with variants like *BRCA1* and *BRCA2*, but less well-studied with other less common variants. The optimal local therapy for women with *BRCA*-associated breast cancer remains controversial, but tends to be aggressive and may involve bilateral mastectomies, which may not have any survival advantage. Additionally, surgical management of unaffected women, known to carry a pathogenic cancer-predisposing gene, may vary from surveillance to bilateral mastectomies, too. The oncological safety, and the higher satisfaction of unaffected women and patients with new surgical techniques, like the skin-sparing (SSM) and nipple-sparing (NSM) mastectomies, eased up the process of counselling. In this review, we address the oncological safety of less aggressive surgical options for both; patients and unaffected carriers.

KEYWORDS

hereditary breast cancer, risk-reducing surgery, ipsilateral breast tumor recurrence, ATM, CHEK2, BRCA, PALB2, TP53

1 Introduction

Breast cancer is the most common cancer worldwide and is considered one of the leading causes of cancer-related mortality in both developed and developing countries. In 2020, about 2.3 million women were diagnosed with breast cancer worldwide and 685,000 died of their disease (1). In 2023, almost 300,000 women will be diagnosed with breast cancer in the U.S alone (2). Almost one in five patients with newly diagnosed breast cancer report a family history of breast cancer (3–5). However, smaller fraction may be attributed to an inherited cancer-predisposing gene, mostly in *BRCA1* or *BRCA2* (6). Based on one meta-analysis, the estimated mean cumulative risk for developing breast cancer by age 70 for carriers of the *BRCA1* variant is 57%, whereas the risk for carriers of the *BRCA2* variant is a little lower at 49% (7). However, other studies reported higher cumulative breast cancer risk (72%) to age 80 for *BRCA1* and 69% for *BRCA2* carriers (8). The extent to which other pathogenic variants, like *CHEK2*, *PALB2*, *ATM*, *TP53*, are associated with breast cancer susceptibility varies significantly (9, 10).

Molecular diagnostics, like next generation sequencing (NGS), is becoming affordable and is widely utilized to detect variants in cancer predisposing genes (11, 12). For patients without *BRCA1/2* variants, breast-conserving surgery (BCS), with or without neoadjuvant chemotherapy, followed by radiation therapy, is the treatment of choice for most patients; it offers similar survival to that of mastectomy (13–16). More recent study claimed even better survival outcome with BCS followed by radiation therapy, compared to mastectomy (17–20). In a recent study that used the Surveillance, Epidemiology and End Results (SEER) database which identified 205,788 women with breast cancer diagnosed from 1988 to 2018, patients who underwent BCS and radiotherapy had higher competing risk of breast cancer recurrence (adjusted hazard ratio [HR]: 1.996, 95% CI: 1.925-2.069, p<0.001) and lower competing risk of breast cancer-specific death (BSD) when compared to mastectomy (adjusted HR: 0.584, 95% CI: 0.572-0.597, p<0.001) (21). Another study that also used the SEER database reached almost similar conclusions (22). Additionally, BCS provides better quality of life; a recent study concluded that patients treated with BCS were more satisfied with their cosmetic outcome compared to those who had mastectomy with or without reconstruction (23).

In this review, we discuss surgical treatment options for patients with breast cancer known to have a high-penetrant cancer-predisposing gene, like the *BRCA1* and *BRCA2*, and address the oncological safety of less aggressive surgical options, for both patients and unaffected carriers.

2 The prevalence of germline mutations

Depending on population studied and method of testing, 5-15% of breast cancer patients are carriers of one of the increasingly recognized hereditary predisposition genes. Multiple studies have evaluated the prevalence of pathogenic (PV) or likely pathogenic variants (LPV) in breast cancer patients; majority of such studies were retrospective and from single institution. In a large industry sponsored study, over 35,000 women with breast cancer underwent germline genetic testing with a 25-gene panel. PV/LPVs were detected in 9.3% of women tested; 51.5% were in genes other than *BRCA1* or *BRCA2*, including *CHEK2*, *ATM* and *PALB2*. Rates were significantly higher among younger women aged < 40 years (24). In another study, all women 20 years of age or older diagnosed with breast (or ovarian cancer) in the state of California and Georgia in 2013 and 2014, and reported to the SEER registries were reviewed. Over 77,000 patients with breast cancer were included; almost 25% of them had genetic test results. Pathogenic variants were mostly in *BRCA1* (3.2%), *BRCA2* (3.1%), *CHEK2* (1.6%), *PALB2* (1.0%) and *ATM* (0.7%) (25).

We recently reported our experience on 1,310 non-Western patients diagnosed with breast cancer. Patients were tested as per the National Comprehensive Cancer Network (NCCN) guidelines. Age ≤ 45 years was the most common indication for testing, while positive family history of breast, ovarian, pancreatic or prostate cancers, and triple-negative disease were among other frequent indications. Among the whole group, 184 (14.0%) patients had PV/LPVs; only 90 (48.9%) were in *BRCA1* or *BRCA2*, while 94 (51.1%) others had pathogenic variants in other genes; mostly in *APC*, *TP53*, *CHEK2* and *PALB2*. Mutation rates were higher among patients with positive family history (p=0.009); especially if they were 50 years or younger at the time of breast cancer diagnosis (p<0.001). Patients with triple-negative disease had relatively higher rate (17.5%) and mostly in *BRCA1/2* genes (71.4%) (26).

3 Patients at risk

Several international guidelines, including the American Society of Clinical Oncology (ASCO) (27), the NCCN (28), the American Society for Radiation Oncology (ASTRO) (29), and the European Society for Medical Oncology (ESMO) (30), attempted to select patients at higher risk for carrying PV/LPVs. Most of these guidelines were based on consensus, and not a result of randomized clinical trials. The NCCN guidelines are updated frequently and often such updates might not be closely followed by practicing community oncologists. The most recent criteria were expanded to include older patients (50 instead of 40 years), and all patients with triple negative disease regardless of their age (Table 1). However, the recent introduction of poly ADP ribose polymerase (PARP) inhibitors to treat patients with *BRCA1/2* variants resulted in more expansion of the testing guidelines to include all patients who may potentially benefit from certain anti-cancer therapy used in the setting of *BRCA1/2* variants. A randomized phase-3 trial (OlympiAD) showed that olaparib, a PARP inhibitor, when compared to palliative chemotherapy, in human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer patients, with pathogenic germline *BRCA1/2* variants, was associated with better progression-free survival (PFS) (31). Similar results were reported using talazoparib, another PARP inhibitor (32). More recently, PARP inhibitors were also tried in the setting of high-risk early-stage breast cancer with germline pathogenic

TABLE 1 Recommendations for germline genetic testing*.

Age^	Gender^	Ancestry^	Treatment^ Indication	Pathology^	Family History^
All patients ≤ 50 years	All male patients	All patients with Ashkenazi Jewish Ancestry	Systemic treatment decisions using PARP inhibitors for MBC	Triple-negative breast cancer	Breast cancer at age ≤50 years
					Male breast cancer
				Multiple primary breast cancers (synchronous or metachronous)	Ovarian cancer
					Pancreatic cancer
			Adjuvant treatment decisions with olaparib for high-risk, HER2-negative EBC	Lobular breast cancer with personal or family history of diffuse gastric cancer	Prostate cancer with metastatic, or high- or very-high-risk group
					≥3 Total diagnoses of breast cancer in patient and/or close blood relatives
					≥2 Close blood relatives with either breast or prostate cancer (any grade)

*As per the National Comprehensive Cancer Network (NCCN) guidelines.
^ Regardless of any other risk factor.
PARP, Poly ADP ribose polymerase; MBC, Metastatic breast cancer; HER2, Human epidermal growth factor receptor-2; EBC, Early breast cancer.

BRCA1/2 variants (Olympia trial). When compared to placebo, adjuvant olaparib for one year was associated with significant improvement in distant (dDFS) and invasive (iDFS), disease-free survivals, and possibly overall survival (OS), too (33).

Given this expansion in the indications for genetic testing, it's estimated that almost two-thirds of breast cancer patients will have at least one indication for genetic testing. However, many studies had shown that the current testing guidelines are restrictive and only a fraction of eligible patients are tested (34, 35). Additionally, several other studies had shown that the prevalence of PV/LPVs in the other non-tested patients are high enough to justify testing all patients in a testing approach known as "universal testing" (36). This approach was adopted by the American Society of Breast Surgeons, which called for testing all breast cancer patients regardless of their age, personal or family history of cancer.[37]

4 Surgery for the diseased breast

Options for the diseased breast varies and can range from BCS (followed by radiation therapy) to many forms of mastectomies. Each option has its own advantages and obviously some potential setbacks (37).

4.1 BCS versus mastectomy

Tumor's characteristics, including size and site, and patient's characteristics, like breast size, may determine the extent of surgery; mastectomy versus BCS, regardless of the existence of *BRCA1/2* variants. Patients with newly diagnosed early-stage breast cancer who carry a PV/LPV in *BRCA1* or *BRCA2* are often advised to undergo mastectomy, which can be skin-sparing or nipple-sparing. BCS was never compared, in a randomized study, to mastectomy in this setting. Much of our knowledge, however, is based on small retrospective studies and pooled analysis of such studies.

In one systematic review that included 3,807 patients in 23 observational studies, differences in outcomes between mastectomy and BCS among breast cancer patients with *BRCA1/2* variants were analyzed. Patients were young with a median age at breast cancer diagnosis of 41 years; 2,200 (57.7%) had *BRCA1* variants while 1,212 (31.8%) had *BRCA2*. BCS was performed on 2,157 (56.7%) while 1,408 (41.5%) patients had mastectomy. Risk of loco-regional relapse (LRR) was significantly higher in the BCS group (HR: 4.54, 95% CI: 2.77-7.42, p<0.001). However, disease-specific recurrence (HR: 1.58, 95% CI: 0.79-3.15, p=0.200), disease recurrence (HR: 1.16, 95% CI: 0.78-1.72, p=0.470), contralateral breast cancer (HR: 1.51, 95% CI: 0.44-5.11, p=0.510), and death (HR: 1.10, 95% CI: 0.72-1.69, p= 0.660) were not higher in the group who underwent BCS (38).

In another systematic review of 18 studies that compared BCS and mastectomy, OS at 5, 10, and 15 years were comparable (83%, 86.0%, and 83.2%) with mastectomy, and with BCS (88.7%, 89.0% and 83.6%), respectively. However, the ipsilateral breast cancer recurrence rates at 5, 10, and 15 years were significantly lower with mastectomy (3.4%, 4.9%, and 6.4%, respectively) than with BCS group (8.2%, 15.5%, and 23%, respectively). Researchers concluded that BCS can be offered for select patients with *BRCA1/2* mutation after proper counseling and with intensive follow-up (39).

Patient's satisfaction for cosmetic results should always be balanced against oncological safety. The need for adjuvant radiation therapy following BCS and the possible increase in the risk of complications that may lead to a possible subsequent mastectomy with immediate breast reconstruction should always be addressed with patients when considering BCS versus mastectomy.

4.2 BCS in *BRCA1/2* vs sporadic breast cancer

Several other studies had attempted to answer the question of the oncological safety of BCS by comparing the outcomes of patients with *BRCA1/2* mutation to a control group of patients with sporadic breast cancer. In one retrospective study that

reviewed the clinical and pathological records of 501 patients who underwent BCS in China between 2005 and 2018, 63 patients had *BRCA1* or *BRCA2* variants. After a median follow-up of 61 months for carriers and 70 months for noncarriers, the DFS (p=0.424) and the OS (p=0.173) were not significantly different. Interestingly, there was no difference between the two groups in ipsilateral breast tumor recurrence (p=0.348). However, CBC was significantly worse in carriers; 9.5% versus 0.68%, p<0.001 (40). No significant difference in ipsilateral-breast tumor recurrence (IBTR) was also reported in another Chinese study (41).

In another meta-analysis that included 13 studies with 701 BRCA-mutation carriers and 4,788 controls, IBTR was significantly higher in BRCA-mutation carriers (RR: 1.589; 95% CI 1.247-2.024; p<0.001). As expected, risk of recurrence increased as the follow up increases; (RR: 1.601; 95% CI 1.201-2.132) with 10 or more years of follow up and (RR: 1.505; 95% CI 1.184-1.913) with median follow up of 7 or more years. However, overall survival in three included cohort studies found no evidence to suggest a deterioration in OS in patients with BCS (38). Multiple other studies had confirmed the high rate of IBTR in *BRCA1/2* carriers treated with BCS compared to matched controls with sporadic breast cancer (42).

5 Risk-reducing mastectomy

Compared with non-carriers, patients with *BRCA1/2* mutation have a higher risk for contralateral breast cancer with *BRCA1*-mutation is associated with higher risk compared to those with *BRCA2*. Several studies had compared outcomes of women who underwent risk-reducing mastectomies with those who opted to continue on surveillance (43). Surgical decision-making process is quite complex and should take into consideration several risk-modifying factors including age at first breast cancer diagnosis, the use of adjuvant endocrine therapy and planned, or already performed oophorectomy. Younger patients who have not received adjuvant endocrine therapy or undergone oophorectomy, might be at higher risk for ipsilateral breast cancer recurrence (IBCR) and CBC, and thus might benefit from a more aggressive surgical approach. Women with strong family history, like those with family member diagnosed or died, with breast cancer at younger age, tend to choose mastectomy, while younger patients aged 30 or less are more likely to choose surveillance. Anxiety and fear of getting a second breast cancer are significantly lower following RRM, which impacts positively on the quality of life of such patients (44). Several surgical options are available to manage the contralateral breast but mostly nipple-sparing, skin-sparing mastectomy, which is usually associated with excellent cosmetic and oncological results.

5.1 Skin-sparing and nipple-sparing mastectomies: how effective and how safe?

In skin-Sparing mastectomy (SSM), a radial, axillary or an inframammary incision is utilized, much of the breast skin is spared but carefully dissected off breast tissue with removal of the entire breast glands to create a pocket that facilitates immediate breast reconstruction with implant or autologous graft. Nipple-sparing mastectomy (NSM) is similar to SSM, but the nipple-areola complex (NAC) is preserved, as well (45, 46). Both techniques are increasingly utilized in clinical practice and are associated with superior cosmetic outcomes and better patients' satisfaction compared to mastectomy (47–50). In addition to the usual complication encountered with other types of breast reconstructions, NAC necrosis is the main complication of NSM and tends to be higher among smokers, obese and those with large breasts, and following radiotherapy (51, 52).

However, one of the main concerns associated with both SSM and NSM is the risk of local breast cancer recurrence at the NAC secondary to occult nipple involvement or a second new primary cancer in the retained breast tissue (53–57). Such risk is obviously higher among patients who carry a pathogenic germline breast cancer predisposing genes. Breast cancer recurrence at the NAC, often referred to as "oncologic safety" can be a concern. Several studies, mostly retrospective ones, attempted to answer the question in two groups; the affected patients who underwent contralateral prophylactic surgery, and among unaffected carriers.

The oncologic safety of SSM and NSM was initially studied in the setting of sporadic breast cancer. In a 2010 meta-analysis of 9 studies that enrolled 3,739 patients, rates of local recurrence in SSM did not differ significantly from those who underwent non-SSM (53). Another meta-analysis of 20 studies involving 5,594 women with early-stage breast cancer did not detect any differences in local recurrence, DFS or OS between those receiving SSM compared to those receiving conventional mastectomy without reconstruction (54). Another large systematic review of 17 retrospective studies included 7,107 patients; majority (85.4%) of them had the procedure for invasive carcinoma. Following a median follow up of 48 months (range 25-94), the mean rates of local recurrence was 5.4% (0.9-11.9), and recurrence involving the NAC was 1.3% (0-4.9) (55). Another large retrospective study from Korea that involved 944 patients, reached similar conclusions. Multicentricity or multifocality, negative hormone receptor, or HER2-positive subtype, high histologic grade, and extensive intraductal component, were independently associated with cancer recurrence at the NAC after NSM (56).

Several other studies addressed issues related to oncologic safety among patients harboring a pathogenic cancer-predisposing gene. In one study, researchers examined tissues from 62 NACs from 33 women (25 *BRCA1*, 8 *BRCA2*) who underwent mastectomy between 1987 and 2009 at Mayo Clinic. Atypical hyperplasia, carcinoma in situ, or invasive carcinoma were not found in any of the 33 prophylactic mastectomy specimens performed. However, 2 (7%) of the 29 breasts with cancer, and available tissue, had malignant findings, and 1 (3%) had atypia in the NAC (57).

More recently, Rocco et al. reviewed 9 studies reported on the incidence of primary breast cancer following NSM in *BRCA1/2* unaffected carriers who undergo prophylactic bilateral mastectomy. From an oncological point of view, NSM appears to be a safe option for BRCA mutation carriers, with low reported rates of new breast cancers. Additionally, the procedure was associated with low rates

of postoperative complications, and high levels of satisfaction and postoperative quality of life (58). In another study, researchers reviewed 114 NSM performed from 2008 to 2019 on patients with breast cancer in 105 *BRCA1/2* carriers (56 *BRCA1*, 47 *BRCA2*, and two women with both mutations). Five (4.4%) patients had positive nipple margins on final pathology and all underwent nipple excision. Systemic therapy was offered to 76% patients; 65 (62%) with chemotherapy and 48 (46%) received endocrine therapy. Patients were followed up for a median of 70 months (range 15-150), no patient had a recurrence in the retained NAC or at the site of a nipple excised for a positive margin. The rate of locoregional recurrence outside the nipple and distant recurrence were also low at 2.6% and 3.8%, respectively (59).

In another study from 9 major institutions in the US, researchers retrospectively reviewed their experience on 548 prophylactic NSM performed in a cohort of 346 patients with *BRCA1* or *BRCA2* variants. Unilateral risk-reducing NSM secondary to a concurrent, or prior cancer in the contralateral breast, were performed on 144 (41.6%) patients, while bilateral prophylactic NSM were performed on 202 (58.4%) patients. With median and mean follow-up of 34 and 56 months, respectively, no ipsilateral breast cancers were reported after prophylactic NSM. Similarly, breast cancer did not occur in any patients undergoing bilateral risk-reducing NSM (60).

6 Moderate penetrance genes

The recent advances in NGS technologies resulted in an increase use of multigene panel testing and enabled sequencing of *BRCA1/2* concomitantly with many additional genes. Recent studies suggest that other cancer predisposing genes, including *PALB2, ATM, CHEK2, TP53, RAD51C*, *RAD51D*, and many others, confer variable risks of breast and other cancers (61–63). Rates of such variants are very variable, depending on population studied and testing method utilized. Figure 1 illustrates an example of such variation in a study that used a 25-multi gene panel, and enrolled over 35,000 patients; half of them were non-Western with different ethnic background (24), and a recently published study from our group that enrolled over 1,000 Arab breast cancer patients utilizing a multi-gene panel, too (26). Appropriate counselling and data-driven risk management with appropriate plans for risk-reducing intervention or surveillance for patients with breast cancer and unaffected individuals, are highly needed (64–67).

6.1 *PALB2*

Pathogenic/likely pathogenic *PALB2* variants is associated with high risk for breast cancer, with studies showing a life-time risk of 40-60% (68). One multi-national study that analyzed data from 524 families with *PALB2* PVs in 21 countries concluded that the estimated relative risk (RR) of breast cancer was 7.18 (95% CI, 5.82- 8.85; $p=6.5\times10^{-76}$) (69). A large family-based study reached similar conclusions (70). Additionally, patients harboring PVs of *PALB2* are at higher risk for ovarian cancer and Fanconi anemia

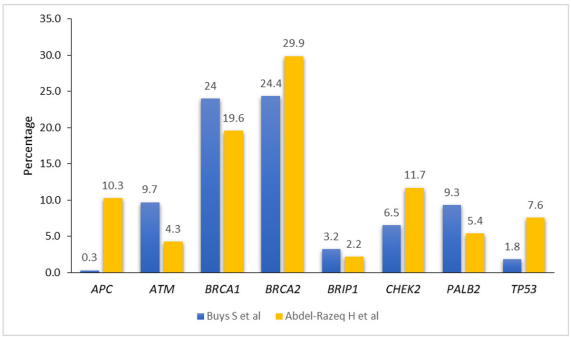

FIGURE 1
Prevalence of pathogenic/likely pathogenic variants among breast cancer patients in different ethnic groups.

which is inherited in an autosomal recessive manner (71). The NCCN guidelines recommend annual mammogram beginning at age 30 years with consideration for breast MRI. Risk-reducing surgery should also be discussed with the patient.

6.2 *CHEK2*

The rate of *CHEK2* germline mutation is higher in certain ethnic groups like the Northern European countries. Certain variants in the *CHEK2* gene (I157T and c.1100delC) are associated with higher risk for breast cancer (72). The cumulative lifetime risk ranges from 28% to 37% (73). While no data available on the benefit of RRM, annual mammogram and breast MRI once a year starting at 40 years of age, are highly recommended. Carriers of *CHEK2* pathogenic variants are at higher risk for colon, prostate, bladder, kidney and thyroid cancers, more so with c1100delC variant (74).

6.3 *TP53*

The *P53* is a tumor suppressor gene that prevents the development of cancer. Patients with germline mutation, Li-Fraumeni syndrome, are at risk for early-onset breast cancer, sarcomas, and other cancers in children and young adults (75, 76). Following cellular stress, like radiation therapy (RT)-associated cell injury, *P53* provides the cell with ability to repair DNA damage through multiple downstream repair pathways. In a small series of 8 patients with breast cancer and germline *TP53* pathogenic variant, 6 of them were treated with radiation therapy following surgery, ipsilateral breast recurrences were reported in three and contralateral breast cancers in three more. RT-induced cancers were reported in two, in addition to three new primary cancers. On the other hand, only one contralateral breast cancer occurred among patients who had not received radiation therapy (77). Several other case reports of RT-associated malignancies supported the recommendation against RT in patients with *TP53* (78–82). As such, mastectomy should be recommended to possibly avoid radiation therapy following BCS.

6.4 *ATM*

Heterozygous pathogenic variant in *ATM* is associated with a 13-33% cumulative lifetime risk for breast cancer (83, 84). Risk-reducing mastectomy is not recommended for carriers; however, it might be considered based on personal and family history. No apparent risk of post-surgery radiation therapy on patients with pathogenic variant. Mammogram with consideration of breast MRI is recommended yearly starting at age 40 years.

7 Conclusions

Germline genetic testing is currently offered for majority of patients with breast cancer, as it informs both preventive and treatment decisions. Available data support the oncologic safety of more conservative surgical approaches in breast cancer patients even with the highest penetrant germline variants like *BRCA1* and *BRCA2*. Unaffected carriers may also be offered active surveillance should they choose so. However, evidence to guide clinical decisions on less frequent, mild to moderate risk variants, is lacking.

Author contributions

HA-R: Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Funding

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The author would like to acknowledge Mrs. Hira Bani Hani, Mrs. Alice Haddadin and Ms. Doaa J. AlSadi for their help in preparing the manuscript.

Conflict of interest

The author declares no commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 04 October 2023
DOI 10.3389/fonc.2023.1272602

OPEN ACCESS

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RECEIVED 04 August 2023
ACCEPTED 15 September 2023
PUBLISHED 04 October 2023

CITATION
Horani M and Abdel-Razeq H (2023) Treatment options for patients with hormone receptor-positive, HER2-negative advanced-stage breast cancer: maintaining cyclin-dependent kinase 4/6 inhibitors beyond progression.
Front. Oncol. 13:1272602.
doi: 10.3389/fonc.2023.1272602

Treatment options for patients with hormone receptor-positive, HER2-negative advanced-stage breast cancer: maintaining cyclin-dependent kinase 4/6 inhibitors beyond progression

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Breast cancer is the most commonly diagnosed cancer in women worldwide. Over the past decade, the treatment paradigm for patients with metastatic breast cancer (MBC) has taken an important shift towards better survival and improved quality of life (QOL), especially for those with hormone receptor (HR)-positive diseases which represent the majority of breast cancer subtypes. The introduction of cyclin-dependent kinase 4/6 (CDK4/6) inhibitors in the upfront therapy of such patients has resulted in dramatic improvement in progression-free survival (PFS) and overall survival (OS), too. However, almost all patients would, sooner or later, develop disease progression and necessitate transition to different lines of treatment that may include chemotherapy. The idea of maintaining CDK4/6 inhibitors beyond disease progression seems attractive, as this approach has the potential to improve outcome in this setting despite the fact that the true benefit, in terms of survival, might not carry the same weight as it initially does. Researchers have been investigating potential mechanisms of resistance and identify possible biological markers for response after disease progression. Much of the available data is retrospective; however, few randomized clinical trials were recently published and few more are ongoing, addressing this point. In this paper, we intend to review the available published studies investigating the potential role for keeping CDK4/6 inhibitors in play beyond disease progression.

KEYWORDS

CDK4/6 inhibitors, palbociclib, ribociclib, abemaciclib, metastatic breast cancer, disease progression, endocrine therapy

1 Introduction

Breast cancer is the most prevalent cancer in women worldwide and one of the leading causes of death among women in the United States and worldwide (1–3). Patients with advanced breast cancer may present with *de novo* metastatic disease in a proportion of patients that varies in different health care systems, significantly more in low-income countries (4). Additionally, a sizable proportion of patients may progress to advanced stages following treatment of early or locally advanced diseases (5).

The majority of breast cancer patients belong to HR+/HER2− subtype (6), which carries a more favorable prognosis compared to the other subtypes (7). Over the years, chemotherapy and endocrine therapy (ET) had been the mainstay of treatment of advanced HR+/HER2− breast cancer. The addition of cyclin-dependent kinase 4/6 (CDK4/6) inhibitors to ET in the treatment of advanced-stage breast cancer has boosted responses and survival outcomes over the past few years, especially in the first-line setting (8). Ribociclib, palbociclib, and abemaciclib have all been approved, based on better disease control and survival benefits when combined with ET and have become the standard of care as first-line treatment for advanced HR+/HER2− breast cancer (9–11). CDK4/6 inhibitors have also produced significant improvements and better outcomes in second-line settings when combined with fulvestrant upon progression on aromatase inhibitor (AI) (12). In this manuscript, we review previous attempts and ongoing trials investigating the role of continuing the same or different CDK4/6 inhibitors, with ET, beyond disease progression.

2 Systemic therapies following progression on CDK4/6-inhibitors:

2.1 Analysis of real-world data

Patients with advanced HR+/HER2− breast cancer whose disease has progressed on frontline CDK4/6 inhibitors with ET have many options for treatment, but no standard of care exists for the next line of systemic therapy. Possible strategies include switching to different class of ET, switching to chemotherapy, as single agent or in combination, or utilizing novel targeted agents. Agents like alpelisib for patients with somatic PIK3CA mutations; elacestrant, a newly approved selective estrogen receptor degrader (SERD); everolimus; a mammalian target of rapamycin [mTOR] inhibitor; and poly (ADP-ribose) polymerase (PARP) inhibitors like talazoparib or olaparib for patients with germline BRCA1 or BRCA2 mutations are widely used (13–16). The optimal sequencing of the above options is not well-established; however, the choice of the next line of treatment depends on many factors including underlying comorbidities, menopausal status, potential adverse effects, molecular profile, presence of specific germline mutations, and the presence or absence of solid indications to start cytotoxic chemotherapy, in addition to patients' preference.

The idea of CDK4/6 inhibitor continuation beyond progression was first studied in several small retrospective studies. In one study, analysis was done on 30 female patients with HR+/HER2-negative MBC treated at the Cleveland Clinic Foundation, who continued CDK4/6 inhibitors after initial progression. The primary endpoint was progression-free survival (PFS) beyond first documented disease progression. Initial ET-CDK4/6 inhibitor regimens received included palbociclib combined with letrozole (67%), fulvestrant (23%), or other ET. Only a minority of patients were on abemaciclib combinations. The median PFS for all patients while receiving CDK4/6 inhibitors and ET combination was 23.5 months (95% CI, 12.8–27.8), and median PFS beyond initial progression was 11.8 months (95% CI 5.34–13.13). Median OS since treatment initiation was around 45.4 months (17).

Two years later, another report was published with a similar concept. The analysis included 87 patients with metastatic HR+/HER2-negative patients who received palbociclib-containing regimens in the metastatic setting and were rechallenged with abemaciclib in combination with ET on progression (18). Palbociclib was combined with AI in the majority of patients (63%); the rest had it combined with fulvestrant. Approximately, a third (36.8%) of the patients switched to fulvestrant and abemaciclib after disease progression on AI and palbociclib. The same ET (AI or fulvestrant) was maintained with switching the CDK4/6 inhibitor to abemaciclib in around 25% of the patients. Only a minority of patients switched to abemaciclib monotherapy. Median PFS was similar for patients who received abemaciclib combined with an ET (5.1 months, 95% CI, 3.2–7.6) compared with patients who received abemaciclib as monotherapy (5.4 months, 95% CI, 1.9–NR). In order to further investigate the potential benefit of abemaciclib, another analysis was done on patients based on treatment with an ET to which they were not exposed, compared to rechallenging with ET with a previous exposure. There were no meaningful differences in both PFS (5.1 vs. 5.7 months) and OS (17.2 vs. 15.3 months). In terms of CDK4/6 inhibitor sequencing and its effect on outcome, median PFS was better in patients receiving sequential CDK4/6 inhibitors (8.4 months, 95% CI, 4.1–NR) compared to 3.9 months (95% CI, 2.9–5.7) in patients receiving non-sequential CDK4/6 inhibitor treatment ($p = 0.0013$) (18). However, one cannot make conclusions based on these statistics as patients on the non-sequential approach would have probably had a more aggressive disease. RB1 alterations and ERBB2 and CCNE1 amplification were detected by gene sequencing in few patients who developed rapid disease progression on CDK4/6 inhibitors; those mutations could be an early indicator for lack of efficacy and primary resistance the CDK4/6 inhibitor class (18).

A recently published analysis of real-world data was conducted at two centers in the United States to determine what systemic therapies were being used following progression on a CDK4/6 inhibitor and compare differences in outcome (19). This study was designed to investigate systemic therapies used in the second-line setting following disease progression on first-line ET-CDK4/6 inhibitor combinations. It also aimed to describe the real-world PFS (RW-PFS) and OS after initiation of second-line modalities. In the analysis, palbociclib was the CDK4/6 inhibitor used in the majority of patients in the first-line setting (88.2%) while the remaining received either ribociclib or abemaciclib. Aromatase inhibitors were the companion ET in around two-thirds of the patients, and fulvestrant with the other third. A total of 839 patients eventually

received second-line systemic therapy and were included in the analysis. The most common second-line therapy was chemotherapy (29.7%), while ET monotherapy was used in 12.4% of the patients, most of which were treated with fulvestrant. The analysis also showed use of targeted agents, like everolimus, in 11.7%, while few others used PARP inhibitors or alpelisib (19). A CDK4/6 inhibitor was continued, alone or combination with ET as a second line, in 302 patients; most of them maintained the same CDK4/6 inhibitors used initially. For patients receiving a CDK4/6 inhibitor in the second-line treatment, the median OS was 35.7 months and the median RW-PFS was 8.25 months. For patients treated with chemotherapy, fulvestrant as single agent, or everolimus, the estimated median RW-PFS was worse: 3.71, 3.25, and 3.32 months, respectively. RW-PFS was significantly better with CDK4/6 inhibitor continuation when it was compared to chemotherapy (HR 0.48, 95% CI 0.43–0.53, $p < 0.0001$), as OS analysis showed benefit with CDK4/6 inhibitor continuation as well (HR 0.30, 95% CI, 0.26–0.35, $p < 0.0001$) (19).

More recently, another real-world data analysis was published from Japan, as investigators explored treatment modalities and their effect on subsequent therapy lines following disease progression on palbociclib-based combinations. Time to treatment failure (TTF) was the main endpoint (20). Three different approaches of CDK4/6 inhibitor sequencing were undertaken. First, both CDK4/6 inhibitor and ET were switched (i.e., palbociclib was replaced by abemaciclib and ET was switched to another agent). Second, only the ET was switched while palbociclib was maintained. Third, only the CDK4/6 inhibitor was switched (abemaciclib replaced palbociclib) while ET was maintained. The analysis included 1,170 patients treated with palbociclib combinations in the first-line setting and beyond. The combination of fulvestrant and abemaciclib was the most commonly used subsequent therapy. Median TTF of the first subsequent ET (as single agent) was 4.4 months (95% CI, 2.8–13.7) while patients on CDK4/6 inhibitor and ET combinations had a TTF of 10.9 months (95% CI, 6.5–15.6). Patients treated with ET and mTOR inhibitor combination had a TTF of 6.1 months (95% CI, 5.1–7.2). A subgroup analysis based on ET-therapy sensitivity showed that TTF for the ET-CDK4/6 inhibitor combinations was relatively long in both ET-sensitive and ET-resistant subgroups (20).

These observational data suggest that it is not uncommon for physicians to proceed with the same or different CDK4/6 inhibitor upon progression on their prior ET-CDK4/6 inhibitor combinations. Table 1 summarizes the outcomes of the abovementioned studies.

3 Systemic therapies following progression on first-line CDK4/6 inhibitors:

3.1 Randomized trials

Three randomized clinical trials trying to answer the same question were recently published. The first was the MAINTAIN trial which is a randomized phase II trial studying the efficacy of maintaining palbociclib with or without ET in patients whose disease had progressed on ET+CDK4/6 inhibitor (21, 22). A total of 119 patients with metastatic HR+/HER2-negative breast cancer (patients could have received up to one line of chemotherapy) were included in the study and were randomized into two arms: the first received (switch) ET combined with ribociclib, and the other arm (switch) ET combined with placebo (60 and 59 patients, respectively); the initial CDK4/6 inhibitor used in the prior line was palbociclib in the majority of patients. Switch ET meant that patients receive fulvestrant as ET in the case of disease progression on a prior AI or receive AI (exemestane) in the case of disease progression on fulvestrant. PFS was the primary endpoint of the study; secondary endpoints included overall response rate (ORR) and OS, among others (22). At data cutoff with a median follow-up of 18 months, PFS was improved in the ribociclib arm when compared to placebo, 5.29 months vs. 2.76 months, respectively, with a hazard ratio of 0.57 and a 95% CI of 0.39–0.95 and a significant p-value of 0.006. Median PFS at 12 months was also improved, 24.6% for the combination arm versus 7.4% for the placebo arm (22).

3.1.1 The addition of immunotherapy

The addition of immunotherapy to the combination of ET+CDK4/6 inhibitors was studied in the PACE trial, which was a multicenter randomized open-label phase III trial conducted prospectively to study the efficacy of palbociclib continuation combined with fulvestrant beyond disease progression on prior AI +CDK4/6 inhibitors, compared to fulvestrant monotherapy, and to study the role of adding immunotherapy (avelumab) to the palbociclib/fulvestrant combination (23). There were a total of 220 patients with metastatic HR+/HER2-negative breast cancer with prior progression on AI and any CDK4/6 inhibitors. Similar to the MAINTAIN trial, patients could have been treated with only one line of chemotherapy in the metastatic setting. Palbociclib was the initial CDK4/6 inhibitor in the vast majority of patients. PFS (palbociclib/fulvestrant vs. fulvestrant monotherapy) was the primary endpoint. PFS for the triplet combination (versus fulvestrant monotherapy) was a secondary endpoint, in addition to objective response rate across all arms (24). In regard to the primary endpoint after a median follow-up of 2 years, the palbociclib combination failed to show benefit as the PFS for the palbociclib/fulvestrant arm was 4.6 months and 4.8 months for the fulvestrant monotherapy arm (HR = 1.11 and a two-sided p-value of 0.62). As for the secondary endpoints, median PFS was numerically better in the triplet arm (8.1 months) but was not statistically significant (hazard ratio of 0.75 vs fulvestrant monotherapy, and a two-sided p-value of 0.23). The overall response rates were 7.3% for the fulvestrant monotherapy arm, 9% for the doublet (fulvestrant and palbociclib) combinations, and 13% for the triplet combinations. The clinical benefit rates were more or less similar between all arms. Adverse effects were consistent with the safety profile accustomed to each agent (24).

Finally, the PALMIRA trial, which was an international, multicenter, randomized, open-label, phase II trial was conducted, aiming to evaluate the efficacy of continuation of palbociclib combined with second-line ET in patients with HR+/HER2– advanced breast cancer after disease progression on palbociclib-

TABLE 1 Summary of non-randomized trials.

Study (reference)	Number of patients	Initial CDK4/6 inhibitor regimen	Primary Endpoint	Arms	Outcome
Samuel Eziokwu A, et al. Retrospective Analysis (17)	30	Palbociclib-containing regimen	PFS*	CDK4/6 inhibitor + switch ET	11.8 months (95% CI, 5.34–13.13)
Wander SA, et al. Retrospective Analysis (18)	87	Palbociclib–AI Palbociclib–fulvestrant	PFS*	Abemaciclib monotherapy	5.4 months (95% CI, 1.9–NR)
				Abemaciclib + ET	5.1 months (95% CI, 3.2–7.6)
				Sequential CDK4/6 inhibitor	8.4 months (95% CI, 4.1–NR)
				Non-sequential CDK4/6 inhibitor	3.9 months^
Martin JM et al. Analysis of Real-World data-US (19)	839	Palbociclib (88%), ribociclib, or abemaciclib (12%) AI (2/3) Fulvestrant (1/3)	RW-PFS*	CKD4/6 inhibitor (+/–ET)#	8.25 months^
				Chemotherapy	3.71 months^
				Fulvestrant monotherapy	3.25 months^
				Everolimus	3.32 months^
Masataka Sawaki, et al Analysis of Real-World data -Japan (20)	1,170	Palbociclib-based regimens	TTF	Endocrine monotherapy	4.4 months (95% CI, 2.8–13.7)
				CKD4/6 inhibitor + ET	10.9 months (95% CI, 6.5–15.6)
				ET + mTOR inhibitor	6.1 months (95% CI, 5.1–7.2)

PFS, progression-free survival; AI, aromatase inhibitors; ET, endocrine therapy; RW, real world; TTF, time to treatment failure.
*Beyond initial progression.
#Versus chemotherapy: HR 0.48, 95% CI 0.43–0.53.
^95% CI not reported in the original study.

based first-line combination with ET (25). The analysis included 198 patients who were eligible if they had evidence of clinical benefit to ET+CDK4/6 inhibitors in the first-line setting (i.e., no primary endocrine resistance). Patients were randomly assigned to receive either palbociclib combined with switch ET (fulvestrant or letrozole) or second-line switch ET monotherapy. PFS was the primary endpoint of the trial, secondary endpoints included clinical benefit rate and overall response, among others (26). At data cutoff and after a median follow-up of 8.7 months, median PFS for the two arms were similar, 4.2 months and 3.6 months in the palbociclib/ET and ET monotherapy arms, respectively. Overall response and clinical benefit rates were also similar in the two arms. In terms of safety, the combination arm had more grade 3/4 toxicity (45.2% vs. 8.3%) (26). Table 2 shows a summary of all three trials.

4 Discussion

Though the breast cancer-related mortality has decreased over the past few years (27), it remains one of the leading causes of death among women worldwide (3, 28). Treatment of breast cancer in the metastatic setting have come a long way in improving survival outcomes, especially in patients with HR+/HER2– tumors (Figure 1) (27). The addition of CDK4/6 inhibitors in the frontline setting, and even in subsequent lines after progression on ET, had impeccable results and have become the cornerstone in the treatment of such patients (29). Those drugs are generally well-tolerated (30); neutropenia, leukopenia, thrombocytopenia, anemia, fatigue, diarrhea, and transaminitis are the most frequent adverse effects encountered (31).

All CDK4/6 inhibitors have shown significant improvement in PFS, and some (ribociclib and abemaciclib) have also improved OS when combined with ET in both first- and second-line settings [9, 31]. The notion of maintaining CDK4/6 inhibitors after disease progression is intriguing, and that led many researchers at leading institutions around the world to report patients' real-world outcomes, by switching the ET used and either maintaining the same CDK4/6 inhibitor or switching it to another. Most of the retrospective data discussed above were encouraging, suggesting that some patients may gain some benefit in maintaining CDK4/6 inhibitors upon progression

TABLE 2 Randomized studies comparing CDK4/6 extension beyond progression versus other treatment options.

Study (Reference)	Study design (Number of patients)	Initial CDK4/6 inhibitor regimen	Median follow-up (months)	Arms	PFS* (Months)	HR, p-value, 95% CI
MAINTAIN (21, 22)	Randomized phase II trial (n = 119)	Palbociclib + AI/ fulvestrant	18	Switch ET + switch to ribociclib	5.29 (95% CI 3.02–8.12)	HR 0.57, (95% CI 0.39–0.95) p = 0.006
				Switch ET + placebo	2.76 (95% CI 2.66–3.25)	
PACE (23, 24)	Randomized open-label, phase III trial (n = 220)	Any CDK4/6 inhibitor# + AI	24	Fulvestrant + palbociclib	4.8^	HR = 1.11 (90% CI 0.79–1.55) Two-sided p = 0.62
				Fulvestrant monotherapy	4.6^	
				Fulvestrant + palbociclib + avelumab	8.1^	HR = 0.75 (vs fulvestrant monotherapy) (90% CI 0.50–1.12) Two-sided p = 0.23
PALMIRA (25, 26)	Randomized, open-label, phase II trial (n = 198)	Palbociclib + ET	8.7	Switch ET + palbociclib	4.2 (95% CI 3.5–5.8)	HR 0.8 (95% CI 0.6–1.1) p = 0.206
				Switch ET monotherapy	3.6 (95% CI 2.7–4.2)	

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; ET, endocrine therapy; AI, aromatase inhibitors.
*Beyond initial progression.
#Mostly palbociclib.
^95% CI not reported in the original study.

on prior CDK4/6 inhibitor treatment. However, these data analyses were weak, as for their observational nature, inclusion of heavily pretreated patients, heterogeneous population, and in some, a small number of patients included. In addition, many of the clinical characteristics of treatment arms were lacking in some of these studies.

The MAINTAIN and PACE are two randomized clinical trials that investigated this approach, but the outcome was not the same leaving physicians with loose ends. In the MAINTAIN trial, both the CDK4/6 inhibitor and ET were switched upon progression and ribociclib was used after progression on palbociclib. Ribociclib combined with ET led to a statistically significant improvement in PFS. In an exploratory analysis, based on tumor biomarkers, the efficacy was better in patients who had no ESR1 mutation (ESR1 wild type); median PFS for the ESR1-WT treated with ribociclib was 8.3 months, compared to 2.7 months for those on placebo. Patients in both groups, with mutant ESR1, had similar PFS (32). This was a bit undermined by the small number in those subgroups; however, this would prove an eye opener for some of the following trials and future approaches in dealing with sequencing CDK4/6 inhibitors, and searching for other predictive biomarkers.

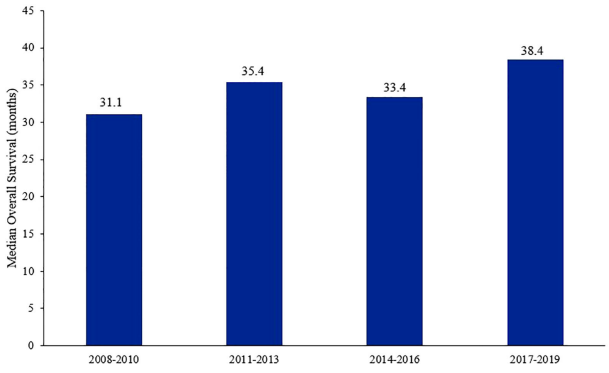

FIGURE 1
Median overall survival of patients with HR+/HER2– metastatic breast cancer over time.

In the PACE trial, a different approach was undertaken as only ET was switched and the CDK4/6 inhibitor palbociclib was maintained in the majority of patients; in addition, a third arm was included with the addition of avelumab; a PD-L1 inhibitor. Maintaining palbociclib upon progression failed to prove beneficial in this trial, and the addition of immunotherapy (avelumab) showed PFS benefit but was not statistically significant; this might trigger more investigation in the near future.

Tumor biomarkers seemed to play an integral role in predicting response. Having certain mutations might carry a potential for more favorable response, as suggested by a subgroup analysis revealing that patients with PIK3CA and ESR1 mutations detected by liquid biopsy when analyzing circulating tumor DNA (ctDNA) had more favorable responses (33), making the argument to keep looking for predictive biomarkers even more powerful.

The PALMIRA trial, which is considered by many as the tiebreaker between the two previous trials, had also failed to

demonstrate PFS benefit with palbociclib continuation. Further studies are ongoing to investigate the potential benefits of this approach. For now, the best course of action may will be sticking to other treatment modalities with proven better efficacy compared to ET monotherapy, including antibody–drug conjugates, targeted agents, or even chemotherapy.

It is worth-mentioning that none of the above trials experimented abemaciclib in the setting of progression beyond ribociclib or palbociclib. It seems that abemaciclib is different in terms of biological and potentially pharmacological characteristics than ribociclib and palbociclib (34), and this might justify switching to abemaciclib upon disease progression on a CDK4/6 inhibitor, which might have a potential role in overcoming resistance acquired to the previous CDK4/6 inhibitor. This approach is being evaluated in the ongoing post-MONARCH phase III trial (35).

Patients with early progression on CDK4/6 inhibitors (defined as disease progression in <6 months) might not be the best candidates for CDK4/6 inhibitors in subsequent lines as many of these patients would have some sort of primary resistance to this family of drugs (36), and potentially a more aggressive nature to the disease. In an attempt to investigate the possible pathways of resistance to CDK4/6 inhibitors, a phase III open-label multicenter trial (PADA-1 trial) was conducted in France investigating the possible implication of the ESR1 mutation on acquiring resistance to treatment in HR+/HER2− breast cancer (first randomized trial to do so). Patients with HR+/HER2− metastatic breast cancer were monitored for changes in ESR1 mutation in the ctDNA in blood while on palbociclib + AI combination therapy in the first-line setting (37). Randomization was based on detected ESR1 mutation status, as those patients with newly detected mutation or increasing mutation burden in the ctDNA with no evidence of disease progression were randomized to either continue with the same treatment or to switch to different ET-CDK4/6 inhibitor combination: fulvestrant with palbociclib. PFS was the primary endpoint in this trial. Out of the 1,000 patients initially recruited, 279 patients developed a rising ESR1 mutation. A total of 172 patients were randomized into two arms: 88 patients switching to the palbociclib + fulvestrant combination and 84 patients who were maintained on the same initial combination (palbociclib + AI). PFS estimated from random assignment in the intention-to-treat analysis was improved in the palbociclib + fulvestrant compared to the palbociclib + AI group (11·9 months vs. 5·7 months, respectively, with a hazard ration of 0·61, and a significant p-value 0·0040) (37).

The end result of the PADA-1 trial supports the approach that early therapeutic targeting of rising blood ESR1-mutation burden could carry significant clinical implications and has the potential benefit to predict primary resistance and possibly shorter survival. Around one-third of patients treated with the AI+CDK4/6 inhibitor combination will develop an ESR1 mutation at some point and subsequently develop resistance; however, there seems a good chance those patients would retain sensitivity to CDK4/6 inhibitors if the ET companion was changed (38). A recent phase II trial showed promising outcomes in patients with advanced HR+/HER2− breast cancer and acquired ESR mutation progressing on prior ET. In this small cohort trial, patients received treatment with a combination of abemaciclib and lasofoxifene (a non-selective estrogen receptor modulator). Most of the patients had disease progression on prior CDK4/6 inhibitor treatment; the median PFS was 13.9 months (95% CI, 8.0–NE), and the clinical benefit rate was 62.1% (39). An ongoing active phase III randomized trial (ELAINE-3) will evaluate the efficacy and safety of this combination against fulvestrant + abemaciclib in ESR1-mutated breast cancer (40).

It will be interesting to see more trials after PADA-1 with a similar design in the near future. To touch on that, an analysis update was recently published from the PACE trial in the most recent American Society of Clinical Oncology (ASCO) annual meeting (2023) (41), with monitoring the burden of circulating tumor cells (CTCs) in the blood, which was done at baseline, at time of first disease assessment, and finally at time of disease progression. Patients were classified into two categories according to the level of circulating tumor cells: indolent (<5 CTCs/7.5 ml) and aggressive (≥5 CTCs/7.5 ml). Baseline tumor cell readings were prognostic, as median PFS was 5.7 months for the indolent group and 3.5 months for the aggressive group. When the median PFS was estimated according to treatment groups, patients treated with fulvestrant monotherapy had PFS of 1.9 months for the "aggressive" group, compared to 8.5 months for the "indolent" ones, while the PFS for patients managed with fulvestrant/palbociclib combination was 4.6 months for the "aggressive" vs. 5.3 months for the indolent. Similarly, median PFS for patients managed with fulvestrant/palbociclib/avelumab triplet was 5.4 months in the "aggressive" vs. 8.3 months in the "indolent" (41). Further investigation of this model in the future or other similarly designed models might predict clinical benefit for either CDK4/6 inhibitor continuation or adding immunotherapy to the equation.

Secondary or acquired resistance to CDK4/6 inhibitors could result from various mutations including a mutation in RB1 leading to activation of other cell-cycle factors, such as E2F and the cyclin E-CDK2 axis. BioPER was a phase II trial exploring potential biomarkers (mainly Rb protein expression) for efficacy of continuing palbociclib beyond disease progression on prior palbociclib–ET combinations. A total of 32 patients were included in the final analysis with median follow-up around 18 months; the clinical benefit rate of maintaining palbociclib combined with physicians' choice of endocrine therapy after disease progression on prior palbociclib-based combination, a primary endpoint, was 34.4% (95% CI, 18.6–53.2). PFS at 6 months was 31.2% (95% CI, 18.7–52.2). The percentage of patients with lost Rb protein expression (<1%) in tumor cells at baseline after disease progression was 13%, which was a biological coprimary endpoint. Treatment in those patients failed to achieve clinical benefit; this finding suggests that switching to another class of drugs might carry better chances for response (42, 43). An exploratory analysis showed significantly worse outcomes in patients with any of the following biomarkers detected: ESR mutation, low Rb protein expression, and high cyclin E1 expression. Detection of CTCs from liquid biopsies was done at different intervals during treatment; interestingly, undetected circulating tumor DNA at day 15 of cycle 1 was associated with significantly longer PFS.

Lastly, a better understanding of patterns of resistance driving loss of response to CDK4/6 inhibitor and/or ET will be essential to guiding

more rational approaches and evidence-based selection of subsequent lines of treatment and improving outcomes for such patients. In addition, testing newer endocrine therapy agents that may possess different biochemical activity and potentially overcoming resistance to older-generation agents might help provide new options for treatment in patients with ET-resistant HR+/HER2− breast cancer, as an example; a phase III (EMBER 3) trial will evaluate the efficacy of a novel SERD "Imlunestrant" with or without abemaciclib, compared to investigator choice of ET in patients with disease progression beyond AI-CDK4/6 inhibitor combinations (44).

5 Conclusions

CDK4/6 inhibitors have changed the natural history of HR+/HER2− metastatic breast cancer. However, all patients will unfortunately progress and a new line of therapy should be introduced. Many drugs, as single agent or in combination, can be used in this setting. Our review showed that most of recently published clinical trials have failed to show meaningful improvement in outcome when CDK4/6 inhibitors continued following disease progression. However, the utilization of liquid biopsy to detect CTCs and ctDNA, and testing for certain biomarkers, may improve our ability to better select anticancer therapy following disease progression on CDK4/6 inhibitors.

Author contributions

MH: Data curation, Writing – original draft, Writing – review & editing, Investigation. HA-R: Data curation, Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Project administration, Supervision.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors would like to thank Mrs. Alice Haddadin and Mrs. Hira Bani Hani for their help in preparing this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 10 October 2023
DOI 10.3389/fonc.2023.1240098

OPEN ACCESS

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RECEIVED 14 June 2023
ACCEPTED 11 September 2023
PUBLISHED 10 October 2023

CITATION
Roheel A, Khan A, Anwar F, Akbar Z, Akhtar MF, Imran Khan M, Sohail MF and Ahmad R (2023) Global epidemiology of breast cancer based on risk factors: a systematic review.
Front. Oncol. 13:1240098.
doi: 10.3389/fonc.2023.1240098

Global epidemiology of breast cancer based on risk factors: a systematic review

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Background: Numerous reviews of the epidemiology and risk factors for breast cancer have been published previously which heighted different directions of breast cancer.

Aim: The present review examined the likelihood that incidence, prevalence, and particular risk factors might vary by geographic region and possibly by food and cultural practices as well.

Methods: A systematic review (2017-2022) was conducted following Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, reporting on epidemiological and risk factor reports from different world regions. Medical Subject Heading (MeSH) terms: "Breast neoplasm" "AND" country terms such as "Pakistan/epidemiology", "India/epidemiology", "North America/epidemiology", "South Africa/epidemiology" were used to retrieve 2068 articles from PubMed. After applying inclusion and exclusion terms, 49 papers were selected for systematic review.

Results: Results of selected articles were summarized based on risk factors, world regions and study type. Risk factors were classified into five categories: demographic, genetic and lifestyle risk factors varied among countries. This review article covers a variety of topics, including regions, main findings, and associated risk factors such as genetic factors, and lifestyle. Several studies revealed that lifestyle choices including diet and exercise could affect a person's chance of developing breast cancer. Breast cancer risk has also been linked to genetic variables, including DNA repair gene polymorphisms and mutations in the breast cancer gene (BRCA). It has been found that most of the genetic variability links to the population of Asia while the cause of breast cancer due to lifestyle modifications has been found in American and British people, indicating that demographic, genetic, and, lifestyle risk factors varied among countries.

Conclusion: There are many risk factors for breast cancer, which vary in their importance depending on the world region. However, further investigation is required to better comprehend the particular causes of breast cancer in these areas as well as to create efficient prevention and treatment plans that cater to the local population.

KEYWORDS

breast cancer, systematic review, epidemiology, risk factors, regional effects

Introduction

Breast cancer (BC) is a major public health issue that affects women all over the world. It is the most often diagnosed cancer and the second biggest cause of cancer-related deaths among women globally (1). Breast cancer occurs at different rates around the world, with Western nations having greater incidence rates than Eastern nations. However, due to lifestyle changes, an increase in longevity, and the adoption of Westernized dietary practices, the prevalence of breast cancer is quickly rising in low- and middle-income countries (1). Several studies have shown that several factors, including age, race, and socioeconomic status, genetic factors like BRCA mutations, hormonal factors like age at menarche, parity, and age at first full-term pregnancy, breastfeeding, and lifestyle-related factors like diet, physical activity, alcohol use, and tobacco use are all associated with an increased risk of breast cancer (2).

Understanding and treating carcinoma of the breast on a global basis depends heavily on epidemiology. Breast cancer is the most prevalent kind of cancer in women globally, and its effects on people's health as well as the general population cannot be overstated (3). We can gather and analyze data using epidemiology to better understand the distribution, risk factors, incidence, fatalities, and variations in the occurrence of breast cancer. The rate of incidence is significantly higher among old-aged women and the median age of breast cancer diagnosis was 63 years from year 2014-2018, which has, increased to 69 years during the years 2015-2019. However, the mortality rate has been reduced by 1.1% during 2013-2019; improving the average life span of the population due to the accessibility and availability of better healthcare facilities and timely diagnosis which has a profound impact on longevity factors. In Pakistan, the incidence of BC is increasing as compared to other Asian countries and the average life span is 67 years, which is less than the Western population. Since 2019, nearly 4 million patients with breast cancer have been living in the United States and the number of metastatic breast tumors revolts to one and a half million by 2021 (4, 5). The ratio of recurrence is almost 20-30% among the women who are treated or considered free of disease (6).

Globally women have been affected by several types of breast cancer, which are differentiated based on hormone levels, aetiology, clinical screening and availability of various treatment options. Commonly, invasive breast cancer types are classified into estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor 2 (HER2). In Asia, the incidence of hormone-positive BC is relatively high as compared to other regions (7).

Determination of risk factors involved in the progression of breast cancer is especially important. Genetic factors such as gene mutations and family history are major threats to the development of cancer in first-degree relatives. Numerous biological processes, including histone modifications, polycomb/trithorax protein complexes, short non-coding or antisense RNAs, and DNA methylation, mediate epigenetic events. These various adjustments are intricately linked. The ability of genes to be expressed throughout typical stages of development is closely conditioned by epigenetic control (8). Histone deacetylases (HDACs) are a class of enzymes that play a critical role in the regulation of gene expression by modifying the acetylation status of histone proteins (9). Changes in the makeup of chromatin and the portability of DNA to DNA transcription factors can result from HDACs changing the acetylation status of histones, affecting the processes that lead to apoptosis (programmed cell death) and the cell cycle and altering the expression and function of hormone receptors such as the ER and PR, which may have an impact on hormone-dependent tumour growth. As a result, oncogenes may be activated or tumour suppressor genes may be silenced, accelerating the growth of cancer (10). Histone and non-histone proteins are acetylated by HDAC inhibitors (HDACi), which have an impact on gene expression, the advancement of the cell cycle, cell migration, terminal differentiation, and cell death. Understanding the anticancer mechanism(s) through which HDACi therapy drives differentiation in cancer may be crucial for understanding how GEF (guanine nucleotide exchange factor) protein regulation by HDAC inhibition influences cell differentiation (11). Age-related risks are closely related to the stage of menopause in women. Most women get affected with tumors at the post-menopausal stage (12). There is a strong association of breast density, obesity and hormonal imbalance with the incidence of breast cancer. Moreover, environmental and lifestyle risk factors, like toxic air pollution, occupational hazards, lack of physical activities, poor diet and smoking are contributing to the onset of BC (13). In leukemia and breast cells, HDAC expression and function are influenced by a variety of environmental variables. It has been demonstrated that environmental endocrine disruptors, change the expression and activity of the HDAC gene in breast cells (14). It is possible that altered HDAC activity plays a role in the emergence of leukemia, including acute myeloid leukemia (AML).

Chemicals known as endocrine disruptors prevent the endocrine system, which is in charge of producing and controlling hormones in the body, from operating normally. These substances have the potential to imitate or obstruct natural hormones, resulting in hormonal imbalances and possibly harmful consequences on health (15). Increased estrogen activity may result from exposure to endocrine disruptors, which may then promote the development of hormone-sensitive breast cancer cells. Certain endocrine-disrupting substances, especially bisphenol A (BPA) and phthalates, have been linked in studies to an increased risk of breast cancer. During the last several decades, there has been an increase in the prevalence of breast cancer worldwide. While many causes have contributed to this increase, endocrine disruptors are one cause for concern (16). Understanding the global epidemiology of breast cancer based on risk factors is essential for developing effective prevention and treatment strategies tailored to local populations. Therefore, this systematic review aims to evaluate the available evidence on the global epidemiology of breast cancer based on risk factors by systematically collecting recent published literature (2017-2022).

Methodology

Search strategy

Systematic review of the literature utilizing PubMed was performed according to PRISMA 2020 guidelines (17) (Figure 1; PRISMA flow diagram), as used in our previous systematic reviews (18, 19). PubMed is frequently suggested in guidelines for systematic reviews and covers a sizable amount of the literature pertinent to our research question. Furthermore, one of the unique features of PubMed is the Medical Subject heading (MeSH) terms, which are employed in PubMed for systematic review literature searches because they raise the standard and dependability of search results. The National Library of Medicine established MeSH words as a regulated vocabulary for indexing and annotating papers, and PubMed is a biological database that incorporates citations to pertinent material (20). By including both index terms from standardized terminologies like MeSH and free-text terms, using MeSH terms enables researchers to conduct more thorough searches (21). MeSH words offer a standardized approach to represent concepts and themes, guaranteeing that all pertinent articles are included in the search and assisting in the identification of pertinent articles (22). By enabling researchers to insert more precise terms associated with the study question, they also aid in the refinement of search results (23).

All publications were retrieved from PubMed in September 2022, with Medical Subject Heading (Mesh) Terms; a new and thoroughly revised version of lists of subject headings compiled by the National Library of Medicine (NLM) for its bibliographies and cataloging. The Mesh term “Breast neoplasm” was used with the Boolean operator “AND” and other related Mesh Terms related to regions/country names and “Epidemiology” to search all the records available from 2017 to 2022.

Study selection

A detailed list of retrieved articles related to BC epidemiology based on risk factors was collected for quantitative analysis. The

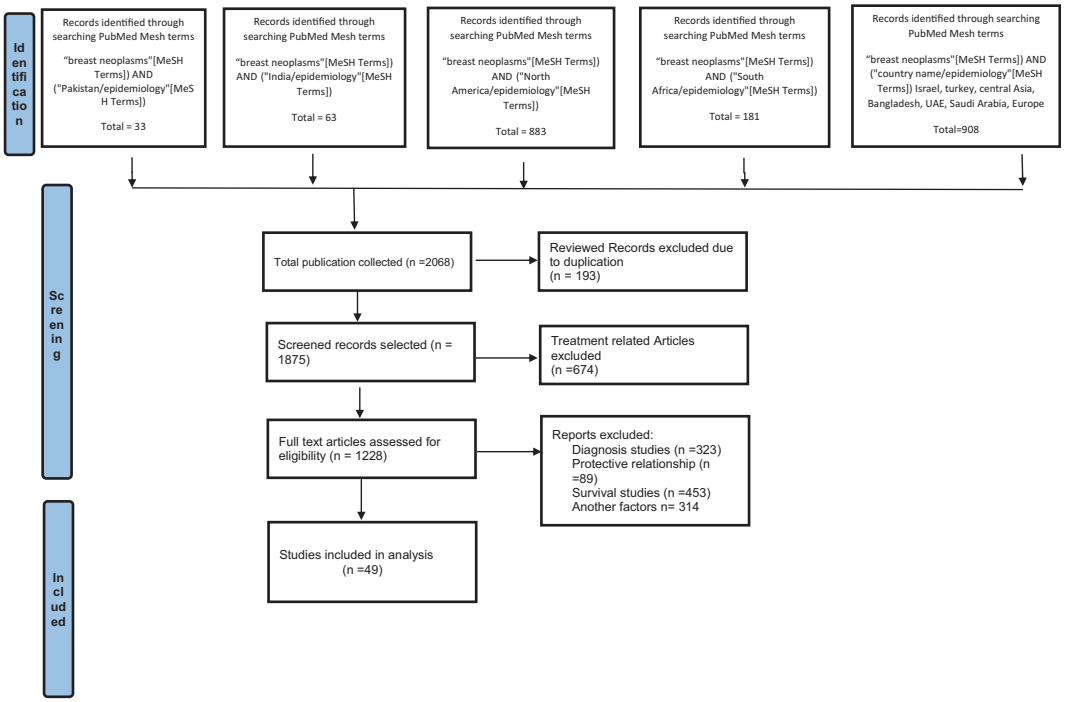

FIGURE 1
PRISMA flow diagram of study selection.

initial screening was based on the title and abstract, while the final inclusion was based on full texts where available. EndNote software was used to combine and sort out duplicated articles based on the inclusion and exclusion criteria. All authors reviewed the retrieved articles and included only those articles, which were fulfilling the following conditions.

Inclusion criteria

Full-text articles published in PubMed Indexed journals, indexed with Mesh Terms as stated above.

Exclusion criteria

Abstracts, short commentaries, and studies focusing on treatment, and/or in languages other than English were excluded. Systematic reviews and letters to the editors were not included in this review. Qualitative studies regarding treatment therapies, survival rates, and diagnostic irregularities were excluded because of their inappropriate focus on the aim of our review.

Data extraction

The first authors of this manuscript independently performed data extraction. All disagreements were discussed and resolved by all other authors in this study. The following data taken from each article was entered into a spreadsheet: Study reference, year published, study design, study region and risk factors.

Quality assessment

Three investigators independently rate the quality of included study as good, fair or poor. Final ratings were determined by consensus among all reviewers, only those studies rated as good or fair were included.

Results

Study selection

An extensive search was conducted in PubMed using advanced search strategies to identify articles related to breast neoplasms in different regions. The search terms utilized were “breast neoplasm” and “Pakistan/epidemiology”, which resulted in 33 articles being extracted. After a rigorous process of inclusion and exclusion criteria, 11 articles on prevalence studies were selected for further analysis.

Similarly, the search terms “breast neoplasms” and “India/epidemiology” were used, resulting in 63 articles being extracted. Out of these, 14 articles were deemed suitable for epidemiological studies after applying the selection criteria. The search terms “breast neoplasms” and “North America/epidemiology” produced a total of 883 articles, and 11 of these were selected for the study. The search terms “breast neoplasms” and “South Africa/epidemiology” produced 181 articles, with 6 being selected. Finally, the search terms “breast neoplasms” and “Israel/Turkey/Central Asia/Bangladesh/UAE/Saudi Arabia/Europe/Epidemiology” produced 908 articles, and 21 were selected for the study.

The above results demonstrate the comprehensive nature of the literature search and thorough application of the inclusion and exclusion criteria.

Study characteristics

An initial search in PubMed utilizing MeSH terms (described above) resulted in the extraction of 2068 articles. Duplicate articles (n=193) were removed, leaving 1875 articles for further review. The remaining articles were evaluated by examining their titles and abstracts, and after applying the selection criteria, 49 studies were included in the present review, as shown in the PRISMA flow chart (Figure 1). The studies selected are summarized in Table 1, which highlights the reference, design, risk factors, sample size and type of the studies included in this systematic review. Table 2 presents the proportion of risk factors in various regions of the World.

Tables 1, 2 present various studies conducted on breast cancer incidence and risk factors in different regions of the world. The findings were discussed based on the study design and risk factors and geographical region.

In the Asian region, studies have found that breast cancer incidence rates are higher in Asian Indian and Pakistani Americans than in non-Hispanic white Americans (24). Risk factors identified include a family history of breast cancer, early menarche, late menopause, positive family history, and obesity (25). Viral infections, genetic mutations, lack of knowledge about breast cancer symptoms and risk factors, and low vitamin D levels are also associated with an increased risk of breast cancer (26–32). The prevalence of BRCA1/2 mutations is higher in Indian breast and/or ovarian cancer patients, and delays in the diagnosis and treatment of breast cancer are associated with poor referral systems (33).

In Africa, inherited mutations in the BRCA1/2 genes have been found to be a significant issue among Nigerian women (48). Low vitamin D status and VDR genetic polymorphisms are associated with an increased risk of breast cancer in Ethiopian women (49, 50).

In the USA, studies also identified risk factors such as use of hair dye and chemical straightener (51), unhealthy plant-based diet (52), obesity and diabetes (53, 57), sugar-sweetened soda (54), certain genetic variations (58), occupational exposure to organic solvents (59), smoking (55) and endocrine-disrupting metals (56) that are associated with increased breast cancer incidence and mortality.

Certain occupations and industries, such as healthcare and the service sector, are also associated with increased risk (60). Weight loss is associated with a reduced risk of breast cancer in postmenopausal women. Studies have also identified genetic variations associated with survival in breast cancer patients.

In Europe, studies have found that joint tobacco smoking and alcohol intake (61), occupational exposure to organic solvents and ambient air emissions of polycyclic aromatic hydrocarbons (63), age, hormonal factors, and family history of breast cancer (64), thyroid gland diseases (65), and employment in certain industries (66) are associated with an increased risk of breast cancer.

TABLE 1 Main results, risk factors and study design of studies associated with breast cancer incidence in various regions of the world.

Reference	Main Results/Findings and Risk Factors	Sample size	Study type/ design
	Asia		
(24)	Breast cancer incidence rates were higher in Asian Indian and Pakistani Americans (AIPA) than in non-Hispanic white Americans (NHW). Family history of breast cancer, reproductive factors	4900 AIPA and 482 250 NHW	Surveillance, Epidemiology and End Results-based study
(25)	Breast cancer was more common among postmenopausal women who had early menarche, late menopause, and a positive family history of breast cancer	326 women	Cross-control study
(26)	Breast density was positively associated with age, body mass index (BMI), and parity, and negatively associated with smoking and oral contraceptive use	477 women	Cross-sectional study
(27)	Breast cancer incidence was projected to increase over time, particularly among women aged 50 years and older.	9771 registered diagnosed cases	Time-trend analysis
(28)	Metaplastic breast carcinoma was associated with worse survival outcomes compared to invasive ductal carcinoma (Histological type of cancer)	42 patients	Retrospective closed Cohort study
(29)	Epstein-Barr virus (EBV), human papillomavirus (HPV), and mouse mammary tumor virus (MMTV) were detected in breast cancer tissue samples, suggesting a possible etiological role of these viruses in breast cancer	tissue biopsies (n = 250)	Case-control study
(30)	P53 overexpression was associated with hormone receptor status and triple-negative breast carcinoma	91 patients	Retrospective study
(31)	Younger breast cancer patients (<40 years old) had more advanced cancer at diagnosis and worse survival outcomes compared to older patients (Age)	1,334 patients	Retrospective study
(32)	Transforming growth factor β1 (TGFβ1) gene polymorphism (T29C) was associated with an increased risk of breast cancer	150 subjects, 80 cases and 70 healthy controls	Case-control study
(33)	The prevalence of BRCA1/2 mutations was higher in Indian breast and/or ovarian cancer patients than non-BRCA mutations	1010 patients	Multi-gene panel screening
(34)	Delays in diagnosis and treatment of breast cancer were associated with lack of knowledge about breast cancer symptoms and risk factors, as well as poor referral systems	269 breast cancer patients	Mixed-methods study
(35)	Obesity was associated with increased oxidative stress in breast cancer patients	30 patients women, 30 healthy control	Cross-sectional study
(36)	Lack of knowledge about breast cancer symptoms and risk factors was common among women in a low socio-economic area of Mumbai	480 women	Community-based study
(37)	Low serum levels of 25-hydroxyvitamin D were associated with an increased risk of breast cancer in Indian women	297 subjects	Case-control study
(38)	The prevalence of breast cancer screening was low among women aged 30-49 years in India, and was associated with higher education, urban residence, and wealth.	336,777 women aged 30-49 years	Secondary data analysis
(39)	Air pollution emissions are associated with a higher incidence and prevalence of breast cancer in the Aktobe region of western Kazakhstan		Retrospective study
(40)	Genetic polymorphisms in the DNA repair genes XRCC1 and XRCC3 may be associated with breast cancer susceptibility in Bangladeshi women	121 breast cancer patients and 133 healthy controls	Case-control study
(41)	Gene-positive breast cancer in UAE had an earlier age of onset, higher rates of bilateral tumors, and lower rates of lymph node involvement compared to gene-negative tumors	309 patients	Retrospective study
(42)	Sedentary lifestyle and unhealthy dietary habits were associated with an increased risk of breast cancer among women attending an oncology day treatment center in Turkey	65 diseased women, 65 healthy women	Case control study
(43)	Younger age at diagnosis was associated with worse outcomes in breast cancer patients, particularly those aged 25 years or younger	137 patients	Histopathological and clinical study
(44)	HER2 over-expressed breast cancer was found to be more aggressive and associated with poorer prognosis in Saudi Arabian women	1867 patients	Retrospective study
(45)	Triple-negative breast cancer was the most common subtype among Saudi Arabian women and was associated with younger age at diagnosis	270 female patients	multi-centric, Cross-sectional study

(Continued)

TABLE 1 Continued

Reference	Main Results/Findings and Risk Factors	Sample size	Study type/ design
(46)	Breast cancer patients in Botswana presented with a more advanced stage of disease and had lower survival rates compared to patients in South Africa and the United States (Late presentation)	Botswana (n = 384, 2011-2015), South Africa (n = 475, 2016-2017), and the US (n = 361,353, 2011-2012)	Retrospective study
(47)	Hormone receptor-positive tumors were the most common subtype of breast cancer in Rwanda, and were more commonly diagnosed at advanced stages	138 patients	Retrospective study
	Africa		
(48)	Inherited breast cancer is a significant issue among Nigerian women, and the BRCA1/2 mutations account for a large proportion of inherited cases	1,136 women, 997 women without cancer	Case-control study
(49)	The prevalence of inherited mutations in breast cancer predisposition genes among women in Uganda and Cameroon is relatively low, with BRCA1/2 mutations being the most common	196 cases and 185 controls	A multigene sequencing panel
(50)	Low vitamin D status and VDR genetic polymorphisms are associated with an increased risk of breast cancer in Ethiopian women	392 female breast cancer patients and 193 controls	Case-control study
	America		
(51)	Hair dye and chemical straightener use are associated with an increased risk of breast cancer in black women, but not in white women	participants (n = 46,709), women ages 35–74	Prospective cohort study
(52)	A healthful plant-based diet is associated with a lower risk of breast cancer, whereas an unhealthful plant-based diet is associated with a higher risk of breast cancer	76,690 women from the Nurses' Health Study (NHS, 1984–2016) and 93,295 women from the NHSII (1991–2017).	Prospective cohort study
(53)	Weight loss is associated with a reduced risk of breast cancer in postmenopausal women (Obesity)	Postmenopausal women (n = 61,335)	Observational study
(54)	Sugar-sweetened soda consumption is associated with an increased risk of breast cancer mortality	927 breast cancer cases	Western New York Exposures and Breast Cancer Study
(55)	Smoking is associated with an increased risk of breast cancer, particularly in hormone receptor-positive tumors, in African American women	67 313 women, 45–75 years of age	Multiethnic Cohort (MEC) study
(56)	Blood levels of endocrine-disrupting metals are associated with an increased risk of breast cancer in American women.	9260 women aged ≥ 20 years	multivariate logistic regression models
(57)	Obesity and diabetes are independently associated with an increased incidence of breast cancer in Louisiana.	Luminal A (n=1,584), TNBC 364 Luminal B 232 and HER2 + 115	retrospective case-control study
(58)	Variations in TNFα, PPARγ, and IRS-1 genes are associated with survival in breast cancer patients.	breast cancer between 1995 and 1999	Prospective cohort study
(59)	Certain occupations and industries, such as healthcare and the service sector, are associated with an increased risk of breast cancer in both women and men	Women 17 865 and Men 492	Occupational Disease Surveillance System cohort
(60)	Exposure to ambient air emissions of polycyclic aromatic hydrocarbons is associated with an increased incidence of breast cancer in American women	N/A	Ecological study
	Europe		
(61)	Joint tobacco smoking and alcohol intake increase cancer risk	19,898 women	Questionnaires
(62)	Long-term consumption of non-fermented and fermented dairy products is not associated with breast cancer risk	33,780 women	Population-based prospective cohort study
(63)	Occupational exposure to organic solvents, including ethanol, is associated with increased breast cancer risk	38,375 breast cancer cases and 191,875 controls	population-based nested case–control study
(64)	Benign breast diseases are associated with age, hormonal factors, and family history of breast cancer	61 617 women	cohort study
(65)	Thyroid gland diseases are associated with increased breast cancer risk	7408 women	retrospective case–control study

(Continued)

TABLE 1 Continued

Reference	Main Results/Findings and Risk Factors	Sample size	Study type/ design
(66)	Employment in certain industries is associated with increased breast cancer risk	845 women	population-based case-control study
(67)	Adherence to healthy lifestyle behaviors is associated with reduced breast cancer risk, and this association is stronger in women without a genetic predisposition to breast cancer	146326 women	COX proportional hazard regression model
(68)	Occupational heat exposure is associated with increased breast cancer risk	1,738 breast cancer cases and 1,910 controls	Case-control study
(69)	Smoking is associated with increased breast cancer risk	102,927 women	Generations Study cohort
	Israel		
(70)	Breast cancer incidence is increasing among younger women (Age)	34,251 women	Cross-sectional study
(71)	Inherited predisposition to breast and ovarian cancer is observed in non-Jewish populations in Israel (Genetic factors)	68 cases	Population study
(72)	Cumulative mammographic density is positively associated with age-specific incidence of breast cancer	200 women	Cohort study
(73)	Passive smoking is associated with increased breast cancer risk in women with NAT2 polymorphism	137 breast cancer patients 274 population-based controls	population-based case-control study

Adherence to healthy lifestyle behaviors is associated with reduced breast cancer risk, and this association is stronger in women without a genetic predisposition to breast cancer (67). In addition, smoking and alcohol intake increases cancer risk (69, 75).

In Israel, studies have found that breast cancer incidence is increasing among younger women (70). Genetic factors, including inherited predisposition to breast and ovarian cancer (71, 73), and mammographic density (72) are also associated with increased breast cancer risk.

The risk factors for breast cancer are subdivided into demographic, genetic, hormonal, lifestyle, and other categories as shown in Table 2. The percentage prevalence by area is also shown in Figure 2, which demonstrates that genetic and societal variables are the most prevalent risk factors for breast cancer in Asia, with a prevalence of 70 and 50, respectively. Additionally important are lifestyle factors, which have a prevalence of 50 and 30, respectively, and hormonal aspects.

With a frequency of 40, hormonal variables are the most common risk factor for breast cancer in Africa. With a prevalence of 20 each, genetic, demographic, and other (Air pollution, Oxidative stress, Infections/Diseases, Occupations) factors are also significant. In America, lifestyle factors are the most significant risk factor for breast cancer, with a prevalence of 80. Genetic, demographic, and hormonal factors also contribute, with a prevalence ranging from 20 to 40.

In Europe, other factors such as oxidative stress, infections, diseases, and occupations have the highest prevalence, with a prevalence of 80. Hormonal, genetic, demographic, and lifestyle factors also play a role, with a prevalence ranging from 20 to 60.

In Israel, genetic and demographic factors have an equal prevalence of 40, followed by hormonal and lifestyle factors with a prevalence of 20 each. Other factors have a prevalence of 20.

Discussion

Patients with breast cancer have multiple risk factors associated with their disease (76). Depending on the characteristics of specific geographical regions, certain risk factors either modifiable or non-modifiable have variable influences on the health of women. Early identification of modifiable factors helps develop strategies to reduce the incidence of Breast cancer whereas other factors such as age, gender, and family history are not in an individual's control to avoid breast cancer risk (77). Hormone positive breast tumor is quite common among Asian women. Figure 2 shows that approximately 50% of women have imbalanced hormonal levels, which increases the chances of BC development whereas in Europe and Africa, the estimated prevalence of BC due to hormonal abnormalities is 40%. Various risk factors contribute to the progression of breast tumors at various levels. All regions discussed in this review showed variable data on individual factors associated with the prevalence of BC all over the world. Presence of mutant genes (BRCA1 and BRCA2) can increase the incidence of BC up to 80% of women populations as compared to non-mutant genes (78). Few mutant genes (CHEK2, PTEN, CGH1, STK1 and PALB2) do not impose much influence on the occurrence of BC. Despite this genetic variability, a few genes (RAD52, OCT4, FASL, IGFIR, APE1, BARD1, IL4, and IL21) pose a protective impact and decrease the risk of developing BC. Chances of BC are significantly high if the patient has a positive BC family history even in men. Overall, the prevalence of BC in males is quite low but family history increases the risk in males as well. This trend is confirmed in various studies conducted in different regions of the world. We discussed association of various risk factors with specific geographical regions in the following sections.

TABLE 2 Risk factors associated with breast cancer in different regions of the world and populations.

Reference	Region	Age	Obesity	Breast Density	Genetic Mutation /Family History	History of Cancer	Hormonal Imbalance	Pregnancy	Lifestyle Factors \diet	Smoking/ Alcohol	Drug Abuse	Infections/ Diseases	Air Pollution/ Occupation
Asia		Demographic			Genetic		Hormonal Imbalance		Lifestyle			Others	
(24)	Asian Indian, Pakistani Americans	+				+							
(25)	Southern Punjab, Pakistan	+	+			+	+		+				
(27)	Karachi, Pakistan	+		+									
(26)	Karachi, Pakistan	+		+									
(28)	Karachi, Pakistan		+		+					+	+		
(29)	Pakistan											+	
(30)	Lahore, Pakistan				+		+						
(31)	Karachi, Pakistan	+											
(32)	Rawalpindi, Pakistan				+								
(33)	India				+								
(34)	North East India	+					+						
(35)	India		+										
(36)	Mumbai, India		+				+	+					
(38)	India	+	+					+	+				
(39)	Western Kazakhstan												+
(40)	Bangladesh				+								
(41)	UAE	+				+						+	
(42)	Turkey								+				
(43)		+											
(44)	Saudi Arabia				+								
(45)		+											

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TABLE 2 Continued

Reference	Region	Age	Obesity	Breast Density	Genetic Mutation /Family History	History of Cancer	Hormonal Imbalance	Pregnancy	Lifestyle Factors \diet	Smoking/ Alcohol	Drug Abuse	Infections/ Diseases	Air Pollution/ Occupation
Asia		Demographic			Genetic		Hormonal Imbalance		Lifestyle			Others	
(46)	South Africa	+					+		+				
(47)	Rwanda	+					+						
(48)	Nigeria				+								
(50)	Ethiopia								+				
(51)	North America								+				
(52)									+				
(53)			+										
(54)									+				
(55)										+			
(74)									+				
(56)													+
(57)			+									+	
(58)					+								
(59)													+
(60)													+
(61)	Denmark								+	+			
(62)	Sweden								+				
(63)	Denmark									+			+
(64)	Sweden	+			+		+						
(65)	Germany											+	
(66)	UK												+
(67)	Spain												+
(75)	UK												+
(69)	Poland									+			

(Continued)

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TABLE 2 Continued

Reference	Region	Demographic				Genetic			Hormonal Imbalance		Lifestyle				Others		
		Age	Obesity	Breast Density	Genetic Mutation /Family History	History of Cancer	Hormonal Imbalance	Pregnancy	Lifestyle Factors \diet	Smoking/ Alcohol	Drug Abuse	Infections/ Diseases	Air Pollution/ Occupation
Asia													
(70)		+					+						
(71)					+								
(72)				+									
(73)					+					+			

Asia

A person's demographic group or a particular subset of the population can have an impact on the occurrence, distribution, assessment, and management of breast cancer through certain characteristics. Demographic considerations can shed light on the patterns and trends in the incidence of breast cancer in various communities (79). Age, weight, and breast density are highly correlated with the incidence of BC (80). According to a study in Pakistani Asian women, younger females are more affected by BC and its prevalence increased from 70% to 130% among females aged 30 to 34 years and among the age group 50-64 years, the percentage prevalence increased from 23.1% to 60.7% (26) (Table 1). Particularly, the incidence of metastatic BC and high-grade BC in young females has escalated in the past few years. The frequency, grade at being diagnosed, and available treatments for breast cancer can all be influenced by socioeconomic factors like income, education, and access to the hospital (81). Due to the socioeconomic problems in Asian countries, early diagnosis and timely screening is not accessible (31). People from rural areas have faulty beliefs and feel hesitation at the time of mammographic inspection (82). This reluctant behavior is a major reason for the increased incidence of BC at a young age. According to certain studies, married women might receive a better prognosis than single or divorced women (83). In adolescents, 86% of patients are diagnosed with invasive ductal carcinoma, 16.8% have luminal A and 30.5% patients have luminal B cancer. 30% of patients were affected by HER2+ whereas only 15% showed diagnosis with triple negative BC (32). Late diagnosis in developing countries drastically increased the progression to late stage tumor. In a recent study, Prevalence of stage III cancer was 62% whereas 24.8% patients were diagnosed with stage II cancer (47).

Breast cancer risk is heavily influenced by hereditary variables, and several genetic variants are known to dramatically enhance the risk of developing the illness. BRCA1 and BRCA2 gene mutations are the most well-known genetic changes linked to breast cancer. These genes are crucial for preserving the stability of the genetic material in the cell since they are involved in mending damaged DNA. The chance of developing breast and ovarian cancers is considerably increased by inheriting a deleterious mutation in either the BRCA1 or BRCA2 gene (84). An association has been observed between genetic mutations and the risk of BC. In Asia, Approximately 70% of patients have genetic polymorphism, DNA repair, overexpression of p53, presence of BRCA1 and BRCA2, and other hereditary characteristics (Figure 2), whereas the risk of BC in other regions due to genetic mutation is comparatively low. High occurrence of breast cancer due to the genetic mutation in BRCA1 and BRCA2 genes in Asian women is directly related to first-degree relatives (85).

Africa

A complex interaction of factors, including genetics, way of life, socioeconomic circumstances, healthcare infrastructure, and cultural beliefs, characterizes the epidemiology of breast cancer in Africa. The female hormones progesterone and estrogen can affect

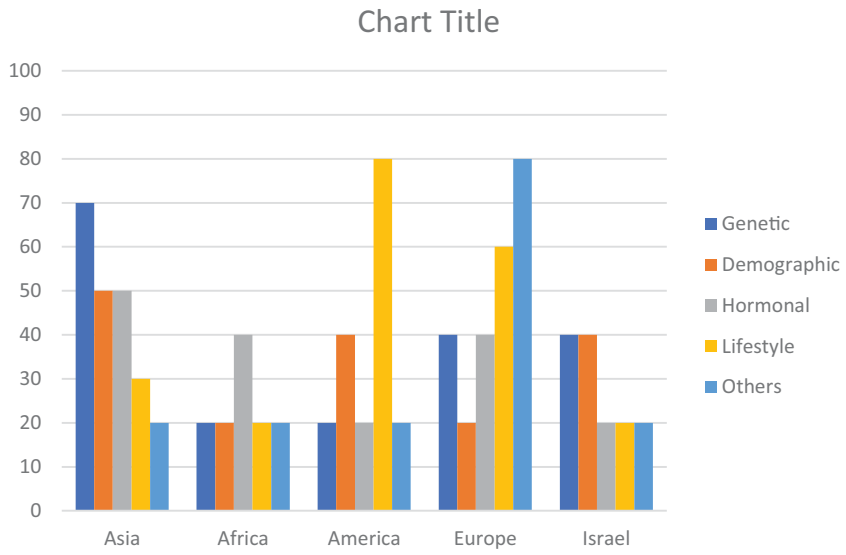

FIGURE 2
Region-wise percentage prevalence of risk factors. Demographic (Age, Obesity, Breast Density), Genetic (Genetic Mutation, Family History, History of Cancer), Hormonal (Hormonal Imbalance, Pregnancy), Lifestyle (Lifestyle Factors, diet, Smoking/Alcohol, Drug Abuse), Other (Infections/Diseases, Air Pollution/Occupation).

the development of breast tissue and cells, and both their levels and activities are linked to an increased risk of breast cancer. A hormone called estrogen promotes the growth and upkeep of female reproductive tissues. High amounts of estrogen or continuous exposure to estrogen can raise the likelihood of breast cancer because it can encourage cell development in the breast. Imbalance of hormonal profile in the female population is the major risk factor for developing BC (86). Proliferation of cancer cells can be aggressive if estrogen and progesterone levels are not up to the mark. Breast cancer risk has been linked to long-term usage of combination hormone replacement therapy (estrogen and progestin) during menopause (87). Premenopausal and postmenopausal stages are highly linked with the occurrence of BC (88). Existing research on breast cancer in Africa is characterized by a limited collection of studies. According to the limited collections of studies conducted in Africa have been shown that 40% involvement of hormonal factors in the prevalence of BC. In addition, other factors; including Infections/Diseases, Air Pollution/Occupation, have been found to equally contribute to the occurrence of breast cancer within the African population. However, it is important to note that the lack of resources in many African regions poses significant challenges to collecting precise and comprehensive data. To gain a more comprehensive understanding of the distinct patterns of breast cancer in different African locations and to tailor therapeutic interventions accordingly, a more extensive and rigorous research effort is warranted.

America

Susceptibility of inherited mutations in America and Africa is modest however; nearly one-third of the female population of Europe and Israel is under threat of BC progression due to genetic mutations (Figure 2). If a person contains dangerous mutations in breast cancer-related genes, genetic testing can reveal this. For the evaluation of risks, prevention tactics, and screening advice, this information may be essential (89).

Lifestyle modifications impart beneficial effects on women's health. Women who are exposed to smoking, containing toxic aromatic compounds and consuming alcohol, are more prone to developing breast cancers (61, 63). Physical inactivity on a regular basis is linked to an increased risk of breast cancer. It has been demonstrated that regular physical activity lowers the incidence of breast cancer (90). Most of the population of America and Europe have a sedentary lifestyle and unhealthy eating habits (51) and the affected population with BC is 80% and 60% respectively (Figure 2). Prevalence of lifestyle risk factors in other geographical regions is very low which may involve certain social and ethical problems (91).

Based on race and ethnic origin, the incidence rate is higher in black women as compared to white women (92). A recent surveillance and epidemiology study demonstrated that Asian Indian and Pakistani women who reside in the United States have a high degree of BC incidence ratio as compared to non-Hispanic white women (24). Based on age, young and late menopausal age are most affected by this life-threatening disease because of imbalanced hormonal profiles. Other factors including late pregnancy, use of contraceptive pills and hormonal therapies for conception alter the normal levels of estrogen and progesterone, which are the main hormones involved in the growth of BC.

Obesity is linked with majority of chronic diseases including breast carcinoma. A higher risk is observed in menopausal women who are obese and have a sedentary lifestyle as compared to the females having normal BMI (93). Unhealthy eating habits, consumption of Trans fats and dawn-to-dusk working hours affect the normal physiological processes of our body and increase

the risk of developing cancer. Physical activities like walking and aerobic exercises help to reduce the threat of BC to a greater extent (94).

Europe

Air pollution, drug abuse and infections have a deleterious influence on the European population that affected 80% of the population (Figure 2). Occupational hazards thrust including exposure to organic solvents and fumes of dangerous gases are more prominent causes of health problems in Europe (75). Moreover, noise pollution is also a crucial risk factor that is associated with the etiology of BC (75).

In addition to physical workouts, a healthy diet and consumption of essential vitamins reduce the risk of BC. Several studies have shown that intake of vitamin D with treatment has positive outcomes in cancer patients thus slowing the progression of the disease whereas its deficiency can increase the BC risk (95). Moreover, consumption of alcohol and smoking is linked with a higher incidence of BC and it is evident by various studies (69). Occupational toxic exposure and air pollution are also contributing factors in the occurrence of BC all over the world because of global climate alterations (96).

Quercetin (QCT), a flavonoid derived from many fruits and vegetables, is endowed with manifold biological properties, such as the ability to elicit a strong inhibitory effect on the growth of several tumor cell lines (97). Quercetin may aid in preventing DNA deterioration in cells and thwarting the formation of cancer cells by lowering oxidative stress (98). The BRCA genes' expression may be affected by quercetin, perhaps improving their capacity for DNA repair (99). Research has been done on quercetin's potential to lessen breast density, which could, in turn, reduce the risk of breast cancer. According to certain studies, quercetin can modify estrogen metabolism and affect hormone levels, which may affect the composition and density of breast tissue (100). QCT has been proposed as an auxiliary molecule when combined therapy, when given along with many chemotherapeutic medications, such as topotecan, cisplatin, and sorafenib, in the treatment of various malignancies (97). According to this review, genetic and hormonal risk factors contributed 40% toward prevalence of BC in women but lifestyle modification factors 60% associated with BC.

Obesity and breast cancer have a complicated and varied association. Insulin resistance and persistent low-grade inflammation are both linked to obesity. These elements can foster a body environment that is conducive to the growth of breast cancer (101). Due to increasing breast density, people may find it harder to identify breast tumors or abnormalities, which can delay diagnosis and treatment. Compared to non-obese patients, obese breast cancer patients are more likely to have a cancer recurrence and are at a higher risk of dying from the disease (102). Obesity has an inverse relation with menopause age that contributes to the development of Breast cancer (103). An observational study conducted in Europe and America explained that the risk of BC due to obesity was lower at premenopausal age as compared to postmenopausal age (53).

Israel

In Israel, breast cancer is by far the most prevalent type of cancer among women. Because of variables like longer life expectancies, altered reproductive habits, and lifestyle choices, prevalence rates have been continuously increasing. The mortality rate has been declining, nevertheless, in part because of breakthroughs in therapy, early detection, and screening techniques. Women between the ages of 50 and 74 can receive mammograms through Israel's national breast cancer screening program. The goal of this initiative is to identify breast cancer early, when it can be treated more successfully. The decreasing mortality rates have been attributed to routine screening and early diagnosis (104). Particularly among Ashkenazi Jewish women, Israel's population is distinct in that some genetic variants are relatively common. This population has a greater prevalence of BRCA1 and BRCA2 gene mutations, which increases the chance of getting breast and ovarian cancer and reported elevated carrier frequency of 0.9% in the Ashkenazi Jewish population, a specific BRCA1 mutation known as 185delAG is also occasionally seen in non-Jewish patients with a distinct haplotype (105). Breast density is another factor associated with the incidence of BC as females with dense breasts are at a higher chance of developing BC as compared to those with less dense breasts (72). Early diagnosis by mammography is significantly difficult in dense breasts, which leads to the progression of late-stage BC. On the other hand, certain diseases such as diabetes, hypertension, insulin intolerance, multiple sclerosis and polycystic ovary syndrome (PCOS) also increase the risk of BC (106). Our data showed that in Israel demographic and genetic factors are predominant (40%) among the population as compared to other risk factors, which associated with occurrence of BC only 20% (Figure 2). For the most up-to-date details about breast cancer epidemiology in Israel, it is crucial to study the most recent sources, including Israeli health authorities, cancer registries, and research organizations.

Conclusion

This paper has reviewed studies of incidence, prevalence, and risk factors for breast cancer in India, Pakistan, Kazakhstan, Turkey, USA, Europe and the United Arab Emirates. The evidence shows that diet, obesity, and genetic factors, such as BRCA mutations and DNA repair gene polymorphisms vary from region to region. These findings emphasize the need to be aware of the particularities of each region of the world with respect to breast cancer risk. This will facilitate early detection and improve prognosis. Our study provides valuable insights into the epidemiology, risk factors, and outcomes of breast cancer in various populations and highlights the need for further research and intervention efforts to reduce the burden of breast cancer in these regions.

Study limitations

Not all world regions were included and study methodologies varied. In addition, the traditions, customs, and genetic backgrounds of the residents of different geographic regions is only superficially known. Thus, more specific research is needed that specifically target distinct populations or examine particular risk factors in order to enhance the comprehensiveness and accuracy of findings.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

Author contributions

AR played a major role in the literature search, collecting and screening of abstracts, full-text articles, data extraction and quality assessment of the included studies and prepare the first draft of the manuscript, AK provide the Idea and design for systematic review, supervised and guided AR in data collection and reporting and review and revised the manuscript. FA and ZA: screening of abstracts, full-text articles, data extraction and quality assessment of the included studies and revision of the manuscript. MA, MK, MS, RA: critical revision of manuscript and resolution of discrepancies. All authors made significant intellectual contributions and approved the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 13 October 2023
DOI 10.3389/fonc.2023.1274340

OPEN ACCESS

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RECEIVED 08 August 2023
ACCEPTED 28 September 2023
PUBLISHED 13 October 2023

CITATION
Chen H, Du P, Yang T, Xu X, Cui T and Dai Y (2023) Hepatitis C virus infection is associated with high risk of breast cancer: a pooled analysis of 68,014 participants. *Front. Oncol.* 13:1274340. doi: 10.3389/fonc.2023.1274340

Hepatitis C virus infection is associated with high risk of breast cancer: a pooled analysis of 68,014 participants

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Introduction: Breast cancer is the most common malignancy among women. Previous studies had shown that hepatitis C virus (HCV) infection might serve as a risk factor for breast cancer, while some studies failed to find such an association.

Methods: In this study, we presented a first attempt to capture and clarify this clinical debate via a cumulative analysis (registration ID: CRD42023445888).

Results: After systematically searching and excluding the irrelevant publications, five case-control or cohort studies were finally included. The synthetic effect from the eligible studies showed that patients with HCV infection had a significantly higher prevalence of breast cancer than non-HCV infected general population (combined HR= 1.382, 95%CI: 1.129 to 1.692, P=0.002). There was no evidence of statistical heterogeneity during this pooled analysis (I^2 = 13.2%, P=0.33). The sensitivity analyses confirmed the above findings. No significant publication bias was observed among the included studies. The underlying pathophysiological mechanisms for this relationship might be associated with persistent infection/inflammation, host immune response, and the modulation of HCV-associated gene expression.

Discussion: Though the causal association between HCV infection and breast cancer did not seem quite as strong, screening for HCV might enable the early detection of breast cancer and help to prevent the progression of the disease. Since the topic of this study remains a matter of clinical debate, further studies are still warranted to validate this potential association.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42023445888

KEYWORDS

breast cancer, hepatitis C virus, cumulative analysis, risk, prevalence

Abbreviations: CI, Confidence interval; HCV, hepatitis C virus; HR, Hazard ratio; NOS, Newcastle–Ottawa Scale.

Introduction

According to the Cancer Statistics 2023 (1), breast cancer is still the most common malignancy among women, accounting for 31% of new diagnoses of female cancers. The estimated new cases of breast cancer are predicted at 297,790 which is more than two-fold of new diagnoses of lung and bronchus cancers (120,790 cases). Besides, breast cancer contributes the second greatest number of deaths in women, accounting for 15% of estimated deaths (43,170 cases) (1). There are projected to be more than 3 million new cases of breast cancer every year by 2040, as well as more than 1 million deaths per year from the disease (2). The frequency and the death of breast cancer are various in different races. It is reported that black women are 4% less likely to develop breast cancer than white women, but the mortality in black women is 40% higher than in white women (1). The tumorigenesis of breast cancer may be influenced by a variety of risk factors, these include, but are not limited to age, family history and hereditary factors, early menarche and late menopause, delayed or nulliparous fertility, long-term hormone replacement therapy, mammary gland hyperplasia and mammary duct ectasia, and environmental and lifestyle factors (3–6). With the progress of research, more and more risk factors have been identified for the development of breast cancer, such as the concomitant diseases, i.e., depression (7), meningioma (8), and endometriosis (9).

According to the current evidence, virus infection is significantly associated with the development of multiple malignancies, e.g. hepatitis virus and hepatocellular carcinoma (HCC), Epstein-Barr virus and nasopharyngeal carcinoma, human papillomavirus and cervical cancer, human T-lymphotropic virus 1 and T-cell lymphoma, and human cytomegalovirus (HCMV) and glioblastoma (10, 11). Interestingly, both Epstein-Barr virus and human papillomavirus infections are the risk factor for breast cancer (12, 13). Type C viral hepatitis is one of the common viral-mediated infectious diseases deriving from the liver. Mounting studies have implied that chronic hepatitis C virus (HCV) infection may cause the tumorigenesis of HCC as well as the extrahepatic malignancies (i.e., gastrointestinal cancers, lymphoma, lung cancer, urologic malignancies, and gynecologic cancers) on the account of the persistent inflammation induced by HCV infection (14–19).

In recent years, there has been an increasing amount of attention paid to the potential association between HCV and the risk of breast cancer (20). A nationwide cohort developed by CHENG et al. (21) demonstrated that untreated HCV infection (hazard ratio [HR]= 1.701: 95% CI: 1.205-2.4) was associated with the incidence of breast cancer. The authors further observed that a higher risk of breast cancer was detected in those patients who were <49 years (HR= 2.193: 95% CI: 1.097-4.384) (21). However, several related studies did not support such a positive relationship between HCV and breast cancer. Swart A et al. (22) showed that the number of breast cancer in the HCV-positive cohort was comparable to that of the control group. In line with Swart A's findings, a few relevant studies also failed to find a significant association between HCV infection and the high risk of breast cancer (23, 24). These studies were designed to investigate the prevalence of breast cancer in patients with HCV. On the contrary, Liu et al. (25) conducted a study that investigated the prevalence of HCV in breast cancer patients. The results showed that the prevalence of HCV in patients with breast cancer was not significantly higher than that of the cancer-free inpatients (25).

Based on the above evidence, the association between chronic HCV infection and the risk of breast cancer is still controversial. Therefore, a systematic review and meta-analysis of the current evidence is urgently needed to evaluate this potential link between HCV-infected persons and the development of breast cancer. In this study, we presented a first attempt to capture and clarify this unrevealed clinical issue via a cumulative analysis.

Methods

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were followed for the present systematic review and cumulative analysis. Supplementary Table 1 listed the PRISMA checklist. In addition, this study also registered with the PROSPERO (ID: CRD42023445888). More details of the methodology of this cumulative study could be found in PROSPERO. The following literature search, study selection, inclusion/exclusion criteria, and data extraction were conducted by two authors independently. Any ambiguities could be resolved by a third author.

Data Sources and search strategy

Four commonly used electronic databases, i.e., MEDLINE (PubMed), the EMBASE, Cochrane Library, and PsychINFO databases, were systematically retrieved to identify the qualified studies. Those potential studies covered the period between the inception of the four databases and May 1, 2023. This review only included English-language studies. Based on searches in the MEDLINE database, the following terms were used in combinations: ((((((((((((((((((((((((((((((((((((("Breast Neoplasms"[Mesh]) OR (Breast Neoplasm)) OR (Neoplasm, Breast)) OR (Breast Tumors)) OR (Breast Tumor)) OR (Tumor, Breast)) OR (Tumors, Breast)) OR (Neoplasms, Breast)) OR (Breast Cancer)) OR (Cancer, Breast)) OR (Mammary Cancer)) OR (Cancer, Mammary)) OR (Cancers, Mammary)) OR (Mammary Cancers)) OR (Malignant Neoplasm of Breast)) OR (Breast Malignant Neoplasm)) OR (Breast Malignant Neoplasms)) OR (Malignant Tumor of Breast)) OR (Breast Malignant Tumor)) OR (Breast Malignant Tumors)) OR (Cancer of Breast)) OR (Cancer of the Breast)) OR (Mammary Carcinoma, Human)) OR (Carcinoma, Human Mammary)) OR (Carcinomas, Human Mammary)) OR (Human Mammary Carcinomas)) OR (Mammary Carcinomas, Human)) OR (Human Mammary Carcinoma)) OR (Mammary Neoplasms, Human)) OR (Human Mammary Neoplasm)) OR (Human Mammary Neoplasms)) OR (Neoplasm, Human Mammary)) OR (Neoplasms, Human Mammary)) OR (Mammary Neoplasm, Human)) OR (Breast Carcinoma)) OR

(Breast Carcinomas)) OR (Carcinoma, Breast)) OR (Carcinomas, Breast)) AND (((((“Hepacivirus”[Mesh]) OR (Hepatitis C virus)) OR (Hepatitis C viruses)) OR (HCV)) OR (Hepatitis C)). A manual search of the reference lists was also conducted to identify further eligible studies. The features of the included studies were displayed in Table 1, presenting the characteristics of the included studies.

Assessments of HCV and breast cancer

HCV infection and breast cancer were confirmed and classified by using the International Classification of Diseases (ICD) codes and standards of the World Health Organization (WHO). The diagnosis of HCV infection was validated based on the presence of anti-HCV seropositivity for at least 6 months or liver histology. The diagnosis of breast cancer was confirmed by histopathology, clinical expression, and mammography.

Inclusion criteria

Any studies reporting the association between HCV infection and breast cancer were considered to be eligible, reporting either the prevalence of breast cancer in HCV patients or the prevalence of HCV in breast cancer patients. In addition, those studies providing a hazard ratio (HR), odds ratios (OR), or relative risk (RR) with the 95% confidence intervals (CI) that reported the relationship between HCV and breast cancer were also considered to be eligible. The scientific question for guiding this study was: Is there a positive association between HCV infection and breast cancer? The inclusion criteria for this study followed the PICOS standard: Patient (HCV infection patients with breast cancer or breast cancer with HCV), Intervention (diagnosis of breast cancer or HCV), Comparison (compared with the control subjects: either healthy population without HCV infection or those diagnosed with benign breast diseases), Outcome (the prevalence of breast cancer or HCV infection), and Study design (any study designs).

Exclusion criteria

The exclusion criteria used in this study were: (a) the types of study belonged to review, comments, or case reports; (b) duplicated data derived from the same samples or the same scientific question; (c) non-human experimental studies; (d) since the present study is designed for evaluating whether HCV is an independent risk factor for breast cancer, thus those study samples presented with co-infected with both HBV and HCV being removed.

Data extraction

To extract the essential data from each included study, we designed a data collection form. The following items were extracted, including the first authors’ names, publication year, country/region, study design, mean age of the participants, the number of breast cancer cases in the HCV group and the non-HCV group or HCV cases in the breast cancer group and the non-cancer group, HR with its 95%CI, and the variable adjustments.

Quality assessment

Newcastle-Ottawa Scale (NOS) was applied to assess the methodological quality of cohort studies or case-control studies. The NOS checklist includes nine items, in which gains scores of 0–3, 4–6, and 7–9 represent low quality, moderate quality, and high quality, respectively.

TABLE 1 Characteristics of the five included studies.

Study	Study area	Study design	Mean age (years)	Study group case/ total	Control group case/ total	HR with 95%CI	Variable adjustment
Larrey (13) 2010	France	Case–control	21-84	17/294	5/107	1.24 (0.47-3.27)	NA
Su (12) 2011	Chinese Taipei	Cohort	A broad age	56/234	1760/8862	1.21 (0.96-1.52)	Age, residential area, occupation, urbanization, and income
Hwang (14) 2014	USA	Cohort	51.5 ± 15.95	3/35	105/2295	1.87 (0.62-5.62)	HIV, injection drug use, hemodialysis, hemophilia, and other liver conditions
CHENG-1 (15) 2022	Chinese Taipei	Cohort	A broad age	NA/14584	NA/14584	1.701 (1.205-2.4)	Liver cirrhosis, COPD, ESRD, DM, hypertension, dyslipidemia, cardiovascular events, and stroke
CHENG-2 (15) 2022	Chinese Taipei	Cohort	< 49 years	NA/18230	NA/14584	2.193 (1.097-4.384)	Liver cirrhosis, COPD, ESRD, DM, hypertension, dyslipidemia, cardiovascular events, and stroke
Loosen (15) 2022	Germany	Cohort	48.4 ± 19.2	NA/7667	NA/15706	1.04 (0.6-1.81)	Age, diabetes, obesity

S, Study group: patients with HBV or HCV infection; C, Control group; the healthy general population without HBV/HCV infection; NA, Not available; GC, Gastric cancer; HR, Hazard ratio; CI, Confidence interval; COPD, Chronic obstructive pulmonary disease; ESRD, End-stage renal disease; DM, Diabetes mellitus.

Statistical methodology

In order to conduct this cumulative analysis, STATA version 13.0 for Windows (Stata Corp LP, College Station, USA) was used. A quantitative assessment of the strength of the association between HCV infection and breast cancer was conducted by combining the overall HRs with 95% CIs for all the included studies. A two-tailed *P* value of 0.05 was assumed to indicate statistical significance. Statistical tests for heterogeneity were conducted using I^2 statistics and Cochrane *Q* statistics. Heterogeneity was considered substantial (statistical significance) when $I^2 > 50\%$ or the *P*-value of the *Q* test < 0.10. Rather than a fixed-effects model, a random-effects model was applied in this study due to a high probability of variability in study design and demographic characteristics. To further identify the potential sources of heterogeneity between studies, sensitivity analyses were conducted. For an evaluation of publication bias, the funnel plot, Begg's rank-correlation test, and Egger's regression asymmetry test were conducted.

Results

Literature search

A flow chart of the selection process for identifying the eligible articles could be found in Figure 1. During the initial search of the four databases, 705 articles were detected. After validating the duplicates and those studies did not examine the targeted research question, non-clinical studies, review articles, comments, and case reports, 637 publications were removed and the remaining 68 potential articles were retrieved for the full-text review. Among the remaining studies, 63 publications were eliminated due to lacking a control group, failure to meet the inclusion criteria, inappropriate grouping, and insufficient outcome data. Finally, five studies (21, 26–29) were included in this cumulative analysis. Of note, CHENG et al.'s study (21) provided additional data related to the young age of the patients, which was set as CHENG-1 and CHENG-2.

Study characteristic

The publication date of the eight included studies ranged from 2010 to 2022. The age of the participants ranged from 21 to 84 years. In the aspect of geographical area, three, three, and two studies were conducted in Asia, Europe, and Africa, respectively. The study design of the eight included studies was either cohort or case-control. The sample size ranged from 158 to 32,814, with a total of 68,014 participants. The variable adjustments in the included studies included age, residential area, occupation, urbanization, income, human immunodeficiency virus (HIV) infection, injection drug use, hemodialysis, hemophilia, chronic obstructive pulmonary disease (COPD), end-stage renal disease (ESRD), diabetes mellitus (DM), hypertension, dyslipidemia, cardiovascular events, stroke, and obesity. The characteristics and the HR with 95%CI of the eight included studies were summarized in Table 1.

Study quality

According to the scoring criteria of the NOS, three of the included studies were judged to be of high quality and the remaining two included studies were of moderate quality. In all, 60% (3/5) of the included studies were considered to have high methodological quality. Supplementary Table 2 provided a detailed scoring of the study quality.

Cumulative analysis

As shown in Figure 2, the synthetic effect from five included studies showed that a significantly higher prevalence of breast cancer was observed in patients with HCV infection than those with negative anti-HCV tests (pooled HR = 1.382, 95%CI: 1.129 to 1.692, *P*=0.002) by conducting a random-effects model. There was no evidence of statistical heterogeneity during this combined analysis (I^2 = 13.2%, *P*=0.33). These results suggested that the association between HCV infection and the risk of breast cancer was explicit.

Sensitivity analysis

In order to determine how an individual study influenced a newly calculated overall HR, a sensitivity analysis was conducted. As shown in Table 2 and Figure 3, the positive association between HCV infection and risk of breast cancer was consistent after removing any one of the included studies. The new HR ranged from 1.207 (95%CI: 0.96 to 1.455, *P*<0.001) to 1.422 (95%CI: 1.032 to 1.812, *P*<0.001). Besides, there was no substantial change in the heterogeneity test after eliminating anyone from the study (I^2 ranged from 0.0% to 4.4%, all $P > 0.1$). Based on these results, it appeared that no single study dominated the pooled HR and heterogeneity among studies.

Publication bias

As shown in Figure 4, both the Begg's and Egger's tests demonstrated that there was no significant publication bias was observed among the included studies (Begg's, $P > |z| = 0.707$; Egger, $P > |t| = 0.471$, 95%CI: -1.801 to 3.247).

Discussion

According to the available published data, several studies have assessed the association between HCV infection and the development of breast cancer. However, the relevant studies presented with the inconsistent results on this relationship. In this

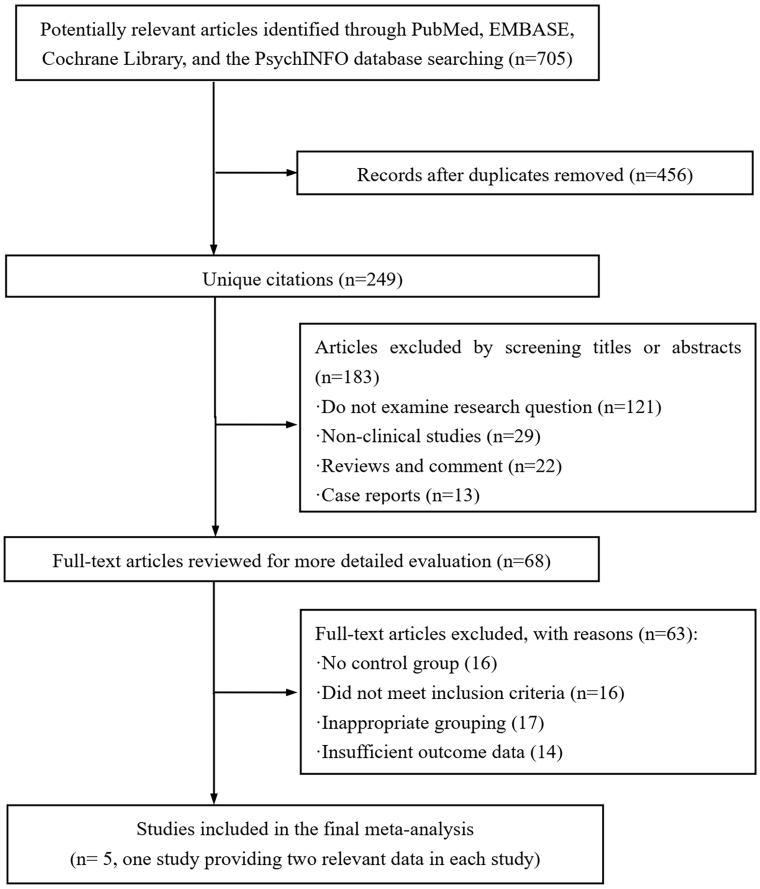

FIGURE 1
Flow chart of study selection.

study, we firstly to clarify this conspicuous issue by quantifying the HR from each related study through a meta-analysis. Based on the combined HR from the five included studies reporting the prevalence of breast cancer in HCV-infected patients, the results revealed that that anti-HCV positive patients were at 1.38-fold higher risk of the development of breast cancer than the healthy population without HCV infection with a statistical significance (synthetic HR= 1.38, 95%CI: 1.129 to 1.692, *P*=0.002). No substantial heterogeneity was identified in this pooled analysis. Subsequent sensitivity analysis and variable adjustments also

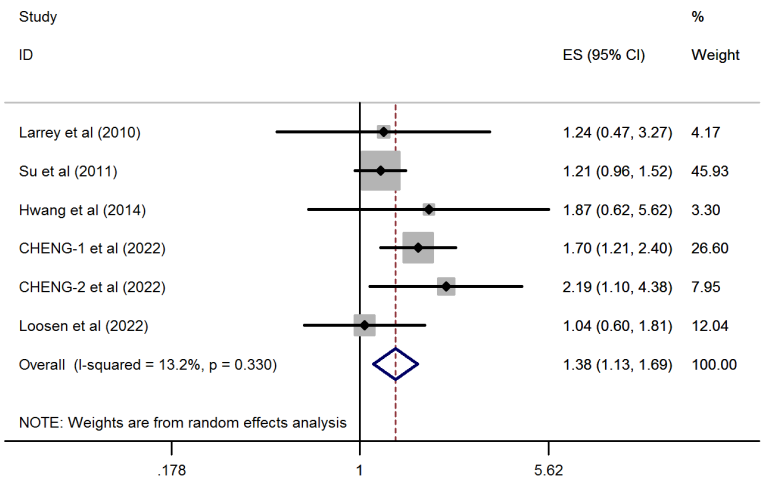

FIGURE 2
Forest plots of the pooled analysis of the included studies reporting the prevalence of breast cancer in patients with chronic HCV infection.

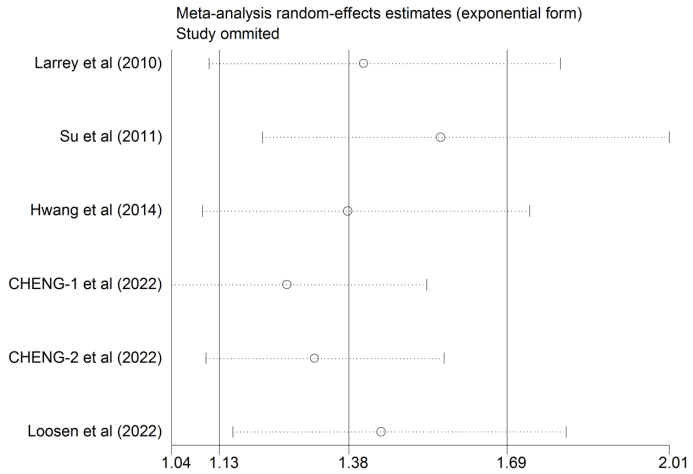

FIGURE 3
Sensitivity analysis after each study was excluded by turns.

confirmed this finding. Based on the above evidence, a positive association between HCV infection and breast cancer development was detected.

Since there was a positive association between HCV infection and the risk of breast cancer, the potential pathophysiological mechanisms of HCV-induced breast cancer should be noted. For example, anti-HCV positivity was found to be correlated to the development of secondary breast cancer (30). Hussein et al. reported that the prevalence of HCV seropositivity was 6-fold greater in women with breast cancer (<45 years) than in adults of the same age without breast cancer diagnoses (31). Similar to Hussein et al.'s findings, a previous case-control study (27) also suggested that HCV-positive women who age <50 years had a 2-fold greater risk of developing breast cancer than the HCV-negative women with comparable age (OR = 2.03, 95%CI = 1.23 to 3.34). Therefore, it is imperative to uncover the pathomechanisms of the HCV-mediated breast cancer. According to the current evidence, the underlying mechanisms that existed in this potential relationship might be associated with multiple etiologies, including persistent infection/inflammation, host immune response, and the modulation of HCV-associated gene expression (27, 30).

The HCV infection promoted and maintained chronic inflammation in the infected sites, mainly in the liver but also in some organs and tissues other than the liver (32). This is due to viral antigens and genomes that have been detected in extra-hepatic tissues (33). Persistent inflammation induced by HCV infection causes the cancerous transformation of the extra-hepatic organs, which may be correlated to the response to a progressive reorganization of their structure (33). On the other hand, chronic inflammation may cause the genetic instability and arise genetic and epigenetic alterations in cells, resulting in carcinogenesis. It was reported that HCV infection could cause lymphoproliferation by inducing cytokine production (34), while aberrant lymphoproliferation had the potential to transfer as the tumor infiltrating lymphocytes (TILs) (35). TILs are the recognized oncogenic factors for the development and progression of breast cancer (36). Therefore, HCV-mediated inflammatory cytokines may induce an indirect carcinogen for breast cancer.

It was suggested that HCV could maintain persistent infection through immune evasion mechanisms (37). Thus, systemic impairment of immune function induced by HCV might also play role in the tumorigenesis of breast cancer. As reported, HCV-associated antigens, genome or replicative sequences were

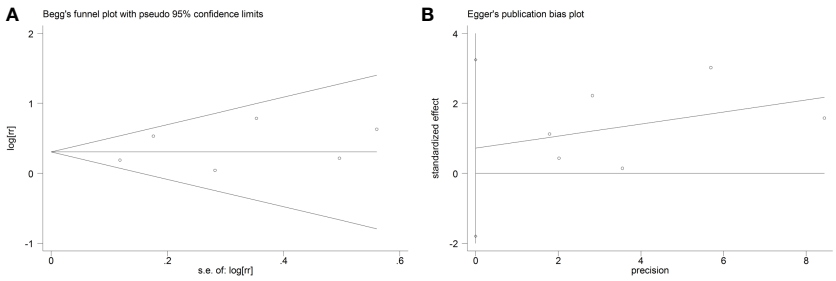

FIGURE 4
Publication bias analyses. **(A)** Begg's test; **(B)** Egger's test.

TABLE 2 Sensitivity analysis in the five included studies reporting HCV and risk of breast cancer.

Study omitted	RR (95% CI) for remainders	Heterogeneity	
		I^2	*P*
Larrey et al. (2010)	1.291 (1.039, 1.542) *P*<0.001	4.4%	0.382
Su et al. (2011)	1.422 (1.032, 1.812) *P*<0.001	0.0%	0.492
Hwang et al. (2014)	1.275 (1.045, 1.504) *P*<0.001	0.0%	0.41
CHENG-1 et al. (2022)	1.207 (0.96, 1.455) *P*<0.001	0.0%	0.745
CHENG-2 et al. (2022)	1.262 (1.031, 1.493) *P*<0.001	0.0%	0.561
Loosen et al. (2022)	1.32 (1.073, 1.567) *P*<0.001	0.0%	0.48

HR, hazard ratio; CI, confidence interval.

detected in T- and B-lymphocytes (38), indicating HCV might involve in the host immune response. Chronic HCV infection may induce immunocompromised status on account of the neutrophil or T-cell dysfunction (39). As a result of chronic antigenic stimulation by HCV, B lymphocytes expand clonally, producing monoclonal and polyclonal antibodies and producing immune complexes (40). Mounting evidence suggests that HCV not only plays an oncogenetic role in cancer development but is also involves in immunity and autoimmunity disorders (41). There are pathological, biochemical, and immunological abnormalities associated with HCV, which indicate its potential tumorigenicity (42).

Modulation of HCV-associated gene expression might also play role in HCV-mediated breast cancer. Attallah et al. (43) suggested that HCV infection in breast cancer patients was correlated to high levels of serum fibronectin and circulating HCV-NS4 expressions. HCV was also found to inactivate the cancer suppressor proteins, such as retinoblastoma proteins (Rb) and p53, affecting cell cycle, cell viability, and genome stability (44). HCV nonstructural proteins causing breast cancer progression might be associated with the downregulation of Rb (43). Moreover, HCV nonstructural proteins could form a complex with Rb, resulting in the reduction of Rb and ultimately induced cancer cell proliferation (45). It was reported that HCV could encode several viral proteins, i.e., Core- and NS5A, thus interacting with intracellular cascades pathways and functioning in the oncogenesis of breast cancer (33). In addition to the above potential pathophysiological mechanisms, metabolic alterations subsequent to HCV infection, might also play roles in the induction of breast cancer (21, 46). As reported, several HCV-mediated metabolic events could not be reversed, even after viral clearance (46). The metabolic factors might involve in the development of HCV-associated breast cancer.

For the first time, we tried to clarify the controversial clinical findings with the topic: "Is HCV infection a risk factor for breast cancer?". We found that patients with HCV infection had a significantly higher prevalence of breast cancer than non-HCV healthy controls. The carcinogenic effects on breast cancer development induced by HCV infection might be associated with the persistent infection/inflammation, immune escape, and the modulation of HCV-associated gene expression. However, HCV might be not a strong promoter of breast cancer due to only 1.38-fold higher risk was detected. The causal association between HCV infection and breast cancer remains further investigation due to the results were derived from limited included studies. Besides, the study sample and study design varied across the included studies, which might interfere the exact association between HCV infection and breast cancer. Since a positive association between HCV infection and risk of breast cancer is detected, patients with or without treatment for HCV might affect the risky of breast cancer development. Among the five included studies, only one study (Larrey et al.) (26) reported the relationship between past or ongoing treatment of HCV (70%) or never treated HCV (30%) and the risk of breast cancer. However, Larrey et al.'s study did not show the independent prevalence of breast cancer in patients with past/ongoing treatment of HCV or never treated HCV. Therefore, we could not judge what was the difference on the strength of the association between HCV infection and risk of breast cancer in the two groups. Chronic HCV is currently treatable with several direct-acting antivirals (DAAs) that can target various HCV genotypes, stages of liver disease, and comorbidities (47). At present, DAAs and interferon-free and ribavirin-free regimens are used for the treatment of HCV infection. Since HCV infection may increase the risk of breast cancer, it is speculated that patients with HCV antiviral therapies may have a low risk of breast cancer than those without HCV treatment. This hypothesis was evidenced by several studies demonstrated that antiviral therapies for HCV might improve the outcomes of HCV-associated extrahepatic diseases, such as cardiovascular risk profile (48) and renal function (49). Of note, however, a case series study (50) demonstrated that a possible relationship between treatment with DAAs and development of extrahepatic malignancies, including breast cancer. Therefore, further studies are needed to explore whether the specific treatments for HCV will increase or reduce the risk of breast cancer.

Conclusion

In summary, the present cumulative study demonstrated that patients with HCV infection were at a 1.38-fold higher risk of the development of breast cancer than the healthy population with a statistical significance. However, it should be acknowledged that the direction of causality between HCV infection and risk of breast cancer was not so clear due to limited studies were included and all of them had a retrospective design. Therefore, future prospective, well-designed cohorts with large samples and strict inclusion criteria are still warranted to better validate the relationship between HCV infection and breast cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

HC: Data curation, Investigation, Writing – original draft. PD: Formal Analysis, Writing – review & editing. XX: Data curation, Writing – original draft. TC: Methodology, Writing – review & editing. YD: Writing – original draft, Writing – review & editing. TY: Supervision, Writing – review & editing.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the grants from the Science and Technology Planning Project of Guangzhou (No. 201904010401) and the Science and Technology Project of Panyu District, Guangzhou (No. 2020-Z04-006).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1274340/full#supplementary-material

SUPPLEMENTARY TABLE 1
PRISMA Checklist of 2020.

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 17 October 2023
DOI 10.3389/fonc.2023.1284926

OPEN ACCESS

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RECEIVED 29 August 2023
ACCEPTED 04 October 2023
PUBLISHED 17 October 2023

CITATION
Xu R, Yin P, Wei J and Ding Q (2023) The role of matrix stiffness in breast cancer progression: a review.
Front. Oncol. 13:1284926.
doi: 10.3389/fonc.2023.1284926

The role of matrix stiffness in breast cancer progression: a review

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The significance of matrix stiffness in cancer development has been investigated in recent years. The gradual elastic force the extracellular matrix imparts to cells, known as matrix stiffness, is one of the most important types of mechanical stimulation. Increased matrix stiffness alters the biological activity of cells, which promotes the growth of numerous malignancies, including breast cancer. Comprehensive studies have demonstrated that increasing matrix stiffness activates molecular signaling pathways that are closely linked to breast cancer progression. There are many articles exploring the relationship between mechanism hardness and breast cancer, so we wanted to provide a systematic summary of recent research advances. In this review, we briefly introduce the mechanism of matrix stiffness in breast cancer, elaborate on the effect of extracellular matrix stiffness on breast cancer biological behavior and signaling pathways, and finally, we will talk about breast cancer treatment that focuses on matrix stiffness.

KEYWORDS

matrix stiffness, mechanical stimulation, extracellular matrix, breast cancer, signaling pathways

1 Introduction

Breast cancer is one of the most common malignancies among women. One of the most obvious signs of breast cancer is tissue hardening, which can be detected by palpating malignant nodules (1, 2). The stiffness of normal healthy breast tissue is approximately 0.2 kPa, while that of breast cancer tissue is over 4 kPa (3). In addition to the increased stiffness of breast cancer tissues, adjacent matrix tissues are also affected. A study in an animal model demonstrated that when breast tissue become invasive, the adjacent matrix tissue is also much stiffer than the distant normal tissue (1). These phenomena arise from abnormal changes in the structure and composition of the tumor extracellular matrix (ECM) in breast cancer (2).

The ECM is an intricate network of three-dimensional macromolecules consisting mainly of collagen, non-collagen, elastin, proteoglycans and glycosaminoglycans (4); it offers appropriate chemical signals and mechanical stimulation to regulate cell shape,

metabolism, function, migration, proliferation, and differentiation (4, 5). Mechanical stimulation involves compression, matrix stiffness, and hydrodynamics (6). Moreover, matrix stiffness, also known as rigidity or modulus of elasticity, is defined as the resistance of a material to deformation by a force applied at a very slow rate (quasi-static) (7). Stiffness is an intrinsic material property of tissues, and increased tissue hardness is the most obvious and recognized mechanical abnormality in tumors, including breast cancer (8).

With the application of atomic force microscopy (AFM) and the continuous refinement of 3D culture techniques, the study of matrix stiffness has become more feasible (9, 10). Hydrogels are good candidates in the study of ECM physical properties using 3D modeling (11). Various types of hydrogels have been used in the study of matrix stiffness, including polyacrylamide hydrogels, hyaluronic acid hydrogels, collagen hydrogels, gelatin hydrogels, etc. (12). Based on the fact that the matrix stiffness is a constant state of transformation with the dynamics of the ECM, more advanced stimuli-responsive hydrogels were synthesized (13). Stimuli-responsive hydrogels can adjust their stiffness in response to external physical or chemical stimuli to better mimic the *in vivo* environment in matrix stiffness studies (11, 13). In addition, to better approach the treatment of breast cancer from the aspect of stromal stiffness, various 3D experimental models have been developed, mainly including cancer cell lines, 3D spheroids, *in vivo* patient-derived xenografts (PDX), and *in vitro* patient-derived organoids (PDO) (14–17). For example, PDOs have been established from breast cancer, and this model can be used to predict drug response in cancer patients, which in turn informs the patient's treatment regimen (17). 3D spheroids also have extensive use in exploring the role of matrix stiffness in breast cancer invasion (18).

The formation of tumors mainly depends on the balance between increased matrix stiffness and matrix degradation (19). Collagen accumulation and pathological collagen cross-linking are the major causes of increased ECM stiffness in breast cancer (7). Analysis of human breast tissue samples has revealed that the transition from non-malignant tissue to invasive ductal carcinoma (IDC) corresponds to significant collagen deposition, resulting in stromal stiffening (20). In addition, computational analysis of mammographic images has shown that dense breast tissue has a stiffer matrix, contains more linearized and bound collagen, and is associated with a higher risk of breast cancer (21, 22). Degradation of breast cancer matrix is mainly dependent on the regulation of lysyl oxidase (LOX), lysyl oxidase like-1-4 (LOXL 1-4), and matrix metalloproteinases (MMPs), which are extracellular matrix remodeling enzymes (23, 24). LOX promotes the cross-linking of elastin and collagen in the ECM and prevents collagen degradation, which promotes breast cancer progression (25). Furthermore, MMPs remodel the ECM by degrading ECM proteins, which in turn promote breast cancer metastasis (26). Thus, an excessively stiff matrix or excessive matrix degradation can promote the progression of breast cancer.

Matrix stiffness is closely related to malignant breast cancer phenotypes, including proliferation, metastasis, invasion, and drug resistance. There are many articles exploring the relationship between matrix stiffness and breast cancer, so we want to provide a systematic summary of recent research advances. In this review, we systematically introduce the major causes of breast stiffening and summarize the role of matrix stiffness in breast cancer initiation and progression and its potential applications. This may provide clues for studying matrix stiffness in breast cancer and exploring its clinical applications in breast cancer treatment.

2 Formation of matrix stiffness in breast cancer

The stiffness of cancer tissue is mainly determined by cancer and stromal cells (27). Matrix deposition and cross-linking are the two major causes of breast cancer stiffening (28, 29) (Figure 1). Cancer cells and stromal cells are jointly involved in matrix deposition and cross-linking and determine matrix stiffness (27).

2.1 Matrix deposition

Among all stromal cells, cancer-associated fibroblasts (CAFs) are the most efficient in depositing and remodeling the ECM in the tumor microenvironment (30, 31). Stromal cells with high alpha-smooth muscle actin (aSMA) expression are known as cancer-associated fibroblasts (CAFs) (32). Through Notch signaling, interaction between cancer cells and fibroblasts can advance the CAF phenotype in breast cancer (30). Cancer cells can also promote the transformation of fibroblasts into CAFs by secreting TGFβ, which in turn further promotes tumor progression through ECM remodeling (33, 34). During breast cancer progression, up to 80% of stromal cells acquire the CAF phenotype (35). CAFs synthesize and secrete collagen procollagen molecules, which are processed and arranged to form collagen fibers. As fibrillar collagen (both type I and type III) is progressively deposited in the ECM, the normal ECM gradually transforms into dense fibrous tumor stroma (36, 37). Except for CAFs, other stromal cells play an important role in causing increased matrix stiffness, including macrophages. Macrophages secrete a variety of soluble factors that induce ECM deposition, thereby stiffening the extracellular matrix (20). In addition, during breast cancer progression, breast cancer epithelial cells gradually lose epithelial markers to acquire mesenchymal markers and mesenchymal cell-like properties through epithelial-mesenchymal transition (EMT), which in turn exerts a function like that of CAFs, synthesizing and secreting collagen, leading to stromal deposition promoting an increase in stromal stiffness (38). In summary, both stromal cells and breast cancer cells undergoing EMT can promote increased matrix stiffness through matrix deposition.

2.2 Matrix cross-linking

CAFs and cancer cells highly express LOX/LOXs (39), which are amine oxidases that mainly regulate covalent cross-linking between ECM collagen and elastin (40, 41). In breast cancer, LOX and collagen

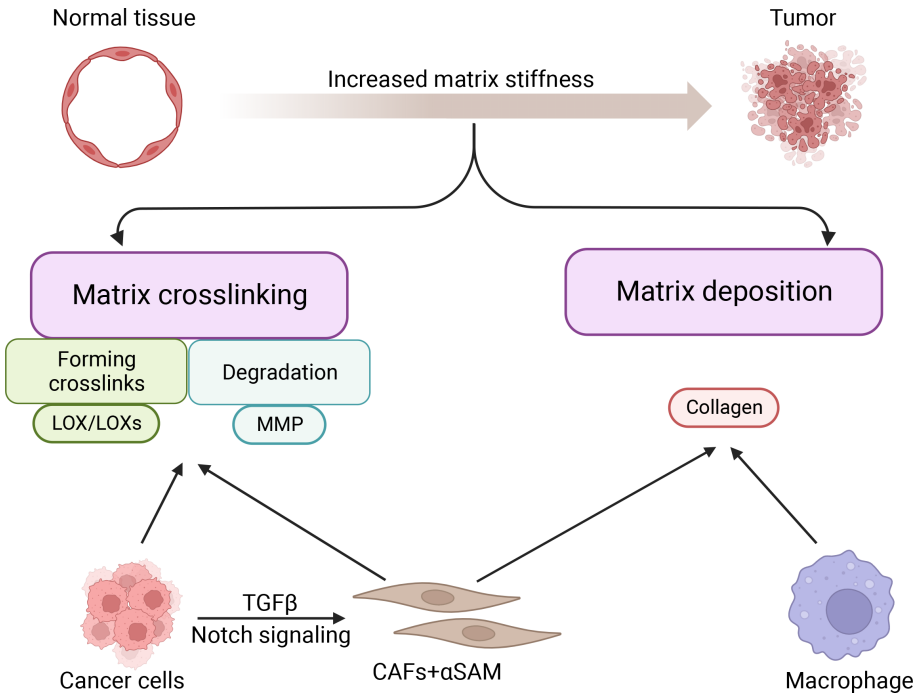

FIGURE 1
Formation of matrix stiffness in breast cancer.

influence the architecture of the ECM and create a favorable microenvironment for tumor development and progression (42).

LOX was reported to promote fibrosis of breast tissue through collagen cross-linking, leading to increased matrix stiffness in breast tumors (43, 44). Increased matrix stiffness can induce the assembly of focal adhesions and up-regulate GFR-dependent PI3K signaling, ultimately leading to tumor progression (1). Furthermore, breast cancer cells and CAFs can synthesize and secrete proteolytically active MMPs (45), which can degrade almost all proteins in the ECM when metal ions are used as cofactors (46). However, when collagen is cross-linked, MMPs are unable to break down the collagen, thus increasing matrix stiffness (47). The phenomenon of collagen cross-linking leading to increased matrix stiffness in cancer cells is widespread. For instance, when highly expressed in pancreatic cancer cells, tissue transglutaminase (TG2) crosslinks proteins to stiffen the pancreatic tumor tissue (48). However, TG2 promotion of breast tumor matrix cross-linking has not been elucidated. In conclusion, matrix cross-linking is essential for enhancing the stiffness of cancer tissues (29).

3 Initiation and progression of breast cancer regulated by matrix stiffness

Increased matrix stiffness leads to breast malignancy and contributes to the malignant phenotypes of breast cancer by promoting breast cancer proliferation, metastasis, invasion, immune evasion, stemness, and drug resistance through the regulation of breast cancer and stromal cells (Figure 2). With the development of 3d culture technology, it has become possible to simulate different matrix stiffnesses using hydrogels, making it possible to study *in vitro* how matrix stiffness affects cell signaling pathways. Matrix stiffness affects tumor and non-tumor cells through multiple molecular signaling pathways in breast tumors that promote tumor progression (Table 1).

3.1 Tumorigenesis of breast cancer promoted by matrix stiffness

Mammary density (MD) is associated with an overall increased lifetime risk of malignancy. Increased mammary density is primarily caused by the deposition of fibrillar collagen (68). It has been shown to result in an increase in stromal stiffness which disrupts the physiologic breast morphogenesis (69, 70). Even a small increase in matrix stiffness results in activation of Rho GTPase and induces collagen matrix contraction to disrupt tissue structure. Rho GTPase also activates the ROCK pathway, which can lead to malignant changes in the breast (49). Collagen cross-linking leads to matrix stiffening promotes integrin aggregation, enhances PI3K activity, and induces oncogene-initiated invasion of epithelial cells (1).

Mammary epithelial cells in cultured soft matrix can grow into normal epithelial tubules; however, in hard matrix, they exhibit an abnormal tumor-like morphology (71). Study of epigenomic changes show that increased stromal stiffness leads to increased nuclear ruffling and lamellipodia-associated chromatin, ultimately inducing a tumor phenotype (72). In a mouse experiment, it was also found that increased matrix stiffness increased mammary tumorigenesis by about three times (73). So, increased matrix stiffness is inextricably linked to breast cancer initiation.

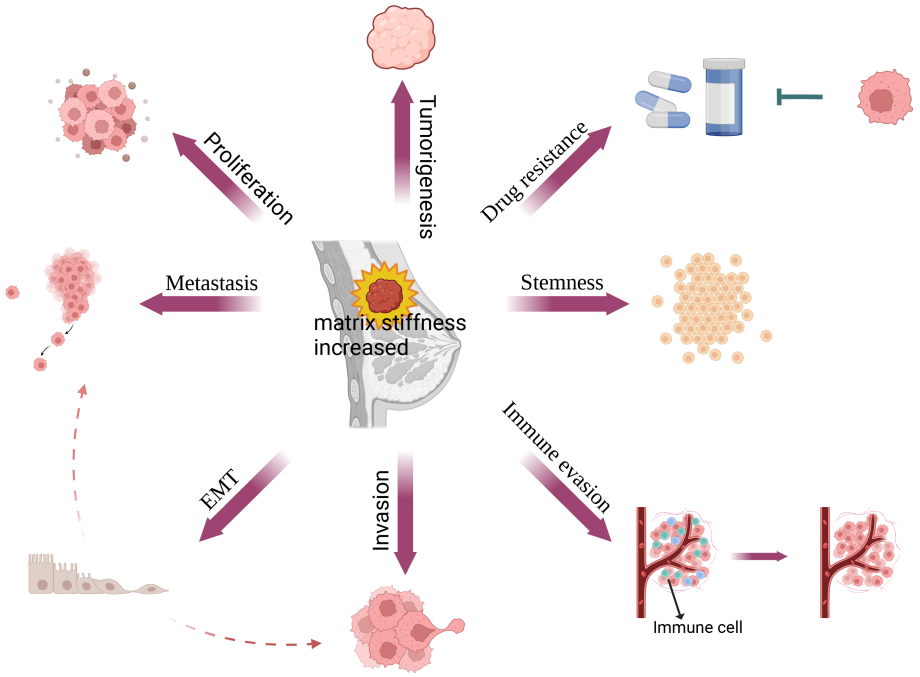

FIGURE 2
Relationship between matrix stiffness and breast cancer. In breast cancer, matrix stiffness can affect its proliferation, EMT, metastasis, invasion, immune evasion, stemness and drug resistance, thus further promoting fibrosis and the progression of breast cancer.

3.2 Proliferation of breast cancer cells regulated by matrix stiffness

The uncontrolled proliferation of cancer cells is one of the dominant features of cancer (74). Mesenchymal stem cells (MSC) in a stiff matrix can differentiate into cancer-associated fibroblasts (CAF) with increased expression of the yes-associated protein (YAP) (50). YAP, an important regulatory molecule in the Hippo pathway, is phosphorylated to enter the nucleus to transcribe anti-apoptotic and pro-proliferative genes, thereby regulating cell proliferation and apoptosis and controlling organ size (75–77). In the Wnt pathway, nuclear YAP can promote cell proliferation by up-regulating β-catenin expression (51, 52). In addition, an increase in mammary gland density is often accompanied by an increase in matrix stiffness. Regions with high breast density have increased stromal collagen and epithelial cell contents (78). When NMuMG mammary epithelial cells are cultured on a hard substrate, Wnt3a increases the integrin-linked kinases (ILK)-mediated Frizzled-1 expression and thus promotes epithelial cell proliferation through the integrin signaling pathway (53). Provenzano et al. simulated increased matrix stiffness by increasing the matrix collagen density. They found that matrix stiffness promoted the proliferation of breast cancer cells through FAK-Rho and FAK-Ras-ERK signaling networks (54). Similar conclusions have been reached in animal experiments. Injecting breast cancer cells cultured in a stiffer matrix into mice can form larger tumors (79). In conclusion, matrix stiffness drives breast cancer cell proliferation.

3.3 Invasion and metastasis of breast cancer cells regulated by matrix stiffness

Changes in matrix stiffness significantly affect the cytoskeletal structure and ability of breast cancer cells to invade and metastasize. By analyzing PAM50 tumor subtypes, Adam et al. found that compared to the less aggressive luminal A and normal-like subtypes, the more aggressive subtypes such as basal, human epidermal growth factor receptor 2 (HER2), and luminal B, had stiffer matrix and poorer overall survival. They suggested that increased matrix stiffness enhances breast cancer invasion (79). Mechanistically, integrins play important roles in this process (Figure 3). Integrin receptors activate insulin receptors (IR) by forming β1 and β3 integrins and IR complexes. IR activates the PI3K/AKT/mTORC1 signaling axis to promote breast cancer cell metastasis (55). Moreover, matrix stiffness can directly activate integrin β1 and focal adhesion kinase (FAK), which accelerates focal adhesion (FA) maturation and induces downstream cascades of intracellular signals in the RhoA/ROCK pathway. ROCK isoforms differentially regulate the RhoA/ROCK1/p-MLC and RhoA/ROCK2/p-cofilin pathways in a coordinated fashion to modulate breast cancer cell motility in a substrate stiffness-dependent manner through integrin β1-activated FAK signaling (58). Moreover, with the activation of EGFR and PLCγ1, the expression of Mena, a protein associated with metastasis in breast cancer, is up-regulated in a stiff matrix. High Mena expression further increases matrix stiffness by depositing fibronectin via α5 integrin (56, 57). In conclusion, integrins are important in promoting the invasive metastasis of breast cancer cells.

TABLE 1 List of Matrix Stiffness Affecting Breast Cancer.

Phenotype	Signaling Pathway	Effect on Cells	References
Tumorigenesis	ROCK signaling pathway	Rho GTPases activation	(49)
	Integrin signaling pathway	Integrin/PI3K activation, oncogene initiation	(1)
Proliferation	YAP signaling pathway	YAP/MLC upregulation, PASP secretion	(50)
	Wnt signaling pathway	β-catenin upregulation	(51, 52)
	Integrin signaling pathway	ILK-mediated Frizzled-1 upregulation	(53)
	FAK signaling pathway	FAK-Rho upregulation, Ras-MAPK activation	(54)
Invasion/ Metastasis	Integrin signaling pathway	Integrin/IR/PI3K/AKT/mTORC1 activation	(55)
		EGFR/PLCγ1 activation, Mena upregulation	(56, 57)
	ROCK signaling pathway	RhoA/ROCK1/p-MLC and RhoA/ROCK2/p-cofilin in a coordinate fashion to modulate breast cancer cell motility	(58)
	TWIST1 signaling pathway	Promoted EMT, TWIST1 nuclear transportation	(59, 60)
Stemness	Integrin signaling pathway	Integrin/ILK/PI3K/Akt activation	(61)
	\	TAZ/NANOG dissociate, SOX2 and OCT4 upregulation	(62)
	YAP signaling pathway	YAP nuclear translocation	(63)
Drug resistance	YAP signaling pathway	Promoted EMT, YAP nuclear transportation	(64)
		Merlin/MST/LATS inactivation, ILK/YAP upregulation	(8)
Immune evasion	\	PDL1 upregulation	(65, 66)
	\	Diminish T cells permeation and migration	(67)

The symbol (\) represents none.

One of the key processes that promotes the progression of metastasis in cancer cells is the epithelial-mesenchymal transition (EMT). EMT, the process by which epithelial cells lose polarity, intercellular adhesion, acquire migratory, and invasive properties to become mesenchymal cells, is thought to play a key role in initiating the metastatic cascade response. Thus, EMT allows cancer cells to leave the primary tumor, invade the surrounding ECM, enter the blood and lymphatic vessels, and spread to all body parts (80). When matrix stiffness increasing, cells in the matrix gradually develop an EMT phenotype, indicating that they are more likely to undergo invasive and metastatic spreading (81, 82).

TWIST1 is a basic helix-loop-helix (bHLH) transcription factor that promotes tumor metastasis by initiating EMT and degrading ECT (59, 60). Furthermore, the increase in matrix stiffness causes TWIST1 to move toward the nucleus, directly affecting the EMT program. G3BP2 is a TWIST1 binding protein and tyrosine residue Y103 is present in its binding sequence. In a soft matrix, there is a strong tendency for the two to interact; however, in a stiffened matrix, TWIST1 dissociates from G3BP2 and is transferred to the nucleus (81). Fattet et al. have shown that increased matrix stiffness activates extracellular signal-regulated kinases (ERK) and ribosomal S6 kinase1 (RSK1). Activated ERK/RSK1 phosphorylates the ephrin Receptor EPHA2 at serine 897 (S897). Moreover, phosphorylated EPHA2 activates LYN Kinase to form an EPHA2/LYN complex. This complex phosphorylates Y103 in the TWIST1-G3BP2 binding sequence, leading to TWIST1-G3BP2 dissociation (83). Additionally, it has been demonstrated that during matrix stiffness, activated integrins phosphorylate Y103 through tyrosine kinases, which eventually prevents TWIST1 from binding to G3BP2 (81). Additionally, a study found a positive correlation between TWIST1 expression and tumor stiffness in patients with breast cancer (84). Barriga et al. found that high tissue stiffness promoted EMT triggering neural crest migration, and this study in turn confirmed *in vivo* that higher matrix stiffness increased the propensity of cells to undergo EMT, leading to distant metastasis (85). Generally, increased matrix stiffness promotes breast cancer metastasis by activating the EMT through a mechanical conduction pathway.

In addition, there is a phenomenon in the process of cancer recurrence and metastasis, which is that breast cancer recurrence and metastasis are usually detected in tissues that are softer than normal breast or primary breast tumors (such as bone marrow, liver, brain, and lung) (86). Therefore, the soft microenvironment can promote the survival of disseminated breast cancer cells at the secondary site. When breast cancer cells were cultured on the soft matrix mimicking the site of metastasis, they were found to remain dormant for a long time to escape the killing effects of chemotherapy drugs. Soft matrix can also induce chemical resistance in breast cancer by increasing autophagy, making metastatic breast cancer more difficult to treat (87).

3.4 Stemness of breast cancer cells regulated by matrix stiffness

Cancer stem cells (CSC) are a small subpopulation of cancer cells that maintain their self-renewal and undifferentiated abilities. Breast cancer stem cells (BCSC) can self-renew, differentiate, drive

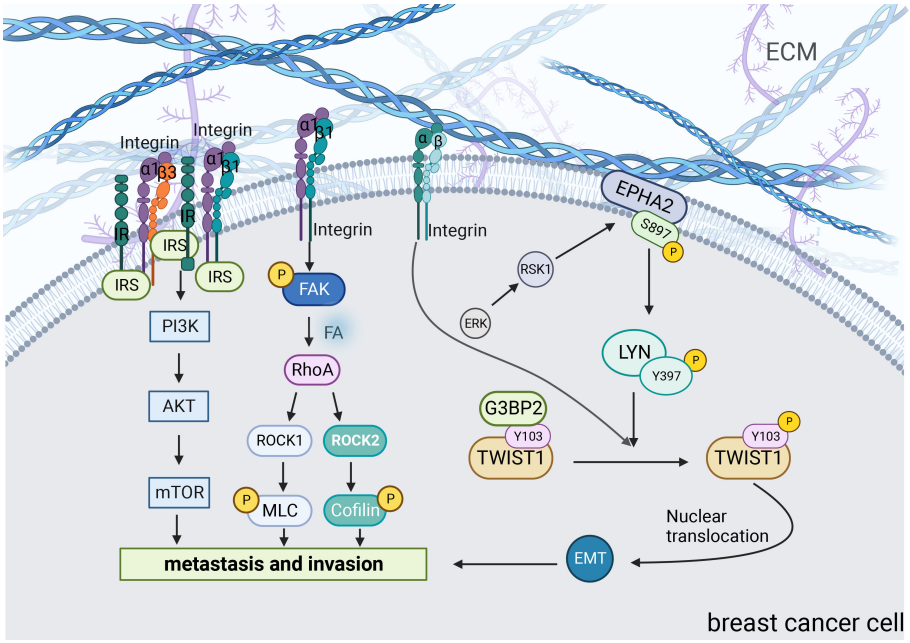

FIGURE 3
Signaling pathways associated with increased matrix stiffness leading to breast cancer invasion and metastasis. Matrix stiffness activates many mechanoreactive signaling pathways in cells through transmembrane proteins including integrins. Pathways such as PI3K, ROCK and TWIST1 play a major role in this transduction. Central players in these signaling pathways can connect with other molecules and ultimately translate changes in the ECM into relevant biological changes.

tumor progression, and mediate drug resistance and metastasis (88). Pang et al. investigated the relationship between matrix stiffness and BCSC by detecting CSC markers CD44, Nanog, and CD49f. They found that the expression of all these markers increased when the matrix stiffness increased. By comparing the expression of CD44 at different matrix stiffness values, they found that BCSCs were preferentially located in a stiff microenvironment (61).

BCSCs were mainly regulated by ILK. In the presence of increased matrix stiffness, ILK regulates BCSC development via the PI3K/Akt pathway and promotes angiogenesis in tumor cells, ultimately contributing to tumor metastatic spread (61). A recent study found that cells cultured on hard polyacrylamide hydrogels (9 kPa) had a significantly higher proportion of BCSCs compared to cells cultured on soft polyacrylamide hydrogels (0.5 kPa; matching the compliance of normal mammary glands). Exploration of the mechanism revealed that when matrix stiffness was increased, TAZ dissociated from NANOG, promoting the transcription of SOX2 and OCT4, which in turn increased the proportion of BCSCs in the breast cancer and promoted the stemness phenotype of breast cancer (62). In another study, by using three different hydrogels, Matrigel, collagen I, and fibrinogen gels, to simulate three different matrix compositions, collagen, laminin, and fibronectin, respectively, it was found that the increased matrix stiffness due to different matrix compositions had different effects on the stemness of breast cancer (89). Yan Li et al. cultured breast cancer cells using different stiffness of polyacrylamide hydrogels and found that increased matrix stiffness promotes YAP nuclear translocation, which in turn promotes BCSCs maintenance (63). Matrix stiffness may be important for the induction and maintenance of CSC; however, this requires further investigation.

3.5 Drug resistance of breast cancer cells regulated by matrix stiffness

Drug resistance is one of the most important factors affecting breast cancer treatment outcomes (90). Improving the sensitivity of breast cancer cells to chemotherapeutic agents is essential to improve the survival rate of patients with breast cancer. In addition, the ability to achieve effective drug concentrations at the tumor site is also essential for the treatment of cancer. Most chemotherapeutic agents are dose-dependent, and chemotherapeutic agents need to pass through the tumor vasculature system, cross the vessel wall to enter, and pass through the interstitial space of the tumor to reach the cancer cells to exert their therapeutic effects. However, when stromal stiffness increases, the extravascular hydrostatic pressure, or interstitial pressure (IFP), increases within the tumor, resulting in inhibited drug extravasation. On the other hand, increased stromal stiffness leads to vascular compression, resulting in inadequate perfusion within the tumor, further reducing drug concentration. More unfortunately, when stromal stiffness is increased, the dense ECM further impedes the effective diffusion of chemotherapeutic agents, ultimately making it difficult to achieve effective concentrations and reducing the efficacy of chemotherapeutic agents (91–93).

The responsiveness of primary breast cancer cells to chemotherapeutic agents is altered after they are removed from

the host microenvironment and transferred to hard-surface cultures *in vitro*. The activities of PTX and DOX were strongly correlated with matrix hardness. Substrates that are too hard can reduce the activities of PTX and DOX, leading to drug resistance (94). In addition, the activity of targeted drugs for breast cancer treatment can be influenced by stromal stiffness. Lapatinib is an orally administered small-molecule epidermal growth factor tyrosine kinase inhibitor. It is primarily used to treat HER2(human epidermal growth factor receptor-2)-amplified breast cancer. Furthermore, the ratio of HER2 phosphorylation decrease with increasing matrix stiffness and was negatively correlated with lapatinib insensitivity (95).

Sorafenib is a small-molecule tyrosine kinase inhibitor with anti-angiogenic activity that has been used to treat hepatocellular and renal cancers (96). Hepatocellular cancer cells on stiff substrates show resistance to sorafenib compared to those on soft substrates (97). The same phenomenon has been observed in breast cancer cells (98). Breast cancer cells cultured on harder substrates were more resistant to sorafenib (99).

Moreover, the EMT affects the sensitivity of breast cancer cells to chemotherapy. Notably, increased matrix stiffness promotes the nuclear translocation of YAP, triggering EMT and increasing drug resistance. However, only the MDA-MB-231 cell line showed drug resistance with increased simulated matrix stiffness during the experiment (64). This suggests that the effect of matrix stiffness on drug resistance is related to the cell line. Additionally, matrix stiffness can regulate YAP's translocation, dephosphorylation, and transcriptional activity by increasing ILK expression, ultimately leading to increased drug resistance in breast cancer cells (8). In summary, targeting matrix stiffness is a prospective strategy for improving the efficacy of chemotherapy.

In addition to chemotherapy, radiotherapy is also an important treatment for breast cancer. One study showed that low doses of radiation had no significant effect on tumor cell migration when matrix stiffness was increased, but when high doses of radiation were changed, tumor cell adhesion increased and migration rate decreased significantly. On soft substrates, low doses of radiation can reduce the migration rate of tumor cells. These results indicate that the radiosensitivity of tumors on hard substrates is dose dependent (100). But the results are not widely accepted. Rieken et al. suggested that radiation promotes tumor migration by inducing integrin overexpression (101). In conclusion, the mechanism of the influence of matrix stiffness on radiosensitivity is still unclear, and some conclusions are still controversial, which may be closely related to radiation dose, radiation time and cell types (93).

In addition, the targeted therapies of breast cancer could also be affected by matrix stiffness. Lapatinib is a targeted drug for the treatment of HER2-amplified breast cancer (102). Increased matrix stiffness leads to YAP overexpression, which in turn modulates the Hippo pathway and reduces the efficacy of lapatinib (95, 103). In conclusion, matrix stiffness has an impact on multiple treatments for breast cancer, including chemotherapy, radiotherapy and targeted therapy.

3.6 Immune evasion of breast cancer cells regulated by matrix stiffness

Immunotherapy is a novel modality for the treatment of breast cancer. However, breast cancer is considered a low-immune reactive cancer. The key to immunotherapy is the interaction between the programmed death-1 receptor (PD-1) and programmed death ligand 1 (PD-L1). Previous studies revealed a positive association between high PD-L1 expression and matrix stiffness. High PD-L1 expression in breast cancer is associated with poor prognosis (65, 66). On a physical level, when collagen crosslinks and matrix stiffness increases, T cells have difficulty penetrating the matrix and their ability to migrate in the matrix is greatly diminished, thus limiting the further role of T cells in the tumor (67). Therefore, reversing immune evasion in breast cancer remains a challenge.

4 Therapy for breast cancer by targeting matrix stiffness

As the study of matrix stiffness has intensified, new directions for breast cancer treatment have been provided. Matrix targeting in breast cancer can be broadly divided into two types:1) Reducing the source of matrix stiffness. 2) Blocking the effect of matrix stiffness on the downstream pathways (Table 2).

To reduce the source of matrix stiffness and collagen cross-linking, ECM enzymes, such as LOX/LOXLs, MMPs, and CAFs, can be used to directly block the excessive synthesis of certain ECM components. For example, 4-methylumbelliferone (MU) can significantly inhibit the synthesis and accumulation of hyaluronic acid (HA, a matrix component), which promotes tumor cell metastasis (110). β-Aminopropionitrile (BAPN) acts as a LOX inhibitor and suppresses breast cancer proliferation and metastasis by inhibiting collagen cross-linking (25, 104, 105). Tetrathiomolybdate (TM), a LOX inhibitor, belongs to a group of copper chelators that inhibit LOX activity by binding to and depleting copper. A phase IIa TM study is underway in breast cancer patients at an intermediate to high risk of recurrence (25).

Prinomastat is a selective oral matrix MMP -2, -9, -13 and -14 inhibitor. The drug has been shown to prevent angiogenesis and tumor development in a range of preclinical models, including those of colon, breast, lung, melanoma, and glioma (106). Growth factors, including TGF-β, PDGF, and VEGF, can also be used as targets to block the increase in matrix stiffness. Pirfenidone (PFD) is a potent TGF-β inhibitor approved for treating pulmonary and renal fibrosis (111). For example, Hamidreza et al. showed that PFD reduced breast cancer epithelial-mesenchymal transition and globule formation by targeting CAFs (107).

Downstream receptors of matrix stiffness, such as integrins, FAK, Rho GTPase, and AKT, can be used as therapeutic targets. Seon-Ok Lee et al. found that fomes fomentarius ethanol (FFE) could inhibit MDA-MB-231cells motility and growth, by reducing the expression of

TABLE 2 List of conversion therapy drugs.

Categorizations		Drugs	Mechanism	References
Extracellular matrix remodeling enzymes inhibitors	LOX inhibitors	BAPN	Inhibit collagen cross-linking	(104, 105)
		TM	Copper chelator	(25)
	MMPs inhibitors	Prinomastat	MMP -2, -9, -13 and -14 inhibitor	(106)
Targeted drugs	TGF-β	PFD	Inhibition of TGF-βexpression in CAFs	(107)
	Akt	FFE	Reduce phosphorylated Akt	(108)
	HER2-Src-α6β4 integrin	Lapatinib	Inhibit HER2 activity	(109)
Others		MU	Inhibit HA synthesis	(110)

MMP-9 and phosphorylated Akt (108). Furthermore, the complex formation of HER2-Src-α6β4 integrin influences the targeted therapy with lapatinib. Cuiying Liu et al. explored how stiffness regulated the response of breast cancer cells to lapatinib. They found that, on the stiff substrate, the HER2 is difficult to combine with β4 integrin molecules, constructing fewer complexes of HER2-Src-α6β4 integrin. Consequently, free HER2 molecules were inhibited by lapatinib. In addition, as early as 2002, the concept of "biomechanopharmacology" was first proposed (109). The development of this field will provide new ideas for future treatments.

5 Conclusions and perspectives

The role of the ECM in tumorigenesis has been increasingly studied, and changes in matrix stiffness have also been considered as factors contributing to disease development. This review begins with an introduction to the mechanical microenvironment in breast cancer. We then elaborated on the effect of extracellular matrix stiffness on breast cancer's biological behavior and signaling pathway. Finally, we discuss the transformation treatments for matrix stiffness in breast cancer.

In addition, several questions remain unanswered. Can matrix hardness be integrated into clinical research? Is there an interaction between the various mechanical stimuli? Can mechanical stimuli such as matrix stiffness be measured quantitatively? Are there signaling pathways other than those mentioned above? Research into the effects of matrix hardness on signaling pathways is only beginning, and the effects of matrix stiffness on biological pathways, such as transcription, post-transcriptional modification, translation, and post-translational modification, need to be further investigated. In addition, we also noted that antibody-drug conjugates (ADCs) are gradually becoming a novel treatment for breast cancer. However, studies on the aspect of ADCs related to matrix stiffness are still relatively scarce, and further studies are needed to explore the relationship between the two subsequently. Although studies on the effects of matrix stiffness on breast cancer are already underway, our understanding of the mechanisms involved is limited to the tip of the iceberg. We will be able to develop new therapeutic options through a better understanding of matrix stiffness. We believe that concerted efforts by researchers are required to address these questions.

Author contributions

RX: Writing – original draft. JW: Writing – review & editing. QD: Writing – review & editing. PY: Writing – original draft.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

All authors would like to thank Biorender (www.biorender.com) for Figures editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 24 October 2023
DOI 10.3389/fonc.2023.1292211

OPEN ACCESS

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RECEIVED 11 September 2023
ACCEPTED 12 October 2023
PUBLISHED 24 October 2023

CITATION
Yao L, Chen J and Ma W (2023) Decoding TROP2 in breast cancer: significance, clinical implications, and therapeutic advancements. *Front. Oncol.* 13:1292211. doi: 10.3389/fonc.2023.1292211

Decoding TROP2 in breast cancer: significance, clinical implications, and therapeutic advancements

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Breast cancer is a heterogeneous disease characterized by distinct molecular subtypes, varied prognoses, and differential treatment responses. Understanding the molecular landscape and identifying therapeutic targets, such as trophoblast cell-surface antigen 2 (TROP2), is vital. TROP2 is notably overexpressed in breast cancer, playing a significant role in tumor growth, invasion, metastasis, and treatment resistance. While significant progress has been made in targeting TROP2 in breast cancer, several challenges and knowledge gaps remain. These challenges include the heterogeneity of TROP2 expression within breast cancer subtypes, resistance to its targeted therapies, potential off-target effects, limited therapeutic agents, and identifying optimal combination treatments. Integrating findings from clinical trials into clinical practice further complicates the landscape. This review article delves deep into TROP2 in breast cancer, highlighting its expression patterns, clinical implications, and therapeutic advancements. By understanding the role of TROP2, we can pave the way for personalized treatments, and transform the landscape of breast cancer care.

KEYWORDS

breast cancer, heterogeneity, TROP2 (Trophoblast cell-surface antigen 2), therapeutic target, clinical trials

1 Introduction

Breast cancer remains the most commonly diagnosed cancer among women globally, accounting for approximately 30% of all new cancer diagnoses in women annually. Predictions for 2023 estimate 297,790 new invasive breast cancer cases, 55,720 new cases of ductal carcinoma *in situ* (DCIS), and an expected 43,700 breast cancer-related deaths (www.cancer.org). These statistics underlie the ongoing efforts to evolve and refine breast cancer treatment strategies.

Notably, breast cancer is a heterogeneous disease (1) with distinct molecular subtypes such as hormone receptor-positive (HR+), human epidermal growth factor receptor 2-positive (HER2+), and triple-negative breast cancer (TNBC) (2–4). Each of these subtypes exhibits unique molecular features, clinical behavior, and treatment response profiles (5, 6). Consequently, this diversity mandates tailored therapeutic strategies for effective patient outcomes.

Although progress has been made in breast cancer management, obstacles like treatment resistance and paucity of therapeutic options persist (7–9). Within this realm, trophoblast cell-surface antigen 2 (TROP2), a transmembrane glycoprotein comprising 323 amino acids, emerges as a promising candidate (10). TROP2 overexpressed is prevalent in multiple cancer types, including breast cancer, especially in the TNBC subtype (10–12). Studies have demonstrated that TROP2's downregulation delays TNBC cell and tumor growth, underlying its oncogenic significance in breast cancer (13, 14). Furthermore, TROP2 upregulation correlates with various aggressive tumor characteristics, such as enhanced tumor growth, invasion, metastasis, and resistance to treatment (12, 15, 16).

However, translating the potential of TROP2 into effective therapeutic strategies are challenging. Heterogeneity in TROP2 expression within breast cancer subtypes can affect treatment response and clinical outcomes (17, 18). Other hurdles include resistance to TROP2-targeted therapies, potential off-target effects, and the limited arsenal of agents that specifically target TROP2 (12, 19, 20). These challenges are compounded by the intricacies of conducting clinical trials and bridging the gap between laboratory findings to clinical implementation.

It is pivotal to decode the complexities of TROP2's role in breast cancer for progress in personalized treatment and overcoming resistance. Understanding the expression patterns of TROP2, prognostic relevance, and therapeutic innovations offers avenues for better-targeted therapies, optimizing therapeutic response, and enhancing breast cancer patient outcomes (18, 21, 22).

Despite accumulating evidence on TROP2's therapeutic potential in breast cancer, a significant knowledge gap regarding its precise role and therapeutic application challenges (23). Addressing these gaps is essential for realizing the full promise of TROP2 as a therapeutic target in breast cancer and improving patient outcomes.

In this review, we endeavor to shed light on TROP2 in breast cancer, focusing on its expression patterns, clinical implications, and therapeutic progress. We explore the variability of TROP2 expression among different breast cancer subtypes and its correlation with clinicopathological factors. Additionally, we discuss the prognostic value of TROP2 expression, its association with treatment response, and its potential as a predictive biomarker. The latest therapeutic innovations targeting TROP2, including monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and immunotherapeutics, are also examined. Finally, our focus shifts to prospective avenues and challenges in harnessing TROP2 therapeutically. Emphasizing the necessity for more in-depth research to elucidate TROP2's molecular mechanisms and navigate the obstacles in developing effective TROP2-targeted therapies, we aim to uplift patient outcomes and reshape breast cancer treatment paradigms.

2 Molecular landscape and therapeutic targeting strategies in breast cancer

2.1 Overview of breast cancer subtypes

The heterogeneity of breast cancer manifests as distinct molecular subtypes, each with specific molecular characteristics and clinical behaviors. These subtypes significantly influence treatment approaches and outcomes. The major subtypes include HR+, HER2+, and TNBC, each has its own therapeutic considerations (6, 24).

In a recent groundbreaking multi-omics study conducted by Jin et al. (25), the intricate molecular landscape of breast cancer, with a specific focus on the HR+/HER2- subtype, was underscored, shedding light on its profound implications for therapeutic responses and outcomes. This study unveiled an immunogenic subtype enriched with immune cells, signifying the potential benefits of immunotherapy for this specific breast cancer subtype.

Therapeutic considerations are different for each subtype. HR+ breast cancers, primarily driven by estrogen receptor (ER) and/or progesterone receptor (PR) are amenable to endocrine therapies. These receptors serve as therapeutic targets, and as such, endocrine therapies are highly effective in this subtype (26). Hormone-based treatments, such as selective estrogen receptor modulators and aromatase inhibitors, play a pivotal role in managing HR+ breast cancers. Similarly, HER2+ breast cancers are targetable with HER2-directed therapies. Targeted therapies, including HER2-directed monoclonal antibodies like trastuzumab and pertuzumab, have revolutionized the treatment of HER2+ breast cancers (27). These targeted treatments specifically inhibit HER2 signaling, leading to improved outcomes. In contrast, TNBC, which lacks ER, PR, and HER2 expression, presents a formidable challenge in treatment due to the absence of precisely targeted therapies. Current approaches for TNBC include conventional chemotherapy and ongoing research into novel therapies, including immunotherapy and targeted agents (28).

In summary, the treatment landscape for breast cancer is significantly influenced by the specific molecular subtype, with each subtype requiring distinct therapeutic strategies. Understanding the molecular intricacies of breast cancer, particularly the HR+/HER2- subtype, is crucial for optimizing therapeutic responses and outcomes, including the potential benefits of immunotherapy, as emphasized in the recent multi-omics study by Jin et al. (25).

2.2 Therapeutic targets in breast cancer

Though traditional treatments such as surgery, chemotherapy, radiation therapy, and hormonal therapy have undoubtedly

improved outcomes for many breast cancer patients. However, the persisting challenges such as treatment resistance (28–30), and disease recurrence (31), necessitate the identification of novel therapeutic targets. By homing in on the molecular driver of tumor progression, targeted therapies promise precision and efficacy, minimizing treatment-related toxicities (32–34).

2.3 TROP2 as a potential target

TROP2, a 323 amino acids transmembrane glycoprotein (10), is prominently overexpressed in various epithelial cancer types, including breast cancer, especially the TNBC subtype (10–12). Its oncogenic attributes in breast cancer, such as driving tumor growth and progression, have been documented (13, 14). Elevated TROP2 expression is linked with aggressive tumor characteristics, including enhanced tumor growth and metastasis (12, 15, 16), making it a promising therapeutic target. Furthermore, while TROP2's expression in normal tissues is subdued (35), its pronounced expression in breast cancer presents a potential therapeutic window (14, 36).

2.4 Role of TROP2 in breast cancer progression

TROP2's involvement in breast cancer progression spans various facets, from promoting cell proliferation to resisting therapies (12). Crucially, it activates several tumorigenic signaling pathways, like the Wnt/β-catenin and EGFR-linked MAPK/ERK and PI3K/Akt pathways (12, 37). Furthermore, its influence extends to matrix metalloproteinases, which facilitate cancer cell invasion (12, 38), and it also plays a role in maintaining CSCs, known for their association with tumor recurrence and therapy resistance (39). The multifaceted roles of TROP2, as elucidated through these pathways, underscore its potential as a therapeutic target (Figure 1).

This diagram illustrates the multifaceted involvement of TROP2 in various oncogenic signaling cascades. TROP 2 activates the ERK1/2-MAPK axis, promoting malignant transformation and driving tumorigenesis. Additionally, it modulates the Notch pathway, influencing stem cell functions and potential tumor differentiation and hierarchy (40). TROP2 also interacts directly with nuclear β-catenin, propelling cell proliferation, a hallmark of cancer (41). A comprehensive understanding of these pathways, as depicted, offers insights into potential therapeutic targets in TROP2-driven cancers.

3 Expression patterns and clinical significance of TROP2 in breast cancer

3.1 Heterogeneity of TROP2 expression within breast cancer subtypes

TROP2 exhibits notable overexpression in TNBC, a subtype distinguished by its aggressive phenotype, establishing it as a pivotal therapeutic target and prognosis biomarker (18, 36). Conversely, TROP2 overexpression in HR+ breast cancer is more subdued but prominently pronounced in HER2+ breast cancer, suggesting a potential avenue for combination therapies targeting both TROP2 and HER2 (13, 18, 42). Additionally, luminal B (ER+, or PR-, HER2-) breast cancer, known for its less favorable prognosis relative to luminal A (ER+, PR+, HER2-), also displays significant TROP2 overexpression (22). Understanding the variability of TROP2 expression across subtypes is imperative for tailoring treatments effectively.

3.2 Clinical implications of TROP2 expression

Elevated TROP2 expression levels in breast cancer correlate with unfavorable prognostic markers, including larger tumor size,

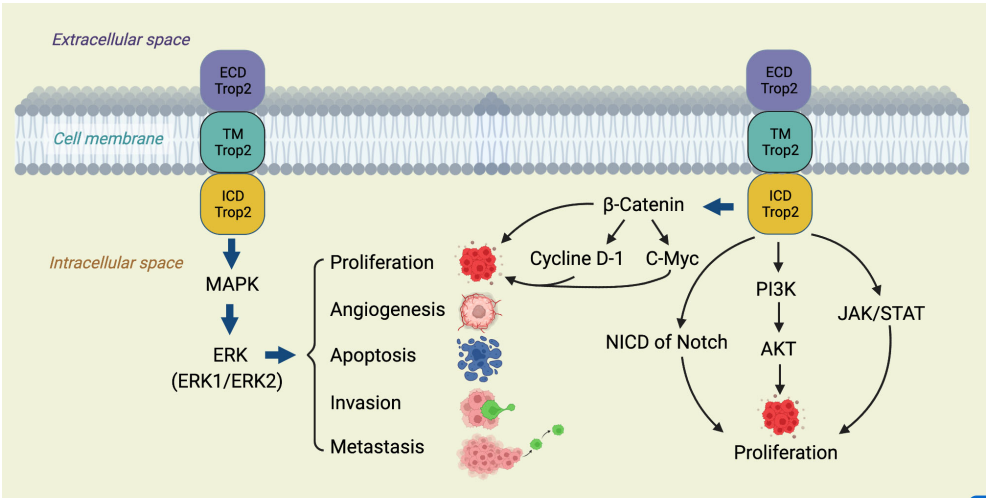

FIGURE 1
TROP2's central role in tumorigenic signaling pathways.

and increased risk of recurrence (43, 44). Specifically, in TNBC, heightened TROP2 levels are linked to increased tumor aggression and resistance to chemotherapy (45–47). Furthermore, TROP2's potential as a therapeutic target is highlighted in HER2+ breast cancer, where combined treatment modalities may enhance outcomes (18, 36, 42). The integration of TROP2 expression analysis into treatment decisions holds promise for optimizing therapeutic strategies.

3.3 Correlation between TROP2 expression and clinicopathological factors

Studies on the associations between TROP2 expression and clinical-pathological characteristics in TNBC present mixed findings. While some studies found no substantial correlation between TROP2 levels and clinicopathological factors in breast cancer, like age, histologic subtype, tumor grade, stage, lymphovascular invasion, or tumor-infiltrating lymphocytes (TILs) levels (36), the significance of TROP2 as a prognostic factor in breast cancer remains undeniable. Further investigations in this domain could refine personalized treatment strategies.

3.4 TROP2 as a predictive biomarker

TROP2 stands out as a promising predictive biomarker in breast cancer, with its expression levels informing on treatment response. Elevated TROP2 expression levels in tumors have been associated with resistance to specific therapies, such as chemotherapy and endocrine treatments (48). Notably, in the realm of targeted therapies, TROP2-expression has shown potential in enhancing responsiveness to drugs like Sacituzumab Govitecan in specific breast cancer subtypes (48). However, mechanisms of resistance, potentially linked to the upregulation of multidrug resistance proteins, are an area warranting further investigation. The intricate relationship between TROP-2 and other cellular pathways emphasizes the need for a comprehensive approach to leveraging its potential as a therapeutic target.

In summary, TROP2's expression patterns and implications in breast cancer solidify its stature as both a prognostic and predictive biomarker. As we advance our understanding, the insights gathered can guide clinical decisions, promote personalized treatments, and improve patient outcomes.

4 TROP2-targeted therapies in breast cancer clinical trials

Numerous clinical trials are currently investigating TROP2-targeted therapy for breast cancer, including mAbs, ADCs, and CAR T-cell therapies. These therapies hold great promise for advancing breast cancer treatment by targeting TROP2 specifically and effectively.

4.1 mAbs and ADCs targeting TROP2

Several TROP2-targeted mAbs are under evaluation, with a focus on improving therapeutic efficiency while sparing healthy cells (49, 50). Notably, Liu et al. reported promising results with T-cell-redirecting bispecific antibodies (TRBAs) targeting TROP2 and CD3, which suppressed tumor growth in both TNBC cell lines and primary tumor cells (51).

Promising ADCs under investigation include PF-06664178 (46, 52), IMMU-132 (46, 53, 54), and DS-1062a (46, 55). These agents target TROP2-expressing cancer cells and are under clinical evaluation for their therapeutic potential in TROP2-positive breast cancer.

4.1.1 PF-06664178

Developed by Pfizer, PF-06664178 represents a cutting-edge ADC drug that utilizes a humanized IgG1 mAb targeting TROP2, a prominent antigen on breast cancer cells. The mechanism of this ADC is intriguing: once it binds to TROP 2 and is internalized by the cancer cell, it is directed to the lysosomes, it is within these cellular compartments that the ADC releases its cytotoxic payload, the auristatin-based compound known as Aur0101 (38). Preliminary studies investigating PF-06664178 have yielded encouraging outcomes. These initial findings depict a drug with modest antitumor activity, which has sparked significant interest in the scientific and medical communities. As a result, more comprehensive evaluations and clinical trials are now underway to determine its therapeutic potential and safety profile in treating TROP2-positive breast cancer patients (52).

4.1.2 Sacituzumab Govitecan

Sacituzumab Govitecan (Trodelvy or IMMU-132) is an FDA-approved ADC tailed for TROP2-positive cancers, particularly TNBC. Its mAb component specifically targets TROP2, delivering the cytotoxic agent SN-38 directly to the tumor cells. Clinical trials have demonstrated its effectiveness for metastatic TNBC patients, positioning it as a promising frontline treatment (56–58).

4.1.3 Datopotamab deruxtecan

Dato-DXd, or DS-1062a, represents a promising addition to the landscape of TROP2-targeted therapies in breast cancer. This innovative ADC pairs a TROP2-targeting antibody with topoisomerase I inhibitor payload, creating a highly specific and effective therapeutic approach (57). The rationale behind Dato-DXd lies in its dual mechanism of action. The TROP2-targeting antibody ensures precise binding to TROP2-expressing breast cancer cells, delivering the therapeutic payload with pinpoint accuracy (59). The attached topoisomerase I inhibitor disrupts the cancer cell's DNA replication and repair processes, leading to cell death. Ongoing

clinical trials are examining its potential for treating advanced breast cancer (37).

4.1.4 SKB264

SKB264, also known as AKB264, represents another noteworthy member of the TROP2-targeted ADC family. It shares the same mAb as IMMU-132, which targets the TROP2 receptor in breast cancer cells, is being investigated for its potential therapeutic effects. Current clinical trials aim to ascertain its effectiveness in various breast cancer stages, particularly in advanced forms (49). The key feature of SKB264 lies in its potential to harness the specificity of the TROP2-targeting antibody, ensuring precise binding to TROP2-expressing breast cancer cells (60).

4.2 CAR T-cell therapy

CAR T-cell therapy is emerging as a promising approach for TROP2-positive cancers, including breast cancer. Chen et al. developed a CAR targeting Trop2 (T2-CAR) with different co-stimulatory intercellular domains and found that T2-CAR T cells exhibited robust cytotoxic activity against Trop2-positive cells *in vitro*. Moreover, these T2-CAR T cells produced a plethora of effector cytokines upon antigen stimulation (61). Interestingly, when a CD27 intercellular domain was incorporated, the antitumor activity of T2-CAR T cells was enhanced, especially in tumor-bearing mouse models. These CD27-based T2-CAR T cells demonstrated a higher survival rate in the spleens and tumor tissues of tumor-bearing mice and exhibited upregulated IL-7Rα expression and downregulated PD-1 expression, indicating a multifaceted mechanism of enhanced killing effect.

In another study, Zhu et al. demonstrated that CAR T-cells equipped with a fully human single-chain variable fragment (scFv) targeting TROP2 effectively killed TROP2-positive pancreatic cancer cells and inhibited tumor growth in xenograft models. These findings suggest that TROP2-CAR T-cells, including breast cancer, can be a potent therapeutic strategy for TROP2-positive cancer types. In another study, Zhao et al. developed bi-specific CAR T-cells targeting TROP2 and PD-L1 and showcased their superior tumoricidal activity in both *in vitro* and *in vivo* settings (62). Collectively, these results indicate the potential of bi-specific CAR T-cells as an emerging immunotherapeutic strategy for TROP2-positive cancers, including breast cancer. As CAR T-cell therapy continues to evolve, further research and clinical investigations are crucial to realize its full therapeutic potential.

In conclusion, TROP2-targeted therapies are gaining momentum as potential treatments for breast cancer. Ongoing clinical trials will continue to define the role of these therapies in the treatment landscape. For a comprehensive list of ongoing clinical trials focusing on TROP2 inhibitors in TNBC, please refer to Table 1.

TABLE 1 Current recruiting clinical trials involving TROP2 inhibitors in TNBC.

Study Title	Interventions	Phase	Study Design	Number Enrolled	NCT Number	Primary Completion
A Study of ZEN003694 and Talazoparib in Patients with Triple Negative Breast Cancer	ZEN003694, Talazoparib	Phase 2	Allocation: Non-Randomized, Intervention Model: Parallel Assignment, Masking: None (Open Label), Primary Purpose: Treatment	179	NCT 03901469	November 2023
Avelumab With Binimetinib, Sacituzumab Govitecan, or Liposomal Doxorubicin in Treating Patients with Stage IV or Unresectable, Recurrent Triple Negative Breast Cancer	Anti-OX40 Antibody PF-04518600, Avelumab, Binimetinib (and 3 more…)	Phase 2	Allocation: Randomized, Intervention Model: Parallel Assignment, Masking: None (Open Label), Primary Purpose: Treatment	150	NCT 03971409	June 30, 2024
First-in-human Study of DS-1062a for Advanced Solid Tumors (TROPION-PanTumor01)	Datopotamab Deruxtecan (Dato-DXd), Steroid Containing Mouthwash, Non-Steroid Containing Mouthwash	Phase 1	Allocation: Randomized, Intervention Model: Sequential Assignment, Masking: None (Open Label), Primary Purpose: Treatment	890	NCT 03401385	January 1, 2025
A Study of Dato-DXd With or Without Durvalumab Versus Investigator's Choice of Therapy in Patients with Stage I-III Triple-negative Breast Cancer Without Pathological Complete Response Following Neoadjuvant Therapy (TROPION-Breast03)	Dato-DXd, Durvalumab, Capecitabine, Pembrolizumab	Phase 3	Allocation: Randomized, Intervention Model: Parallel Assignment, Masking: None (Open Label), Primary Purpose: Treatment	1075	NCT 05629585	September 20, 2027

(Continued)

TABLE 1 Continued

Study Title	Interventions	Phase	Study Design	Number Enrolled	NCT Number	Primary Completion
A Study of Dato-DXd Versus Investigator's Choice Chemotherapy in Patients with Locally Recurrent Inoperable or Metastatic Triple-negative Breast Cancer, Who Are Not Candidates for PD-1/PD-L1 Inhibitor Therapy (TROPION-Breast02)	Dato-DXd, Paclitaxel, Nab-paclitaxel (and 3 more…)	Phase 3	Allocation: Randomized, Intervention Model: Parallel Assignment, Masking: None (Open Label), Primary Purpose: Treatment	600	NCT 05374512	December 3, 2025
Fudan University Shanghai Cancer Center Breast Cancer Precision Platform Series Study-Neoadjuvant Therapy	Dalpiciclib, Pyrotinib, SHR-A1811 (and 13 more…)	Phase 1 Phase 2	Allocation: Randomized, Intervention Model: Parallel Assignment, Masking: None (Open Label), Primary Purpose: Treatment	716	NCT 05582499	September 2024
Sacituzumab Govitecan in Primary HER2-negative Breast Cancer	Capecitabine, Carboplatin, Cisplatin, Sacituzumab govitecan	Phase 3	Allocation: Randomized, Intervention Model: Parallel Assignment, Masking: None (Open Label), Primary Purpose: Treatment	1332	NCT 04595565	March 30, 2027

5 Prospects and challenges in TROP2-targeted therapies

The future of breast cancer treatment sees promise in advanced interventions such as ADCs, next-generation CAR T-cell therapies, and novel immunotherapeutic interventions. Integrating TROP2-targeted therapies with conventional treatments could enhance efficacy and patient outcomes. Crucial steps ahead include biomarker validation, understanding resistance mechanisms, and refining therapeutic avenues through rigorous clinical trials and translational research. Integrating TROP2-targeted therapy into personalized medicine and ensuring equitable access are important objectives to enhance patient care.

Nevertheless, targeting TROP2 in breast cancer treatment is not devoid of challenges. Ensuring therapy specificity is vital to minimize toxicity in normal tissues. The varied nature of breast cancer subtypes necessitates strategies capable of addressing each subtype effectively. Key challenges lie in surmounting treatment resistance, pinpointing predictive biomarkers, and gaining an in-depth understanding of TROP2 signaling dynamics. Addressing these aspects will pave the way for fully harnessing the potential of TROP2-targeted therapy in breast cancer treatment.

6 Concluding remarks

TROP2's role in breast cancer, highlighted by its pronounced overexpression and pivotal function in tumor dynamics, establishes it as a compelling therapeutic target. With an array of promising TROP2-centric interventions, from mAbs, ADCs, to CAR T-cell therapy, the landscape of treatment, especially for TNBC, is evolving. The endorsement of Sacituzumab Govitecan by the FDA for metastatic TNBC highlights this potential. However, the journey is not without obstacles, with resistance emergence, of breast cancer subtypes variation, and the need for reliable biomarkers being foremost. To truly harness the promise of TROP2-based interventions, these challenges mandate focused research. The horizon of TROP2-targeted strategies shines brightly with promise for advancing breast cancer therapeutics.

Author contributions

LY: Conceptualization, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing, Methodology, Writing – original draft. JC: Data curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. WM: Conceptualization, Formal Analysis, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank the American Cancer Society (https://www.cancer.org) and the clinical trials (https://www.clinicaltrials.gov) platform to make the data set publicly available.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

ACS	American Cancer Society
ADC	Antibody-drug conjugate
CAR	Chimeric antigen receptor
CSCs	Cancer stem cells
DCIS	Ductal carcinoma in situ
DFS	Disease-free survival
ECD	Extracellular domain
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinase
EMT	Epithelial to mesenchymal transition
HER2	Human epidermal growth factor receptor 2
HR	Hormone receptor
mAbs	Monoclonal antibodies
MAPK	Mitogen-activated protein kinase
NICD	Intracellular domain of the notch protein
MMP	Matrix metalloproteinase
OS	Overall survival
PI3K	Phosphoinositide 3-kinases
PR	Progesterone receptor
scFv	Single-chain variable fragment
SIT	Short intracellular tail
STAT	Signal transducers and activators of transcription
TD	Transmembrane domain
TNBC	Triple-negative breast cancer
TROP2	Trophoblast cell-surface antigen 2

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 04 December 2023
DOI 10.3389/fimmu.2023.1266450

OPEN ACCESS

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RECEIVED 25 July 2023
ACCEPTED 16 November 2023
PUBLISHED 04 December 2023

CITATION
Lan H-R, Chen M, Yao S-Y, Chen J-X and Jin K-T (2023) Bispecific antibodies revolutionizing breast cancer treatment: a comprehensive overview.
Front. Immunol. 14:1266450.
doi: 10.3389/fimmu.2023.1266450

Bispecific antibodies revolutionizing breast cancer treatment: a comprehensive overview

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Breast cancer (BCa) is known as a complex and prevalent disease requiring the development of novel anticancer therapeutic approaches. Bispecific antibodies (BsAbs) have emerged as a favorable strategy for BCa treatment due to their unique ability to target two different antigens simultaneously. By targeting tumor-associated antigens (TAAs) on cancer cells, engaging immune effector cells, or blocking critical signaling pathways, BsAbs offer enhanced tumor specificity and immune system involvement, improving anti-cancer activity. Preclinical and clinical studies have demonstrated the potential of BsAbs in BCa. For example, BsAbs targeting human epidermal growth factor receptor 2 (HER2) have shown the ability to redirect immune cells to HER2-positive BCa cells, resulting in effective tumor cell killing. Moreover, targeting the PD-1/PD-L1 pathway by BsAbs has demonstrated promising outcomes in overcoming immunosuppression and enhancing immune-mediated tumor clearance. Combining BsAbs with existing therapeutic approaches, such as chemotherapy, targeted therapies, or immune checkpoint inhibitors (ICIs), has also revealed synergistic effects in preclinical models and early clinical trials, emphasizing the usefulness and potential of BsAbs in BCa treatment. This review summarizes the latest evidence about BsAbs in treating BCa and the challenges and opportunities of their use in BCa.

KEYWORDS

breast cancer, bispecific antibodies, targeted therapy, immunotherapy, tumor specificity

1 Introduction

Breast cancer (BCa) remains a significant global health concern, demanding the development of innovative and effective therapeutic strategies (1). Bispecific antibodies (BsAbs) have emerged as a promising approach to treating BCa, offering unique capabilities for targeted therapy and immunomodulation (2). BsAbs are engineered molecules designed to bind two antigens simultaneously (3). This feature targets tumor-associated antigens (TAAs) on BCa cells while engaging immune effector cells or blocking critical signaling pathways. By harnessing this dual targeting ability, BsAbs can enhance tumor specificity and induce robust immune responses against BCa cells (3).

Several preclinical and clinical studies have demonstrated the potential of BsAbs in BCa treatment. For instance, BsAbs targeting HER2 have shown the ability to redirect immune cells to HER2-positive BCa cells, resulting in a potent tumor cell-killing (4, 5). Additionally, BsAbs targeting immune checkpoint molecules, such as PD-1 or PD-L1, have shown promising results in overcoming immunosuppression and enhancing immune-mediated tumor clearance (6, 7). Combining BsAbs with conventional therapies, including chemotherapy or targeted agents, has also shown synergistic effects in preclinical models and early clinical trials (8, 9). These combination strategies hold great potential for improving treatment outcomes in BCa patients. Therefore, BsAbs represent a promising therapeutic approach in BCa treatment (10). Their ability to simultaneously target tumor cells and engage the immune system offers the potential for enhanced tumor specificity and improved anti-cancer activity (11).

Further research and clinical investigations are warranted to optimize BsAb design, dosing, and combination strategies. By harnessing the potential of BsAbs, we may witness significant advancements in managing BCa and ultimately improve patient outcomes (12, 13). This review aims to explore the potential of BsAbs in treating BCa by examining its mechanisms of action, preclinical and clinical evidence, and future prospects.

2 Breast cancer

BCa is considered a complex and heterogeneous breast tissue disease (14). It is one of the most frequent malignancies in women but can also occur in men, although it is less common (15). Understanding the different subtypes of BCa is essential for tailoring treatment approaches to individual patients (16). This information can help in accurate diagnosis, treatment planning, and patient prognosis (17).

2.1 Molecular subtypes of breast cancer

Human epidermal growth factor receptor 2 (HER2)-positive BCa is characterized by the overexpression of the HER2 protein (18). These tumors grow more rapidly and have a poorer prognosis (19). However, targeted therapies such as trastuzumab (Herceptin) have significantly improved outcomes for patients with HER2-positive BCa (20). Triple-negative breast cancer (TNBC) is another BCa subtype characterized by the absence of and HER2 expression, estrogen receptor (ER), and progesterone receptor (PR) (21). This subtype is more aggressive and has fewer targeted treatment options available, accounting for 10-15% of BCas (22). Luminal A and Luminal B BCas are subtypes characterized by the presence of hormone receptors (ER and/or PR) (23). Luminal A tumors have a low proliferative rate and tend to have a better prognosis (24). In contrast, Luminal B tumors have a higher proliferative rate and are associated with a slightly worse prognosis than Luminal A (24).

2.2 Histopathologic classifications

One subtype of BCa is ductal carcinoma *in situ* (DCIS), originating in the milk ducts (25). It is considered non-invasive, as the abnormal cells are confined to the ducts and have not spread to nearby tissues (26). However, if left untreated, DCIS can progress to invasive BCa. The most common subtype of invasive BCa is invasive ductal carcinoma (IDC), accounting for approximately 70-80% of cases (27). IDC begins in the milk ducts and invades the surrounding breast tissue. This subtype can be further categorized based on hormone receptor status and HER2 expression (28). Invasive lobular carcinoma (ILC) is another subtype that starts in the milk-producing glands (lobules) and can spread to other parts of the breast and beyond (29). It accounts for approximately 10-15% of invasive BCas and has distinct characteristics and patterns of growth compared to IDC (30). Inflammatory BCa (IBC) is a rare and aggressive subtype (31). It accounts for approximately 1-5% of BCa cases (32). Unlike other subtypes, IBC presents symptoms such as redness, swelling, and warmth in the breast, giving it a distinct appearance (33). Immediate and aggressive treatment is required for IBC.

Advances in research and molecular profiling have helped identify these subtypes and develop targeted treatments, leading to improved outcomes for patients with BCa (34–36). Each subtype has unique characteristics and responses to specific therapies.

3 Bispecific antibodies: structures and mechanisms of action

BsAbs are a class of engineered antibodies that can simultaneously bind to two targets, often two distinct antigens or receptors (37). They are designed to redirect immune cells or deliver therapeutic payloads to specific cells or tissues, offering a versatile approach to treating various human disorders. BsAbs have gained significant attention and promise in medicine due to their unique targeting abilities and potential applications in treating multiple diseases (38). The design of BsAbs involves combining specific binding domains from two different mAbs into a single molecule. This allows them to interact with two different targets simultaneously, facilitating various therapeutic strategies (39).

3.1 Different forms of bispecific antibodies

The world of BsAbs is a burgeoning field offering a versatile array of therapeutic possibilities. BsAbs come in various constructions, each tailored to address specific medical needs. Some, like the IgG-like BsAbs, closely mimic natural antibodies, exemplified by catumaxomab's application in ovarian cancer (40). Others, such as CrossMabs, connect different antibody fragments to target multiple antigens simultaneously, as seen with CEA-TCB in colorectal cancer (41). T-cell engagers like blinatumomab recruit and activate T-cells to combat leukemia (5). Dual-variable-domain (DVD) antibodies incorporate two antigen-binding domains within a single heavy chain, with RG6110 being a candidate for HER2-positive tumors (42). Moving beyond, tri-specific antibodies, exemplified by AFM13 in Hodgkin lymphoma, target three antigens for even greater specificity (43). BsAbs can be conjugated to cytotoxic drugs, like ABBV-838 for solid tumors (44), or combined with checkpoint inhibitors, e.g., Epcoritamab for B-cell malignancies (45). Immune stimulators, such as Cibisatamab with a 4-1BB agonist for colorectal cancer (46), and applications beyond cancer, like Emicizumab for hemophilia A (47), further illustrate the diversity and promise of BsAbs. These innovations can potentially revolutionize targeted therapies across a spectrum of diseases, marking a pivotal era in biotechnology. Several forms of BsAbs, including full-length IgG-like antibodies, bispecific T-cell engagers (BiTEs), and dual-variable domain immunoglobulins (DVD-Igs) are shown in Figure 1 (39, 48, 49).

3.2 Bispecific antibodies mechanism of action

The primary application of BsAbs lies in cancer immunotherapy (50). BsAbs can enhance the anti-tumor immune response by targeting cancer cells and engaging the immune system (44). Here, it has been discussed how BsAbs can induce anti-tumor immune responses and affect tumor cells. BsAbs are designed to recognize two distinct antigens: one on the surface of tumor cells and another on immune cells. This dual targeting allows BsAbs to bridge the gap between tumor and immune cells, bringing them into close proximity (11). The antigen recognized on the tumor cell surface by one arm of the BsAb is often a specific marker associated with the tumor. This binding can trigger various mechanisms for tumor cell killing. In this context, it has been revealed that BsAbs can induce apoptosis in tumor cells by cross-linking them with immune cells, such as cytotoxic T cells or natural killer (NK) cells (51). This activates the immune cells to release cytotoxic molecules like perforin and granzymes, which damage the tumor cell membrane and lead to cell death. The binding of the BsAb to the tumor cell can also recruit immune cells, particularly NK cells, to the tumor site (52). These NK cells can recognize the Fc portion of the BsAb and induce antibody-dependent cell cytotoxicity (ADCC), leading to the lysis of the tumor cell (52). Some BsAbs are engineered to activate the complement system, a part of the immune system that can cause cell lysis. When the BsAb binds to the tumor cell and activates complement, it forms a membrane attack complex (MAC) that punches holes in the tumor cell membrane, resulting in cell death (53, 54). By binding to an antigen on immune cells, the other arm of the BsAb can activate these immune cells. For example, it can engage with T cells and provide a co-stimulatory signal that enhances their activation and proliferation. This helps boost the immune response against the tumor. BsAbs can also influence the TME. They can help reduce immunosuppressive factors and promote an inflammatory response within the tumor, making it more susceptible to immune attack (55). Moreover, BsAbs can facilitate the uptake and presentation of tumor antigens by antigen-presenting cells (APCs), such as dendritic cells (DCs). This can lead to a more robust adaptive immune system activation, including T-cell responses (56).

One prominent example is the approval of Blinatumomab, a BiTE antibody, for treating relapsed or refractory B-cell acute lymphoblastic leukemia (57). Blinatumomab binds to CD19 on cancer cells and CD3 on T cells, enabling T cells to recognize and eliminate malignant B cells (57). This approach has shown remarkable efficacy in clinical trials, improving patient outcomes (58). Specific targeting therapy using lymphokine-activated killer (LAK) cells treated with BsAbs appeared to be a promising and effective form of adoptive immunotherapy for malignant glioma (59). In a phase I clinical trial, four ovarian cancer patients were treated with autologous lymphocytes coated with a bispecific F(ab')

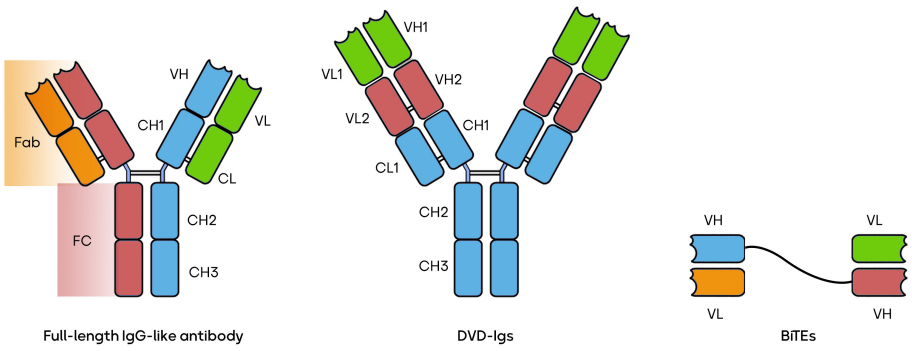

FIGURE 1
Three types of BsAbs.

2 antibody. Fortunately, no serious side effects were reported. However, it was noted that the patients developed human anti-murine antibodies, primarily targeting the idiotype of monoclonal antibody (MOv18) (60). This finding suggests an immune response against the murine components of the BsAb used in the treatment. Monitoring and managing immune responses to therapeutic antibodies is essential in developing such treatments to ensure their safety and efficacy. Further research and clinical trials may address these immune responses to improve the treatment's outcomes.

In addition to cancer therapy, BsAbs have shown potential in various other human disorders. For example, in autoimmune diseases such as rheumatoid arthritis, BsAbs can be engineered to simultaneously bind to an antigen expressed on the surface of auto-reactive B cells and CD3 on T cells, facilitating the depletion of these pathogenic B cells (61). This approach helps restore immune balance and reduce inflammation.

Furthermore, BsAbs hold promise in infectious disease treatment (62). They can be designed to target viral antigens and recruit immune cells, such as NK cells or macrophages, to eliminate infected cells via NK cell-mediated ADCC (62, 63). This approach has been explored for HIV, hepatitis B and C, and other viral infections (62, 64, 65). Neurology is another area where bi-specific antibodies have shown potential (66–68). A study reported a hypothesis that using anti-CD3 activated T cells (ATCs) armed with a chemically heteroconjugated anti-CD3 × polyclonal anti-CMV BsAb (CMVBi) could effectively target and eradicate CMV-infected cells (69). Even at low arming doses of CMVBi, the researchers found that specific cytotoxicity (SC) against CMV-infected target cells was significantly enhanced compared to unarmed ATCs, especially at various effector-to-target ratios (E:T). Armed ATCs demonstrated substantial killing of CMV-infected targets while sparing uninfected cells. Additionally, co-cultures of CMVBi-armed ATCs with CMV-infected targets triggered the release of cytokines and chemokines from the armed ATCs. This strategy represents a potential non-major histocompatibility complex restricted approach to prevent or treat CMV-related infections following organ or allogeneic stem cell transplantation (69).

BsAbs can be developed to target specific proteins involved in neurodegenerative disorders like Alzheimer's disease or Parkinson's disease (70, 71). By binding to the pathological proteins and engaging immune cells or facilitating clearance mechanisms, BsAbs can potentially halt disease progression or reduce the accumulation of toxic aggregates (72, 73).

The efficacy of single-chain variable fragment (scFv) versus bivalent targeting for T cell-mediated killing of TAAs can vary depending on several factors, including the specific target antigen, the construct's design, and the immune response context (74). ScFv-based constructs consist of a single chain of variable regions of an antibody, while bivalent constructs typically include a dimeric or multimeric format with dual antigen-binding sites (75, 76). The choice between these constructs often depends on the antigen density on the surface of target cells and the need for avidity. In cases where the TAA is highly expressed, bivalent targeting can enhance T cell activation and cytotoxicity due to increased antigen crosslinking, potentially leading to a more potent killing (77). However, for targets with lower antigen density or minimizing off-target effects is crucial, scFv-based constructs may be preferred as they provide specificity while reducing the risk of off-target binding (78).

An investigation has revealed that the IgG-[L]-scFv BsAb platform significantly improves the ability of T cells armed with BsAbs to combat tumors. Compared to the separate administration of BsAbs and T cells, using BsAb-armed T cells, known as EATs, led to reduced tumor necrosis factor-alpha (TNF-α) release, quicker tumor infiltration, and strong antitumor responses. The effectiveness of EAT therapy *in vivo* was influenced by factors like the dose of BsAbs used for arming, the quantity of EAT cells per injection, the total number of EAT doses, and the treatment schedule's intensity. Importantly, the antitumor potency of EATs remained intact even after cryopreservation and EATs employing γδ T cells were demonstrated to be both safe and as effective as αβ T cell-based EATs. This research highlights the potential of EATs as a promising avenue for cancer treatment (79).

The development and approval of BsAbs have been relatively slow despite over 25 years of engineering and clinical trials due to several challenges, including complex design, manufacturing, and safety concerns. BsAbs require precise engineering to ensure proper targeting and minimal off-target effects, making their development more time-consuming and resource-intensive. Additionally, manufacturing BsAbs can be challenging, as they often involve the production of two different binding domains within a single molecule. Safety concerns, such as cytokine release syndrome (CRS) and on-target/off-tumor toxicities, have also slowed their progress through clinical trials (80).

Collectively, BsAbs represent a powerful therapeutic approach with diverse applications in human disorders. Their ability to simultaneously target multiple antigens or receptors provides enhanced specificity and efficacy compared to traditional mAbs. As research and development in this field continue to advance, BsAbs hold great promise for improving the treatment outcomes of various diseases and transforming the landscape of medicine.

4 Known bispecific antibodies in breast cancer treatment

Numerous BsAbs are currently undergoing development and possess diverse designs that hold significance concerning BCa. BsAbs dedicated to BCa entails agents that effectively direct immune recognition toward cancer cells, aim at specific cancer antigens, and target the microenvironment associated with the disease (Figure 2). These BsAbs are being meticulously crafted for their potential utilization as antibody-drug conjugates and as molecular cues to guide engineered T-cells toward their intended targets (81) (Table 1). MM-111's ability to simultaneously bind to both HER2 and HER3 receptors provides a means to disrupt downstream signaling pathways, while ertumaxomab enhances the interaction between immune effector cells and tumor cells (99). These BsAbs hold promise as they target multiple pathways involved in HER2-positive cancers, potentially overcoming

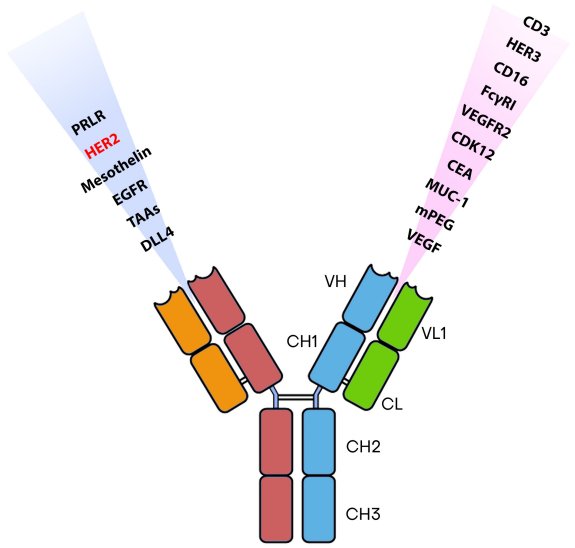

FIGURE 2
The most commonly identified antigens for designing BsAbs.

resistance. Furthermore, using activated T cells armed with anti-HER2 BsAbs (HER2Bi-aATC) presents another avenue for treatment. This approach leverages the power of the immune system to target HER2-expressing cancer cells directly (10).

4.1 Preclinical studies

To assess safety and efficacy, a study combines MM-111 with trastuzumab, a standard HER2-targeted therapy. The research includes a dose-escalation phase and an expansion cohort, aiming to identify the right treatment dosage. Preliminary results indicate that the ongoing study intends to improve treatment options for HER-2-positive advanced BCa patients (100).

HER2-targeted immunotherapy has revolutionized the treatment of HER2-positive BCa, offering multiple strategies to combat the disease (101, 102). Monoclonal antibodies (mAbs) like trastuzumab have long been the standard of care, effectively targeting HER2 overexpression (103). The combination of trastuzumab, pertuzumab, and paclitaxel has shown promising results as a frontline therapy for advanced HER2-positive BCa (104). However, resistance to anti-HER2 antibodies remains challenging, necessitating the development of alternative approaches (105). Researchers have created a bispecific anti-HER2 antibody, TP_L, to address this issue by combining trastuzumab and pertuzumab.

Pertuzumab is an additional humanized antibody with a different target site on HER2 than trastuzumab. This novel antibody, TP_L, preserves the binding characteristics of both of its parent antibodies and exhibits pharmacokinetic properties similar to conventional immunoglobulin G molecules. TP_L demonstrates superior capabilities in blocking HER2 heterodimerization compared to the combination of pertuzumab and trastuzumab. This heightened performance may be due to steric hindrance or the induction of a conformational change in the HER2 protein. Importantly, TP_L proves effective in inhibiting HER2 signaling even in BCa cell lines that have developed resistance to trastuzumab. In both laboratory and animal experiments, TP_L surpasses trastuzumab and pertuzumab combined in suppressing the growth of these trastuzumab-resistant BCa cell lines. Notably, TP_L treatment successfully eliminates well-established trastuzumab-resistant tumors in mice. These findings strongly suggest that trastuzumab-resistant breast tumors rely heavily on HER2 signaling. They also indicate that a comprehensive blockade of HER2 heterodimerization could be a viable therapeutic approach. TP_L's unique potential to overcome trastuzumab resistance underscores its promise as an attractive treatment option in clinical settings. Further exploration and evaluation of TP_L's efficacy are warranted for its consideration as a valuable therapeutic strategy (82). Other potential solutions are MM-111 and ertumaxomab, offering distinct mechanisms of action (10).

T cell bispecific antibodies (TCBs) are engineered molecules that can bind to T cell receptor (TCR) components and TAAs, such as HER2 or tumor-specific antigens (TSAs) (106). However, TCBs targeting HER2 have been associated with severe toxicities, possibly due to HER2 expression in normal epithelial cells (107). Researchers investigated an alternative approach by targeting p95HER2, a carboxyl-terminal fragment of HER2 expressed in about 40% of HER2-positive tumors (83). They demonstrated that p95HER2 was not expressed in normal tissues, as confirmed by specific antibody analysis. The researchers successfully engineered a p95HER2-TCB, and their study demonstrated its remarkable effectiveness in combating primary BCas and brain lesions that exhibit p95HER2 expression. What's particularly noteworthy is that, in contrast to TCBs directed at HER2, the p95HER2-TCB did not affect normal, non-transformed cells that do not exhibit HER2 overexpression (83). These findings suggest that targeting p95HER2 with TCBs could offer a safe and effective treatment strategy for a subgroup of HER2-positive tumors by selectively targeting a TSA. The findings pave the way for further research and potential clinical development of p95HER2-TCB as a targeted treatment for HER2-positive BCas expressing p95HER2.

In another investigation, a research team developed four BsAbs by combining anti-HER2 antibodies with anti-CD3 antibodies (84). These BsAbs were created using a genetically encoded noncanonical amino acid. The variations included different valencies and the presence or absence of an Fc domain. The study investigated how these variations influenced the BsAbs' ability to target HER2-expressing cancer cells. The results showed that the different valencies of the BsAbs did not significantly impact their effectiveness in fighting tumors. However, the Fc domain enhanced the BsAbs' ability to induce cytotoxic activity against the cancer cells. Unfortunately, the Fc domain also triggered T-cell activation in a manner unrelated to the presence of the target antigen. The study demonstrated that the BsAbs efficiently redirected T cells to eliminate all cancer cells expressing HER2, including those with low levels of HER2 expression. This was observed in laboratory experiments conducted *in vitro* and animal models (rodent xenografts) (84). This study offers valuable insights into the structural characteristics of BsAbs that impact their

TABLE 1 The most important BsAbs in treating BCa.

BsAbs	BsAbs Targets	Details of study	Outcomes	Ref
TP_L	HER2 epitops BsAb Sources: trastuzumab and pertuzumab	*In vitro* BT-474 SK-BR-3 HCC-1954 MDA-MB-231 MDA-MB-468 and MCF-7 *In vivo* female BALB/c mice	•Superior blocking action against HER2 heterodimerization compared to the combination of trastuzumab and pertuzumab •Effectively inhibits HER2 signaling in trastuzumab-resistant BCa cell lines •Outperforms trastuzumab plus pertuzumab in inhibiting the growth of trastuzumab-resistant BCa cell lines •Eradicates established trastuzumab-resistant tumors in mice	(82)
p95HER2-TCB	P95HER2 and CD3ϵ	*In vitro* MCF7 MCF10A Jurkat cells *In vivo* Humanized xenograft models	•Potent anti-tumor effects on primary BCas and brain lesions that express p95HER2 •Unlike TCBs targeting HER2 the p95HER2-TCB had no impact on nontransformed cells that do not overexpress HER2	(83)
Four types of BsAbs	HER2 and CD3 IgG-based bsAbs	*In vitro* SKBR3 Her2 3 +; MDA MB453 Her2 2 +; MDA MB231 Her2 1 +; MDA MB468 Her2 0 *In vivo* xenograft NGS mice model	•Different valencies of the BsAbs did not significantly impact their effectiveness in fighting tumors •Fc domain enhanced the BsAbs' ability to induce cytotoxic activity against the cancer cells •The Fc domain also triggered T-cell activation in a manner unrelated to the presence of the target antigen •The BsAbs efficiently redirected T cells to effectively eliminate all cancer cells expressing HER2 including those with low levels of HER2 expression	(84)
BiMAbs	HER2/EGFR/CEA/EpCAM and αCD3/αCD28 IgG1-Fc based format	*In vitro* MCF-7 HT-1080/FAP	•Effectively activated T cells and induced cytotoxicity only in the presence of tumor cells •Combination treatment with αTAA–αCD3 BiMAb and co-stimulatory αTAA–αCD28 or αTAA–TNFL fusion proteins significantly enhanced T cell activation proliferation activation marker expression cytokine secretion and tumor cytotoxicity	(85)
HER2-BsAb	HER2 and CD3	*In vitro* HCC1954 *In vivo* BALB-$Rag2^{-/-}$IL-2R-γc-KO (DKO) mice	•Promoted of T-cell infiltration and suppression of tumor growth mainly when used in conjunction with human PBMC or ATC	(86)
BAb	CEA and HER2 Murine IgG1 subclass	*In vitro* SKOv3-CEA-1B9 *In vivo* Double-positive tumour-bearing nude mice	•Enhanced tumor localization compared to single-specificity antibodies	(87)
DF3xH22	MUC-1 and HER2	*In vitro* R75-1 MCF-7 BT-20 T-47D SKBR-3	•Mediated the phagocytosis of MUC-1-expressing target cells •Inducing ADCP	(88)
BsAb; mPEG × HER2	mPEG and HER2 Anti-HER2 scFv and anti-DNS scFv	*In vitro* MCF7/HER2 ($HER2^{high}$) and MCF7/neo1 ($HER2^{low}$) *In vivo* BALB/c nude mice	•One-step formulation of PLD using mPEG × HER2 enhanced tumor specificity increased drug internalization and improve the anticancer activity of PLD against HER2-overexpressing and doxorubicin-resistant BCa	(89)
TC-BsAb	EGFR and HER2	*In vitro* BT-474 and SK-BR-3 *In vivo* female BALB/c nude mice	•Demonstrated significantly greater potency in inhibiting the growth of BCa cell lines compared to trastuzumab cetuximab and the combination of trastuzumab plus cetuximab	(90)
Anti-EGFR/VEGFR2 BsAb	EGFR and VEGFR2 Cetuximab IgG linked to the scFv of ramucirumab via a glycine linker	*In vitro* MDA-MB-231 BT-20 MDA-MB-468 BT549 and HS578 T *In vivo* female athymic nude mice	•Inhibited EGFR and VEGFR2 in TNBC cells disrupting the autocrine mechanism •Inhibited ligand-induced activation of VEGFR2 and blocked the paracrine pathway mediated by VEGF secreted from TNBC cells in endothelial cells	(91)

(Continued)

TABLE 1 Continued

BsAbs	BsAbs Targets	Details of study	Outcomes	Ref
HB-32	DLL4 and VEGF Derived from Bevacizumab and H3L2 was use as the parental mAb The anti-DLL4 antibody (H3L2) was generated using the hybridoma technique and humanized transformation	*In vitro* MDA-MB-231 cells *In vivo* BALB/c nude mice	•Effectively inhibited the proliferation migration and tube formation of HUVEC which are involved in angiogenesis •HB-32 inhibited the proliferation of BCa cells and induces tumor cell apoptosis more effectively than treatment with an anti-VEGF antibody or an anti-DLL4 antibody alone	(92)
HER2xPRLR bispecific ADC	HER2 and PRLR A fully human mAb to human PRLR and "in-house trastuzumab"	*In vitro* HEK293 cells	•Significantly enhanced the degradation of HER2 and the cell-killing activity of a noncompeting HER2 ADC—in BCa cells that coexpressed HER2 and PRLR	(93)
PRLR-DbsAb	PRLR and CD3	*In vitro* MDA-MB-231 MCF-7 and SKBR-3 cells *In vivo* Female NOD/SCID mice	•Activated T cells and stimulated the release of antitumor cytokines •Showed significant inhibition of tumor growth and increased survival compared to traditional mAb treatment	(94)
MDX-21	HER2 and FcγRI (CD64)	*In vitro* SK-BR-3 BT-20 T-47D	•Induce phagocytosis and cytolysis of BCa cells by human MDMs •Induced ADCP and ADCC •Combining MDX-H210 and G-CSF did not demonstrate significant therapeutic efficacy regarding clinical responses •Isolated neutrophils from patients undergoing G-CSF treatment displayed high cytotoxicity in the presence of MDX-210	(95)
MesobsFab	Mesothelin and FcγRIII (CD16)	*In vitro* BT-474 HCC1806 SK-BR-3 and MDA-MB-231 *In vivo* Humanized xenograft models	•Facilitated the recruitment and infiltration of NK cells into tumor spheroids •Induced ADCC •Elicited dose-dependent cell-mediated cytotoxicity against mesothelin-positive tumor cells •Induced cytokine secretion •Reduced cell invasiveness	(96)
HER2bsFab	HER2 and FcγRIII (CD16) Fab-like BsAb	*In vitro* SK-OV-3 SK-BR-3 BT-474 MCF-7	•Effectively inhibited the growth of HER2-high tumors by recruiting resident effector cells expressing mouse FcγRIII and IV •Showed superior inhibition of HER2-low tumor growth compared to trastuzumab	(97)
BsAb	HER2 and FcγRIII (CD16) A trivalent anti-erbB2/anti-CD16 BsAb	*In vitro* SKBR3 cells	•Activated NK cells to enhance anti-tumor immune responses	(98)

functionality. Additionally, it underscores the promising potential of BsAbs as a therapeutic choice for BCa patients, particularly those with low or varied HER2 expression. By proficiently targeting cancer cells that express HER2, even those with minimal HER2 levels, BsAbs present a promising avenue for enhancing BCa treatment.

A study aimed to improve the efficacy of T cell-recruiting BsAb (BiMAb) for solid TAAs in carcinomas has been challenging compared to hematologic malignancies (85). The researchers put forward a hypothesis that the combination of co-stimulatory Bispecific Monoclonal Antibodies (BiMAb) with αTAA–αCD3 BiMAb could bolster T cell activation and their ability to multiply, thus improving the targeting of tumor antigens that are expressed weakly or heterogeneously. Various combinations of αTAA–αCD3 and αTAA–αCD28 BiMAb in a tetravalent IgG1-Fc format were examined, targeting multiple BCa antigens like HER2, epithelial cell adhesion molecule (EpCAM), carcinoembryonic antigen (CEA), and epidermal growth factor receptor (EGFR). Additionally, they explored bifunctional fusion proteins of αTAA–tumor necrosis factor ligand (TNFL) superfamily members, including 4-1BBL, OX40L, CD70, and TL1A. To evaluate the functionality of these BiMAbs, the researchers conducted tests using co-cultures of tumor cell lines and purified T cells in monolayer and tumor spheroid models. The results revealed that αTAA–αCD3 BiMAb effectively activated T cells and induced cytotoxicity only in the presence of tumor cells, signifying a strict reliance on cross-linking. Furthermore, the combination treatment of αTAA–αCD3 BiMAb with co-stimulatory αTAA–αCD28 or αTAA–TNFL fusion proteins led to a significant enhancement in T cell activation, proliferation, activation marker expression, cytokine secretion, and their ability to target and destroy tumor cells (85).

Moreover, co-stimulation of BiMAb decreased the minimum needed dose for T-cell activation. The co-stimulation is able to inhibit immune-suppressive effects of interleukin (IL)-10 and tumor growth factor (TGF)-β on T cell activation and the formation of memory cells (108). Furthermore, using immune checkpoint inhibitors (ICIs) intensified the co-stimulation facilitated by BiMAb. This effective co-stimulation could be achieved by targeting a secondary BCa antigen or fibroblast

activation protein (FAP) expressed on another type of target cell (109). In tumor spheroids derived from pleural effusions of BCa patients, the presence of co-stimulatory BiMAb proved to be crucial for activating tumor-infiltrating lymphocytes (TILs) and eliciting cytotoxic anti-tumor responses against BCa cells. In a broader context, the study showcased that co-stimulation significantly enhanced the ability of T cell-activating BiMAb to eliminate tumors while still relying on the recognition of TAAs. This approach has the potential to offer a more localized activation of the immune system with heightened effectiveness and reduced peripheral side effects, presenting promising prospects for enhancing immunotherapy in the treatment of solid tumors (85).

On the other hand, some studies have warned about targeting CD28 with mAbs and its fatal toxicities (110, 111). A phase I clinical trial of TGN1412, a superagonist anti-CD28 mAb, revealed severe and unexpected toxicities in healthy volunteers, highlighting the need for extreme caution when conducting trials with such agents. The rapid onset of a systemic inflammatory response, including CRS, organ failure, and a dramatic depletion of immune cells, underscored the potential dangers of novel immunomodulatory therapies. This study serves as a stark warning about the importance of rigorous preclinical evaluation and the careful design of early-phase clinical trials, emphasizing the necessity of close monitoring and promptly addressing adverse events to ensure the safety of participants. These findings indicated the imperative for thoroughly understanding and mitigating potential toxicities before advancing such therapies into human trials (112).

In this regard, a novel HER2/CD3 BsAb platform called HER2-BsAb also was designed (86). HER2-BsAb preserves the antiproliferative effects of trastuzumab, an established HER2-targeted therapy, while recruiting and activating non-specific circulating T-cells. This recruitment and activation of T-cells promote tumor infiltration and eradicate HER2-positive tumors, even those resistant to standard HER2-targeted therapies (113). In *in vitro* studies, it has been established that HER2-BsAb could have cytotoxicity against tumors. The effectiveness, measured by EC_{50} (half-maximal effective concentration), is directly related to the level of HER2 expression on the surface of various human tumor cell lines. This correlation holds regardless of the lineage or type of the tumor, emphasizing the versatility of HER2-BsAb. Crucially, the cytotoxic effects mediated by HER2-BsAb appear to be relatively resistant to PD-1/PD-L1 inhibition. This suggests that HER2-BsAb may remain effective even with ICIs. Furthermore, HER2-BsAb has demonstrated a remarkable ability to promote the infiltration of T-cells and suppress tumor growth, especially when combined with human peripheral blood mononuclear cells (PBMCs) or activated T-cells. The compelling antitumor properties observed in both *in vivo* and *in vitro* settings provide strong support for advancing the clinical development of HER2-BsAb as a potential cancer immunotherapeutic. By leveraging the unique capabilities of BsAbto engage T-cells and target HER2-positive tumors, HER2-BsAb holds potential as a valuable addition to the treatment arsenal for HER2-positive solid tumors, including those resistant to standard HER2-targeted therapies (86).

An investigation explored the expression of carcinoembryonic antigen (CEA) and HER2 in BCa and evaluated the potential of a BsAb termed BAb targeting both antigens for improved tumor uptake and residence time. Immunohistochemistry was initially performed on primary breast tumors, revealing that 65% of cases were positive for CEA, 19% for HER2, and 12% expressed both antigens. A BAb targeting CEA and HER2 was then developed and characterized. In the context of a double-positive tumor model (SKOv3-CEA-1B9), it was observed that the BAb displayed comparable internalization patterns to the 35A7 F(ab')2-PDM despite its dual specificity. Interestingly, the BAb exhibited a notably higher degree of uptake in comparison to the FWP51 F (ab')2-PDM, with the disparity becoming more pronounced 72 hours post-injection (7.3 ± 2.1% as opposed to 1.4 ± 0.5% of the injected dose per gram of tissue). This investigation postulates that the concurrent targeting of two distinct TAAs, namely, CEA and HER2, on the same cellular entity via a Bispecific Antibody (BsAb) can potentially augment tumor localization when contrasted with single-specificity antibodies. Such an approach bears promise for enhancing the effectiveness of antibody-based therapeutic interventions in the context of BCa (87).

The potential of a mAb, DF3, and its BsAb DF3xH22 in mediating phagocytosis and cytolysis of MUC-1-expressing BCa cells was examined by monocyte-derived macrophages (114). MUC-1 is frequently expressed in adenocarcinomas, including 80% of BCas, while HER2 is overexpressed in approximately 30% (88, 115). The expression of MUC-1 and HER2 exhibits partial overlap but lacks coordination. Consequently, concurrently targeting both antigens with antibodies may broaden the scope of patients eligible for immunotherapeutic interventions. The study outcomes revealed that Monoclonal Antibody (MAb) DF3 and Bispecific Antibody (BsAb) DF3xH22 both facilitated Antibody-Dependent Cellular Phagocytosis (ADCP). MAb DF3 exhibited a more pronounced ADCP activity than BsAb DF3xH22, while neither antibody induced ADCC. Interestingly, the inclusion of interferon-gamma (IFN-γ) in monocyte-derived macrophage cultures led to a suppression of ADCP in contrast to the presence of GM-CSF alone. Immunohistochemical analysis of primary BCa tissues depicted a partially overlapping yet non-coordinated expression pattern of MUC-1 and HER2 across the 67 cases examined. Based on these findings, the authors recommend simultaneously targeting MUC-1 and HER2 in BCa due to their partially overlapping expression profiles. MAb DF3 and BsAb DF3xH22 effectively facilitate target cells expressing MUC-1 phagocytosis. Further investigations are required to ascertain whether this antibody-triggered phagocytosis leads to sustained and specific T-cell activation against MUC-1 (114).

The study aimed to improve the therapeutic efficacy of PEGylated liposomal doxorubicin (PLD) in patients with HER2-overexpressing BCa. PLD is often ineffective in these patients due to their intrinsic low sensitivity to doxorubicin (89). The researchers developed a humanized BsAb (BsAb; mPEG × HER2) targeting methoxy-polyethylene glycol (mPEG) and HER2. The primary objective of this study was to augment the specificity, internalization, and anticancer efficacy of PEGylated Liposomal Doxorubicin (PLD) in cancer cells characterized by HER2 overexpression. Through a one-step formulation process, the investigators integrated PLD with mPEG × HER2 to create

liposomes specifically targeted to HER2. These liposomes exhibited stability under conditions of both 4°C in phosphate-buffered saline (PBS) and 37°C in the presence of serum. The inclusion of αHER2/PLD, denoting the targeted liposomes, facilitated receptor-mediated endocytosis and increased doxorubicin accumulation within HER2-amplified BCa cells (MCF7/HER2). The cytotoxicity of αHER2/PLD was notably elevated, demonstrating more than a 200-fold enhancement in MCF7/HER2 cells and a 28-fold increase in drug-resistant MDA-MB-361 cells characterized by a deletion in the *TOP2A* gene. In an *in vivo* mouse model featuring tumor-bearing mice, αHER2/PLD exhibited a specific accumulation of doxorubicin in the nuclei of cancer cells. Compared to untargeted PLD, this targeted approach resulted in significantly enhanced antitumor efficacy against both MCF7/HER2 and MDA-MB-361 tumors. Importantly, αHER2/PLD demonstrated cardiotoxicity similar to that of PLD in both human cardiomyocytes and murine models. The findings of this investigation propose that the one-step formulation of PLD employing mPEG × HER2 represents a straightforward method to heighten tumor specificity, increase drug internalization, and enhance the anticancer activity of PLD against BCa cases characterized by HER2 overexpression and resistance to doxorubicin. This approach can potentially ameliorate the limited sensitivity of HER2-positive BCa to PLD and subsequently improve treatment outcomes (89).

Researchers have developed an anti-EGFR/HER2BsAb called TC-BsAb to address the limitations of anti-HER2 therapies. TC-BsAb is engineered by combining trastuzumab with cetuximab, an anti-EGFR chimeric antibody (90). The administration of TC-BsAb results in the internalization of both EGFR and HER2 receptors, in contrast to trastuzumab and cetuximab when used individually or in combination, which fail to induce the internalization of HER2. This observation suggests that TC-BsAb operates through a distinct and unique mechanism compared to the individual antibodies. In both *in vitro* and *in vivo* experiments, TC-BsAb displayed a notably higher efficacy in inhibiting the proliferation of BCa cell lines when compared to trastuzumab, cetuximab, or the combination of trastuzumab and cetuximab. These findings indicate the potential of TC-BsAb as a promising therapeutic approach for BCa treatment. It is essential to emphasize that further investigations and clinical trials are imperative to substantiate the effectiveness and safety of TC-BsAb in BCa patients. Nonetheless, developing BsAbs, such as TC-BsAb, opens new avenues for enhancing treatment outcomes in BCa cases characterized by HER2 overexpression and addresses the limited response to current therapeutic modalities (90).

The study's findings reveal that EGFR and vascular endothelial growth factor receptor 2 (VEGFR2) are frequently overexpressed in TNBC and cooperate in an autocrine and paracrine manner to facilitate tumor growth and angiogenesis (116). While mAbs targeting EGFR (e.g., cetuximab) and VEGFR2 (e.g., ramucirumab) have received FDA approval for various cancer types, they are not currently sanctioned for treating BCas. In TNBC, VEGF-A secreted by cancer cells exerts paracrine effects by promoting angiogenesis in endothelial cells and simultaneously stimulates cancer cell growth via autocrine signaling (117). To interrupt this autocrine/paracrine loop and concurrently target the EGFR-mediated tumor growth signaling and the VEGFR2-mediated angiogenic pathway, the investigators devised a BsAb, specifically an anti-EGFR/VEGFR2 BsAb. Utilizing a glycine linker, this BsAb was created by combining the IgG backbone of cetuximab with the scFv of ramucirumab. The physicochemical characterization of the anti-EGFR/VEGFR2 BsAb demonstrated its ability to bind to both EGFR and VEGFR2 with a binding affinity similar to that of the parental antibodies. The BsAb exhibited anti-tumor activity *in vitro* and *in vivo* using TNBC models. Mechanistically, the anti-EGFR/VEGFR2 BsAb directly inhibited EGFR and VEGFR2 in TNBC cells, thus disrupting the autocrine mechanism in a TNBC xenograft mouse model. Additionally, it blocked ligand-induced activation of VEGFR2 and thwarted the paracrine pathway mediated by VEGF, which was secreted from TNBC cells and impacted endothelial cells. These innovative findings underscore the multifaceted mechanisms by which the anti-EGFR/VEGFR2 BsAb impedes tumor growth. Consequently, further investigation is warranted to explore its potential as a targeted antibody therapeutic for TNBC treatment (91).

Resistance to therapies targeting VEGF-A and VEGF-R2 is observed in many tumor models (118). In light of this, it has been found that blocking both the DLL4-Notch and VEGF signaling pathways simultaneously can have a synergistic effect in inhibiting tumor blood vessel density and function, ultimately reducing tumor growth (119). A bispecific mAb named HB-32 has been successfully developed, targeting human DLL4 and VEGF. HB-32 has demonstrated high binding affinity to VEGF and DLL4 (120). *In vitro* experiments have shown that HB-32 effectively inhibits the proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVEC), which are involved in angiogenesis. Furthermore, *in vivo* xenograft studies using BCa cells (MDA-MB-231) have been conducted. These studies have demonstrated that HB-32 inhibits the proliferation of BCa cells and induces tumor cell apoptosis more effectively than treatment with an anti-VEGF antibody or an anti-DLL4 antibody alone. These findings suggest that the BsAb HB-32 holds promise as a potential treatment for BCa. By targeting DLL4 and VEGF, HB-32 exhibits enhanced anti-tumor effects compared to single-targeting antibodies. However, further research and clinical trials are necessary to fully evaluate the efficacy and safety of HB-32 as a therapeutic option for BCa (92).

The prolactin receptor (PRLR) plays a significant role in certain breast and prostate cancers, making it an attractive target for cancer treatment (121). However, previous attempts to block PRLR have shown limited effectiveness despite being safe (122). In another investigation, the trafficking and internalization of cell surface proteins targeted by antibody-drug conjugates (ADCs) were compared (93). Specifically, the trafficking of HER2, the ado-trastuzumab emtansine (T-DM1) ADC's target, was compared to that of PRLR, another potential target in BCa (113). The researchers found that PRLR undergoes rapid and constitutive internalization and efficiently traffics to lysosomes, where it is degraded. They also discovered that the cytoplasmic domain of PRLR plays a crucial role in promoting its internalization and degradation. Interestingly, when the PRLR cytoplasmic domain was transferred to HER2, it

enhanced the degradation of HER2. Based on these findings, the study showed that low levels of cell surface PRLR (approximately 30,000 receptors per cell) were sufficient for effective killing by a PRLR ADC. In contrast, higher levels of cell surface HER2 (approximately 106 receptors per cell) were required for cell killing by a HER2 ADC. Moreover, the investigators demonstrated that the non-covalent linkage of HER2 to PRLR at the cellular membrane, achieved by using a BsAb capable of binding to both receptors, led to a significant enhancement in HER2 degradation and the cytotoxic effect of a non-competing HER2 ADC. In BCa cells where HER2 and PRLR were coexpressed, a HER2xPRLR bispecific ADC exhibited superior cell-killing activity compared to a HER2-specific ADC. These results underscore the pivotal role of intracellular trafficking in determining the efficacy of ADC targets. They suggest that tethering an ADC target to a rapidly internalizing protein, such as PRLR, can heighten the internalization process and the cell-killing potential of ADCs. This novel approach promises to enhance the therapeutic effectiveness of ADC-based treatments in BCa and potentially other cancer types (93).

Another study developed a novel BsAbs, PRLR-DbsAb, which can simultaneously target PRLR and CD3 on the surface of T cell (94). By engaging the immune system, this antibody enhances the body's natural defenses against cancer cells expressing PRLR. PRLR-DbsAb successfully activated T cells and stimulated the release of antitumor cytokines that help kill BCa cells. Animal studies using mouse models further demonstrated the potential of PRLR-DbsAb as a therapeutic option, showing significant inhibition of tumor growth and increased survival compared to traditional mAb treatment (94). These findings highlight the promise of immunotherapy, explicitly targeting PRLR, as a potential avenue for effective cancer treatment. However, further research and clinical trials are necessary to fully explore the therapeutic potential of PRLR-DbsAb and its impact on human patients with PRLR-expressing cancers.

MDX-210 is a BsAb designed to target HER2 and Fc gamma receptor I (FcγRI) (123). Notably, HER2 is overexpressed in approximately 30% of BCa patients, and FcγRI is present on the surface of specific immune cells. In an examination of the capacity of MDX-210, its partially humanized counterpart MDX-H210, and the parental mAb 520C9 (anti-HER2/neu) to induce phagocytosis and cytolysis of BCa cells by human monocyte-derived macrophages (MDMs), the results revealed that both MDX-210 (via FcγRI) and 520C9 (via FcγRII) facilitated similar levels of antibody-dependent cellular phagocytosis (ADCP) and ADCC. MDX-H210, the partially humanized variant of MDX-210, exhibited equivalent ADCP activity compared to MDX-210. Confocal microscopy corroborated that the dual-labeled cells represented bona fide phagocytosis. It was noted that ADCP and ADCC were more pronounced when MDMs were pre-incubated with granulocyte-macrophage colony-stimulating factor (GM-CSF) compared to macrophage colony-stimulating factor (M-CSF). The study established that MDX-210 was as effective as the parental antibody 520C9 in stimulating phagocytosis and cytolysis by MDMs *in vitro*. Furthermore, MDX-210 and MDX-H210 demonstrated similar levels of ADCP activity (95). These findings support the ongoing clinical investigations of MDX-210 and its partially humanized derivative as potential treatments.

TNBC poses a significant medical challenge due to its unfavorable prognosis and limited therapeutic options (124). Mesothelin, a membrane protein with limited normal tissue expression but frequently elevated levels in a substantial portion of TNBC cases, has garnered attention as a promising target for therapy (125). Overexpression of mesothelin in breast tumors is linked to reduced disease-free survival and an increased incidence of distant metastases (125). To explore an immunotherapeutic approach based on BsAb, which simultaneously targets mesothelin and engages CD16, a Fab-like bispecific format named MesobsFab was employed (96). *In vitro* experiments utilized two TNBC cell lines characterized by varying surface mesothelin expression levels and distinct epithelial/mesenchymal phenotypes. The results indicated that MesobsFab effectively facilitated the recruitment and infiltration of natural killer (NK) cells into tumor spheroids, elicited dose-dependent cell-mediated cytotoxicity against mesothelin-positive tumor cells, triggered cytokine secretion, and mitigated cell invasiveness. MesobsFab also induced cytotoxicity in quiescent human PBMC, primarily through its NK cell-mediated ADCC activity. In *in vivo* experiments, the therapeutic efficacy of MesobsFab correlated with the density of mesothelin on the target cells (96). These findings underscore the significance of mesothelin as a pertinent therapeutic target, particularly in the subset of TNBC cases characterized by mesothelin overexpression, which is associated with dismal overall and disease-free survival rates. Moreover, this study highlights the potential of MesobsFab as an antibody-based immunotherapeutic agent for TNBC, demonstrating its capacity to augment immune-mediated anti-tumor responses and curb tumor invasiveness.

Trastuzumab is a well-established treatment for HER2-positive metastatic BCas, but various factors often limit its efficacy (126). A BsAb called HER2bsFab with a moderate affinity for HER2 and a unique, high affinity for FcγRIII was designed for BCa treatment (97). *In vitro* characterization of HER2bsFab showed that its major mechanism of action is ADCC, as no remar HER2-driven effect was detected. HER2bsFab demonstrated potent ADCC activity at very low concentrations against HER2-high, HER2-low, and trastuzumab-refractory cell lines. *In vivo*, studies have shown that HER2bsFab effectively inhibited the growth of HER2-high tumors by recruiting resident effector cells expressing mouse FcγRIII and IV. Importantly, HER2bsFab showed superior inhibition of HER2-low tumor growth compared to trastuzumab. Additionally, engagement of FcγRIIIA by HER2bsFab was not dependent on the V/F158 polymorphism and induced more robust activation of NK cells upon recognition of target cells. Overall, HER2bsFab exhibited potent anti-tumor activity against HER2-low tumors while overcoming most of the Fc-related limitations of trastuzumab. By combining its specificity and affinity for both HER2 and FcγRIIIA, HER2bsFab has the potential to expand the eligibility of patients for BCa immunotherapy, offering a promising approach to overcome the limitations of current treatments. However, further research and clinical trials are necessary to

validate the effectiveness and safety of HER2bsFab in BCa patients (97).

In a study, a trivalent BsAb targeting HER2 and CD16 was developed. This BsAb was designed to physically cross-link immune cells, specifically NK cells, to tumor cells, promoting cellular cytotoxic mechanisms and enhancing anti-tumor immune responses (98). The BsAb was engineered with bivalent arms that specifically bind to the extracellular domain of ErbB2, a receptor overexpressed in certain tumors, and monovalent Fab fragments that redirect NK cells. The functionality of the BsAb was confirmed through its ability to bind to both SKBR3 tumor cells and NK cells in a bispecific manner. One advantage of this trivalent BsAb is its molecular size, which falls between that of a diabody (smaller antibody fragment) and a whole antibody. This size is expected to provide benefits such as better tissue penetration due to the smaller size and slower clearance from circulation compared to complete antibodies. Collectively, this novel trivalent BsAb holds promise as a therapeutic agent for targeting ErbB2-positive tumors and activating NK cells to enhance anti-tumor immune responses. Further improvements and evaluations are warranted to optimize its efficacy and potential clinical applications (98).

4.2 Clinical studies

This section discussed the most important clinical studies in BCa patients treated with various types of BsAbs (Table 2). As mentioned earlier, MDX-H210 is a BsAb composed of antigen-binding fragments (F(ab') fragments) of mAb H22, which binds to FcγRI, and mAb 520C9, which targets HER2. This BsAb has demonstrated tumor cell lysis *in vitro* and mouse models expressing human FcγRI. FcγRI is a potent signaling molecule that is expressed on monocytes, macrophages, immature DCs, and granulocyte colony-stimulating factor (G-CSF)-stimulated polymorphonuclear cells (PMN) (132, 133). An investigation focused on using myeloid cells, specifically FcγRI (CD64)-expressing monocytes/macrophages and G-CSF-primed neutrophils, as effector cells for tumor cell cytotoxicity mediated by specific immunoglobulin receptors (127). *In vitro* experiments demonstrated that MDX-210 effectively induced lysis of HER2 overexpressing BCa cell lines. Further assays revealed that FcγRI-positive neutrophils were a significant population of effector cells during G-CSF therapy. Building on these preclinical findings and a previous study at Dartmouth, a phase I clinical trial was conducted in BCa patients to test the combination of G-CSF and MDX-210. In this study, patients receiving G-CSF were treated with escalating single doses of MDX-210. The therapy was generally well tolerated, although some patients experienced fever and short periods of chills, which correlated with elevated plasma levels of IL-6 and TNF-α. Following MDX-210 administration, a temporary decrease in total white blood count and absolute neutrophil count (ANC) was observed. However, *in vitro* experiments showed that isolated neutrophils from patients undergoing G-CSF treatment displayed high cytotoxicity in the presence of MDX-210. These findings suggest a potential role for G-CSF and BsAb in immunotherapy for BCa. By harnessing the cytotoxic capabilities of FcγRI-expressing myeloid cells, specifically neutrophils, in combination with HER2 targeting, this approach holds promise for enhancing anti-tumor immune responses. Further research and clinical trials are needed to assess the efficacy and safety of this combination therapy (127).

In another phase I clinical trial, the primary objective was to investigate the utilization of the humanized BsAb MDX-H210 in conjunction with G-CSF in patients afflicted with metastatic BCa (MBCa) displaying overexpression of HER2 (128). The study encompassed several key aims, which encompassed establishing the maximum tolerated dose (MTD) of MDX-H210 when administered alongside G-CSF, characterizing the pharmacokinetic profile of MDX-H210 when used in combination with G-CSF, assessing the treatment's toxicity, biological effects, and its potential therapeutic efficacy. The treatment regimen involved administering MDX-H210 weekly for three doses, followed by a 2-week hiatus and an additional three weekly doses. A total of 23 patients were recruited for this trial, and the doses of MDX-H210 were incrementally escalated from 1 mg/m2 to 40 mg/m2, with the MTD not being reached. The adverse effects linked to the combination of MDX-H210 and G-CSF were relatively manageable, and no dose-limiting toxicity was observed. Common side effects included fever in 19 patients, diarrhea in 7 patients, and allergic reactions in 3 patients, none of which necessitated the discontinuation of therapy. The beta-elimination half-life of MDX-H210 spanned from 4 to 8 hours at doses up to 20 mg/m2. A significant release of cytokines IL-6, G-CSF, and TNF-α was observed after administering the BsAb. Flow cytometric analysis indicated the binding of MDX-H210 correlated with the disappearance of circulating monocytes within 1 hour of infusion. The plasma of most patients showed significant levels of human anti-BsAb after the third infusion. However, this cohort of heavily pre-treated patients observed no objective clinical responses. Although the study did not demonstrate significant therapeutic efficacy regarding clinical responses, it provided valuable information regarding the toxicity profile, pharmacokinetics, and biological effects of MDX-H210 in combination with G-CSF. Further studies may be warranted to explore alternative treatment strategies or combinations to improve outcomes for patients with MBCa overexpressing HER2 (128).

A phase I clinical trial was conducted to assess various aspects of KN026, a novel BsAb with the unique property of targeting two distinct HER2 epitopes, akin to the mechanisms of action of trastuzumab and pertuzumab. This study primarily focused on examining its safety profile, pharmacokinetics, initial therapeutic effectiveness, and the potential of certain biomarkers to predict its activity. The clinical trial was carried out on a group of female patients afflicted with MBCa characterized by HER2 overexpression, who had previously exhibited disease progression while undergoing anti-HER2 therapies (129). KN026 was administered as a standalone treatment, with varying dosages of 5 mg/kg once weekly, 10 mg/kg once weekly, 20 mg/kg once every two weeks, or 30 mg/kg once every three weeks. The trial adhered to a dose escalation procedure based on the "3 + 3" rule, followed by a subsequent expansion of dose levels. A total of 63 patients were recruited for this study. The adverse events associated with KN026

TABLE 2 The most important clinical studies using BsAbs in BCa.

BsAbs	BsAbs Targets	Details of study	Outcomes	Ref/NCT
Combination of G-CSF and MDX-210	HER2 and FcγRI	*In vitro* *In vivo* Phase I clinical trial	•Effectively induced lysis of HER2 overexpressing BCa cell lines •The therapy was generally well tolerated although some patients experienced fever and short periods of chills which correlated with elevated plasma levels of IL-6 and TNF-α •A decrease in total WBC count and ANC •Isolated neutrophils from patients undergoing G-CSF treatment displayed high cytotoxicity in the presence of MDX-210	(127)
Combination of G-CSF and MDX-210	HER2 and FcγRI	Phase I clinical trial	•Common side effects included fevers in 19 patients diarrhea in 7 patients and allergic reactions in 3 patients which did not necessitate discontinuation of therapy •The beta-elimination half-life of MDX-H210 ranged from 4 to 8 hours at doses up to 20 mg/m2 •Release of cytokines IL-6 G-CSF and TNF-α •Increasing human anti-BsAb after the third infusion •No objective clinical responses	(128)
KN026	HER2 (domain II and IV) From heavy chains of pertuzumab and trastuzumab27 with a common light chain	KN026-CHN-001 Phase I first-in-human multicenter open-label single agent dose-escalation and dose-expansion study	•Increased ORR and median PFS in patients with co-amplification of HER2/CDK12	(129) NCT03619681
HER2 BATs	HER2 and CD3 Two cross-linked mAbs	Phase II clinical trial	•Increased Th1 cytokines Th2 cytokines and chemokines were observed after HER2 BATs infusions •Enhanced adaptive and innate antitumor responses Immune consolidation with HER2 BATs after chemotherapy increased the proportion of patients who remain stable at four months and improves the median OS for both HER2-HR$^+$ and TNBC patient groups	(130) NCT01022138
HER2Bi armed anti-CD3–activated T cells in combination with low-dose IL-2 and GM-CSF	HER2 and CD3 BsAb sources: Trastuzumab heteroconjugated to OKT3	Phase I clinical trial	•Increasing OS •Increasing IFN-γ and Th1 cytokines in the patient's blood indicating enhanced immune responses. These infusions induced •Inducing antigen-specific T cell and antibody responses against HER2 CEA and EGFR	(131) NCT00027807

treatment, which were attributed to the intervention itself, included symptoms such as fever (referred to as pyrexia), diarrhea, elevated levels of aspartate aminotransferase, and increased alanine aminotransferase levels in the blood. Notably, severe (Grade III) treatment-related adverse events were observed in only four patients, indicating that the safety profile of KN026 was generally manageable. An analysis of the relationship between the exposure to the drug and the observed response supported the identification of recommended doses for phase II trials, which were determined to be either 20 mg/kg administered once every two weeks or 30 mg/kg once every three weeks. In a subset of 57 patients, these doses yielded objective response rates (ORR) of 28.1% and a median progression-free survival (PFS) of 6.8 months, with a 95% confidence interval spanning from 4.2 to 8.3 months. Furthermore, translational research conducted on a subgroup of 20 patients who exhibited HER2 gene amplification provided valuable insights. This research confirmed that the concurrent amplification of the CDK12 gene, which is involved in the regulation of the cell cycle, in conjunction with HER2, served as a promising biomarker for predicting a more favorable response to KN026 treatment. Patients demonstrating co-amplification of HER2 and CDK12 achieved an ORR of 50% and a median PFS of 8.2 months, in stark contrast to patients who lacked this co-amplification, where the ORR was 0% and the median PFS was limited to 2.7 months. This noteworthy discovery underscores the potential utility of HER2/CDK12 co-amplification as a predictive biomarker, offering a means of identifying patients who are more likely to experience positive therapeutic outcomes when treated with KN026 (134). Therefore, KN026, a BsAb targeting HER2, exhibited a favorable safety profile and achieved therapeutic efficacy that was comparable to the combination of trastuzumab and pertuzumab, even in patients who had undergone extensive prior treatment. The presence of co-amplification of HER2 and CDK12 may serve as an important predictive biomarker for identifying patients with a greater likelihood of responding positively to KN026 therapy (129).

In a phase II clinical trial, the study investigated the effectiveness of anti-CD3 × anti-HER2 BsAb equipped activated T cells, referred to as HER2 BATs, in patients with metastatic BCa who lacked HER2 overexpression, including those with HER2-estrogen and/or progesterone receptor-positive (HR$^+$) tumors as well as those with TNBC (130). The primary objective of the trial was to extend the

typical duration of disease progression following the ineffectiveness of first-line therapy, with secondary objectives focusing on enhancing overall survival and stimulating immune responses. The trial enrolled 24 patients with HER2-HR$^+$ BCa and 8 patients with TNBC. The HER2-HR$^+$ patients had an average of 3.75 prior lines of chemotherapy, while the TNBC patients had an average of 2.4 prior lines of chemotherapy. Patients received HER2 BAT infusions on a weekly basis for three weeks, with an additional booster dose administered after 12 weeks. Among the 32 patients who could be evaluated, eight maintained stable disease four months after the first infusion. Notably, no dose-limiting toxicities were observed during the course of treatment. Tumor markers declined in 13 out of 23 patients with available tumor marker data. The median OS for the entire patient cohort was 13.1 months (with a 95% confidence interval ranging from 8.6 to 17.4 months). Specifically, HER2-HR+ patients exhibited a median OS of 15.2 months (95% CI: 8.6 to 19.8 months), while TNBC patients had a median OS of 12.3 months (95% CI: 2.1 to 17.8 months). Within patients who had either chemotherapy-sensitive or chemotherapy-resistant disease following prior chemotherapy, the median OS was 14.6 months (95% CI: 9.6 to 21.8 months) and 8.6 months (95% CI: 3.3 to 17.3 months), respectively. Moreover, the study observed significant increases in interferon-γ immunospots, Th1 cytokines, Th2 cytokines, and chemokines following the infusions of HER2 BATs, indicating an enhancement in both adaptive and innate antitumor responses (130). These findings suggest that employing HER2 BATs for immune consolidation after chemotherapy increases the proportion of patients who maintain stable disease at the four-month mark and improves the median OS for both the HER2-HR+ and TNBC patient groups. The study also underscores the enhancement of adaptive and innate antitumor responses. Future investigations exploring the combination of HER2 BATs with checkpoint inhibitors or other immunomodulators may offer further potential for improving clinical outcomes.

In a phase I clinical trial, researchers studied the effects of infusing HER2 BATs (HER2-targeted adoptive T cells) in 23 women with HER2 0–3+ MBCa. The median OS for these patients was 37 months. Specifically, the patients with HER2 3+ tumors had a median OS of 57 months, while those with HER2-negative (0–2+) tumors had a median OS of 27 months. This suggests that HER2 BAT infusions may positively impact survival, especially in patients with HER2 3+ tumors. Additionally, HER2 BAT infusions significantly increased IFN-γ ELISpots responses and Th1 cytokines in the patient's blood, indicating enhanced immune responses. These infusions induced antigen-specific T-cell and antibody responses against HER2, CEA, and EGFR. These immune responses could also be transferred to other patients using immune ATC (adoptive T cell therapy) expanded from individuals who had received HER2 BAT infusions. This study suggests that HER2 BAT infusions may improve survival in women with HER2-positive metastatic BCa by enhancing immune responses against cancer-related antigens (131).

5 Challenges and opportunities

The use of BsAb in BCa treatment presents both challenges and opportunities. This section summarized the most significant challenges and achievements of treating BCa with BsAbs (Figure 3).

5.1 Challenges

One of the primary challenges in developing BsAbs for various disease types revolves around the potential occurrence of CRS and autoimmune toxicities when administering BsAbs targeting CD3 and co-stimulation receptors (135, 136). These challenges are categorized into two areas of concern: "on-target/on-tumor" and "on-target/off-tumor" toxicities (137). The "on-target/on-tumor"

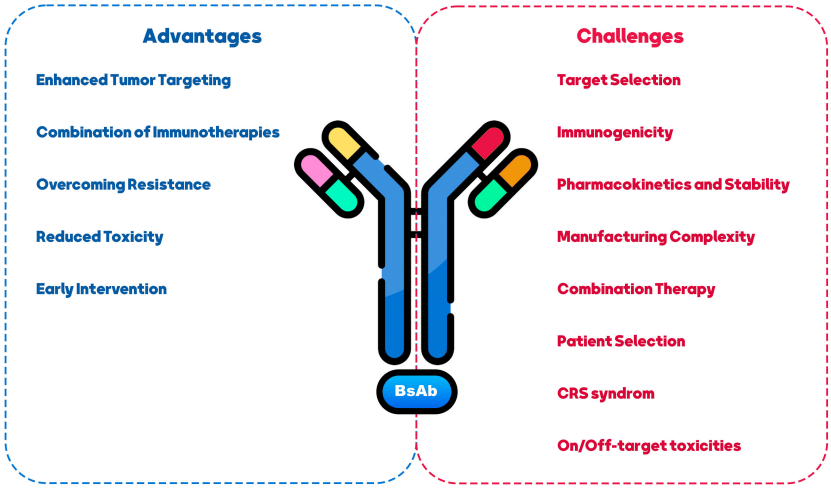

FIGURE 3
Challenges and opportunities in using BiAs as a therapeutic approach for breast cancer treatment.

toxicity typically arises from the engagement of the tumor antigen with the T-cell receptor (TCR), leading to cytokine release. In such cases, strategies like steroid administration, drug dosage, and distribution adjustments can often effectively manage this toxicity. Conversely, CRS is often driven by transient increases in pro-inflammatory cytokines such as TNFα, IL-6, IFNγ, and CCL2. Conversely, addressing the "on-target/off-tumor" toxicity of CD3-based BsAbs on normal tissue poses a greater challenge. This challenge is influenced by factors like the distribution of the target, the level of its expression on normal tissue, and the cellular localization of the target (138).

Animal models that predict BsAb-driven toxicities have proven unreliable in forecasting toxicities in human patients. The severity of CRS may correlate with the expression level of the target antigen in normal tissues. Ongoing clinical trials have observed histological changes such as lymphocytic infiltrates, acute inflammatory responses, and single-cell necrosis following the infusion of CD3 platform effector-based BsAbs (138). Ultimately, the outcomes of these clinical trials will provide valuable insights into the choice of effector cells to be targeted *in vivo* and the optimal dosing schedule, whether it involves a single or multiple administrations.

Identifying appropriate antigen targets in BCa is also challenging (138). BCa is a heterogeneous disease with various subtypes, and the target choice should consider each subtype's specific characteristics (139). Selecting targets highly expressed on cancer cells and having functional relevance in promoting tumor growth or survival is essential (140). Moreover, generating BsAbs can sometimes lead to immunogenicity concerns (141). Introducing non-human components or creating novel antibody formats can potentially trigger immune responses in patients (142). Careful design and engineering strategies are necessary to minimize immunogenicity risks and ensure the safety and efficacy of BsAbs. BsAbs may exhibit altered pharmacokinetic profiles compared to traditional mAbs, such as rapid clearance, reduced half-life, or increased susceptibility to degradation, which can impact their efficacy (143, 144). Addressing these challenges through appropriate modifications, such as antibody half-life extension technologies, can enhance their stability and therapeutic potential (145). The production of BsAbs can be more complex than mAbs due to their dual-targeting nature (146). Manufacturing may require advanced techniques, including antibody engineering, purification, and quality control (147). Developing scalable and cost-effective manufacturing strategies is essential to facilitate BsAb therapies' widespread availability and affordability (148). BCa treatment often involves a multi-modal approach, combining different therapeutic agents (149). BsAbs offer opportunities for combination therapy by targeting multiple pathways simultaneously (150, 151). However, the selection and timing of combination therapies should be carefully evaluated to maximize synergistic effects and minimize potential toxicities (58). Personalized medicine approaches should be considered when using BsAbs in BCa treatment (152). Identifying patients most likely to benefit from BsAb therapy based on biomarkers, genetic profiling, or other predictive factors can optimize treatment outcomes and minimize unnecessary side effects (153, 154).

5.2 Opportunities

Despite the mentioned challenges, BsAbs in BCa treatment presents several opportunities. BsAbs can improve tumor targeting by simultaneously binding to cancer cells and immune cells, redirecting the immune system to attack the tumor (37). This approach can overcome the limitations of tumor heterogeneity and increase the precision and effectiveness of treatment (155). BsAbs can be combined with other immunotherapeutic agents, such as ICIs or cancer vaccines, to enhance anti-tumor immune responses (100, 155, 156). Synergistic effects may be achieved by activating multiple immune pathways and overcoming immunosuppressive mechanisms within the TME. Resistance to targeted therapies is a significant challenge in BCa treatment (157). BsAbs can potentially target multiple signaling pathways simultaneously, addressing resistance mechanisms and improving treatment responses in resistant or refractory BCa cases (7, 158). BsAbs have the advantage of explicitly targeting cancer cells and sparing normal cells, potentially reducing off-target toxicities associated with non-specific treatments (80). This selective targeting may improve patient safety profiles and tolerability (159). BsAbscan can be utilized in earlier stages of BCa, including minimal residual disease or adjuvant settings, to prevent relapse and improve long-term outcomes (106, 160). The ability to engage the immune system and eradicate minimal residual disease may lead to more persistent anti-tumor immune responses, improving survival.

6 Concluding remarks

In conclusion, BsAbs offer exciting opportunities for BCa treatment by leveraging their unique targeting capabilities and the potential to engage the immune system. Addressing CRS, target selection, immunogenicity, manufacturing complexity, and patient selection will be critical to realizing the complete therapeutic.

Author contributions

H-RL: Writing – original draft. MC: Writing – original draft. S-YY: Writing – original draft. J-XC: Writing – original draft, Writing – review & editing. K-TJ: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of China (grant no., 82104445 to H-RL), Zhejiang Provincial Science and Technology Projects (grants no. LGF22H160046 to H-RL, and LGF22H160017 to MC), and Jinhua Municipal Science and

Technology Projects (grants no.2021-3-040 to K-TJ, and, 2021-3-046 to H-RL).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 06 December 2023
DOI 10.3389/fonc.2023.1282536

OPEN ACCESS

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RECEIVED 24 August 2023
ACCEPTED 27 October 2023
PUBLISHED 06 December 2023

CITATION
Ansari MY, Qaraqe M, Righetti R, Serpedin E and Qaraqe K (2023) Unveiling the future of breast cancer assessment: a critical review on generative adversarial networks in elastography ultrasound.
Front. Oncol. 13:1282536.
doi: 10.3389/fonc.2023.1282536

Unveiling the future of breast cancer assessment: a critical review on generative adversarial networks in elastography ultrasound

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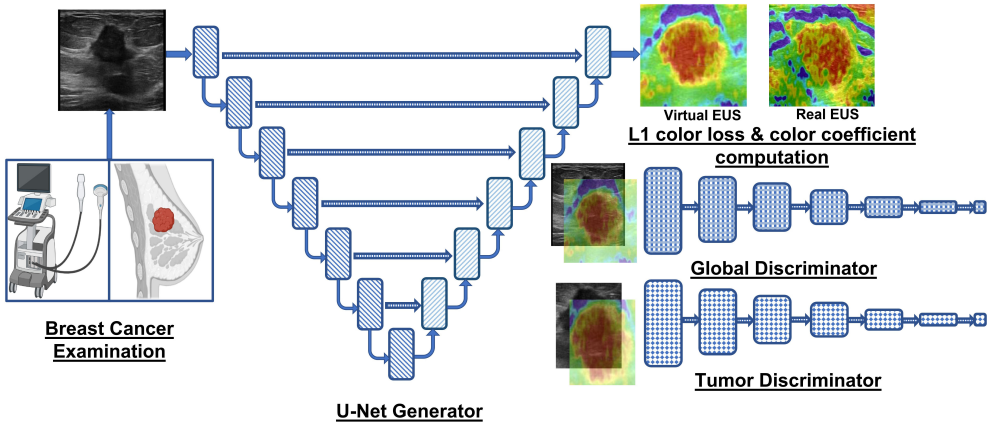
Elastography Ultrasound provides elasticity information of the tissues, which is crucial for understanding the density and texture, allowing for the diagnosis of different medical conditions such as fibrosis and cancer. In the current medical imaging scenario, elastograms for B-mode Ultrasound are restricted to well-equipped hospitals, making the modality unavailable for pocket ultrasound. To highlight the recent progress in elastogram synthesis, this article performs a critical review of generative adversarial network (GAN) methodology for elastogram generation from B-mode Ultrasound images. Along with a brief overview of cutting-edge medical image synthesis, the article highlights the contribution of the GAN framework in light of its impact and thoroughly analyzes the results to validate whether the existing challenges have been effectively addressed. Specifically, This article highlights that GANs can successfully generate accurate elastograms for deep-seated breast tumors (without having artifacts) and improve diagnostic effectiveness for pocket US. Furthermore, the results of the GAN framework are thoroughly analyzed by considering the quantitative metrics, visual evaluations, and cancer diagnostic accuracy. Finally, essential unaddressed challenges that lie at the intersection of elastography and GANs are presented, and a few future directions are shared for the elastogram synthesis research.

KEYWORDS

generative adversarial networks, elastography ultrasound, breast cancer diagnosis, enhancing pocket ultrasound, computer-aided diagnosis, artificial intelligence in medical imaging, medical image synthesis, image-to-image translation



GRAPHICAL ABSTRACT
Overview of the GAN framework employed by Yao et al. (20) and Yu et al. (40) for accurate breast lesion elastogram synthesis, aiding in accurate diagnosis of detected lesions in US image.

1 Introduction

Ultrasound (US) imaging is commonly applied across diverse clinical environments for visualizing various anatomical regions within the human body. US modality operates on the principles of reflection and scattering of highfrequency ultrasound waves from different types of soft tissues (of varying echogenicity) within the human body. US imaging presents numerous advantages that make it a favorable alternative to other medical imaging modalities (e.g., X-ray (1), magnetic resonance imaging (MRI) (2), computed tomography (CT) (3), and histopathology images (4, 5)). These advantages include its cost-effectiveness, patient safety, widespread availability, exceptional diagnostic efficacy, user-friendliness, portability, and, notably, its radiation-free nature (6).

Elastography Ultrasound (EUS) adds additional information regarding tissue elasticity to the conventional gray scale Ultrasound (also known as the B-mode US) (7). In a typical EUS, a tissue compression mechanism is used along with the transducer to assess tissue stiffness or elasticity. The response of tissue to mechanical deformation or vibration is processed and visualized as a color-coded map to quantify tissue stiffness. The type of algorithm used to generate the elasticity color map depends on the elastography technique utilized in EUS. For instance, strain-based elastography (8) (i.e., mechanical deformation) utilizes correlation-based methods, which calculate the displacement or strain by comparing pre-compression and post-compression US images. Shear wave elastography (9) employs time-of-flight methods, measuring the time shear waves (generated by the transducer) take to propagate through the tissue. Acoustic radiation force impulse (10) applies a localized acoustic radiation force to the tissue and measures the resulting tissue displacement using cross-correlation or speckle tracking algorithm. Model-based elastography techniques (11, 12), employ mathematical models to estimate tissue stiffness based on the data acquired from the US images. Subsequently, the strain information in the generated elastogram about the region of interest (ROI) is studied by radiologists to diagnose diseases such as liver fibrosis (13), breast lesions (14, 15), prostate cancer (16), thyroid nodules (17), and musculoskeletal disorders (18, 19). Specifically in the case of breast cancer, the elastogram allows the radiologists to accurately identify stiffer ROI (i.e., malignant lesions), minimizing the removal of benign lesions and damage to healthy tissues in biopsies. Moreover, the lesion shape, infiltration pattern, and elasticity analysis of surrounding tissue may provide important information regarding the extent and aggressiveness of the carcinoma, thereby guiding treatment decisions. Altogether, B-mode ultrasound provides anatomical information, and elastography adds the perspective of tissue stiffness or elasticity, increasing the clinical utility of US.

The integration of elastogram into the US enhances its clinical applicability and utility but introduces several new challenges. B-mode US is subjective to the radiologist's experience and expertise. The sensitivity to human subjectivity and expertise increases significantly for EUS because of additional factors during US capture, such as probe position, applied pressure, and frequency of mechanical compression (20). Furthermore, radiologists require additional training to accurately interpret the elasticity information and differentiate pathologies (i.e., types of tissue) in the color-coded heatmaps. The elastograms are also influenced by signal attenuations, which degrades the quality of EUS for deep-body tissues. Therefore, radiologists need to be familiar with the artifacts in EUS to provide accurate diagnoses while correlating their findings with the patient's clinical history.

Deep learning algorithms have revolutionized the analysis of US images because of their automatic nature, ability to extract task-relevant features (i.e., reduced dependence on domain knowledge), state-of-the-art performance, and end-to-end nature (21). However, the well-known neural network-based methodologies face challenges due to the composition and noise in US images, which are typically absent in real-world natural images (22, 23). To elaborate, the typical grainy texture of US images is due to the

salt-pepper or speckle noise arising from the interference of reflected sound waves (24). In addition, US images may also contain reverberation artifacts due to the sound waves echoing from two strong anatomical structures, resulting in duplicate structures (25). Furthermore, a bone or calcification can prevent the passage of sound waves, leading to incomplete visualization of the underlying tissues (26). Apart from the noise and artifacts in US images, two different anatomical structures (e.g., pancreas and liver) may appear to be the same depending on the probe position and view of the US, making US analysis challenging for radiologists and deep learning models without probe location metadata.

Recently, Yao et al. (20) have proposed a scheme to generate EUS images (i.e., elastograms) from the conventional B-mode US using a GAN to improve breast cancer diagnosis and the utility of pocket US. In this critical review, we thoroughly examine the work carried out by Yao et al. (20) in the field of EUS image synthesis. The review incorporates various crucial aspects, including a thorough comparison with relevant prior studies in medical image synthesis, a concise overview of the GAN methodology for EUS synthesis, an extensive analysis of the results, and a comprehensive discussion of the unaddressed challenges and potential future directions in EUS generation. By critically evaluating this methodology, our aim is to provide an insightful analysis of the current state-of-the-art in the synthesis of EUS images while also shedding light on the areas that require further investigation and improvement.

The remainder of this critical review is structured as follows: Section II specifies the contributions of Yao et al. (20) and the impact of synthesized EUS. Section III provides an overview of the state-of-the-art medical image synthesis and compares it with the methodology proposed by Yao et al. (20). Section IV describes the GAN methodology, loss functions, and metrics for evaluating the generated V-EUS. Section V presents an analysis of the vital results that support the claims of Yao et al. (20). Section VI discusses un-addressed challenges and essential future directions. Finally, section VII concludes the critical review.

2 Contributions and impact

The key contributions of the methodology proposed by Yao et al. (20) are next summarized. First, the manuscript proposes a GAN for synthesizing virtual EUS (V-EUS or synthesized EUS) from B-mode US. Notably, the authors provide an alternative to conventional EUS generation which could improve the clinical impact of portable US (27, 28). Second, the methodology enhances the GAN network with a tumor discriminator module and a color balancing module, allowing the network to differentiate between the tumor and healthy tissue while ensuring the V-EUS possesses a color distribution that aligns with the actual EUS image. Third, the proposed GAN model is meticulously trained and evaluated using an extensive patient cohort from fifteen medical centers. The dataset comprises 4580 cases, with 2001 images utilized for training, 500 images for internal validation, and 1730 cases from 14 centers for external validation. Furthermore, 349 extra cases of pocket US are employed to evaluate the generalizability in pocket US setups. Fourth, the generated V-EUS images undergo comprehensive testing using quantitative metrics (e.g., image similarity) and qualitative analysis (i.e., visual evaluation). The applicability of the V-EUS is also demonstrated in real-world scenarios, such as improving breast cancer diagnosis, generating elastograms for deep tissues, and improving the diagnostic effectiveness of pocket US.

The impactful contributions of Yao et al. (20) advance academic knowledge, influence existing usage and protocols of the US for breast cancer diagnosis, improve the standard of healthcare in society, and inspire new research frontiers. Also, the authors propose an additional tumor discriminator, which takes the tumor area as the input and determines the authenticity of the tumor region. Additionally, the L1 loss between the V-EUS and real EUS is reweighed using a computed color coefficient to account for color rarity in elastograms. These innovations allow the GAN framework to render color-accurate elastogram of tumor and neighboring tissue, which can also be extended to synthesize EUS of tumors in abdominal organs (e.g., hepatocellular carcinoma) with appropriate training data. The successful reconstruction of V-EUS by the GAN framework, despite the prevalent noise and artifacts in breast US images, significantly impacts the existing protocols of the US breast cancer diagnosis. Particularly, the generation of accurate elastograms for deep-seated tumors, where conventional elastography setups yield suboptimal results due to signal attenuation, signifies a breakthrough. Moreover, the integration of the GAN with pocket US devices can make elastography accessible on portable US platforms, which was not possible earlier due to limited hardware and computational power. Subsequently, the availability of V-EUS for pocket US holds profound societal implications as it can improve the diagnostic accuracy of breast cancer in small clinics and mobile mammography units while providing malignancy information of the detected tumors, thereby shrinking the time duration of the diagnostic protocols and allowing for early and effective treatment. Lastly, a noteworthy impact of this research lies in its potential to inspire innovative GAN variants tailored for elastography generation of other anatomical structures to improve the diagnosis of other carcinomas and fibrosis in a prompt, cost-effective, and timely manner.

3 Literature comparison

Deep learning models have achieved notable success in classifying, segmenting, and detecting relevant ROI in medical images and other modalities of data (2, 29–33). Recently, neural networks have been employed to upscale low-resolution medical images, transform medical imaging modalities, enhance visualization, and improve diagnostic accuracy. Muckley et al. (34) present key learnings from the 2020 fastMRI challenge, which aimed at accelerating the development of neural network architectures for MR image reconstruction while providing a fair open-access comparison to the research community. The manuscript highlights that error characterization and AI-generated hallucinations are critical challenges in evaluating MR

images generated by neural networks. Qu et al. (35) propose the WATNet architecture to generate 7T MRI (i.e., improved anatomical details) from 3T MR images by combining information in spatial and wavelet domains. Notably, the WAT modules learn the scaling and translational parameters for each pixel in the feature map based on the wavelet coefficients, allowing the network to scale different regions of the feature map based on the contrast and edge information in the frequency domain. The WAT module can also serve as a prior for other image synthesis tasks such as CT to MRI conversion. Similarly, Li et al. (36) propose a two-stage deep learning framework, employing 3D-UNet and convolutional LSTM, to accurately reconstruct thin-section MR images from thick-slice MR images, specifically targeting brain MRI super-resolution. High-level methodology analysis reveals that these works employ conventional fully convolutional network (FCN) designs for image reconstruction and superresolution tasks. However, compared to FCN architectures, GAN-based approaches offer several advantages. GANs facilitate sophisticated implicit feature learning within the generator, enabling the network to capture complex patterns from medical images. Moreover, the adversarial training paradigm further enhances the network's ability to learn and generate realistic and high-fidelity medical images.

Recently, GANs have been employed to add an extra dimension to histopathological images. Rivenson et al. (37) employed GANs to transform wide-field autofluorescence images into their corresponding stained versions. An exhaustive evaluation of the GAN on the salivary gland, thyroid, kidney, liver, and lung, involving different stains, shows that virtual staining can circumvent labor-intensive and costly histological staining procedures without any significant differences from the real stained images. Inspired by this application to enhance histopathology, researchers have employed GANs to generate EUS without requiring conventional US setup. Zhang et al. (38) propose a GAN framework, termed AUE-Net, with a U-Net generator equipped with attention mechanism and residual connections for a compelling depiction of elastograms for thyroid nodules. The spatial attention module is utilized at the beginning of the U-Net to identify the nodule regions, and a color attention module is used at the end to create a color attention map for EUS. Moreover, the loss function of the network is augmented to account for the color difference between the real and generated elastograms, forcing the generator to produce images with a color distribution that overlaps real elastograms. Despite the significant contributions of AUE-Net, Yao et al. (20) present essential improvements to the methodology design, evaluation, and application of GANs for elastogram generation. Specifically, the use of a tumor discriminator enables the network to identify tumor areas with higher precision relative to the spatial attention module, which is reflected in the qualitative analysis of the generated elastograms. Additionally, Yao et al. (20) enhance the color loss by using the lab color space with a mathematically derived color coefficient to account for color rarity. Moreover, the authors evaluate the quality of generated elastograms based on improved breast cancer diagnostic accuracy, elastography of deep-seated tumors, and improvement in diagnostic effectiveness of pocket US, which were omitted in the evaluation of AUE-Net for the elastography of thyroid nodule. In a complementary study, He et al. (39) investigate the suitability of using a GAN-based approach (i.e., SRRFNN) to improve lateral resolution in the radiofrequency (RF) data (i.e., up-sample RF data perpendicular to acoustic beam), consequently improving the elastogram quality in ultrasound strain elastography. However, the V-EUS (20) generation approach is a preferable end-to-end solution because it generates elastograms directly from conventional B-mode US rather than upsampling the lateral resolution to improve quality. As an extension to the contributions of Yao et al. (20), Yu et al. (40) utilize the same GAN framework and dataset to show the feasibility of V-EUS in augmented reality (AR-EUS) for improved diagnosis of breast cancer with pocket US. The quantitative and blind evaluation of elastograms in augmented reality shows no significant discrepancies between the AR-EUS and real EUS, establishing the authencity of AR-EUS. Table 1 summarizes the state-of-the-art methods in medical image synthesis that laid the pathway for GAN framework proposed by Yao et al. (20).

4 Methodology overview

GANs are a new class of neural network architectures that excel at generating high-fidelity new data (e.g., elastograms from US images). In terms of architecture, GANs differ significantly from the conventional FCNs because they contain two subnetworks, which are trained adversarially to enhance the capability of the system to generate realistic data instances. To elaborate, a brief description of the components of GANs is next presented. Graphical Abstract describes the neural network architectures of the generator and discriminator within the GAN framework proposed for elastogram synthesis.

4.1 Generator

In EUS synthesis, the generator is an encoderdecoder architecture that generates realistic synthetic elastograms (i.e., V-EUS. Specifically, U-Net architecture (45) is a popular choice for a generator because of its capability to capture multi-scale features and low-level features (through skip connections) to generate elastograms. The encoderdecoder design of the U-Net allows for parameter savings due to shrinking spatial dimensions of the feature maps in the deeper layers of the encoder, thereby providing computational savings. Yao et al. (20) employ the vanilla U-Net architecture with tuned channel count in the encoder and decoder for the generation of elastograms.

4.2 Discriminator

The discriminator of the GAN framework is an FCN that receives the output of the generator (i.e., elastogram) or real EUS as input and performs binary classification. Yao et al. (20) employ a

TABLE 1 Literature overview of the state-of-the-art methods for medical image synthesis.

Reference (Year)	Task	Dataset Information	Core Methodology	Remarks
Rivenson et al. (2019) (37)	Virtual staining	Whole slides of 211,475 and 59,344 of Liver and Kidney tissues	GAN framework, U-Net generator combined with an FCN discriminator	**Pros:** GAN-generated virtual staining can provide similar results as conventional staining, providing time and cost-saving **Cons:** The GAN framework is not validated for other contrast-generating methods multiple excitation and emission wavelengths
Muckley et al. (2020) (34)	MR image reconstruction	7,299 clinical brain scans subsampled k-space data	Comparative analysis of networks for MR image reconstruction for fastMRI challenge 2020	**Pros:** Deep learning methodologies decrease the minimum requirement for MR image reconstruction set by parallel imaging and compressed sensing methods **Cons:** Pseudo-regular sampling of the MR data lacks realism and is not equivalent to the perfectly equidistant sampling pattern used on MRI systems
Qu et al. (2020) (35)	Image enhancement (Image super-resolution)	15 pairs of 3T and 7T brain images	WATNet, an encdoer decoder network with wavelet priors and conditional normalization	**Pros:** Wavelet coefficient can allow learning feature map normalization weights **Cons:** Other tasks, such as MRI to CT and T2 images from T1 translation have not been explored in the work
He et al. (2020) (39)	RF super resolution	50 human subjects, 50-90 frames per patient	Super-resolution radio-frequency neural network (SRRFNN) inspired by a super-resolution GAN	**Pros:** Laterally upsampled RF data processed by SRRFNN performs better than conventional bi-cubic interpolation approach **Cons:** The method does not utilize the actual high-frequency US data using novel beam-forming technology for training
Li et al. (2021) (36)	MR image reconstruction	305 paired brain MRI samples with a thickness of 1.0 mm and 6.5 mm	3D U-Net followed by a convolutional LSTM network for MRI slice refinement	**Pros:** Practical and clinical value of generated thin MRI is higher than other voxel-based morphometry **Cons:** The quality of reconstruction is directly dependent on the accuracy of statistical parametric mapping (SPM)
Dalmaz et al. (2022) (41)	MRI to CT translation, MRI missing slices generation	IXI dataset (53 subjects), BRATS dataset (55 subjects) (42), multi-modalpelvic MRI-CT dataset (15 subjects) (43)	ResViT architecture with vision transformers' block at the bottleneck and convolution operators in the encoder and decoder of the GAN generator.	**Pros:** Convolutional and transformer branches within a residual bottleneck of the generator preserves both local precision and contextual sensitivity **Cons:** Architecture needs further validation with unpaired sets of medical images using cycle consistency loss.
Ozbey et al. (2022) (44)	MRI to CT translation	IXI dataset (40 subjects), BRATS dataset (55 subjects) (42), multi-modal pelvic MRI-CT dataset (15 subjects) (43)	Adversarial diffusion modeling using conditional diffusion for capturing and correlating the image distributions.	**Pros:** Cycle-consistent architecture is used with coupled diffusive and non-diffusive components to bilaterally translate between imaging modalities. **Cons:** Adversarial loss in diffusion models introduce training instability and suboptimal convergence
Zhang et al. (2022) (38)	Elastogram generation	726 thyroid US elastography images of 397 patients	AUE-Net GAN framework, U-Net generator with spatial and color attention.	**Pros:** L1 loss can be added to the generator loss for improving the color distributions of generated elastograms **Cons:** The method does not perform qualitative evaluation of the generated elastograms
Yu et al. (2023) (40)	Elastogram generation	4580 breast cancer cases from 15 medical centers	GAN with a U-Net generator, global and local tumor discriminator, with L1 loss and color coefficient	**Pros:** AR-EUS improves the diagnosis accuracy of pocket US **Cons:** The GAN framework has been only validated for the Chinese population

sophisticated discriminator paradigm derived from conditional GAN, which adds the B-mode US image as an additional input (i.e., prior knowledge) to the discriminator network, enhancing its ability to differentiate between real or V-EUS. The authors also add a local tumor discriminator to the framework to further enhance the capability of the system to distinguish between real or fake tumor areas and their elastograms, thereby improving the estimation of elasticity for the tumor region.

4.3 Adversarial training

GANs are trained in an iterative adversarial fashion to allow the generator to produce high-quality synthetic samples. In the initialization phase, the generator produces synthetic samples with a distribution similar to the training data using random noise or B-mode US images. In the first step, the discriminator is trained on real and V-EUS samples with the goal of learning to

differentiate the two classes accurately. In the second step, the generator is trained to create realistic synthetic samples which the discriminator can classify as real. The adversarial training process allows the generator to perform implicit feature learning, enabling it to detect complex patterns and structures. In the context of EUS generation, the generator does not have information regarding the tissue elasticity explicitly available in the US images; rather, it implicitly learns the complex patterns and correlations between the US images and the desired V-EUS.

4.4 Loss function

In the methodology details outlined by Yao et al. (20), the discriminator loss function is the average of tumor and global cross-entropy losses for accurately classifying real or V-EUS. The generator loss function is formulated to maximize the probability of the discriminator classifying generated samples as real. Furthermore, color loss (i.e., L1 loss) between the VEUS and ground truth weighed by color rarity coefficient is added to generator loss for accurate color distribution of the elastograms.

4.5 Evaluation metrics

Yao et al. (20) perform a thorough quantitative analysis of V-EUS to validate the GAN framework. Particularly the Structural Similarity Index (SSIM), Mean Absolute Percentage Error (MAPE), and Contrast-to-Histogram Correlation (CHC) are used for quantifying the difference between V-EUS and real EUS. An elaborate explanation of these metrics is provided in the Supplementary Materials.

A comprehensive qualitative analysis is conducted subsequent to the quantitative analysis, employing a blind evaluation with the Tsukuba scoring system. This evaluation involves radiologists with diverse levels of experience, ensuring a thorough and unbiased assessment of the V-EUS relative to real EUS. The qualitative analysis validates that the generated EUS has a matching visual appearance to real EUS and gathers feedback from radiologists regarding their preferences. This is crucial for the success of V-EUS because radiologists should be able to incorporate it into their diagnostic workflows and make accurate diagnoses without additional training. Thus, the positive outcomes of the qualitative analysis add to the clinical credibility of the methodology proposed by Yao et al. (20).

5 Analysis of results

This section analyzes whether the results presented by Yao et al. (20) support the claims made by the authors. First, the authors highlight that the proposed GAN framework results in SSIM, MAPE, and CHC scores of 0.903, 0.304, and 0.849, respectively, indicating that numerical metrics show a high overlap in distributions between the real and V-EUS. The preferable SSIM and CHC values are due to the use of the color coefficient and color loss, augmented with the tumor discriminator loss, allowing the GAN to put additional emphasis on the elasticity of the tumor region and overall color distribution. The choice of these quantitative metrics is in line with the literature for the synthesis of CT, MRI, and retinal color fundus images (46). However, SSIM evaluates the V-EUS by comparing local patterns of pixel intensities and does not account for global variations in quality. Similarly, MAPE may lead to misinterpretation of errors because the absolute percentage difference does not provide insights regarding the overestimation or underestimation of elasticity. The quantitative analysis would be more meaningful if Yao et al. (20) incorporated metrics such as multi-scale SSIM, which compares both local and global aspects of the image. Furthermore, Yao et al. (20) omit Frechet Inception Distance (FID) from their quantitative analysis, which is a key metric to evaluate the quality of the GAN-generated images as shown by Zhang et al. (38) for elastogram synthesis of the Thyroid. Nevertheless, the results show that the strain ratio (SR) computed from real and V-EUS leads to statistically similar AUC for diagnosing breast tumors, suggesting that V-EUS can replace real EUS in diagnostic scenarios. Additional stratified analysis of breast cancer diagnosis for tumors of varying sizes and at different locations results in similar performance between real and V-EUS, suggesting that V-EUS can overcome the human subjectivity in capturing the EUS by eliminating the variables such as probe position, applied pressure, and frequency of mechanical compression. The stratified analysis also successfully conveys to the readers that the GAN framework generalizes across tumor sizes and locations, which is critical for real-world deployment.

Second, the results validate the GAN's generalizability with 1730 breast cancer cases across fourteen other medical centers with varying imaging and clinical settings, showing that the GAN framework is independent of perturbations in imaging and clinical settings. Particularly, the authors evaluated the SSIM, MAPE, CHC, and diagnostic AUC for each of the fourteen centers and compared them with the inter-validation performance to assess model generalizability. This thorough analysis of the GAN framework across different medical centers is unique to the study conducted by Yao et al. (20) and is missing from other studies for elastogram synthesis (38). However, the validation sets are completely based on the Chinese population, requiring further validation for other ethnic groups. In line with these results, the authors also show that the GAN can generate V-EUS from low-resolution pocket US images. Adding the V-EUS to the pocket US allows radiologists to improve breast cancer diagnosis by up to 5%, indicating that V-EUS improves the clinical utility of pocket US. Altogether, the outcomes indicate that V-EUS can improve the accessibility and diagnostic accuracy of low-resolution pocket US.

Third, the results incorporate human feedback and evaluation to bridge the gap between computational metrics and human perception. Yao et al. (20) are the first in the literature to perform a novel qualitative analysis to support the quantitative results and usage of V-EUS in radiological workflows. This form of exhaustive qualitative analysis is missing from previous studies for elastogram synthesis (38) and medical image modality translation (34, 35, 41, 44). Specifically, the authors perform a blind evaluation test to compare the preference of junior and senior radiologists between

real and V-EUS. Involving radiologists with different experiences allows authors to gather insights into their contrastive preferences in diagnostic workflows. For instance, the study showed that junior radiologists preferred V-EUS over the real EUS for breast cancer diagnosis using the BI-RADS score, thereby validating the feasibility of V-EUS in day-to-day usage for radiologists. The authors also show that V-EUS can be generated for deep-seated tumors (i.e., depth greater than 20 mm) without artifacts. In contrast, 25.9% (62 of 239) of real EUS display artifacts due to signal degradation at greater anatomical depths. This is a significant breakthrough as real EUS with elasticity artifacts could not be used in practice for diagnosing diseases in deep-seated tissues and tumors. Subsequently, V-EUS opens the possibility of carcinoma diagnosis in deep body tissues, which are currently diagnosed by high-definition 3D imaging modalities (i.e., CT or MRI).

Exhaustive analysis of the results and methodology also reveals that Yao et al. (20) provide sufficient details for the reproducibility and validity of the work. Notably, the methodology clearly explains and details the different components of the GAN framework, including network hyperparameters, training hyperparameters, loss function, metrics, etc. Additionally, open-source implementation of the GAN framework is available on GitHub for verifying the results. Furthermore, the dataset used for training the networks is available upon request after agreeing to terms and conditions. However, the lack of clear documentation and comments in the code makes it challenging for the users to decipher the details in the training and evaluation of the network. Overall, the manuscript makes the methodology and results transparent to the scientific community.

5 Challenges and future directions

This section highlights the unaddressed challenges and gaps in the literature for synthesizing EUS from B-mode US images. Yao et al. (20) evaluate the malignancy of tumors based on SR. The SR is defined as the ratio of average tumor elasticity and a reference region. The generated EUS is decoded by quantizing the image into 256 pseudo-color levels, representing varying elasticity. However, the authors do not justify whether 256 elasticity values are sufficient for representing the underlying elasticity distribution of breast tissues through experiments or evidence from the literature. Furthermore, the SR is computed without providing any specific guidelines for selecting the reference region. These oversights in generating the SR raise concerns about whether the generated V-EUS can effectively model the physical independent information of the underlying breast tissue. Even though the authors show that the effectiveness of SR extracted from V-EUS in diagnosing breast cancer is similar to real EUS, further validation is necessary to clarify whether the other physical properties of the tissue, such as viscoelasticity, anisotropy, homogeneity, or heterogeneity are correctly modeled. Thus, as the first step, we recommend a comprehensive phantom study for the quantitative validation of V-EUS. By gathering feedback from medical experts regarding the biomechanical properties of the V-EUS, the research community can better understand the utility and potential clinical applications of V-EUS.

One critical pitfall of the GANs is class leakage. The groundbreaking work by Salimans et al. (47) demonstrates that GAN-generated images, initially intended to represent a specific class, exhibit the inclusion of properties and attributes from unrelated classes. This blending of features across distinct modes within the training distribution poses a significant concern, as it may lead to the generation of V-EUS images that display interpolations between malignant and benign tumors. The presence of these intermediate or outlier V-EUS images has the potential to misguide radiologists, resulting in erroneous diagnoses and suboptimal outcomes during biopsies. Such outcomes include harm to healthy tissues or the recurrence of carcinoma. To tackle this challenge, we encourage researchers to draw inspiration from techniques developed for feature disentanglement. Notably, prior studies have successfully enforced disentangled learning from noise vectors by incorporating a regularization term that penalizes the network when modifying a single element leads to changes in multiple features within the generated image. Similarly, we propose adopting regularization strategies to penalize the network for the intermixing of attributes originating from different modes of the training distribution in the generated V-EUS.

Accurate quantitative evaluation of GAN-generated medical images represents a significant challenge within image synthesis literature. This challenge arises due to the limitations of conventional metrics, SSIM, which primarily provides a high-level comparison of images based on luminance and contrast. However, pixel-wise metrics like MAPE may assign low values to blurry generated images, failing to adequately capture the visual quality of synthesized V-EUS images. Consequently, researchers like Yao et al. (20) are compelled to undertake comprehensive qualitative studies to assess image fidelity. In a pioneering study, Zhang et al. (48) have shown that the deep features of neural networks can serve as a foundation for developing perceptual metrics. To elaborate, the authors introduce learned perceptual image path similarity (LPIPS), which achieves better agreement with human perception than conventional metrics like SSIM. Given these advancements, we recommend that researchers embrace the state-of-the-art perceptual metrics for conducting quantitative evaluations of GAN methodologies applied in elastogram generation.

Another critical limitation of deep learning methodologies in medical practice is the black-box nature of neural networks. The network explainability information is critical for radiologists to trust the synthesized output, address any biases, and account for significant errors in the elastograms. To ensure the reliability and interpretability of the generated V-EUS, medical practitioners need to understand the underlying components of the B-mode US that contribute to the network's decision-making process. GANs learn the mapping between the B-mode US and the V-EUS by implicit feature learning through an adversarial training process, elevating the need to understand the mapping between the US and the synthesized EUS. Recent advancements have demonstrated the integration of explainability techniques into neural network architectures for the fusion of MRI and CT scans (49). Building upon this progress, it is feasible to develop explainable GAN frameworks as an extension to the work conducted by Yao et al.

(20). The enhanced transparency will enable medical professionals to foster trust and improve the clinical utility of V-EUS.

One of the strengths of the Yao et al. (20) methodology is the inclusion of a dataset that spans multiple medical centers, thus ensuring the evaluation of their GAN across diverse imaging and clinical parameters. However, the population demographic in these hospitals is limited to Chinese patients, thereby restricting the evaluation of the GAN's performance to this demographic. Consequently, the generalizability of the proposed GAN network to other populations with potentially distinct lesion characteristics, such as those with deeper lesions compared to the Asian demographic, remains unexplored. Yao et al. (20) have conducted validation experiments specifically focusing on tumors located at several depths up to 20 mm. While these findings provide valuable insights into the performance of the GAN framework at varying depths, it is crucial to conduct further evaluations across different racial populations. Such evaluations would shed light on the ability of the GAN to generate V-EUS images of breast lesions with varying spread and depth distributions in populations beyond the Chinese demographic, contributing to a more comprehensive understanding of the GAN's capabilities and limitations.

6 Conclusion

To summarize, we perform a comprehensive critical review of the GAN-based methodology equipped with color loss for the generation of realistic EUS images for breast lesion diagnosis. Specifically, we briefly review the methods in image reconstruction and medical image super-resolution to understand the progress in deep learning, which has led to the GAN-based methodologies for elastogram generation from B-mode US. Moreover, we analyze whether the claims are well-supported by quantitative and qualitative evaluations. Finally, we highlight the unaddressed challenges and the future directions in elastogram synthesis. As a whole, the critical review provides a clear understanding of the current cutting-edge deep learning framework for the V-EUS generation while paving the pathway for the upcoming research in elastography synthesis.

Author contributions

MA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. MQ: Conceptualization, Formal analysis, Supervision, Validation, Writing – review & editing. RR: Conceptualization, Formal analysis, Supervision, Writing – review & editing. ES: Conceptualization, Supervision, Writing – review & editing. KQ: Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

This publication was made possible by the support of Texas A&M at Qatar Research Initiative. The statements made herein are solely the responsibility of the authors.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1282536/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 07 December 2023
DOI 10.3389/fonc.2023.1292268

OPEN ACCESS

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RECEIVED 11 September 2023
ACCEPTED 20 November 2023
PUBLISHED 07 December 2023

CITATION
Tollens F, Baltzer PAT, Froelich MF and Kaiser CG (2023) Economic evaluation of breast MRI in screening - a systematic review and basic approach to cost-effectiveness analyses.
Front. Oncol. 13:1292268.
doi: 10.3389/fonc.2023.1292268

Economic evaluation of breast MRI in screening - a systematic review and basic approach to cost-effectiveness analyses

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Background: Economic evaluations have become an accepted methodology for decision makers to allocate resources in healthcare systems. Particularly in screening, where short-term costs are associated with long-term benefits, and adverse effects of screening intermingle, cost-effectiveness analyses provide a means to estimate the economic value of screening.

Purpose: To introduce the methodology of economic evaluations and to review the existing evidence on cost-effectiveness of MR-based breast cancer screening.

Materials and methods: The various concepts and techniques of economic evaluations critical to the interpretation of cost-effectiveness analyses are briefly introduced. In a systematic review of the literature, economic evaluations from the years 2000-2022 are reviewed.

Results: Despite a considerable heterogeneity in the reported input variables, outcome categories and methodological approaches, cost-effectiveness analyses report favorably on the economic value of breast MRI screening for different risk groups, including both short- and long-term costs and outcomes.

Conclusion: Economic evaluations indicate a strongly favorable economic value of breast MRI screening for women at high risk and for women with dense breast tissue.

KEYWORDS

breast cancer screening, breast MRI, abbreviated breast MRI, MR-mammography, cost-effectiveness analysis, economic evaluation

Abbreviations: AB-MRI, Abbreviated breast MRI; *BRCA (gene)*, Breast cancer (gene); CAD, Canadian dollar; CHEERS, Consolidated Health Economic Evaluation Reporting Standards; DBT, Digital breast tomosynthesis; DCIS, Ductal carcinoma in situ; GDP, Gross domestic product; ICER, Incremental cost-effectiveness ratio; LYG, Life years gained; MISCAN, Microsimulation Screening Analysis; QALY, Quality-adjusted life year; QoL, Quality of life; USD, US-dollar; WTP, Willingness to pay.

Introduction

Breast cancer is the most common cancer and the leading cause of cancer-related death in women worldwide with an estimated 2.3 million incident cases and 685,000 deaths in 2020, despite significant advances in therapeutic options and widespread screening programs (1, 2). Diagnosed at an early stage, localized breast cancer, much like colorectal cancer, is associated with excellent 5-year survival rates of approximately 99% (3). Due to the lack of symptoms in an early stage, screening for breast cancer is particularly promising and relevant.

For conventional screening programs, reductions in breast cancer mortality have been demonstrated (4–6), even though the positive results have been a matter of scientific discussion: some authors critically remark the high number of false positive cases (7, 8) and the imperfect sensitivity of mammography. Other authors derive benefits in survival predominantly from advances in breast cancer therapy and an effect of overdiagnosis (9). On top, the risk of radiation-induced cancers must be considered (10).

Among the various modalities applied in breast imaging, breast MRI is accepted to have the highest sensitivity in detecting breast cancer independent from breast density (11). Concerns on specificity and high costs, among other reasons, have averted breast MRI from taking a prominent role in screening.

The most recent multi-center studies have demonstrated that breast MRI does not suffer from reduced specificity compared to conventional mammography (12–14). However, reader experience, quality assurance and continuous monitoring are considered prerequisites for optimizing the diagnostic performance of breast MRI.

While evidence on the superior diagnostic performance of breast MRI in screening women at high risk has been available for several years (15–17), prospective multi-centric data for women with dense breasts have become available only recently and have confirmed superior sensitivity of 95.2% - 95.7% and reduced interval cancer rates of MRI-based screening compared to conventional approaches (18–20). Specificity increased in subsequent screening rounds (incidence rounds) as compared to the first screening round (prevalence round). In general, MRI-detected cancers were smaller than tumors detected by conventional mammography (21), and biologically aggressive cancers are more likely to be detected by MRI (22).

Besides requirements of efficacy, safety, and acceptance of screening, costs and potential benefits of screening programs need to be economically balanced (23). Innovative screening programs and expensive diagnostic tests are required to not only provide superior efficacy but also favorable economic effects (24). As a consequence, both short- and long-term costs and outcomes of screening are increasingly assessed by economic evaluations in order to capture their economic potential and to direct healthcare resource allocation accordingly. Cost-effectiveness analyses have evolved as an established framework for estimating economic value of innovative screening measures based on economic modeling and represent a prerequisite to establish funding by health insurance funds in various healthcare systems (25).

There are various methodological approaches with different outcome categories reported, hampering comparability of the findings and misleading economically inexperienced readers (26, 27).

However, for the various diagnostic modalities in breast imaging, each with different diagnostic potential and financial burden, cost-effectiveness analyses are particularly valuable and may help identify the most efficient medical care for each risk group.

Firstly, we introduce various methodologies of economic evaluations, explain the different approaches of outcome measurement and aim at developing a conceptual understanding of economic evaluations. Secondly, in the systematic review of the literature, the latest available evidence on cost-effectiveness of MRI-based breast cancer screening is discussed and evaluated.

A brief guide to economic evaluations

In health economics, evaluations are conducted to systematically compare different diagnostic or therapeutic strategies, e.g. the standard of care versus an innovative technique. Not only the costs of medical interventions can be considered but a certain "value" can be assigned to the outcomes. Capturing the value of diagnostic radiology can be challenging since diagnostic techniques only indirectly affect health care outcomes (28).

Empirical studies on the economic value of long-term patient journeys and health services administration are often not feasible due to associated costs and time constraints, and controlled experiments may be difficult to implement due to ethical and medical concerns. To overcome these limitations, the contemporary methodology is based on economic modeling and theoretical decision analysis that are applied to simulate the alternating diagnostic or therapeutic pathways, including all relevant medical costs and associated outcomes (24).

Measurement of costs

Various perspectives can be assumed to estimate costs, e.g. the perspective of the healthcare system, society or healthcare provider (29). Depending on the perspective, different costs have to be considered, e.g. direct medical costs including costs of treatment and personnel, indirect medical costs including transportation costs and intangible costs including non-monetary factors such as quality of life. For example, absence from work due to disease may result in productivity losses on the level of the economy that can be expressed in monetary terms.

Measurement of outcomes

There are various outcome categories applicable in economic modeling: Outcomes can be measured in monetary terms, in natural units such as mmHg blood pressure reduction, or life years gained. However, the heterogeneity of outcomes intrinsically limits comparability and transferability of the consecutive results.

Considering changes in life expectancy (life years gained) allows comparisons across various conditions. However, differences in quality of life (QoL) are neglected, e.g. due to side effects of

therapies. Therefore, quality-adjusted life years (QALYs) have evolved as a reference standard as well as generic means of outcome measurement. QALYs include both the quantity as well as quality of life time, obtainable by multiplying life time with quality of life (29). This way, generic outcomes can be compared between different diseases and therapies. Hence, QALYs have become the gold standard in measuring health care outcomes in cost-effectiveness- or cost-utility analyses.

When estimating outcomes in cost-effectiveness modeling, a practical concern is the availability of input variables to construct valid economic models. Data on quality of life are still scarce for many conditions, and the methodological variability of valuing utilities may limit their validity (30). It may be difficult to quantify the quality of life of any health state. Literature on quality of life is growing, and an increasing number of prospective study designs include QoL-measurements as well.

Types of economic evaluations

There are various types of economic evaluations (29): In cost-benefit analyses, outcomes are expressed in monetary terms. Cost-effectiveness analyses try to relate costs to natural outcomes such as reduction of cholesterol levels or life years gained. Cost-utility analyses use quality-adjusted life years as generic outcomes and are considered a reference standard as they enable comparisons across conditions based on a common denominator, i.e. QALYs. In the literature, the terms cost-effectiveness analysis and cost-utility analysis are often used interchangeably.

Decision analysis and Markov-Models

For an economic evaluation based on modeling, a decision tree is necessary including the therapeutic or diagnostic strategies and representing all possible outcomes. Each branch of the decision tree is assigned a predefined probability.

For each branch of the decision tree, a Markov-Model is used as a state-transition model to simulate costs and effects over a predefined time horizon (31). The Markov states are mutually exclusive and collectively exhaustive which means they represent all possible and necessary disease states. Patients may freely transition from one Markov state to another after each cycle, as they receive treatments or experience changes in health states. A Markov model is "memoryless", which means that the transition probabilities assigned to each Markov state do not depend on the history of prior Markov states but only on the current disease state. A fixed cycle length is chosen depending on the modeled disease entity. Associated costs and outcomes are assigned to each Markov state.

Economic modeling of breast cancer screening incorporates not only the costs and outcomes of screening tests and follow-ups, but also stage-dependent costs of therapeutic pathways and consecutive reductions in quality of life. True positive and true negative results are included as well as false negative and false positive findings.

An exemplary decision tree and Markov model to simulate breast cancer screening is depicted in Figure 1. All possible diagnostic outcomes are included: True positive, false negative, true negative and false positive findings. The set of Markov states needs to be differentiated enough to represent the variety of all possible disease states. However, it also needs to be simple enough to be based on valid estimates of input variables, in order to prevent from getting lost in assumptions on subgroups and pre-conditions. From a practical point of view, identification of valid point estimates for the input variables is crucial and depends on the quality of the underlying evidence.

Microsimulation models

While Markov models offer a means to simulate cohorts of patients based on cohort averages, and the simulation is memoryless by its classical definition ("Markov assumption"), microsimulation models represent an alternative technique.

Microsimulation is used to model individual patients' histories that are characterized by predefined variables and sets of rules (32), which is computationally more demanding. Due to their higher complexity, microsimulation models usually require a more thorough design, detailed and epidemiological input data as well as model validation. For instance, Microsimulation Screening Analysis (MISCAN) models have been designed to examine various cancer entities (33).

Incremental cost-effectiveness ratios and willingness to pay

When comparing alternative health care strategies, e.g. an innovative technique versus the established standard of care, additional costs per certain outcome are calculated and expressed as the ICER:

$$ICER = \frac{incremental\ \ cost}{incremental\ \ effectiveness}$$

$$= \frac{cost\ \ alternative\ \ therapy - cost\ \ standard\ \ therapy}{effectiveness\ \ alternative\ \ therapy - effectiveness\ \ standard\ \ therapy}$$

The ICER as a measure of cost-effectiveness can be used by decision makers to direct resource allocation in healthcare systems. The adoption of a new medical procedure is favored when the ICER falls below the WTP-threshold. There is a substantial global heterogeneity in the value of health and the resulting WTP for medical services. Thresholds differ between countries, healthcare systems and individual contexts, and depend on various factors such as reimbursement schemes, availability of services and resources, individual preferences and cultural factors. For instance, developing countries may not be comparable to developed countries, and there is substantial heterogeneity even within Western industrialized countries.

In the United States, a WTP-threshold between US-dollar (USD) 50,000 and USD 200,000 per QALY gained has been discussed, whereas a threshold of £ 20,000 - 30,000 has been adopted for the United Kingdom (34, 35). The world health organization has proposed to use the gross domestic product (GDP) per capita as a threshold that indicates high cost-effectiveness and 3 x the GDP indicating cost-effectiveness (36).

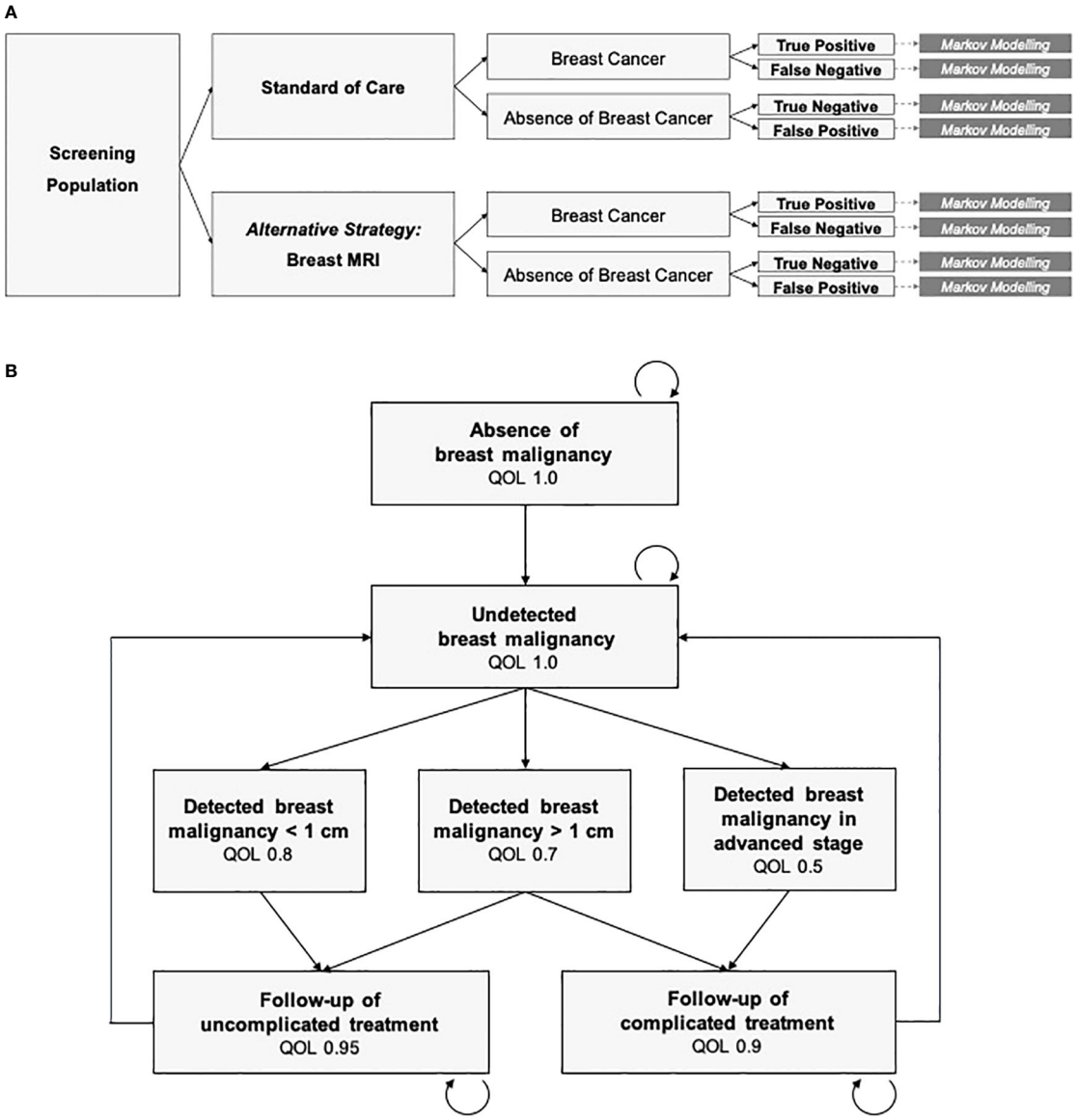

FIGURE 1
Decision analysis and economic modeling. **(A)** Decision tree including the diagnostic strategies (standard of care vs. breast MRI), ground truth (breast cancer vs. absence of breast cancer) and the diagnostic outcomes. Markov Modeling is conducted for each branch of the decision tree. **(B)** The Markov Model is defined by the mutually exclusive and collectively exhaustive Markov states, cycle length, transition probabilities, and the costs and quality of life (QOL) assigned to each state. Mortality is included in any state.

In a cost-effectiveness plane, incremental costs and effects of various strategies are displayed. In case a strategy achieves superior outcomes at reduced costs, the strategy is preferred (dominant strategy). In case a strategy achieves superior outcomes, but is associated with increased costs, the ICER is reflected by the slope of the line (Figure 2).

Sensitivity analyses

Sensitivity analyses are conducted in order to address variability of the input parameters and uncertainty in the model design, and to estimate robustness of model outcomes (37). Input variables are point estimates and often represent the population average. In a deterministic sensitivity analysis, an input variable is varied within a predefined range and the model outcomes are computed. For instance, a range of costs per breast MRI has been reported for different health care systems and providers, and depending on reader experience, sensitivity and specificity of breast MRI may vary. These uncertainties can be addressed by simulating outcomes for a range of possible input values.

However, most variables can not only be expressed by a population average, but follow a probability distribution in the respective population. Therefore, a Monte Carlo simulation is conducted by randomly assuming values from the probability distribution of every variable in the model simultaneously. In a probabilistic sensitivity analysis, the resulting cost-effectiveness is simulated for a significant number of iterations, e.g. 30,000 iterations.

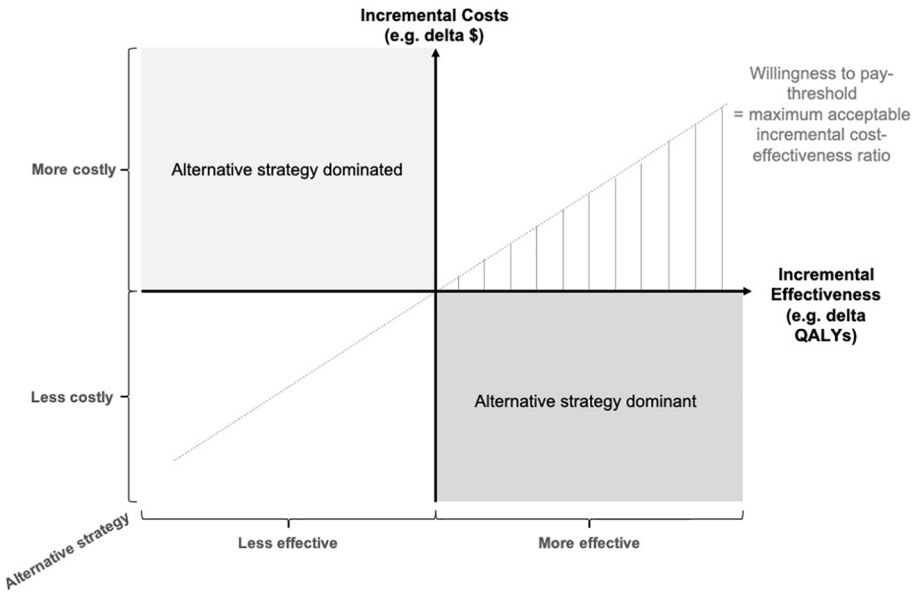

FIGURE 2
Cost-effectiveness plane. The incremental costs (e.g. in $) and incremental effects (e.g. in quality-adjusted life years, QALYs) of the alternative strategy, e.g. breast MRI, are computed to locate the strategy on the cost-effectiveness plane. In case it is more costly and less effective than the standard of care, the alternative strategy is dominated. In case of smaller costs and additional effectiveness, the alternative strategy is dominant. In the case of more effectiveness but more costs, the incremental cost-effectiveness ratio (ICER) has to be smaller than the willingness to pay (WTP) - threshold to be economically preferable.

Quality assurance and checklists

In order to maintain a high standard of quality, extensive recommendations on the methodological conduct of cost-effectiveness analyses have been defined (38). Checklists are available to evaluate adherence to these recommendations. For instance, the Consolidated Health Economic Evaluation Reporting Standards (CHEERS) Statement (39) is widely applied to ensure appropriate reporting (Supplementary Table S1).

Materials and methods: systematic review

In a systematic review of economic evaluations on breast MRI screening, the PubMed database was scanned for literature between January 1, 2000 and November 25th, 2022 (Figure 3). Key-words included "breast MR*", "breast cancer screening", "MR-mammography", "magnetic resonance imaging screening", and "cost effective", "cost-benefit", "economic evaluation", "cost-utility", or "cost". Economic evaluations including cost-effectiveness, cost-utility and cost-benefit analyses on breast cancer screening that applied screening MRI were included into analysis.

Results

In total, 1418 studies were identified on PubMed, 1360 were excluded during screening of abstracts and 33 were excluded during full-text analysis (Figure 3). Finally, 25 articles on economics of breast MRI screening of different risk groups were included into further analysis. The CHEERS checklist was used to assess the quality of the included studies (Supplementary Table S2). Various indications of MRI-based screening have been established in the past years (Table 1).

Cost-effectiveness of breast MRI in high-risk screening

The superior diagnostic performance of breast MRI in women at high risk of breast cancer has repeatedly been demonstrated over the past three decades. The most recent prospective multi-center trials confirm a superior sensitivity of 90 - 93% and a specificity of 89 - 98% in women at high risk of breast cancer, whereas mammography achieved a sensitivity of 33 - 50% and a specificity of 97 - 99% (15–17).

Based on the broad evidence available for several years, a number of economic evaluations have assessed the cost-effectiveness of breast MRI in the high-risk group (Table 2) (42–44, 48–60). The associated cost per breast MRI examination decreased over the previous years. For instance, in 2006 Plevritis et al. assumed a cost of USD 1,038, whereas contemporary analyses assume costs of USD 314 (48). They demonstrated that screening with mammography and MRI could be cost-effective especially for middle-aged breast cancer gene 1 (BRCA 1) mutation carriers vs. BRCA 2 mutation carriers with dense breast tissue at an ICER of USD 55,420 vs. USD 98,454 per QALY, respectively (48). In Canadian women with mutations in the BRCA 1 or 2 gene, alternating screening with conventional mammography and breast MRI every six months compared to annual mammography

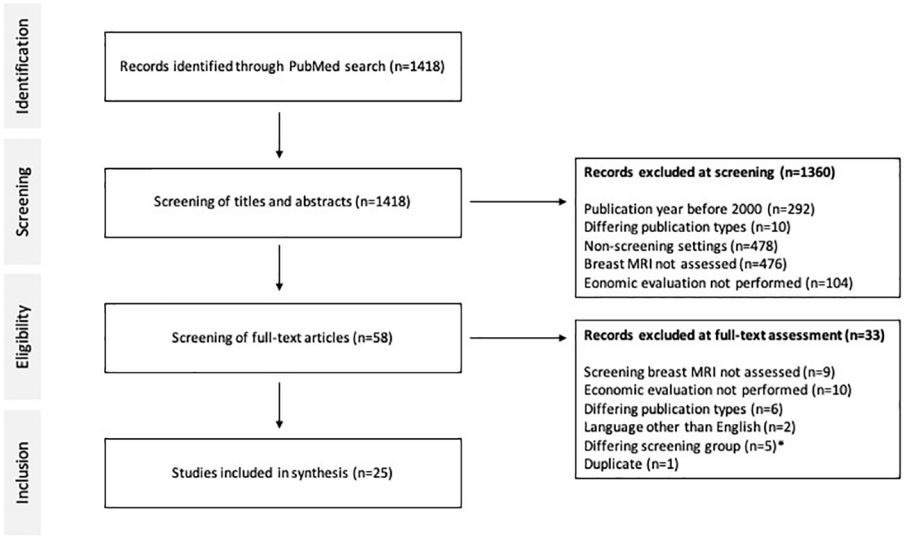

FIGURE 3
PRISMA diagram and literature search. Economic evaluations between 2000 and 2022 on breast MRI in screening of breast cancer were included. * Exceptional patient subgroups such as dialysis patients and childhood survivors of cancer or lymphoma were excluded.

TABLE 1 International recommendations for breast MRI in breast cancer screening stratified for risk groups.

Risk groups	ACS (2007 and 2015) (40, 41)	ACR (2017, 2018 and 2021) (42–44)	EUSOMA (2010) (45)	EUSOBI (2016 and 2022) (46, 47)
High risk	Annual MRI screening for: - *BRCA* mutation carriers and first-degree relatives - women with lifetime risk > 20-25% - Women with Li-Fraumeni, Cowden, Bannayan-Riley-Ruvalcaba syndromes and first-degree relatives (expert consensus) - women who had mantle radiotherapy under 30 years of age (expert consensus)	Annual MRI screening beginning at age 25-30 for: - women with genetics-based increased risk and untested first-degree relatives - women with history of chest radiation with cumulative dose of > 10 Gy before age 30 - lifetime risk > 20%	Annual screening for: - *BRCA1*, *BRCA2*, and *TP53* mutation carriers and first-degree relatives - women with lifetime risk > 20-30% and unclear mutation status (DoR-B) - women who had mantle radiotherapy under 30 years of age (DoR-B)	MRI-based screening according to national or international guidelines is favored
Intermediate risk	Insufficient evidence for - Women with 15-20% lifetime risk - lobular intraepithelial neoplasia, atypical ductal hyperplasia - heterogeneously or extremely dense breasts - personal history of breast cancer including DCIS	- Annual MRI for women with personal history of breast cancer and dense breast tissue or diagnosed before age 50 - MRI should be considered for women with history of breast cancer, LCIS or atypia on prior biopsy	n/a	Women with extremely dense breasts aged 50 - 70: - Supplemental screening - preferably by MRI - at least every 4 years, preferably every 2-3 years - MRI can be used as a stand-alone technique
average risk	women at lifetime risk< 15%: not recommended	No recommendation due to Insufficient evidence	n/a	n/a

n/a, Not applicable.

alone was cost-effective with an ICER of Canadian dollars (CAD) 50,900 per QALY gained (54).

Addressing the impact of specificity on cost-effectiveness of high-risk screening, Kaiser et al. have simulated the ICER for varying levels of specificity in women with high risk of breast cancer based on annual screening intervals (60). Compared to conventional mammography, breast MRI remained cost-effective at a WTP-threshold of USD 100,000 per QALY as long as the specificity did not drop below 86.7%.

Simulating various screening intervals and combinations of breast MRI and conventional mammography for the Dutch healthcare system, Geuzinge et al. found breast MRI in 18-month intervals between the ages of 35 and 60 years to be most cost-effective at an ICER of € 21,380 per QALY gained (59).

In a recent review including economic evaluations from 2006-2019, Li et al. proposed precision screening strategies tailored to age and individual risk from an economical perspective (61). Mammography and additional breast MRI were predominantly

TABLE 2 Economic evaluations on MRI-based breast cancer screening of women at high risk.

Study	Study population	Economic model; perspective	Comparators	Cost of MRI	Outcome measures	WTP-threshold
Plevritis et al. (48)	*BRCA 1* and *BRCA 2* mutation carriers	Monte Carlo (Microsimulation) Model, U.S. healthcare system	Annual mammography, annual supplemental breast MRI at different age ranges	USD 1,038 (2005)	Costs in USD, QALYs, LYs, ACER (cost/QALY), ICER (cost/QALY)	USD 100,000/QALY gained
Griebsch et al. (49)	Family history of breast cancer or *BRCA 1*, *BRCA 2* or *TP53* mutation carriers or 50% risk of inherited mutation	Markov Chain simulation, NHS, UK healthcare system	Annual breast MRI, mammography, or combination of both	£ 250 - 299 (2003/2004)	Costs in £, costs per cancer detected	n/a
Norman et al. (50)	*BRCA 1* mutation carriers	Markov Model, National Health Service, United Kingdom	No screening, annual mammography, breast MRI or combination of both, for different age groups	£ 224 (2006)	Costs in £, QALYs, ICER (cost/QALY)	£ 20,000/QALY gained
Moore et al. (51)	Women with 15% cumulative lifetime risk or higher	Markov Model, U.S. healthcare system	Annual mammography or breast MRI	USD 966 (2006)	Costs in USD, QALYs, ICER (cost/QALY)	USD 50,000 - 200,000/QALY gained
Taneja et al. (42)	*BRCA 1* and *BRCA 2* mutation carriers, other high-risk characteristics (lifetime risk ≥20%)	Decision analytic Model, U.S. healthcare system	Annual breast MRI, mammography and combination of both	USD 1,038 initially, USD 787 for follow-up screening (2005)	Costs in CAD, life expectancy, QALYs, ICER (cost/QALY)	n/a
Lee et al. (43)	*BRCA 1* mutation carriers	Markov Model, societal perspective	Annual mammography, breast MRI or combination of both	USD 577 (2007)	Costs in USD, LYs, QALYs, ICER (cost/QALY)	USD 100,000 and 50,000/QALY gained
Grann et al. (44)	*BRCA 1* and *BRCA 2* mutation carriers	Markov Model, U.S. healthcare system	Annual mammography with and without breast MRI	USD 1,219 initially, USD 940 for short interval follow-up (2009)	Costs in USD, QALYs, ICER (cost/QALY)	n/a
Cott Chubiz et al. (52)	*BRCA 1* and *BRCA 2* mutation carriers	Markov Monte Carlo Model, U.S. healthcare system	Annual mammography and breast MRI starting at different ages	USD 619 (2010)	Costs in USD, QALYs, ICER (cost/QALY)	n/a
De Bock et al. (53)	*BRCA 1* and *BRCA 2* mutation carriers	Microsimulation Model, Dutch and UK healthcare system	Different combinations of mammography and MRI	€ 227 (2013) or £ 220 (2007)	Costs in € or £, LYG, incremental costs per LYG	€ 20,000/LYG and £ 25,000/LYG
Pataky et al. (54)	*BRCA 1* and *BRCA 2* mutation carriers	Markov Model, Canadian healthcare system	Annual mammography, annual supplemental breast MRI	CAD 277 (2008)	Costs in CAD, QALYs, ACER (cost/QALY), ICER (cost/QALY)	CAD 100,000 and 50,000/QALY gained
Saadatmand et al. (55)	Women with familial risk	Microsimulation Model, Dutch healthcare system	Mammography, breast MRI, at different intervals	USD 485 (2013)	Costs in USD and €, LYG, average and incremental costs/LYG	n/a
Ahern et al. (56)	Women at high risk, different life-time risk thresholds	Microsimulation Model, U.S. healthcare system	Annual or biennial breast MRI and mammography at 6-, 12- or 24-month intervals	USD 728 (2012)	Costs in USD, QALYs, LY, ICER (cost/QALY)	USD 100,000/QALY gained
Obdeijn et al. (57)	*BRCA 1* mutation carriers	Microsimulation model, Dutch screening program	Dutch screening guidelines with MRI and mammography, modified protocol with mammography postponed to age 40	€ 368 (2016)	Costs in €, LYG, incremental costs/LYG	n/a

(Continued)

TABLE 2 Continued

Study	Study population	Economic model; perspective	Comparators	Cost of MRI	Outcome measures	WTP-threshold
Phi et al. (58)	*BRCA 1* and *BRCA 2* mutation carriers aged 60-74	Microsimulation model, Dutch screening program	Annual mammography, breast MRI, different combinations and screening intervals for women with dense breasts or all women	€ 168 (2017)	Costs in €, LYG, costs/LYG	€ 20,000/life year gained
Geuzinge et al. (59)	Women with 20% or more familial risk without a known *BRCA1/2* or *TP53* mutation	Microsimulation model, Dutch healthcare system	Annual mammography, breast MRI, with various intervals and age groups	€ 272 (2018)	Costs in €, LY, QALYs, ICER (cost/QALY)	€ 22,000/ QALY gained
Kaiser et al. (60)	Women at high risk	Markov Model, U.S. healthcare system	Annual mammography, ultrasound, mammography and ultrasound, breast MRI	USD 385 (2021)	Costs in USD, QALYs, ICER (cost/QALY)	USD 100,000/ QALY gained

n/a, Not applicable.

cost-effective for BRCA1 mutation carriers in middle-age groups, whereas additional breast MRI was not cost-effective for BRCA 2 mutation carriers.

Cost-effectiveness of breast MRI in intermediate-risk screening

MRI screening in women with dense breast tissue, i.e. intermediate risk for breast cancer, has recently demonstrated excellent outcomes. In the DENSE trial, women with extremely dense breast tissue were offered supplemental MRI screening in the Netherlands. The cancer detection rate dropped from 16.5 per 1000 examinations in the first round to 5.8 per 1000 in the second screening round (18, 19). At the same time the false positive rate decreased from 79.8 to 26.3 per 1000 examinations.

Kaiser et al. have first demonstrated the favorable economic value of breast MRI as a screening technique in women with extremely dense breast tissue (62) based on the findings from the first round of the DENSE study (Table 3). Compared to conventional mammography, they calculated an ICER of USD 8,798 per QALY gained for biennial screening with breast MRI.

Considering the shift in diagnostic performance of breast MRI in the second screening round, i.e. increased specificity of breast

TABLE 3 Economic evaluations on MRI-based breast cancer screening of women at intermediate risk due to elevated breast tissue density.

Study	Study population	Economic model; perspective	Comparators	Cost of MRI	Outcome measures	WTP-threshold
Kaiser et al. (60)	Women with extremely dense breast tissue	Markov Model, US healthcare system	Biennial breast MRI or mammography	USD 385 (2021)	Costs in USD, QALYs, ICER (cost/QALY)	USD 100,000/ QALY gained
Tollens et al. (63)	Women with heterogeneously and extremely dense breast tissue	Markov Model, US healthcare system	Biennial abbreviated protocol breast MRI or DBT	USD 314 (2021)	Costs in USD, QALYs, ICER (cost/QALY)	USD 100,000/ QALY gained
Tollens et al. (63)	Women with extremely dense breast tissue	Markov Model, US healthcare system	Biennial mammography or breast MRI	USD 314 (2021)	Costs in USD, QALYs, ICER (cost/QALY)	USD 100,000/ QALY gained
Geuzinge et al. (64)	Women with extremely dense breast tissue	Microsimulation model, Dutch screening program	Breast MRI, mammography, and combinations thereof, different screening intervals	€ 272 (2018)	Costs in €, number of breast cancers, life years gained, breast cancer deaths, overdiagnosis, QALYs, ICER (cost/QALY)	€ 22,000/ QALY gained
Wang et al. (65)	Women with heterogeneously and extremely dense breast tissue	Microsimulation model, Dutch screening program	Abbreviated protocol breast MRI, conventional mammography, and combinations thereof, different screening intervals	€ 272 (2019)	Costs in €, breast cancer deaths, LYG, incremental cost/LYG, average cost/LYG	€ 20,000/ LY gained
Tollens et al. (66)	Women with dense breast tissue	Markov model, US healthcare system	Biennial breast MRI, full diagnostic protocols vs. abbreviated protocols	USD 314 (2022)	Costs in USD, QALYs, ICER (cost/QALY)	USD 100,000/ QALY gained

MRI reported by the DENSE study group, as well as the reduced cancer detection rate of the second screening round (incidence round) compared to the first round (prevalence round), Tollens et al. confirmed the cost-effectiveness of breast MRI in this patient collective with a further refined Markov-Model (63). When the reduced false positive rate and cancer detection rate from the second screening round are projected on subsequent screening rounds, the reported ICER dropped from USD 38,849 to USD 13,493 per QALY. The authors concluded that the reduced false positive findings and reduced associated follow-up costs outweighed the reduced cancer detection rate from an economic perspective.

Long-term outcomes were also simulated by microsimulation modeling (MISCAN) based on the DENSE trial data and estimated cost-effectiveness of screening women with extremely dense breasts (64). Comparing biennial MRI to biennial mammography would save 8.6 additional lives per 1,000 women invited and cost € 22,500 per QALY gained. In this simulation, MRI screening alone every 4 years saved 7.6 additional lives per 1,000 women at a cost of € 11,500 per QALY gained.

Examining the economic potential of abbreviating MRI protocols for breast cancer screening patients of intermediate risk, evidence on diagnostic performance is scarce. Comparing abbreviated breast MRI to digital breast tomosynthesis (DBT) in women with dense breasts and extremely dense breasts, the EA1411 ECOG-ACRIN study determined a cancer detection rate of 11.8 per 1000 examinations for abbreviated breast-MRI (AB-MRI) and 4.8 per 1000 for DBT (20). No interval cancers were observed. Comstock et al. reported similar levels of sensitivity, yet reduced levels of specificity of AB-MRI.

A simulation of long-term costs and outcomes by Tollens et al. (66) based on the data of Comstock et al. confirmed the cost-effectiveness of AB-MRI in screening women of intermediate risk for breast cancer, including increased false positive findings of abbreviated examinations. As long as the cost of AB-MRI did not exceed 82% of the cost of a full protocol examination, AB-MRI should be considered the cost-effective alternative.

In women with heterogeneously and extremely dense breasts, MRI screening with abbreviated protocols was cost-effective across a wide range of plausible costs per examination when compared to DBT (67). When varying the assumed cost per examination, abbreviated breast MRI was cost-effective below USD 593 and cost-saving below USD 241 compared to DBT.

Wang et al. used the SiMRiSc microsimulation model to compare different screening scenarios including conventional mammography and abbreviated breast MRI in screening women with dense breasts in the Netherlands (65). Costs associated with implementation of a screening program, the involution of breast tissue over time, and radiation-induced tumors were incorporated as well. Biennial MRI screening from 50 - 65 years plus mammography from 66 - 74 years for women with extremely dense breasts was identified as the optimal strategy at an ICER of € 18,201 per life year gained (LYG). Other screening scenarios applying more extensive MRI screening, e.g. biennial MRI from 50 - 74 years, achieved even more LYG and smaller interval cancer rates, yet at an ICER above the predefined WTP-threshold of € 20,000 per LYG.

Cost-effectiveness of breast MRI in average-risk screening

As of today, data on the diagnostic performance of breast MRI in average-risk collectives is limited.

Screening women with average risk of breast cancer with supplemental breast MRI, including women with dense breast tissue, Kuhl et al. found a supplemental cancer detection rate of 15.5 per 1000 cases, with a median size of MRI-detected tumors of 8 mm and no interval cancers in the collective of 2120 women with an observation period of 7007 women-years (68).

Based on these data, a recent cost-benefit analysis (Table 4) simulating screening costs only has indicated that despite higher costs in the short run, triennial MRI screening of women at average risk could be cost-saving compared to annual mammography after 6 years, assuming costs per MRI of USD 400 (69).

Discussion

Indications of MRI-based screening have gradually been extended over the last 20 years (Table 1) along with increasing evidence on improved cancer detection rates of breast MRI in different risk groups (40, 41, 45–47, 70–72).

With evidence on a high specificity of breast MRI in expert hands (12–14), as well as evidence against adverse effects when using repetitive macrocyclic contrast media-enhanced breast MRI for screening (73), financial concerns represent the main obstacle to an increased application of breast MRI in screening women beyond the subgroup of women at high risk. Along with increasing evidence on the safety and efficacy of MRI-based breast cancer screening in women at intermediate risk, the technique has demonstrated to be cost-effective in a variety of indications and conditions that have not yet been implemented in population screening programs. Randomized controlled studies on MRI-based breast cancer screening in women at average risk are unavailable so far.

TABLE 4 Economic evaluation on MRI-based breast cancer screening of women at average risk.

Study	Study population	Economic model; perspective	Comparators	Cost of MRI	Outcome measures	WTP-threshold
Mango et al. (69)	Women at average risk	Monte Carlo simulation model (cost-benefit analysis), US healthcare system	Triennial breast MRI, annual conventional mammography	USD 550 (2019)	Screening costs in USD	n/a

n/a, Not applicable.

Major determinants of cost-effectiveness in screening of various risk groups using breast MRI have been identified, with examination costs being identified as the most potent driver of cost-effectiveness. Diagnostic performance, incidence and prevalence rates could be identified as major determinants as well. However, due to heterogeneity of the modeling approaches, they often cannot be quantitatively compared.

Impact of diagnostic performance

While early studies on the diagnostic performance of breast MRI in high-risk screening have indicated lower levels of sensitivity and specificity of 46 - 77% and 81 - 95%, respectively (74–76), the most recent prospective multi-center trials confirmed a largely superior sensitivity of 90 - 93% and a specificity of 89 - 98% (15–17). These shifts in diagnostic performance may be attributable to premature technique as well as initially limited experience with the new technique. At the same time, examination costs have gradually declined over the previous years. Therefore, initial economic evaluations need to be interpreted in the light of their input parameters and assumptions.

While excellent sensitivity is considered a prerequisite for effective screening, generally accomplished by breast MRI (15–17), specificity has been identified as a major determinant for the economic success of MRI screening. This has particular importance for breast cancer screening as positive findings often result in invasive procedures such as biopsies and surgeries, often associated with psychological burden and significant costs.

Quality assurance, benchmarking and performance metrics should be monitored when designing future cost-effective screening programs, since optimal specificity relies on high-quality imaging and image interpretation (77, 78). Multicentric evaluation has shown that different decision algorithms, such as the Kaiser score, can substantially help to improve the specificity of breast MRI (79–81) and compensate for reader experience to some degree (82).

Facets of economic evaluations

From an economical point of view, the costs of setting up a screening program and performing the first screening rounds (prevalence rounds) are initially higher, but decrease over time as initial expenses include training, quality assurance and supervision. The prevalence rounds are known to yield more false positive findings, i.e. more recommendations for biopsies as the stability of equivocal lesions cannot be determined without prior imaging.

In subsequent screening rounds, specificity therefore increases and less false positives are observed (15, 19). As a consequence, the costs of MRI-based screening are higher for women entering screening programs. To capture all economic effects of screening, the stage and nodal status of MRI-detected cancers need to be considered in economic modeling as well as reduced costs for treatment and long-term follow-up. Comprehensive economic evaluations need to account for these short- and long-term effects in order to yield valid conclusions.

Other factors that improve cost-effectiveness of breast MRI are high prevalence and incidence, i.e. higher risk of breast cancer, that can be influenced by further refining the screening population by more sophisticated risk models.

Overdiagnosis

Overdiagnosis refers to the detection of clinically insignificant breast cancer that does not have an impact on a woman's life expectancy. Concerns on overdiagnosis have been raised after the incidence of invasive breast cancer and ductal carcinoma *in situ* (DCIS) increased particularly in early mammography screening rounds (9, 83). As the significance of cancer currently cannot be distinguished with any reliability by histology and cannot be identified on an individual level, a number of women potentially receive unnecessary work-up and therapy. However, when adjusting for breast cancer risk and lead time, most plausible estimates of overdiagnosis due to screening mammography range between 1% to 10% (84, 85).

At the same time, underdiagnosis represents a major challenge and many women are underserved by conventional screening as evidenced by breast cancer morbidity and mortality statistics. Underdiagnosis hereby is defined as not detecting a present cancer, i.e. false-negative finding. Notably, MRI preferentially detects more aggressive tumors (22) and may be used to predict the course of disease (86), thereby providing an angle for exploring strategies to escalate or de-escalate treatment.

Modeling overdiagnosis remains a challenge in economic evaluations as evidence on breast MRI screening is limited and accurate numbers are scarce. Several microsimulation studies have incorporated estimates of overdiagnosis (59, 64).

Extrapolation from real-world data

Model-based economic evaluations provide valuable insights by simulating long-term costs and outcomes and by modeling different screening strategies for the purpose of decision analysis. However, the more economic models rely on extrapolations from real-world data, the more the validity of the findings may be limited. The results should therefore be interpreted with caution.

For instance, when various screening intervals are simulated in economic modeling, the outcomes are not directly based on empirical evidence. Economic models should be designed to rely on real-world scientific evidence as much as possible, in order for the results to not represent artificial interrelations depending on the modeling approach. For instance, the longer the screening interval, the lower the costs of screening. If increased interval cancer rates and advanced disease stages of belated diagnoses are not properly accounted for, the resulting ICER may be artificially low for prolonged screening intervals. This could potentially result in an endorsement of longer screening intervals that is not directly based on empirical outcomes, which is why a cautious interpretation is advised (87). Further, prolonged screening intervals may affect

attendance rates of screening and women's' psychological comfort, which might result in unforeseen but relevant economic effects. Therefore, recommendations on the length of screening intervals should not be derived from economic simulations alone.

Along with the development of breast MRI as a screening technique, the methodology of economic evaluations has evolved as well. While early cost-effectiveness analyses applied various techniques such as Monte Carlo simulations based on spreadsheet programs and statistics software, dedicated software for economic modeling has become state of the art for contemporary cost-effectiveness analyses. Markov Modeling has been established as a robust economic approach in contrast to Microsimulation models (e.g. MISCAN) that have a strength in accurately modeling epidemiological contexts.

Note on abbreviation

Examination costs depend on various healthcare policy factors, including reimbursement schemes and organization as well as the funding of a screening program. Since small cost reductions have a significant impact on the cost-effectiveness of a technique, there have been many attempts to streamline workflows, reduce non-value added time, and to reduce acquisition and image reading times of breast MRI.

Abbreviated breast MRI, i.e. restricting the number of sequences in breast MRI to an essential "abbreviated" limit, has been proposed as a means to reduce the costs of MR-based screening. Although initially defined as solely pre- and post-contrast sequences with subtracted and maximum-intensity projection images (71, 72), a variety of abbreviated protocols have recently been proposed in clinical studies (73–76) that reported varying levels of specificity. So far, however, a standard definition of abbreviated protocols has not been achieved, resulting in a heterogeneous diagnostic landscape, highly individual abbreviation approaches (17, 77) and - consecutively - in varying results regarding economic potential and implications.

While, likely due to small study and patient selection bias, a similar diagnostic performance compared to full diagnostic protocols was reported in the majority of retrospective studies (88), abbreviated breast MRI suffered from reduced specificity in the most recent prospective multi-center trial (20). High-level evidence on the diagnostic performance of different degrees of protocol abbreviation remains scarce. This is why caution is advised when implementing abbreviated protocols.

So far, the cost of abbreviated breast MRI has not been assigned a fixed reimbursement. First experiences of implementing abbreviated breast MRI as a self-played, supplemental screening tool in the U.S. have shown that three examinations per hour may be considered feasible instead of one full diagnostic protocol, with a scan time of less than 10 minutes at USD 250 per examination (89).

At the same time, innovative techniques such as parallel imaging and deep learning-based reconstruction algorithms (90, 91) have reduced examination times of full diagnostic protocols that effectively overlap with the definition of AB-MRI without a detrimental effect on diagnostic performance. For example, a full diagnostic protocol at our institution including T2w imaging, DWI and dynamic contrast enhanced sequences with pre- and 5 post-injection series is acquired in less than 10 minutes (Magnetom Sola, 16 channel coil, Siemens Healthineers), which meets the most common requirements of an abbreviated protocol in terms of acquisition time, yet offers access to the full diagnostic accuracy of breast MRI.

Limitations of economic modeling

Economic evaluations are afflicted with well-known methodological constraints. The technique is based on a utilitarian approach (92). Large effects and benefits are valued more than smaller effects regardless of the affected patients and the actual needs of those patients. For instance, treatment-related health outcomes in young patients with mild chronic conditions may be substantially larger than health outcomes of oncologic patients in end-of-life conditions which may raise ethical concerns on equity and fairness.

Model-based analyses rely on a simplification of complex clinical pathways and heterogenous patient groups that have to be translated into economical models. In reality, adherence to screening recommendations varies and women enter screening programs at different points in time, skip or prolong screening intervals and deviate from therapeutic and diagnostic pathways projected in economic models.

The validity of the modeled costs and effects depends on the quality of input data. However, as high-quality data on costs and outcomes are scarce, applicability to different contexts is limited. Many cost-effectiveness analyses lack calibration of input parameters and external validation and are therefore prone to bias (93).

Conclusion

With increasing evidence on the efficacy and safety of MRI-based breast cancer screening, available cost-effectiveness analyses indicate a strongly favorable economic value compared to conventional screening for a variety of risk groups.

MRI screening is expected to be extended from women with high risk of breast cancer towards women with dense breast tissue. Cost-effectiveness of breast MRI screening in women with dense breast tissue could be demonstrated based on the most recent evidence from prospective multi-center trials. However, further studies are necessary to evaluate the outcomes and cost-effectiveness of screening women at average risk.

Author contributions

FT: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. PB: Supervision, Writing – review & editing. MF: Methodology, Supervision, Writing – review & editing. CK:

Conceptualization, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1292268/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 08 January 2024
DOI 10.3389/fonc.2023.1320867

OPEN ACCESS

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RECEIVED 13 October 2023
ACCEPTED 13 December 2023
PUBLISHED 08 January 2024

CITATION
Li C, Zhang P, Lv J, Dong W, Hu B, Zhang J and Zhu H (2024) Axillary management in patients with clinical node-negative early breast cancer and positive sentinel lymph node: a systematic review and meta-analysis. *Front. Oncol.* 13:1320867. doi: 10.3389/fonc.2023.1320867

Axillary management in patients with clinical node-negative early breast cancer and positive sentinel lymph node: a systematic review and meta-analysis

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Background: The omission of axillary lymph node dissection (ALND) or axillary radiation (AxRT) remains controversial in patients with clinical node-negative early breast cancer and a positive sentinel lymph node.

Methods: We conducted a comprehensive review by searching PubMed, Embase, Web of Science, and Cochrane databases (up to November 2023). Our primary outcomes were overall survival (OS), disease-free survival (DFS), locoregional recurrence (LRR), and axillary recurrence (AR).

Results: We included 26 studies encompassing 145,548 women with clinical node-negative early breast cancer and positive sentinel lymph node. Pooled data revealed no significant differences between ALND and sentinel lymph node biopsy (SLNB) alone in terms of OS (hazard ratio [HR]0.99, 95% confidence interval [CI] 0.91-1.08, p=0.84), DFS (HR 1.04, 95% CI 0.90-1.19, p=0.61), LRR (HR 0.76, 95% CI 0.45-1.20, p=0.31), and AR (HR 1.01, 95% CI 0.99-1.03, p=0.35). Similarly, no significant differences were observed between AxRT and SLNB alone for OS (HR 0.57, 95% CI 0.32-1.02, p=0.06) and DFS (HR 0.52, 95% CI 0.26-1.05, p=0.07). When comparing AxRT and ALND, a trend towards higher OS was observed the AxRT group (HR 0.08, 95% CI 0.67-1.15), but the difference did not reach statistical significance (p=0.35, I^2 = 0%). Additionally, no significant differences significance observed for DFS or AR (p=0.13 and p=0.73, respectively) between the AxRT and ALND groups.

Conclusion: Our findings suggest that survival and recurrence rates are not inferior in patients with clinical node-negative early breast cancer and a positive sentinel lymph node who receive SLNB alone compared to those undergoing ALND or AxRT.

KEYWORDS

breast cancer, sentinel lymph node biopsy, axillary lymph node dissection, axillary radiation, axillary management

Introduction

Axillary lymph node dissection (ALND) has been the standard therapeutic approach for breast cancer patients with positive sentinel lymph nodes. However, ALND is associated with various complications, including lymphedema, paresthesia, infections, axillary seromas, and other significant morbidities (1). Currently, sentinel lymph node biopsy (SLNB) is recommended for assessing axillary nodal lymph node status in early breast cancer patients who are clinically node-negative. The National Comprehensive Cancer Network (NCCN) suggests that ALND is not required for breast cancer patients with a negative sentinel node. The role of ALND for early-stage breast cancer patients with a limited number of metastatic sentinel lymph nodes remains controversial. According to the American Society of Clinical Oncology Clinical Practice Guideline, ALND should be considered for women with early breast cancer and one to two positive sentinel lymph nodes who are planning to undergo mastectomy (2). Pepels et al. indicated that ALND was recommended in patients with sentinel micrometastases and unfavorable tumor characteristics (3), while no ALND for patients with sentinel lymph nodes micrometastases resulted in a higher five -year regional recurrence rate compared to ALND (4).

However, Galimberti et al. suggest that ALND may be overtreatment for early-stage breast cancer patients, particularly when the tumor burden in the sentinel lymph nodes is minimal or moderate (5). NCCN also suggests that patients who have T1/T2 tumors, one to two positive sentinel lymph nodes, and plan to undergo whole-breast radiotherapy (RT) following breast-conserving therapy are not recommended for ALND (6). The ACOSOG Z0011 (American College of Surgeons Oncology Group) trial demonstrated that patients with clinical T1/T2 tumors and fewer than three positive sentinel lymph nodes undergoing lumpectomy and whole-breast radiation therapy could avoid ALND without negatively impacting local recurrence, disease-free survival, and overall survival (7).Additionally, the IBCSG 23-01 trial, designed to compare outcomes in patients with one or more sentinel micrometastases (≤2 mm) treated with ALND versus no ALND, showed no significant differences in five-year overall survival and five-year disease-free survival (8). A previous retrospective study also indicated that ALND did not improve either post-mastectomy overall survival or disease-free survival among breast cancer patients with one to three positive sentinel lymph nodes (9).

The AMAROS trial aimed to evaluate whether axillary radiation (AxRT) achieved better regional control and fewer side effects compared to ALND. Finding demonstrated that AxRT offered similar axillary control for patients with T1/T2 breast cancer and positive sentinel lymph nodes, while significantly reducing. the occurrence of lymphedema (10). A retrospective cohort study comparing patients with T1/T2 and less than two macrometastases (>2 mm) who underwent either AxRT or non-AxRT also observed similar overall and disease-free survival rates. There was no statistically significant difference in the five-year outcomes between the two groups (11). Motivated by these finding, we conducted a systematic review to compare outcomes in clinical node-negative early breast cancer patients with sentinel lymph node metastasis who underwent mastectomy or breast-conserving surgery. This meta-analysis aims to assess overall survival, disease-free survival, locoregional recurrence and axillary recurrence according to the type of axillary management(SLNB alone, ALND, or AxRT).

Materials and methods

Study selection

We conducted a systematic search of English literature in PubMed, Embase and Cochrane Library databases up to November 2023. Our search encompassed published data only. Search terms included keywords and MeSH terms such as “breast cancer/breast carcinoma”, “sentinel lymph node biopsy,” “axillary lymph node dissection”, and “axillary radiation,” Two authors (C.Z.L and P.Z) independently reviewed the available literature based on the inclusion criteria. Subsequently, potentially relevant references with sufficient information in their titles and abstracts were retrieved full-text article assessment. If the included studies were based on the same data, we selected the latest published version. Any disagreements were resolved through discussion and consensus among the authors. The study selection process adhered to the PRISMA guidelines.

Study inclusion and exclusion criteria

The inclusion criterial were as follows: (1) Design: randomized controlled trials (RCTs) and retrospective studies. (2) Patient eligibility: Studies enrolling patients with clinical node-negative early breast cancer and positive sentinel lymph node (3) Comparative interventions: SLNB alone versus ALND, ALND versus AxRT, and SLNB alone versus AxRT. (4) Outcomes: Studies reporting on overall survival, disease-free survival, axillary recurrence, and locoregional recurrence. The exclusion criterial included abstracts, reviews, case reports, and articles deemed irrelevant or containing missing data.

Data extraction and management

Following the Cochrane Handbook guidelines, two authors independently extracted data from the included studies. Recorded information included the authors’ names, publication year, number of participants, study design, intervention type, tumor stage, micometastasis or macrometastsis count, adjuvant radiation therapy, follow-up duration (years), outcomes, and the quality of evidence in each study. Any discrepancies were addressed and resolved through discussion or with the assistance of a third author.

Quality assessment in individual studies

The risk of bias in all included studies was evaluated using guidelines in the Cochrane Handbook for Systematic Reviews of

Interventions (https://training.cochrane.org/handbooks) (12). Two authors independently assessed the potential risk of bias, including selection bias, performance bias, detection bias, attrition bias, reporting bias, and confounding bias, and other sources of bias. For RCTs, the GRADEpro GDT (Grading of Recommendation Assessment Development and Evaluation Profiler Guide line Development Tool)was used to assess evidence quality. This online too, available at https://www.gradepro.org, evaluates five factors: risk of bias, inconsistency, indirectness, imprecision, and other considerations. Based on these factors, evidence quality is classified into four levels: high (⊕⊕⊕⊕), moderate (⊕⊕⊕⊖), low (⊕⊕⊖⊖) or very low (⊕⊖⊖⊖). For non-RCTs, the Newcastle-Ottawa Scale (NOS) served as the quality assessment tool (13). The NOS awards stars based on three domains: quality of patient selection (up to four stars), comparability between cases and controls (up to two stars), and adequate ascertainment of exposure (up to three stars). Studies with more than seven stars were considered to have a high level of evidence. Two authors independently assessed the quality of evidence in the included studies, With any disagreements resolved through discussion.

Statistical analysis

We used the Review Manager software (version 5.4), update by the Cochrane Library for Systematic Review, to perform the analysis. The summary statistic of generic inverse variance (overall survival, disease-free survival, axillary recurrence, and locoregional recurrence) was assessed by hazard ratios (HRs). 95% confidence intervals (CIs) were calculated using the fixed-effect model. The statistical heterogeneity of the included studies was quantified and examined using the I^2 statistics. An I^2 value of 0% to 25% indicates low heterogeneity, 25% to50% indicates moderate heterogeneity, 50% to75% indicates large heterogeneity, and 75% to100% indicates huge heterogeneity (14). When heterogeneity was observed, we employed the random-effects model. We conducted the subgroup analysis based on the type of axillary management (SLNB alone, ALND, and AxRT). Sensitivity analysis was performed to identify sources of heterogeneity. A funnel plot was used to assess publication bias in the included studies. A p value less than 0.05 was considered statistically significant.

Results

Study selection

Our initial database search yielded a total of 4,714 studies. After removing 504 duplicate studies, we screened the titles and abstracts of 4,210remaining studies. A total of 4,124 studies were excluded due to irrelevance (non-related studies, review articles, case reports, meta-analysis, or lack of data). At the full-text level, 86 potentially eligible studies were assessed, t of which 60 were ultimately excluded after a thorough review. This left 26 studies involving 145,548 patients for inclusion in the meta-analysis (5, 7, 11, 15–36) The PRISMA flowchart in Figure 1 illustrates this selection process.

Study characteristics

Table 1 outlines the characteristics of the included studies. Eighteen studies were retrospective cohort studies (11, 18–27, 34–36), while the remaining eight were RCTs (5, 7, 28–33). The studies were published between April 2009 to July 2023.Sample sizes range from 121 to 97,314 patients, with a total of 145,548 patients analyzed. Among these patients, 40,156 received SLNB alone, while 105,418 underwent either ALND or AxRT. All included studies were published in English. Twenty-two studies reported overall survival data (5, 7, 11, 15–27, 29, 30, 32, 33, 35). Eleven studies reported disease-free survival data (5, 7, 11, 15, 23, 30–35). Six studies analyzed locoregional recurrence (21, 26, 28, 35, 36), and eight studies reported axillary recurrence (11, 22, 24, 26, 30, 32, 33, 35).

Quality assessment

The NOS was used to assess the quality of evidence in non-RCT trials. Five studies received a rating of seven or more stars, indicating high quality (15, 16, 19, 21, 35).Seven studies received a rating of six stars (11, 20, 22, 23, 26, 34, 36), and six studies were assessed as having four to five stars (See Table 1). The GRADEpro GDT tool was used to classify the evidence of RCTs comparing ALND to SLNB alone for patients with clinical node-negative early-stage breast cancer and positive sentinel lymph nodes. The quality of evidence was high for disease-free survival and locoregional recurrence, and moderate in overall survival (See Table 2).

Effect of ALND versus SLNB alone

Eighteen studies (5, 7, 15–27, 29, 32, 35) reported the overall survival data for patients with SLNB alone versus ALND. The pooled results revealed no statistically significant difference between the groups (HR0.99, 95% CI0.91-1.08; p=0.84), and no significant heterogeneity was observed (I^2 = 30%, p=0.11) (Figure 2A). A potential publication bias was suggested by the the asymmetry of the funnel plot (Figure 3A), Subgroup analysis showed no statistically significant difference in overall survival between SLNB alone and ALND in either RCTs (HR 1.09, 95% CI:0.92-1.28; p=0.33) or retrospective studies (HR 0.96, 95% CI:0.86-1.06; p=0.40). Data pooled from the eight studies (5, 7, 15, 20, 29, 31, 32, 35) demonstrated no substantial difference in disease-free survival between the SLNB alone group and ALND groups (HR 1.04, 95%CI:0.90-1.19; p=0.61). Additionally, the studies showed no significant heterogeneity (I^2 = 0%, p=0.73) (Figure 2B). The funnel plot suggested the presence of publication bias (Figure 3B). Subgroup analysis revealed no significant difference in disease-free survival between RCTs (HR 1.03, 95%CI:0.89-1.19; p=0.72) or retrospective studies (HR 1.09, 95%CI:0.77-1.54; p=0.64).

Five studies evaluated locoregional recurrence (5, 21, 28, 35, 36). Pooled data indicated no statistically significant difference between patients who received SLNB alone and those who underwent ALND (HR 0.76, 95%CI 0.45-1.29; p = 0.31) (Figure 2C).However, the

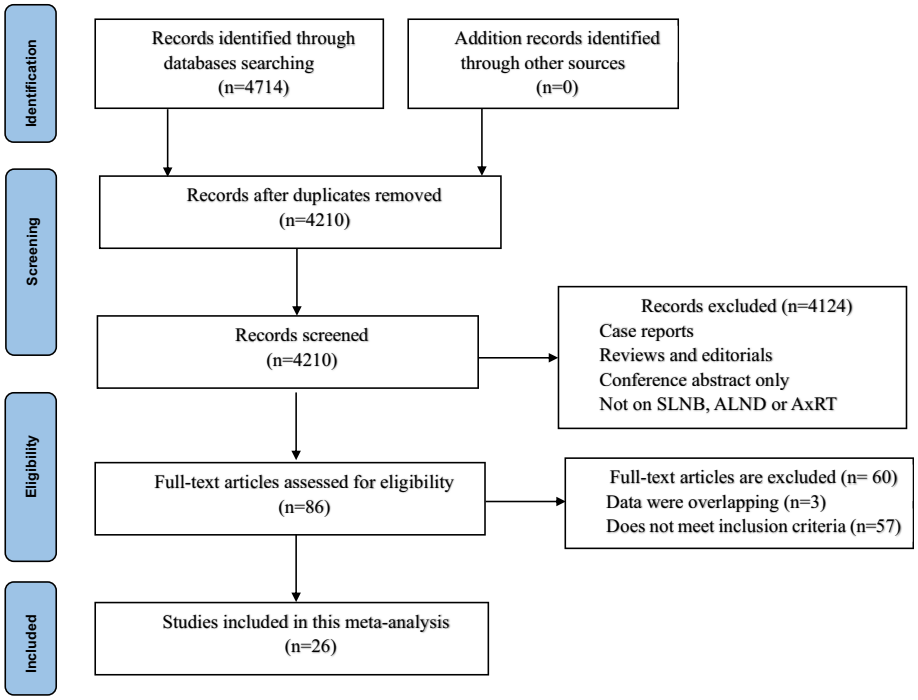

FIGURE 1
Flow diagram of study selection.

asymmetry of the funnel plot (Figure 3C) suggests potential publication bias.

Four studies reported the five-year cumulative incidence of axillary recurrence (22, 24, 32, 35). Although the axillary recurrence rate was higher in the SLNB alone group compared to the ALND group, but this difference was not statistically significant (HR1.01, 95% CI 0.99-1.03; p = 0.35) (Figure 2D).Additionally, the studies showed no significant heterogeneity (I^2 = 41%, p = 0.17). However, only one study reported the 10-year axillary recurrence outcome (32). This study found that axillary recurrence was more frequent among those who received SLNB alone compared to ALND (HR 5.47, 95% CI 1.21-24.63; p=0.013).

Effect of ALND versus AxRT

Four studies (25, 30, 33, 37) compared overall survival between ALND and AxRT. Pooled data analysis revealed no significant difference between the two groups (HR 0.88, 95% CI: 0.67-1.15; p=0.35) (Figure 4A). Additionally, pooling data from three studies (30, 33, 34) assessing disease-free survival also showed no significant difference between ALND and AxRT (HR 0.85, 95% CI: 0.68-1.05; p=0.13). Furthermore, these studies exhibited no significant heterogeneity(I^2 = 0%, p=0.71) (Figure 4B). Two studies (30, 33) reported axillary recurrence. While the axillary recurrence rate was higher in the AxRT group compared to the ALND group, the difference was not statistically significant (HR 0.94, 95% CI: 0.68-1.31; p=0.73) (Figure 4C). There was also no significant between-study heterogeneity(I^2 = 21%, p=0.26).

Effect of AxRT versus SLNB alone

Four studies (11, 25, 35, 37) assessed overall survival by comparing the AxRT group to the SLNB alone group. The results revealed no statistical difference in overall survival between the groups (HR 0.57, 95% CI: 0.32-1.02; p=0.25), with moderate heterogeneity between studies ($\chi 2$ = 4.12, I^2 = 27%, p=0.27) (Figure 5A). The asymmetry of the funnel plot suggests publication bias.

Three studies (11, 25, 35) reported disease-free survival. Patients who received AxRT had a higher rate of disease-free survival than those who underwent SLNB alone (HR 0.52, 95% CI: 0.26-1.05).However, no statistically significant difference was observed between groups (p=0.07). There was moderate heterogeneity among studies ($\chi 2$ = 3.79, I^2 = 47%, p=0.15) as shown in Figure 5B.

Discussion

This meta-analysis aimed to compare the effects of SLNB alone, ALND, and AxRT on various outcomes, including overall survival, disease-free survival, locoregional recurrence, and axillary

TABLE 1 Summary of characteristics of included studies.

Reference	Type of study	SLNB alone/ ALND or AxRT	T stage (T1/T2)	Micro/ Macro	Adjuvant RT (Yes/No)	Follow up (years)	Outcomes	Quality (NOS[a])
			SLNB alone ALND	SLNB alone ALND	SLNB alone ALND			
Tinterri 2023 (29)	RCT	107/218	53/51 47/56	NA NA	NA NA	2.8	OS	RCT
Houvenaeghel 2023 (23)	Retrospective	185/1266	NA NA	NA NA	1123/32 174/9	5.8	OS,DFS	6
Campbell 2023 (32)	RCT	544/544	NA NA	NA NA	482/62 466/73	10	OS,DFS,AR	RCT
Bartels 2023 (30)	RCT	681/744	533/143 612/132	195/419 215/442	681/0 703/41	10	OS,DFS,AR	RCT
Zhou 2022 (19)	Retrospective	1883/1883	740/878 725/862	1883/0 1883/0	596/1287 621/1262	4	OS	7
Kantor 2022 (34)	Retrospective	79/42	48/31 22/20	23/56 9/33	61/18 27/15	2.0	DFS, LRR	6
Sun 2021 (21)	Retrospective	128/201	62/58 82/101	NA NA	68/60 108/93	4.2	OS,LRR	7
Lim 2021 (35)	Retrospective	92/168	41/51 70/28	92/0 168/0	31/61 46/122	5.1	OS, DFS, LRR, AR	7
Ortega 2021 (11)	Retrospective	167/93	NA NA	0/167 0/93	95/72 NA/NA	4.5	OS, DFS, AR	6
Kim 2020 (16)	Retrospective	179/704	83/96 326/378	NA NA	NA NA	4.5	OS	8
Arisio 2019 (26)	Retrospective	211/406	118/82 211/189	155/95 84/322	169/42 335/71	7.0	OS, AR	6
Lee 2018 (20)	Retrospective	1268/3174	NA NA	NA NA	NA NA	3.9	OS	6
Galimberti 2018 (5)	RCT	469/465	322/140 316/142	NA NA	NA NA	9.7	OS, DFS, LRR	RCT
Wu 2018 (37)	Retrospective	11368/2651	4617/6751 1444/1207	NA NA	NA NA	1.9	OS	4
Savolt 2017 (33)	RCT	230/244	157/73 152/92	NA NA	230/0 0/244	8.1	OS, DFS, AR	RCT
Mamtani 2017 (36)	Retrospective	162/190	NA NA	NA NA	NA NA	6.0	LRR	6
Giuliano 2017 (7)	RCT	436/420	303/126 284/134	NA NA	NA NA	9.3	OS, DFS	RCT
Giuliano 2016 (28)	RCT	436/420	303/126 284/134	NA NA	NA NA	9.3	LRR	RCT
Tvedskov 2015 (24)	Retrospective	240/1834	NA NA	NA NA	NA NA	6.3	OS, AR	5
Snow 2015 (17)	Retrospective	60/258	NA NA	NA NA	36/24 147/111	6.3	OS	5
Bonneau 2015 (18)	Retrospective	402/9119	174/228 3665/5454	NA NA	192/210 5426/3677	2.6	OS	4
Park 2014 (27)	Retrospective	197/2384	130/67 1171/1177	NA NA	4/55 439/757	3.5	OS	5
Fu 2014 (25)	Retrospective	106/108	49/47/7 25/53/26	NA NA	59/46 65/28	3.6	OS	4
Yi 2013 (15)	Retrospective	188/673	152/36 445/228	136/52 158/515	NA NA	5.8	OS, DFS	7
Solá 2013 (31)	RCT	121/112	NA NA	NA NA	NA NA	5.0	DFS	RCT

(Continued)

TABLE 1 Continued

Reference	Type of study	SLNB alone/ ALND or AxRT	T stage (T1/T2)	Micro/ Macro	Adjuvant RT (Yes/No)	Follow up (years)	Outcomes	Quality (NOS[a])
			SLNB alone ALND	SLNB alone ALND	SLNB alone ALND			
Bilimoria 2009 (22)	Retrospective	20217/77097	NA NA	3674/16543 6585/70512	NA NA	7.9	OS,AR	6

RCT, randomized controlled trial; SLNB, sentinel lymph nodes biopsy; ALND, axillary lymph node dissection; OS, overall survival; LLR, locoregional recurrence; DFS, disease-free survival; AR, axillary recurrence; NOS, Newcastlee-Ottawa Scale; NA, not available; Micro, micrometasis (<0.2-2.0 mm); Macro, macromeataiss (>2.0 mm). RT, radiation therapy.
a Quality assessment of the observational studies was assessed using the Newcastlee-Ottawa Scale. The quality of the evidence is classified as three levels: high (more than seven stars), moderate (four to six stars), poor (less than four stars).

recurrence, in 145,548 patients with early-stage breast cancer, clinical negative axillary lymph nodes, and positive sentinel lymph nodes. The collected data revealed no significant differences in overall survival, disease-free survival, locoregional recurrence, and axillary recurrence between the SLNB alone group and the ALND or AxRT groups. While the AxRT group showed a higher overall survival rate compared to the ALND group, this difference was not statistically significant. Additionally, no significant disparities were observed in terms of overall survival and disease-free survival between patients who received AxRT and those who received SLNB alone.

Several meta-analyses (38–47) have been conducted to compare the differences in overall survival, disease-free survival, and recurrence rates between ALND and SLNB alone in early-stage breast cancer patients with positive sentinel lymph nodes. However, the impact of ALND remains controversial. Peristeri et al. (38) performed a meta-analysis comparing the effects of SLNB/RT and ALND in five RCTs. Their pooled data showed that the SLNB/RT group had better overall and disease-free survival than the ALND group, with a statistically significant difference in axillary recurrence favoring the ALND group. However, our previous meta-analysis comparing the two approaches in early-stage breast cancer with sentinel lymph node metastasis (43), found no significant differences in overall survival, disease-free survival and locoregional recurrence between the SLNB alone and the ALND group. Similarly, a meta-analysis of Real-World Evidence in the Post-ACOSOG Z0011 trial (39), which included one RCT and six retrospective studies with 8,864 early-stage breast cancer patients with one or two SLN metastases, found no differences between SLNB alone and ALND groups in overall survival, disease-free

TABLE 2 Evaluating the quality of evidence in randomized controlled trials by GRADEpro GDT ALND compared to SLNB alone for patients with clinical node-negative early breast cancer and positive sentinel lymph node.

Patient or population: patients with clinical node-negative early breast cancer and positive sentinel lymph node
Setting: Hospital
Intervention: ALND
Comparison: SLNB alone

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with SLNB	Risk with ALND				
OS – Randomized control trials	514 per 1,000	**545 per 1,000** (485 to 603)	**HR 1.09** (0.92 to 1.28)	6406 (4 RCTs)	⊕⊕⊕◯ Moderate[a]	
DFS - Randomized control trials	512 per 1,000	**522 per 1,000** (472 to 574)	**HR 1.03** (0.89 to 1.19)	6872 (5 RCTs)	⊕⊕⊕⊕ High	
LRR - Randomized control trials	494 per 1,000	**400 per 1,000** (239 to 615)	**HR 0.75** (0.40 to 1.40)	3580 (2 RCTs)	⊕⊕⊕⊕ High	

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
ALND, axillary lymph node dissection**; SLNB,** sentinel lymph node biopsy; **CI,** confidence interval; **HR,** hazard Ratio; **GRADEpro GDT**: Grading of Recommendation Assessment Development and Evaluation Pprofiler Guide- line Development Tool.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

a.I^2 value is 42% as moderate heterogeneity.
ALND, axillary lymph node dissection; SLNB, sentinel lymph nodes biopsy; OS, overall survival; LLR, locoregional recurrence; DFS, disease-free survival; CI, confidence interval; HR, hazard Ratio. The evidence quality was classified into 4 levels: high (⊕⊕⊕⊕), moderate (⊕⊕⊕⊖), low (⊕⊕⊖⊖) or very low (⊕⊖⊖⊖).

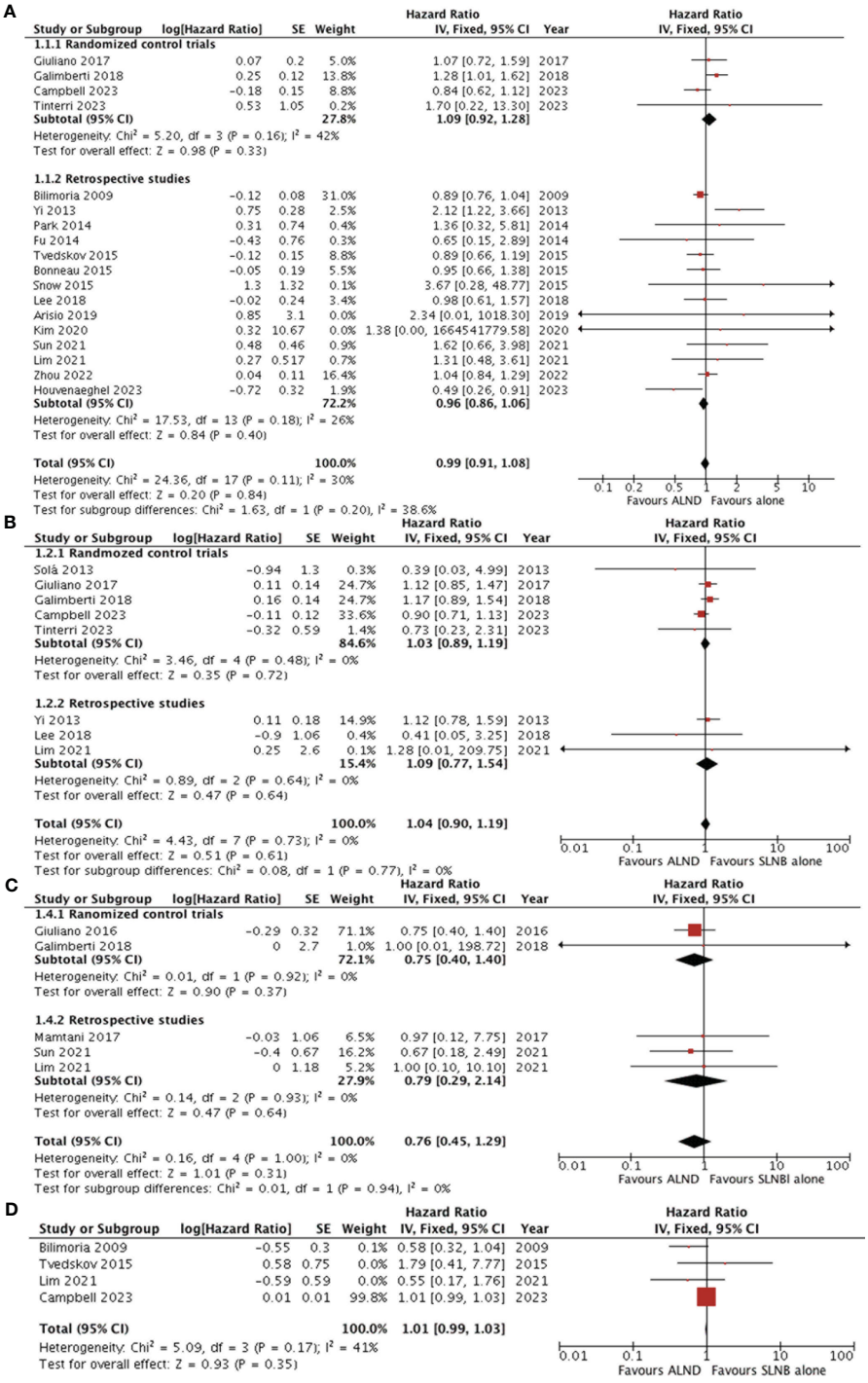

FIGURE 2
Forest plot of ALND versus SLNB alone **(A)** overall survival **(B)** disease-free survival **(C)** locoregional recurrence **(D)** axillary recurrence. ALND, axillary lymph node dissection; SLNB, sentinel lymph node biopsy; CI, confidence interval; SE, standard error; IV, Inverse Variance.

survival, and recurrence rate. However, the incidence rate of lymphedema was significantly lower in SLNB alone group Our systematic review and meta-analysis included eight RCTs and eighteen retrospective cohort studies. The results indicated no statistically significant difference in disease-free survival, overall survival, and locoregional recurrence between ALND and SLNB alone in clinical node-negative early breast cancer patients with positive sentinel lymph nodes. Three studies reported the five-year cumulative incidence of axillary recurrence. The rate was higher in the SLNB alone group compared to the ALND group, but this difference was not statistically significant ($p = 0.36$). However, when the follow-up period was extended to 10 years, Campbell et al. (32) found that that axillary recurrence was more frequent in the SLNB alone group compared to ALND group. Therefore, longer follow-up

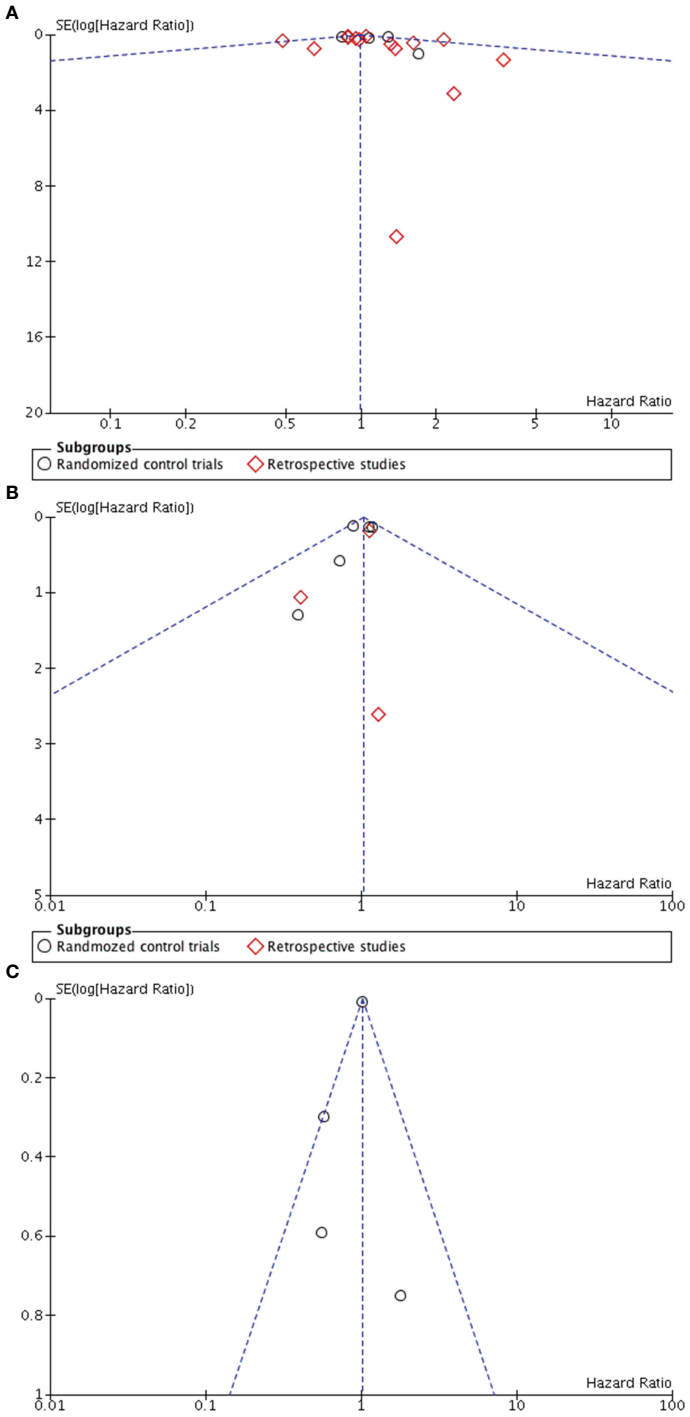

FIGURE 3
Funnel plot in ALND versus SLNB alone **(A)** Funnel plot for overall survival **(B)** Funnel plot for disease-free survival **(C)** Funnel plot for locoregional recurrence.

times are required to definitively compare the axillary recurrence rates between the two groups in this patients population. After ten year of follow-up, the Randomized Controlled EORTC AMAROS trials (30) showed that both AxRT and ALND groups achieved excellent locoregional control and survival in cT1-T2 breast cancer patients with positive sentinel lymph nodes. Additionally, the AxRT group had a lower rate of lymphedema and no difference in quality of life compared to the ALND group. This meta-analysis also confirms no significant differences in disease-free survival, overall survival, and axillary recurrence between patients treated with ALND versus AxRT.

Our review diverges from previous studies in several key aspects. First, by incorporating eight RCTs and 18 retrospective studies involving 145,548 patients, our meta-analysis significantly increase the sample size, leading to more precise and reliable results. Second, we employed the GRADEpro GTD tool to assess evidence

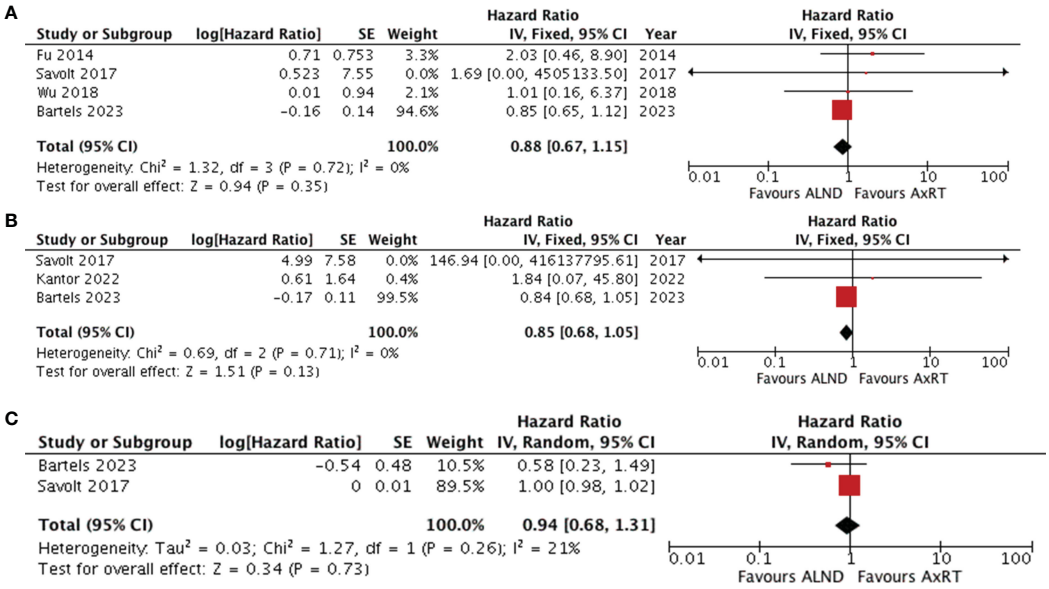

FIGURE 4
Forest plot of ALND versus AxRT **(A)** overall survival **(B)** disease-free survival **(C)** axillary recurrence. ALND, axillary lymph node dissection; AxRT, axillary radiation; CI, confidence interval; SE, standard error; IV, Inverse Variance.

quality within RCTs, revealing high quality for disease-free survival and locoregional recurrence, and moderate quality for overall survival. Furthermore, subgroup analyses based on study type (RCT vs. retrospective) demonstrated that the pooled analysis results remained unchanged, indicating the stability of our finding. Third, and uniquely, our review evaluated the effects of AxRT and SLNB alone in patients with clinical node-negative early breast cancer and positive sentinel lymph nodes, an aspect lacking in prior meta-analysis. However, limitations exist within our meta-analysis. First, our inclusion criteria restricted us to English studies only, potentially introducing publication bias by excluding unpublished data. Second, the NOS tool revealed six non-RCT studies with a, four to five-star rating, including lower-quality evidence. Third, both overall and disease-free survival analyses showed evidence of publication bias. Fourth, moderate heterogeneity was observed among studies regarding disease-free and overall survival when comparing the AxRT and SLNB alone groups. A study by Ortega et al. (11) identified potential sources of this heterogeneity. While removing their study resulted in a significant decrease in heterogeneity for both outcomes, the overall and disease-free survival data also changed substantially (Supplementary Figures 1, 2). Therefore, further careful evaluation of the effect of AxRT versus SLNB alone is required, and these results necessitate confirmation through well-designed prospective studies. Finally, because the lack of sufficient information within the included studies precluded further subgroup analyses based on

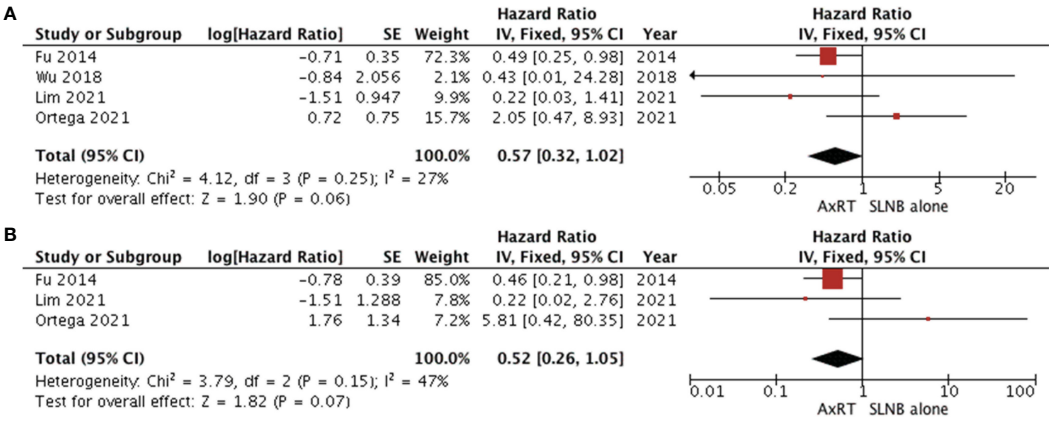

FIGURE 5
Forest plot of AxRT versus SLNB lone **(A)** overall survival **(B)** disease-free survival. AxRT, axillary radiation; SLNB, sentinel lymph node biopsy; CI, confidence interval; SE, standard error; IV,Inverse Variance.

factors like the T1/T2 stage, number of positive sentinel lymph nodes, micrometastasis or macrometastasis, molecular subtype, and age.

Conclusion

This study demonstrates that patients with early-stage breast cancer and positive sentinel lymph nodes who undergo SLNB alone achieve comparable locoregional control and survival to those who receive ALND or AxRT. Our findings suggest that omitting ALND or AxRT may be safe for these patients, although further verification is needed through rigorously designed prospective studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

PZ: Data curation, Formal Analysis, Methodology, Writing – review & editing. CL: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. JL: Data curation, Investigation, Project administration, Writing – review & editing. WD: Formal Analysis, Methodology, Writing – review & editing. BH: Investigation, Methodology, Project administration, Software, Supervision, Writing – review & editing. JZ: Data curation, Investigation, Methodology, Writing – review & editing. HZ: Investigation, Methodology, Project administration, Software, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants from Hebei Province University Basic Research Business Fee Project (Project number: JYG2020003).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1320867/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Forest plot of overall survival after heterogeneity analysis in AxRT versus SLNB lone.

SUPPLEMENTARY FIGURE 2
Forest plot of disease-free survival after heterogeneity analysis in AxRT versus SLNB lone.

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 09 January 2024
DOI 10.3389/fonc.2023.1305545

OPEN ACCESS

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RECEIVED 02 October 2023
ACCEPTED 19 December 2023
PUBLISHED 09 January 2024

CITATION
Zhang H, Hu J, Meng R, Liu F, Xu F and Huang M (2024) A systematic review and meta-analysis comparing the diagnostic capability of automated breast ultrasound and contrast-enhanced ultrasound in breast cancer.
Front. Oncol. 13:1305545.
doi: 10.3389/fonc.2023.1305545

A systematic review and meta-analysis comparing the diagnostic capability of automated breast ultrasound and contrast-enhanced ultrasound in breast cancer

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Objective: To compare the diagnostic performance of automated breast ultrasound (ABUS) and contrast-enhanced ultrasound (CEUS) in breast cancer.

Methods: Published studies were collected by systematically searching the databases PubMed, Embase, Cochrane Library and Web of Science. The sensitivities, specificities, likelihood ratios and diagnostic odds ratio (DOR) were confirmed. The symmetric receiver operator characteristic curve (SROC) was used to assess the threshold of ABUS and CEUS. Fagan's nomogram was drawn. Meta-regression and subgroup analyses were applied to search for sources of heterogeneity among the included studies.

Results: A total of 16 studies were included, comprising 4115 participants. The combined sensitivity of ABUS was 0.88 [95% CI (0.73–0.95)], specificity was 0.93 [95% CI (0.82–0.97)], area under the SROC curve (AUC) was 0.96 [95% CI (0.94–0.96)] and DOR was 89. The combined sensitivity of CEUS was 0.88 [95% CI (0.84–0.91)], specificity was 0.76 [95% CI (0.66–0.84)], AUC was 0.89 [95% CI (0.86–0.92)] and DOR was 24. The Deeks' funnel plot showed no existing publication bias. The prospective design, partial verification bias and blinding contributed to the heterogeneity in specificity, while no sources contributed to the heterogeneity in sensitivity. The post-test probability of ABUS in BC was 75%, and the post-test probability of CEUS in breast cancer was 48%.

Conclusion: Compared with CEUS, ABUS showed higher specificity and DOR for detecting breast cancer. ABUS is expected to further improve the accuracy of BC diagnosis.

KEYWORDS

breast cancer, automated breast ultrasound (ABUS), contrast-enhanced ultrasound (CEUS), diagnosis, meta-analysis

Introduction

Having displaced lung cancer, breast cancer (BC) has become the most frequently diagnosed cancer across the globe and accounts for 1 in 8 of all cancer diagnoses (1). Until 2020, there had been over 2.3 million new cases and 685,000 deaths in BC patients globally (1). Furthermore, the treatment of patients with advanced BC is difficult, and the cure rate is low (2, 3). The relative survival of patients diagnosed with early-stage BC is much higher than that of patients diagnosed with late-stage disease. The 5-year relative survival for BC patients is >99% for stage I disease, 93% for stage II, 75% for stage III, and 29% for stage IV (4). Therefore, early detection, early diagnosis and early treatment are the keys to reducing the mortality rate and improving the prognosis of breast cancer.

Mammography (MG), as the main method of BC screening and diagnosis, has been recognized by most clinicians and radiologists. However, for dense breasts, MG has low sensitivity and specificity, a high probability of false-negative results, uses ionizing radiation and has other shortcomings (5). Ultrasonography has become an important auxiliary imaging method for the diagnosis of breast diseases MG (6). HHUS (handheld ultrasound) has become the most used ultrasound method for the evaluation of breast diseases due to its convenience, high resolution and absence of ionizing radiation (7). However, HHUS has disadvantages, such as a high operator dependence and real-time diagnosis.

To reduce operator dependence, automated breast ultrasound has been developed. Automated breast ultrasound (ABUS) has many advantages over conventional ultrasound. ABUS enables visualization from the skin surface on the breast to the thoracic wall and reserves all the breast volume information on a picture archiving and communication system (8). ABUS has similar diagnostic quality to hand-held ultrasonography in screening. Nevertheless, it can assess the location and size of masses more accurately than HHUS (8).

Contrast-enhanced ultrasound (CEUS) is a pure blood pool imaging technology, which can not only display the morphology of breast lesions but also evaluate the morphology and dynamics of the blood supply to the lesions. Compared with that of US, it has been confirmed that the diagnostic efficiency of CEUS is higher (9). CEUS improves backscattering in the vascular system by injecting contrast agents (gas-filled microbubbles). Therefore, sonographers can make out certain vascular structures and tissues that differ in vascularity in the masses, whereupon one can analyse breast lesions features quantitatively and qualitatively (8).

However, researchers differ in their understanding of the value of ABUS and CEUS in the diagnosis of breast cancer. The diagnostic capability of ABUS and CEUS in BC remains unclear. Therefore, this study evaluated and compared the diagnostic capability of ABUS and CEUS.

Abbreviations: ABUS, automated breast ultrasound; BC, breast cancer; CEUS, contrast-enhanced ultrasound; DOR, diagnostic odds ratio; SROC, symmetric receiver operator characteristic; MG, mammography; TP, true-positive; FN, false-negative; FP, false-positive; TN, true-negative; Se, sensitivity; Sp, specificity; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUC, Area Under Curve; HHUS, handheld ultrasound.

Methods

Search strategy

Two reviewers (ZHY and HJY) independently searched the PubMed, Embase, Cochrane Library and Web of Science databases up to April 2023. The search terms are shown below (Breast Neoplasm OR Breast Tumors OR Breast Cancer OR Malignant Neoplasm of Breast) AND (automated breast volume scan OR automatically generated breast volume scan OR ABVS OR contrast-enhanced ultrasound OR CEUS).

Inclusion and exclusion criteria

The inclusion criteria included the following items: (1) well-defined BC patients included as study subjects; (2) randomized controlled trials divided into two groups, the experimental group with BC patients and the control group using patients with benign lesions; (3) clinical trials involving ABVS or/and CEUS for BC detection; (4) true-positive (TP), false-negative (FN), false-positive (FP), true-negative (TN), sensitivity (Se) and specificity (Sp) shown or figured out according to the literature; and (5) histological examination applied as the gold standard method of diagnosis. The exclusion criteria included following items: (1) animal studies; (2) non-case–control trials; (3) studies without sufficient or experimental data; (4) letters, case reports, guidelines, reviews, and conference abstracts; (5) literature published repeatedly; and (6) studies unrelated to diagnostic means in BC patients.

Data extraction

Two investigators (ZHY and HJY) independently screened the demographic and intervention information from original studies. The extracted information and data were as follows: (1) name of the first author; (2) type of study; (3) region of the author; (4) sample size or number of lesions; (5) age and female/male ratio of experimental participants; (6) year the study was released; (7) gold standard used and (8) the outcome indicators of ABVS and CEUS, including TP, FP, NP, TN, Sp, Se etc.

Statistical analysis

Stata 15.0 software (Stata Corp 4905 Lakeway Drive, TX, USA) was used to compare the diagnostic modalities in studies included in the meta-analysis. The bivariate model was applied to calculate combined sensitivity, specificity, the positive/negative likelihood ratio (PLR/NLR) and the diagnostic odds ratio (DOR). The area under the receiver operator characteristic (ROC) curve estimated the total diagnostic efficacy of ABVS or CEUS in BC patients. Post-

test probability could determine whether the diagnostic probability was increased or decreased in comparison with the pre-test probability, which was assessed from conventional data, trial data or clinical decisions. The statistical heterogeneity based on the included studies was evaluated using the I^2 statistics and Q test. Values of $I^2 < 50\%$ and $P > 0.1$ indicated what could be regarded as inhomogeneity, so a random-effects model was applied for further analysis. Otherwise, a fixed-effect model should be performed. A P value <0.05 indicated a significant difference between samples.

Results

Flow chart and study quality

A total of 5001 studies were searched from four databases (PubMed, Embase, Cochrane Library and Web of Science). After elimination of 1283 duplicate records, 3718 related studies were included. Among these studies, 349 were omitted for being reviews, conference abstracts, meta-analyses, animal studies or case reports, whereas 2062 studies did not have relevant titles and abstracts. The full text of the remaining 130 studies were perused, and 1177 studies were excluded on account of imperfect data. The remaining 16 studies were extracted ultimately on the data extraction requirements. Eight studies used ABUS, and 8 used CEUS. The process of literature screening was performed in Figure 1. The basic characteristics of each study were plotted in Table 1.

ABUS against breast cancer

The random-effects model was applied when the heterogeneity was greater than 50%. The combined sensitivity of ABUS against breast cancer was 0.88 [95% CI (0.73–0.95)], specificity was 0.93 [95% CI (0.82–0.97)], PLR was 11.9 [95% CI (5.1–28.0)], NLR was 0.13 [95% CI (0.06–0.29)], and DOR was 89.09 [95% CI (55.60–142.75)], indicating that ABUS had a high value in the screening of BC (Figures 2A–C).

Publication bias and heterogeneity

Potential publication bias was assessed by the Deeks' funnel plots in the process of detecting BC with ABUS. P value of 0.24 (Supplementary Figure 1) indicated no existing publication bias. There was one study out of the border, representing heterogeneity among included studies, as plotted in Supplementary Figure 2.

Threshold effect

The threshold effect was assessed by the SROC curve plane test. The typical "shoulder arm" was absent, indicating the inexistence of the threshold effect. The area under the SROC curve (AUC) was 0.96 [95% CI (0.94–0.96)], indicating a high diagnostic value of ABUS (Figure 3).

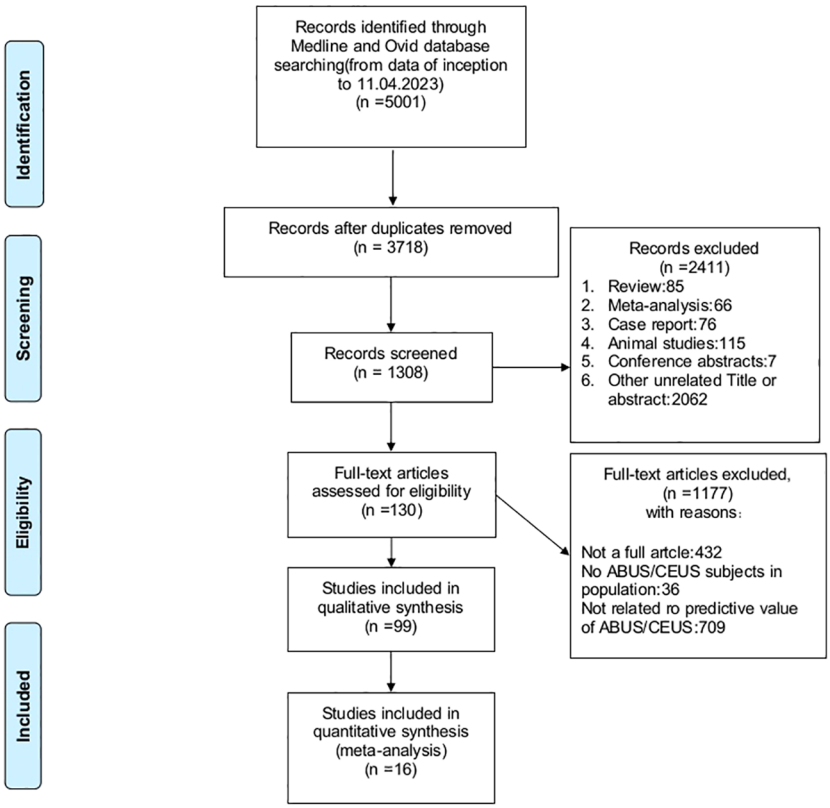

FIGURE 1
Literature screening process of the meta-analysis.

TABLE 1 Basic characteristics of enrolled studies.

References	Year	Study	Region	Sample size	Age (years)	contrast agent		Diagnostic value		
						types	volume	sensitivity	specificity	AUC
Xi Lin (10)	2012	Prospective	China	95	16–78	_	_	100	95	_
Yuanming Xiao (11)	2015	Retrospective	China	273	18-72	_	_	28.95	100	0.7864
Hong-Yan Wang (12)	2012	Prospective	China	239	43.0 ± 12.5	_	_	95.3	80.5	0.948
LIN CHEN (13)	2013	Retrospective	China	219	16-71	_	_	92.5	86.2	_
Weixiang Liang (14)	2017	Retrospective	China	87	43.2 ± 14.5	_	_	81.8	85.2	0.879
Woo Jung Choi (15)	2014	Retrospective	Korea	1866	19-82	_	_	77.78	97.79	_
Jialin Liu (16)	2022	Prospective	China	431	16-82	_	_	92.16	87.05	0.901
Chaoli Xu (17)	2014	Retrospective	China	46	46 ± 1.6	_	_	100	77.8	_
Huiling He (18)	2023	Retrospective	China	26	23-76	SonoVue	4.8ml	85.71	68.42	0.74
Yingying Yuan (19)	2022	Prospective	China	108	53.37 ± 5.15	SonoVue	2.4ml	82.35	70	0.901
Zuopeng Ding (20)	2021	Retrospective	China	109	48.5 ± 10.4	SonoVue	4.8ml	88.46	74.19	0.9084
Natalia Caproni (21)	2010	Retrospective	Italy	43	28-85	SonoVue	5ml	91	72.73	_
Jing Du (22)	2008	Prospective	China	61	23-72	SonoVue	CnTI:3.6ml MFI:1.2ml	93.8	86.2	0.94
Yukio Miyamoto (23)	2014	Prospective	Japan	351	48.5 ± 12.3	Sonazoid	0.015 mL/kg	91.4	85.4	0.886
Daniela Stanzani (24)	2014	Prospective	São Paulo	70	18-78	Definity PESDA	Definity:0.01 mL/kg PESDA: 3ml	92	46.6	_
Caifeng Wan (25)	2012	Prospective	China	91	_	SonoVue	CnTI:3.6ml MFI:1.2ml	82.98	88.64	0.92

CnTI, contrast-tuned imaging; MFI, Microflow imaging; PESDA, perfluorocarbonexposed sonicated albumin.

10.3389/fonc.2023.1305545

Pre-test probability, LR and post-test probability

The relationships among the prior probability, the PLR, the NLR and the posterior probability were assessed via a Fagan graph. When the pre-test probability was set to 20%, the post-test probability of BC was 75%. Moreover, the positive likelihood ratio (PLR) was >10 (PLR = 12), and the negative likelihood ratio (NLR) was > 0.1 (NLR = 0.13), indicating that the ability to diagnose true positives was better (Figure 4).

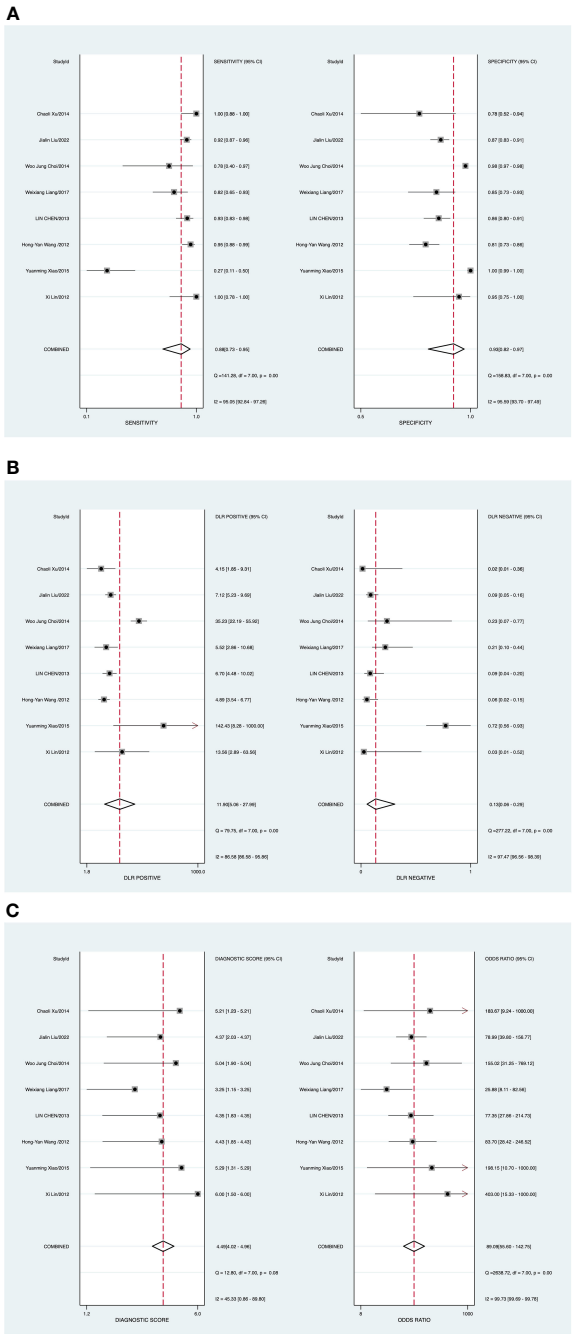

FIGURE 2
(A) Forest plot of sensitivity and specificity of automated breast ultrasound (ABUS) in the diagnosis of breast cancer. **(B)** Forest plot of the diagnosis likelihood ratio (DLR). **(C)** Forest plot of the diagnostic odds ratio (DOR).

Meta-regression and subgroup analysis

Some factors, including a prospective design (prodesign), partial verification bias (fulverif), an adequate description of the study participants (subjdescr), report of method, a broad spectrum of diseases (brdspect), and whether the test results were assigned a value by a blind method, might be relevant to heterogeneity among these ABUS studies. The meta-regression analysis of the above-mentioned factors indicated that prodesign and blinding might be the source of heterogeneity of sensitivity (Supplementary Figure 3).

CEUS against breast cancer

A random-effects model was applied when the heterogeneity was greater than 50%. The combined sensitivity of CEUS against breast cancer was 0.88 [95% CI (0.84–0.91)], specificity was 0.76 [95% CI (0.66–0.84)], PLR was 3.7 [95% CI (2.5–5.5)], NLR was 0.16 [95% CI (0.11–0.21)], and DOR was 23.85 [95% CI (12.59–45.17)], indicating that CEUS had a high value in the screening of BC (Figures 5A–C).

Publication bias and heterogeneity

A *P* value of 0.20 ($P > 0.05$) (Supplementary Figure 4) indicated the absence of publication bias. There was one study outside of the

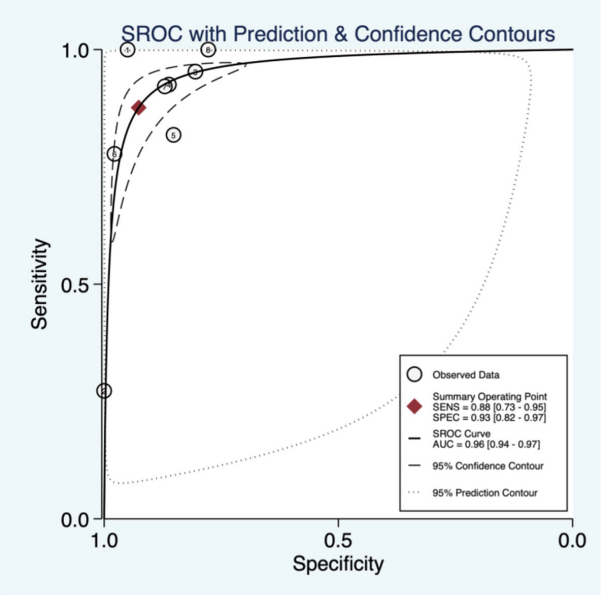

FIGURE 3
Summary of receiver operating characteristics of automated breast ultrasound (ABUS).

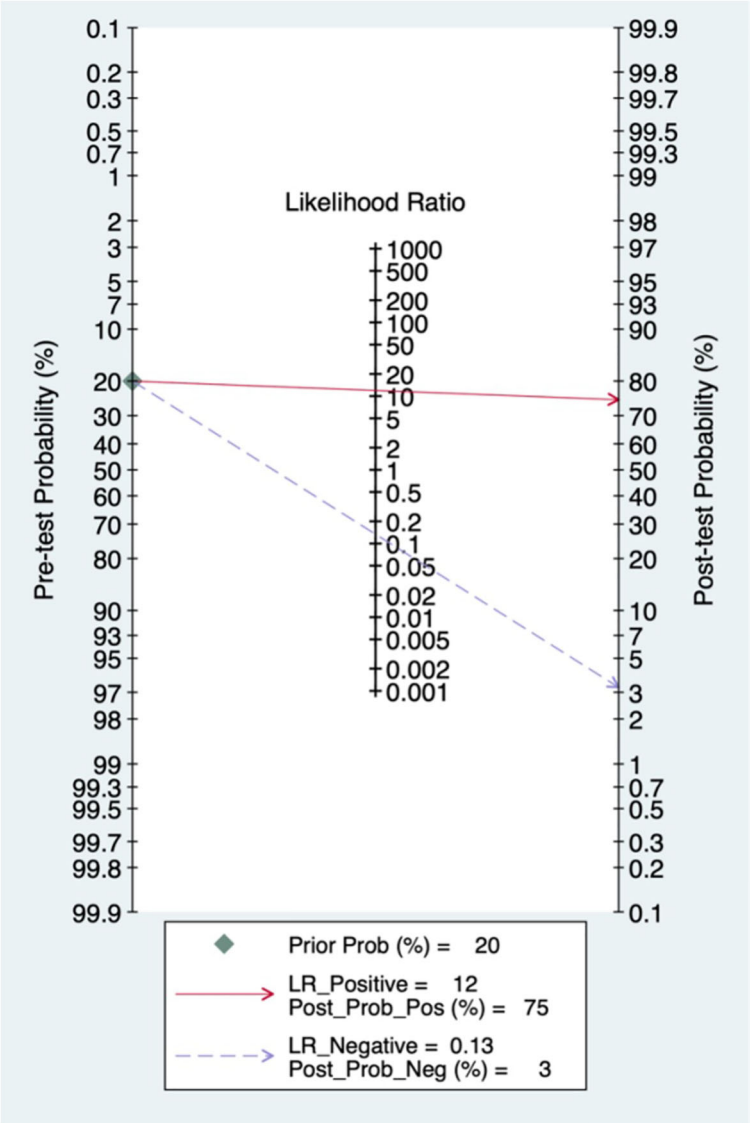

FIGURE 4
Fagan diagram of automated breast ultrasound (ABUS) in the diagnosis of breast cancer.

border, representing heterogeneity among the included studies (Supplementary Figure 5).

Threshold effect

The threshold effect was assessed by the SROC curve plane test. The typical "shoulder arm" was absent, as revealed in Figure 6, indicating the inexistence of a threshold effect. The area under the SROC curve (AUC) was 0.89 [95% CI (0.86–0.92)], indicating a high diagnostic value of ABUS.

Pre-test probability, LR and post-test probability

When the pre-test probability was set to 20%, the post-test probability of BC was 48%. Moreover, the positive likelihood ratio (PLR) was <10 (PLR = 4), and the negative likelihood ratio (NLR) was >0.1 (NLR = 0.16), indicating that the diagnosis could neither be confirmed nor excluded. The diagnostic value of CEUS in BC was limited, as shown in Figure 7.

Meta-regression and subgroup analysis

The meta-regression analysis indicated that prodesign and blinding might be sources of heterogeneity of sensitivity, and no factors were related to sources of heterogeneity of specificity (Supplementary Figure 6).

Comparison of ABUS and CEUS

A comparison of ABUS and CEUS was performed using ROC, sensitivity, and specificity analyses. Among them, ABUS had the best diagnostic value; details are shown in Table 2.

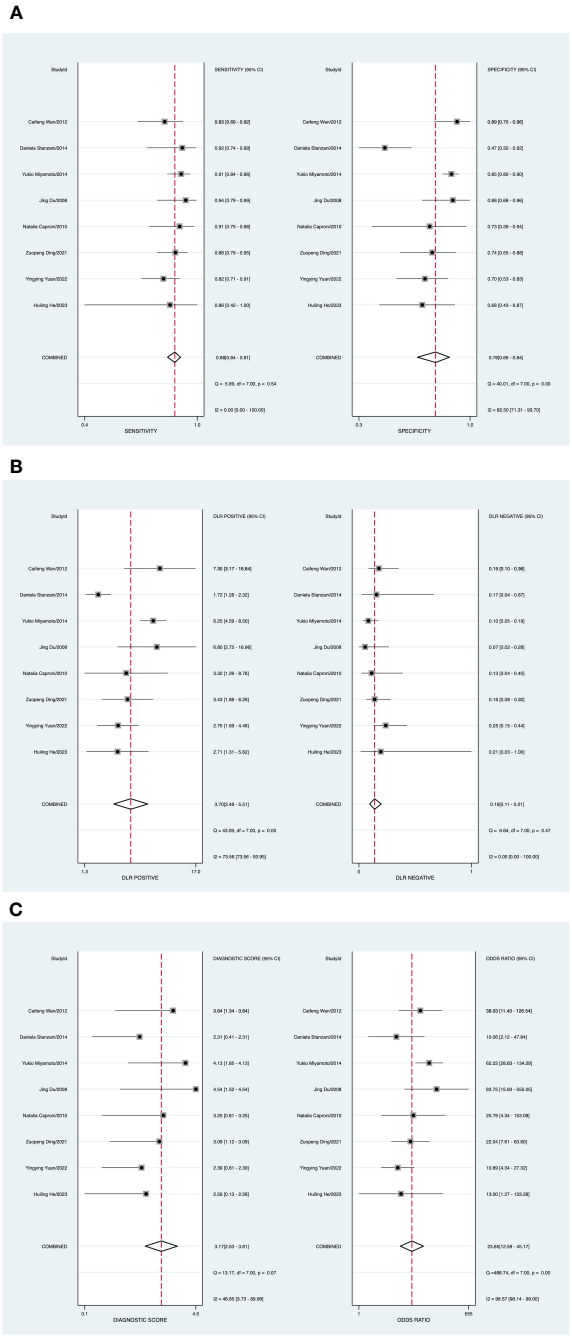

FIGURE 5
(A) Forest plot of sensitivity and specificity of contrast-enhanced ultrasound (CEUS) in the diagnosis of breast cancer. **(B)** Forest plot of diagnosis likelihood ratio (DLR). **(C)** Forest plot of the diagnostic odds ratio (DOR).

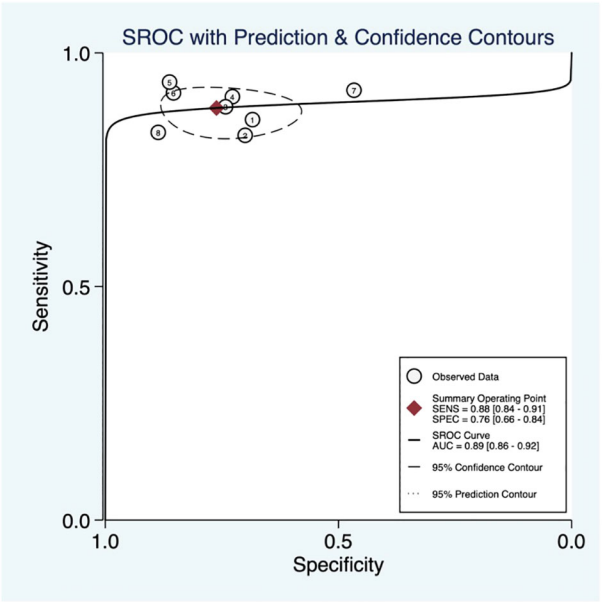

FIGURE 6
Summary of receiver operating characteristics of contrast-enhanced ultrasound (CEUS).

Discussion

The global incidence of BC is increasing each year (26). Early diagnosis can improve the prognosis significantly, especially when the lesions cannot be felt (27). Therefore, early diagnosis and symptomatic therapy in BC patients have weighty significance.

This systematic review and meta-analysis assessed the diagnostic efficiency of ABUS and CEUS in BC. A total of 16 studies, involving 4115 samples, were included in the analysis. Both ABUS and CEUS had a certain diagnostic value for breast cancer, as assessed by DOR. In addition, ABUS has a higher specificity and a larger AUC than CEUS. Moreover, ABUS improved the post-test probability to a greater extent than CEUS. The results showed that the diagnostic performance of ABUS was higher than that of CEUS. It should be noted that according to the search strategy, after screening by the inclusion and exclusion criteria, the final included literatures were mainly from Asian countries. Several studies has reported that for breast cancer dense breast as a risk varying from the lowest to highest sort of density by 4-6 folds, severally (28, 29). The breast density of women arguably in western countries are much lower than in Asian countries (30–33). This may be one reason that the final included literatures mainly focused on Asian countries.

ABUS is a time-saving method and a money-saving method. For breast cancers, the primary screening method is mammography. But its sensitivity is lower for dense breasts. Kim (34) found that mammography had a lower sensitivity in screening lesions of dense breasts as an independent risk factor for breast cancers. ABUS could become a supplementary diagnostic method to mammography when detecting masses in women with dense breasts (34). There are more and more studies for ABUS.

Vourtsis (35) claimed that a three-dimensional automated breast ultrasound system (3D ABUS) used high-frequency ultrasonic transducers and scanned most of the breast at once, which largely addressed the limitations of HHUS.

CEUS is a convenient imaging technique that allows patients to take a more appropriate position and shorter examination time than MRI, and CEUS can also be used in patients with MRI contraindications such as ferromagnetic metal implants. CEUS which is a high-performance, feasible, easy-to-implement, non-irradiating, accessible imaging method has proven to be a valuable complement to breast ultrasound (36). Hu (37) claimed that CEUS could display the

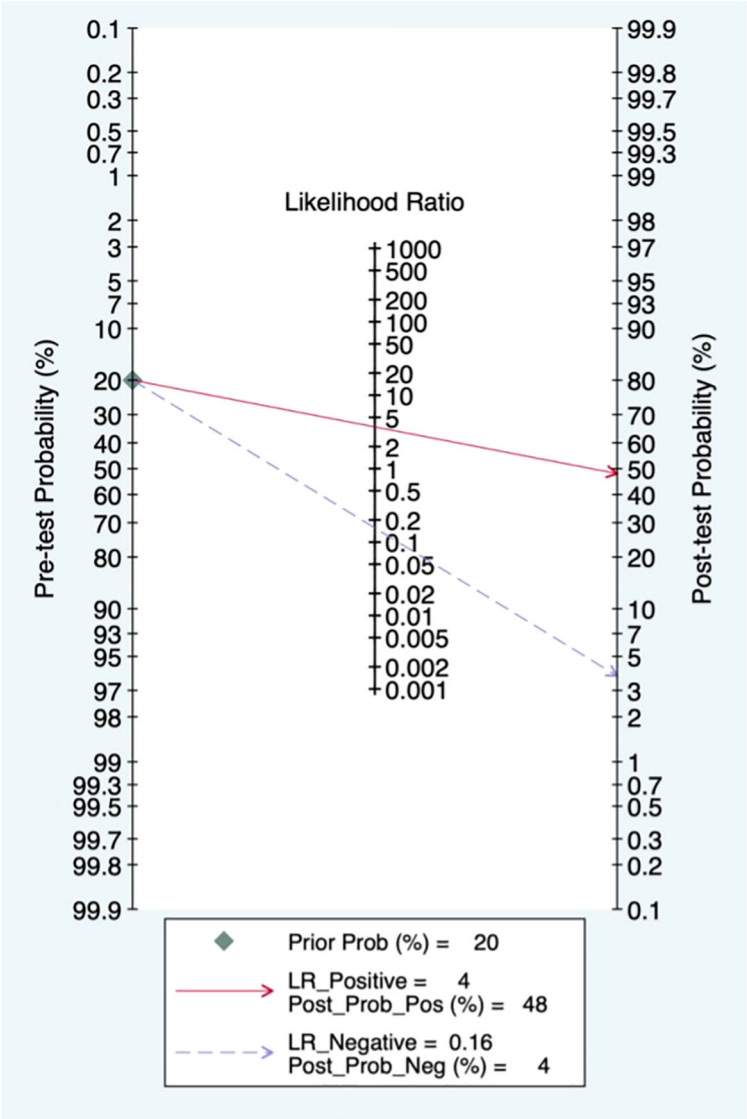

FIGURE 7
Fagan diagram of contrast-enhanced ultrasound (CEUS) in the diagnosis of heart sounds.

features of breast lesions accurately and be helpful for selecting suspected malignant masses for surgery.

ABUS and CEUS also have their own limitations in the application process. ABUS has no ability to evaluate the condition of axillary nodes. Moreover, it still cannot guide the puncture biopsy. ABUS may miss lesions if there is a mass at the outer position of the breast. If the lotion in the ultrasound gel is not distributed homogeneously or even missing an area, air will enter the interspace between the transducer and the skin, inducing the inability to visualize the tissue beneath (38). In addition, the accuracy of CEUS used for detecting ductal carcinoma *in situ* (DCIS) and some rare types of BC is low (37).

The combination of the two may help to improve the ability to diagnose breast cancer. Quan et al. (39) indicated in the recently published literature that ABUS pooled with CEUS had higher precision in the analysis of BC and showed great application value in the judgment of breast cancer. In addition, Yongwei et al. (40) aimed to evaluate the role of ABUS and CEUS in the early prediction of the treatment response to neoadjuvant chemotherapy (NAC) in patients with BC and found that the CEUS-ABUS model could be

TABLE 2 Diagnostic performance of ABUS and CEUS.

Method	AUC	Sensitivity	Specificity	Prior *P*	PLR (%)	NLR(%)
ABUS	0.96	0.88	0.93	20	11.9	0.13
CEUS	0.89	0.88	0.76	20	3.7	0.16

ABUS, automated breast ultrasound; CEUS, contrast-enhanced ultrasound; PLR, the positive likelihood ratio; NLR, the negative likelihood ratio

used clinically to optimize the treatment of patients with breast cancer. However, the current number of studies on this topic is insufficient for a systematic review, which provides direction for our future research.

Limitations

This study has several limitations. Firstly, because of the retrospective studies in this meta-analysis, there was likely to be subject selection bias. For example, most of the studies included were from Asia, especially China, which may cause bias of this research. Secondly, the relatively small sample sizes of the included studies may lead to overestimation of the diagnostic capacity. Thirdly, significant heterogeneity existing among the included reports could reduce the statistical efficiency. It is worth looking into further assessing the diagnostic power of CEUS and ABUS in a large-scale and prospective diagnostic study.

Conclusions

The use of ABUS showed higher specificity and DOR for detecting BC compared with CEUS. ABUS is expected to further improve the diagnostic accuracy of breast cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

HZ: Writing – original draft. JH: Writing – original draft. RM: Writing – original draft. FL: Writing – original draft. FX: Writing – review & editing. MH: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Chengdu Science and Technology Bureau Focuses on Research and Development Support Plan (No. 2019-YF09-00097-SN).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1305545/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Deeks' funnel plot.

SUPPLEMENTARY FIGURE 2
Bivariate boxplot.

SUPPLEMENTARY FIGURE 3
Multiple univariate meta-regression and subgroup analysis. Prospective design: prodesign; fulverif: partial verification bias; subjdescr: adequate description of study participants; brdspect: broad spectrum of disease.

SUPPLEMENTARY FIGURE 4
Deeks' funnel plot.

SUPPLEMENTARY FIGURE 5
Bivariate boxplot.

SUPPLEMENTARY FIGURE 6
Multiple univariate meta-regression and subgroup analysis. Prospective design: prodesign; fulverif: partial verification bias; subjdescr: adequate description of study participants; brdspect: broad spectrum of disease.

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 16 January 2024
DOI 10.3389/fonc.2023.1322078

OPEN ACCESS

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RECEIVED 15 October 2023
ACCEPTED 28 December 2023
PUBLISHED 16 January 2024

CITATION
Zhang C, Zhou F, Zou J, Fang Y, Liu Y, Li L, Hou J, Wang G, Wang H, Lai X, Xie L, Jiang J, Yang C, Huang Y, Chen Y, Zhang H and Li Y (2024) Clinical considerations of CDK4/6 inhibitors in HER2 positive breast cancer. *Front. Oncol.* 13:1322078. doi: 10.3389/fonc.2023.1322078

Clinical considerations of CDK4/6 inhibitors in HER2 positive breast cancer

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Deregulation of cell cycles can result in a variety of cancers, including breast cancer (BC). In fact, abnormal regulation of cell cycle pathways is often observed in breast cancer, leading to malignant cell proliferation. CDK4/6 inhibitors (CDK4/6i) can block the G1 cell cycle through the cyclin D-cyclin dependent kinase 4/6-inhibitor of CDK4-retinoblastoma (cyclinD-CDK4/6-INK4-RB) pathway, thus blocking the proliferation of invasive cells, showing great therapeutic potential to inhibit the spread of BC. So far, three FDA-approved drugs have been shown to be effective in the management of advanced hormone receptor positive (HR+) BC: palbociclib, abemaciclib, and ribociclib. The combination strategy of CDK4/6i and endocrine therapy (ET) has become the standard therapeutic regimen and is increasingly applied to advanced BC patients. The present study aims to clarify whether CDK4/6i can also achieve a certain therapeutic effect on Human epidermal growth factor receptor 2 positive (HER2+) BC. Studies of CDK4/6i are not limited to patients with estrogen receptor positive/human epidermal growth factor receptor 2 negative (ER+/HER2-) advanced BC, but have also expanded to other types of BC. Several pre-clinical and clinical trials have demonstrated the potential of CDK4/6i in treating HER2+ BC. Therefore, this review summarizes the current knowledge and recent findings on the use of CDK4/6i in this type of BC, and provides ideas for the discovery of new treatment modalities.

KEYWORDS

CDK4/6 inhibitor, HER2-positive breast cancer, off-label indications, abemaciclib, palbociclib, ribociclib

1 Introduction

Breast cancer (BC) is now a well-known type of cancer, accounting for 11.7% of all malignancies (1), and is the leading reason for cancer-associated death in women globally (2). At present, breast cancer is classified into five distinct subtypes based on genetic and epigenetic factors. These include luminal A, luminal B, HER2-positive, triple-negative A, and triple-negative B subtypes (3). Human epidermal growth factor receptor 2 positive (HER2+) is a molecular sub-type of BC that causes 15-20% of all BC cases (4). This type of BC is particularly aggressive, often with an uncertain prognosis and a high risk of disease recurrence (5, 6). HER2+ BC is defined as a molecular sub-type that has increased HER2 protein expression by immunohistochemistry (IHC) or has amplified HER2 gene expression by *in situ* hybridization (ISH). The following conditions can indicate HER2+: 1. The IHC result is IHC3+; 2. The IHC result is IHC2+, ISH dual-probe test results show that the HER2/chromosome enumeration probe 17 (CEP17) ratio is maintained at <2.0 and HER2 signal per cell is ≥6.0, or the HER2/CEP17 ratio is ≥2.0 and HER2 signal per cell is ≥4.0 (7).

Targeted therapies can alleviate HER2+ BC, mainly anti-HER2 antibodies such as trastuzumab and pertuzumab, and small molecule tyrosine kinase inhibitors (TKI), such as lapatinib and neratinib (8). The recommended treatment regimen for HER2+ metastatic breast cancer (MBC) is trastuzumab plus pertuzumab and a taxane as primary treatment and trastuzumab emtansine, an antibody-drug conjugate, as the secondary treatment for patients with progressive disease (9–11). Chemotherapy is another treatment option. In the United States, stage II and III HER2+ BC guidelines prescribe neoadjuvant/adjuvant chemotherapy regimen of doxorubicin/cyclophosphamide paclitaxel and docetaxel/carboplatin (12), however, systemic chemotherapy often brings many serious side effects. Despite significant advancements in HER2+ BC treatment over the past 20 years, some early BC patients still experience relapses (13, 14), and some HER2+ MBC patients experience primary or secondary resistance (15, 16). In the end, the majority of HER2+ MBC patients pass away from their illness (17, 18).

Recently, many studies have begun to turn attention to chemotherapy-free regimens that combine targeted therapies with cell cycle inhibitors. According to the past treatment history, cell cycle inhibitors are sensitive to estrogen receptor positive human epidermal growth factor receptor 2 negative (ER+/HER2-) BC. Cell cycle inhibitors are mainly used in this type of BC, and have achieved very good responses in clinical practice. There is also some interest in whether cell cycle inhibitors can be used in HER2+ BC. According to some preclinical and clinical studies, cell cycle inhibitors may be used to treat HER2+ BC in the future, and these results may offer new potential therapeutic approaches and strategies.

In this review, we briefly describe the mechanism of action of CDK4/6i and its current therapeutic efficacy against HER2+ BC. We present clinical trials related to this use that seek to broaden the use of CDK4/6i beyond treating advanced hormone receptor positive (HR+)/HER2- BC.

Abbreviations: BC, Breast cancer; MBC, Metastatic breast cancer; CDK, Cyclin D-cyclin dependent kinase; CDK4/6i, CDK4/6 inhibitors; INK4, Inhibitor of CDK4; RB, Retinoblastoma; pRb, Retinoblastoma protein; ET, Endocrine therapy; HR+/-, Hormone-receptor-positive/negative; ER+/ER-, Estrogen receptor- positive/negative; HER2+/-, Human epidermal growth-factor receptor 2-positive/negative; PR+, Progesterone receptor-positive; FDA, Food and Drug Administration; IHC, Immunohistochemistry; ISH, *In situ* hybridization; CEP17, Chromosome enumeration probe 17; TKI, Tyrosine kinase inhibitors; MBC, Metastatic breast cancer; PDX, Patient-derived xenografts; BM, Brain metastases; BBB, Blood-brain barrier; GBM, Glioblastoma multiforme; EGFR, Epidermal growth factor receptor; HER2-E, HER2-enriched.

2 Mechanism of action of CDK4/6 inhibitors

Normal cells have elaborate regulatory mechanisms to ensure the orderly progress of each phase of the cell cycle. However, cell cycle disorders often lead to cancer development (19). Among them, the cyclin D-cyclin dependent kinase 4/6-inhibitor of CDK4-retinoblastoma (cyclinD-CDK4/6-INK4-RB) is an essential pathway for cancer cells to modulate G1 to S, which is important for many cancer types' initiation, development, and survival (20, 21). When this important pathway is deregulated, cancer cell proliferation increases and leads to many types of cancer occurrence, especially BC (22, 23).

In the cyclinD-CDK4/6-INK4-RB pathway, upstream signaling pathways, such as RAS and PI3K, promote the formation of cyclin D complexes with CDK4/6 by conveying external stimuli to cyclin D expression. This complex results in the phosphorylation of retinoblastoma (RB) protein, which inactivates RB (24). Inactivation of RB reduces RB's repressive control of the E2F family of transcription factors. Inhibition of E2F transcription factors are reduced, E2F is dissociated from RB-E2F complex, and more E2F transcription factor is released (25). On one hand, the released E2F initiates DNA synthesis, leadingthe cell cycle from G1 to S. On the other hand, it promotes the transcription of E-type cyclin, activates CDK2, further phosphorylates RB1, reduces E2F inhibition, releases more E2F, promotes DNA synthesis (26), and forms a positive feedback loop (27). These mechanisms are shown in Figure 1.

As mentioned earlier, when the cell cycle is intact, it can be targeted by CDK4/6i, so CDK4/6i have become anti-tumor drugs. The FDA has highly acknowledged CDK4/6i, primarily abemaciclib, palbociclib, and ribociclib. When combined with targeted therapy, ET is the first choice of treatment for the majority of HR+/HER2- MBC patients (28). Although CDK4/6i are primarily used in HR+/HER2- BC, they also have potential use for other malignancies. For example, melanoma (29, 30), head and neck carcinoma (31, 32), esophageal carcinoma (33, 34), lung cancer (35), liver cancer (36), and other cancers reflect its extensive anti-tumor effect.

3 Clinical trials studying CDK4/6 inhibitors against HER2 positive breast cancer

Considering that extensive anti-tumor effects of CDK4/6i, especially the mechanism of action, cell cycle alternation in HER2+

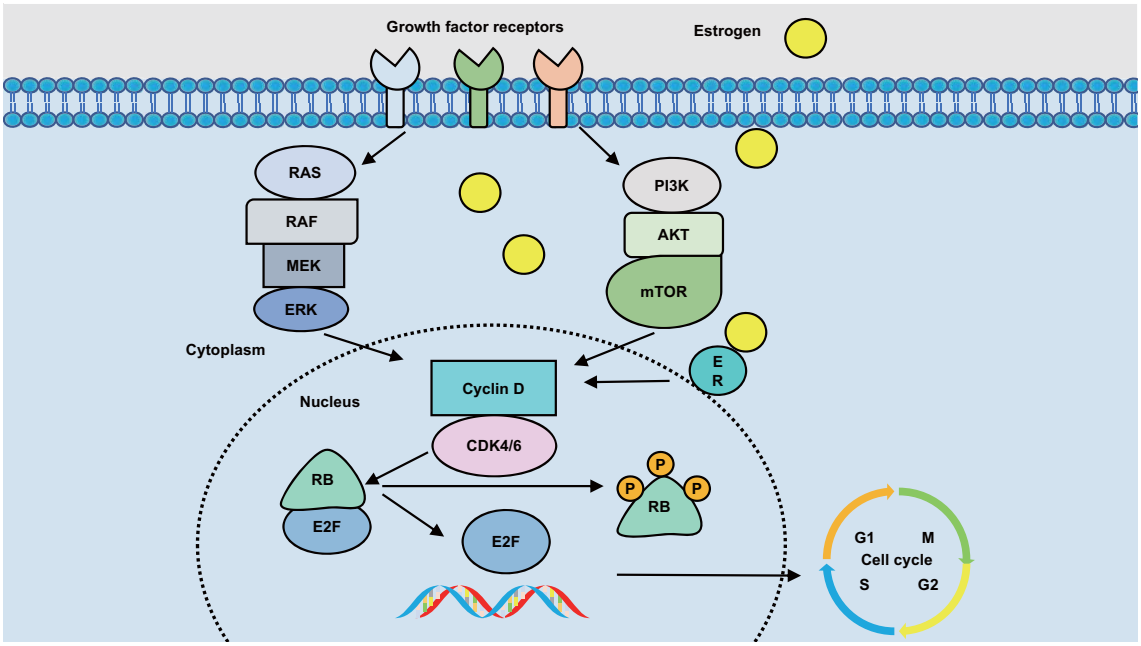

FIGURE 1
CDK4/6 a simple pathway to regulate G1 to S in cancer cells. Description: The transcription of D-type cyclins is influenced by various signaling pathways, including PI3K-AKT-mTOR, RAS-RAF-MEK-ERK, and ER. These pathways induce the expression and stability of D-type cyclins. CDK4/6 acts as a sensor that connects multiple signaling pathways to initiate and progress the cell cycle. CDK4 or CDK6 forms a complex with D-type cyclin, leading to the inactivation of the tumor suppressor Rb in the growth factor receptor pathway or estrogen receptor pathway. Consequently, the cell cycle transitions from the G1 phase to the S phase. Inhibitors of CDK4/6 can arrest the cell cycle in the G0/G1 phase by preventing downstream Rb phosphorylation through the inhibition of CDK4/6. ER estrogen receptor, RAS Ras proteins, RAF Raf kinase, MEK mitogentic effector kinase, ERK Extra cellular-signal-regulated kinases, PI3K phosphoinositide 3-kinase, Akt kinase, mTOR mammalian target of rapamycin, CDK cyclin-dependent kinase, RB retinoblastoma-associated protein, E2F protein.

BC (37), and the cyclinD/CDK 4/6 compound are directly downstream of the HER2 pathway (38), it is reasonable to apply CDK4/6i to HER2+ BC.

3.1 Preclinical trials studying

In some pre-clinical data, CDK4/6i treatment has been shown to remedy HER2+ BC. Nikolai et al. (2016) showed E2F1-driven DNA metabolism and replication of genes. Together with the phosphorylation and activity of the transcriptional coactivator steroid receptor coactivator-3 (SRC-3), E2F1-driven DNA metabolism is regulated by HER2 signaling to enhance BC cell proliferation. Furthermore, employing palbociclib, their analysis found a CDK signaling point that specifies the overlap and divergence of adjuvant pharmacologic targeting. Notably, E2F1 and its target genes are mainly disrupted by lapatinib and palbociclib, which tightly limit *de novo* DNA synthesis (39).

However, preliminary data from some early clinical trials indicate that only one CDK4/6i is ineffective against HER2-overexpressing BC, implying that combination therapy may be tried in HER2+ BC. Studies have found that the combination of small molecule inhibitors of HER2: TKI (e.g., pyrotinib, tucatinib, neratinib, etc.) and CDK4/6i appears to show some unexpected findings in preclinical studies of HER2+ BC. Zhang et al. (40) found that palbociclib improved the effects of pyrotinib in HER2+BC. The findings indicate that the therapeutic regimen of palbociclib and pyrotinib together is highly synergistic and has more antitumor activity than either drug alone. Together they cause a significant decrease in phosphorylated AKT (pAKT) and pHER3 activation, causing G0-G1 arrest, increasing apoptosis, and there is no appreciable increase in toxicity (40). Tucatinib combined with CDK4/6i also showed similar effects. The combined activity of tucatinib with the three approved CDK 4/6i, palbociclib, ribociclib, and abemaciclib, has been demonstrated in HER2+ BC. The combination increases sensitivity to cell inhibition compared to the single agents, while tucatinib and CDK4/6i have no antagonistic interactions, according to cell cycle research (41).

CDK4/6i has also shown a complementary mechanism of action to the dose-dependent effects of TKIs, in particular neratinib and afatinib. CDK4/6i inhibited proliferation/cell viability across multiple compounds in an additive relationship, which was summarized in different HER2 positive models (42). In addition, pre-clinical trials using neratinib and pabociclib in HER2 positive cell lines and patient-derived xenografts (PDX) confirmed the benefits of this combination. It is worth mentioning that the synergistic effect of the combination showed significantly enhanced anti-tumor efficacy, mainly in terms of tumor volume reduction (43). The combination of trastuzumab with abemaciclib also appears to show some therapeutic effect. In a HER2+ PDX

model, no effect on xenograft growth was observed with trastuzumab alone, furthermore abemaciclib alone only inhibited tumor growth without causing regression. Remarkably, the combination of abemaciclib with trastuzumab led to both significant tumor cell growth inhibition and tumor regression (44).

3.2 Clinical trial studies

Several clinical trials studying the use of CDK4/6i and other drugs seem to confirm the safe and effective results observed in preclinical data. In the MonarcHER trial, 273 women with advanced ER+/HER2+ were enrolled and given a treatment combination of fulvestrant, abemaciclib, and trastuzumab, and then compared against standard chemotherapy plus trastuzumab. The study endpoint was reached after a median follow-up interval of 19.0 months. Results showed that the combination of cell proliferation inhibitors increased survival compared with standard chemotherapy, and that adverse reactions were tolerable (45). An NA-PHER2 study with multiple cohorts and multiple sites included 35 patients. The results showed a very interesting phenomenon in the combination of palbociclib with pertuzumab and trastuzumab. They found that Ki67 was lower after 2 weeks of treatment with this combination as well as at the time of surgery (6 weeks after treatment) compared with the beginning of the study (46). From the MonarcHER and NA-PHER2 studies, our hypothesis was that ER+/HER2+ individuals who do not want or cannot take chemotherapy could benefit from simultaneous inhibition of ER, HER2, and RB targets.

The SOLTI-1303 PATRICIA study compared palbociclib with trastuzumab, in combination with ET, to palbociclib with trastuzumab in highly pretreated patients with HER2+ advanced BC. These patients were also highly preconditioned, having received 2-4 lines of an anti-HER2 treatment. The results showed efficacy in this group of ER+/HER+ patients to be encouraging (47). Another phase 1b/2 study showed less consistent results. This combination treatment was safe but had limited resulting activity. The advanced HER2 + patients in this study had intensive pretreatment, including treatment with trastuzumab, pertuzumab, and trastuzumab emtansine. This indicates that patients who are too heavily pretreated in the metastatic setting and who then receive a median of 4 lines of chemotherapy, have a less than satisfactory response (48). These studies suggest that it is uncertain whether or not pretreatment is beneficial for advanced HER2+ BC patients who plan to use the CDK4/6i/anti-HER2 combination treatment. Given the relatively small population sizes in both studies, this may have contributed to some of the differences in results.

The aforementioned results indicate that CDK4/6i and other medications that are used together would provide extra therapeutic benefit for HER2 patients, regardless of pretreatment, therefore more research into this area is needed. Many new CDK4/6i and HER2-targeted medication combination schemes are now being investigated for treating both ER+/HER2+ and HR+/HER2+ breast cancer. These combinations are listed in Tables 1, 2.

4 Patients with HER2+ brain metastasis

Brain metastases (BM) are a common complication for many cancer patients, particularly for those with HER2+ BC (53). These individuals have a higher risk for developing BM (54), with an incidence of about 50%, increasing year by year (55). Patients with this type of breast cancer often have a poor quality of life and poor survival chances (56). Current treatments for such patients include radiotherapy, surgery, and HER2 targeted therapy. Radiotherapy is the main treatment for BM, but it is often associated with neurocognitive decline and has an unclear prognosis (57). HER2 targeted medicines are unable to pass the blood-brain barrier (BBB). Reliable information on how to handle HER2+ BM is lacking. Despite international consensus guidelines recommending a sequential HER2 blockade, it is unclear which anti-HER2 agent is the best choice when BC occurs (58). Therefore, it is necessary to find a systemic therapy that may effectively cross the BBB and avoid the neurocognitive decline caused by radiation therapy.

In some studies, a series of new and highly effective CDK4/6i have been designed and synthesized, which show good BBB permeability in the therapies treating glioblastoma multiforme (59). In contrast, CDK4 and CDK6 inhibitors have been shown to reach high brain concentrations in rodents in preclinical studies and demonstrate the advantages of abemaciclib, which may require lower doses and longer durations than palbociclib (60). Ni et al. (2022) and his colleagues found that combination therapy with tucatinib and abemaciclib could reduce tumor growth and significantly and prolong survival time in mouse models of HER2+ BC with brain metastases, while tucatinib or abemaciclib as monotherapy did not show significant therapeutic benefit (61). Therefore, the use of CDK/6i, either alone or together, may be a potential therapy option for individuals with BM.

The primary goal of the phase II clinical trial NCT02774681 in HER2+ BM was to determine whether palbociclib is effective in HER2+BC patients with BM. In this study, a total of 12 patients were enrolled in a daily oral palbociclib regimen, repeated every 28 days. NCT04334330 is a non-randomized, phase II clinical trial in ER+/progesterone receptor-positive (PR+)/HER2+ BC with BM. This study's main objective was to evaluate the effectiveness of palbociclib, trastuzumab, and pyrotinib in combination with fulvestrant in ER+/PR+/HER2+ BC with BM. The regimen is daily oral palbociclib on days 1 to 21, with intravenous trastuzumab every three weeks, daily oral pyrotinib, and intramuscular fulvestrant every 4 weeks. Cycles were repeated every 28 days. As shown in Table 3.

5 CDK4/6 inhibitors overcome resistance to targeted therapy in HER2 positive breast cancer

The use of HER2 inhibitors, especially in combination, provides significant therapeutic benefits to BC patients, but the response is often limited due to persistent primary or acquired resistance (63–65). There are currently numerous hypothesized pathways for

TABLE 1 Clinical trials studying the application of CDK4/6 inhibitors in HER2+ breast cancer.

Identifier	Study design	Agents and dose	Participants and recruitment period	Estimated/ Actual enrollment	Primary endpoint and duration	Status
NCT03530696	single arm Open label Phase II	Palbociclib: 125mg T-DM1: 3.6 mg/kg	Metastatic HER2+BC and other breast tumors December 6, 2018-December 22, 2022	46	PFS 4 years	Completed No Results Posted
NCT03993964	single arm open label Phase II	Pyrotinib: 400mg SHR6390: 125mg	Metastatic Her2+BC August 15, 2019-October 30, 2020	20	ORR 100 months	Unknown No Results Posted
NCT04293276	single arm open label Phase II	Pyrotinib: ND SHR6390: ND	Metastatic Her2+BC April 1, 2020-August 23, 2021	41	ORR 2 years	Active, not recruiting NCT04293276
NCT03304080	single arm open label Phase I/II	Anastrozole:1 mg Palbociclib:100 mg/125mg Trastuzumab: 6 mg/kg or 8mg/kg Pertuzumab:420mg/840mg	Metastatic Her2+BC December 20, 2017-July 2024	44	DLT MTD CBR 3 months	Active, not recruiting No Results Posted
NCT03284723	Randomized Open Label Phase I	PF-06804103:ND Palbociclib : NDLetrozole : ND	Her2-/HER2+BC November 1, 2017-August 31, 2021	95	DLTs PFS TTP and DR 2 years	Completed Results SubmittedNotPosted
NCT05319873	Randomized Open label Phase Ib/II	Carboplatin : NDDocetaxel : ND Fulvestrant : ND Ribociclib : No Trastuzumab : ND Pertuzumab : ND Tucatinib : ND	Locally advanced/ Metastatic Her2+BC and other breast tumors April 7, 2022- April 1, 2024	18	MTD、pCR 30 days or 58 days	Recruiting No Results Posted
NCT04095390	Randomized Open Label Phase II	Pyrotinib:400 mg SHR6390: 125mg Letrozole: 2.5mg Capecitabine: 500mg	prior trastuzumab-treated advanced HER2+BC September 30, 2019-November 30, 2021	60	ORR 2 months or 3 years	Unknown No Results Posted
NCT02657343 (48)	Non-randomized open label Phase I/II	Ribociclib:300/400/500/600mg T-DM1: ND Trastuzumab: 6 mg/kg Fulvestrant : ND	Advanced/Metastatic Her2+BC March 2016-March 2017 Median follow-up was 12.4months	13	RP2D:400mg CBR : NR mPFS:10.4months 10.9 months	Completed Has Results
NCT03054363 (49)	Single Group Open Label Phase Ib/II	Tucatinib:300mg Palbociclib: 75mg/125mg Letrozole:2.5mg	Metastatic Her2+BC November 2017-April 2020 The median follow-up was 33.6 months	42	Ib mPFS:8.2 months II mPFS:10.0months 4 years	Active, not recruiting Has Results
NCT04778982	parallel arm Open Label Phase II	KN026: 20 mg/kg Palbociclib: 125 mg Fulvestrant:500mg	Metastatic Her2+BC May 25, 2022- March 15, 2023	36	DLT、ORR 24 weeks or 1 year	Terminated No Results Posted
NCT02448420 (47) (PATRICIA II)	Randomized Open Label Phase II	Palbociclib: 125/200 mgTrastuzumab: 8mg/kg or 600mg Endocrine therapy Chemotherapy : NDAntibody-Drug Conjugates: 3.6 mg/kg	Previously-treated Locally Advanced or Metastatic Her2+BC July 2015 - November 2018 No median follow-up time	72	Cohort A:mPFS:4.2 months Cohort B1:mPFS: 6.0 months Cohort B2:mPFS: 5.1 months 6 months or 4 years	Active, not recruiting
NCT05429684	Non-randomized Open label Phase III	Trastuzumab: 6mg/kgPertuzumab:420mg Nab paclitaxel:200mg Pyrotinib:400mg Capecitabine T-DM1:3.6mg/kg Everolimus:4mg CDK4/6 inhibitor:	Advanced Her2+BC January 1, 2021-February 28, 2024	120	ORR、 PDO model inhibition rate six weeks or during the procedure	Recruiting No Results Posted

(Continued)

TABLE 1 Continued

Identifier	Study design	Agents and dose	Participants and recruitment period	Estimated/ Actual enrollment	Primary endpoint and duration	Status
		Palbociclib:125mg AI: Letrozole 2.5mg Anti-PD-1monoclonal antibody:200mg				
NCT03065387	Non-randomized Open label Phase II	Everolimus Neratinib : NDPalbociclib : ND Tra metinib:ND	Advanced Cancer Subjects With HER2 Mutation/ Amplification and other type Mutation/ Amplification October 31, 2017- October 1, 2025-	93	safety and tolerability 、MTD、DLT 28 days or 58 days	Active, not recruiting No Results Posted

BC, Breast Cancer, PFS, progression-free survival, ORR, Objective Response Rate, DLT, Dose-Limiting Toxicity, MTD, Maximum Tolerated Dose, CBR, Clinical Benefit Rate, DLTs, Dose-Limiting Toxicities, TTP, Time to Tumor Progression, DR, Duration of Response, RP2D, Recommended Phase2 Dose, pCR, Pathologic complete response, PDO, Patient-Derived Oranoid. ND, No Dose, NNR, Not reach.

TABLE 2 Clinical trials studying the application of CDK4/6 inhibitors in ER+/HER2+ or HR+/HER2+breast cancer.

Identifier	Study design	Agents and dose	Participants and recruit-ment period	Estimated/ Actual enrollment	Primary endpoint and duration	Status
NCT02675231 (45) (monarcHER)	Randomized Open Label Phase II	(Abemaciclib)LY2835219:150mg Trastuzumab:8mg/kg Fulvestrant:500mg	Locally advanced/ Metastatic HR +/Her2+BC May 31, 2016, and February 28, 2018 The median follow-up was 19.0 months	237	groupA mPFS:8.3months grougB mPFS:5.7months groupC mPFS:5.7months 36 Months	Active, not recruiting Has Results
NCT04224272	Non-randomized Open label Phase II	ZW25:ND Palbociclib : ND Fulvestrant : ND	HR+/Her2+BC June 10, 2020- April 28, 2023	51	DLT、Incidence of AEs 、PFS、Incidence of lab abnormalities 4 weeks or 3.5 years or 6 months	Active, not recruiting No Results Posted
NCT03772353 (50) LORDSHIPS	single arm open label Phase Ib/II	Letrozole:2.5mg Pyrotinib:320mg Dalpiciclib (SHR6390):125mg Fulvestrant : ND	Advanced ER +/HER2+BC February 2019 - June 2020 The median follow-up was 11.4 months	15	ORR: 66.7% mPFS:11.3 months 1 year	Active, not recruiting No Results Posted
NCT02907918	Single arm Open label Phase II/III	Palbociclib:125mg Letrozole:25mg Trastuzumab:2mg/kg or 4mg/kg Goserelin:3.6mg	ER+/HER2+ BC June 30, 2017- August 24,2020	26	Number of Participants With pCR:2 pCR rate:8% 16 weeks	Terminated Has Results
NCT04858516	Single Group Open Label Phase II	Palbociclib : NDExemestane : NDTrastuzumab : NDPyrotinib : ND	ER+/HER2+BC April 30, 2021- April 30, 2024	57	pCR 24 weeks	Not yet recruiting No Results Posted
NCT03709082	Non-Randomized Open Label Phase I/II	Palbociclib:75/mg Letrozole:2.5mg T-DM1:3.6mg/kg	ER+/HER2+ Metastatic BC October 15, 2018- March 12, 2020	3	ORR 5 years	Active, not recruiting No Results Posted

(Continued)

TABLE 2 Continued

Identifier	Study design	Agents and dose	Participants and recruitment period	Estimated/ Actual enrollment	Primary endpoint and duration	Status
NCT03644186 (51)	Randomized Open Label Phase II	Paclitaxel:80mg/m2 Trastuzumab:600mg Pertuzumab:840mg Palbociclib:125mg Letrozole:2.5mg	ER+/HER2+ Early BC April 16, 2019- January 3, 2023 No median follow-up time	144	No pCR No mPFS 16 weeks.	Completed No Results Posted
NCT05076695	Single Group Open Label Phase II	Palbociclib:125mg fulvestrant:500mg trastuzumab: 6mg/kg or 8mg/kg pyrotinib: 400mg	ER+/HER2+ BC October 15, 2021- October 15, 2023	37	pCR 1 year	Recruiting No Results Posted
NCT02947685 (52)(PATINA)	Randomized Open Label Phase III	Palbociclib:125mg trastuzumab: 6mg/kg or 8mg/ kgpertuzumab:420mg or 840mg letrozole:2.5mg Anastrozole:1mg Fulvestrant:250mg Exemestane:25mg	HR+/HER2+ Metastatic BC June 21, 2017- December 30, 2023 No median follow-up time	496	No PFS 24 months	Active, not recruiting No Results Posted
NCT03913234	Single Group Open Label Phase I b/II	Ribociclib:200-600mg Trastuzumab:8mg/kg loading followed by 6mg/kg Letrozole:2.5mg	HR+/HER2+ Advanced BC Actual Study Start Date : June 10, 2019– October 30, 2023	95	PFS 1 year	Recruiting No Results Posted
NCT02530424 (46) (NA-PHER2)	Single Arm Open Label Phase II	Trastuzumab: 6mg/kg or 8mg/kg Pertuzumab:840mg Palbociclib:125mg Fulvestrant500mg	ER+/HER2+ BC May 20, 2015, -February 8, 2016 No median follow-up time	102	Serial measures of Ki67- At baseline Ki67:31·9 week 2:4·3 surgery:12.1 26 weeks	Completed No Results Posted

BC, Breast Cancer, PFS, progression-free survival, ORR, Objective Response Rate, DLT; Dose-Limiting Toxicity, pCR, Pathologic complete response, AEs, Adverse Events, AE, Adverse Event. ND, No Dose, NNR, Not reach.

trastuzumab resistance in BC that is HER2 positive. The primary signaling pathways that HER2 mediates are the RAS/MAPK, PI3K/PKB/Akt, and IL6/JAK/STAT3 pathways. These pathways are crucial for cell growth, differentiation, skeleton construction, cell death, and malignant transformation (66, 67). Among them, PI3K/Akt/mTOR pathway and cyclin D1/CDK4/6/retinoblastoma protein (pRb) axis are important resistance pathways for HER2 targeted therapy (68), as shown in Figure 2.

Over-activation of the PI3K/AKT/mTOR pathway is thought to be among the dominating causes of carcinogenicity, which is linked to various resistance mechanisms to anti-HER2 therapy (69). Pre-clinical evidence has shown that the PI3K/Akt/mTOR pathway contributes to HER2-directed therapy resistance, making it a new target for the treatment of HER2-resistant disease in clinical development (70). This has sparked a number of trials to test whether or not inhibitors of this pathway can overcome HER2-directed therapy resistance. Even though mTOR inhibitors were the main focus of the majority of these trials, they produced encouraging outcomes (71).

Downstream from the HER2 signaling pathway, the Cyclin D-CDK4/6 pathway is important in blocking the HER2 pathway (38). In actuality, HER2 targeted therapy-resistant recurrent tumor cells are susceptible to RNA interference or CDK4/6 inhibitor-mediated cyclin D1 down-regulation (72). When cyclin D1 is activated downstream, trastuzumab and other HER2 targeted medicines become resistant to their effects.

Studies indicate that CDK4/6i and HER2 inhibitors used together yield some intriguing results in the subsequent treatment of HER2+ BC. Goel et al. (2016) used cell line-based mechanistic investigations and clinical transgenic mouse models to discover that CDK4/6i can inhibit RB phosphorylation and decrease tuberin (TSC2) phosphorylation, thereby inhibiting mTORC1/S6K/S6RP activity. Dual inhibition of epidermal growth factor receptor (EGFR)/HER2 and CDK4/6 can more effectively enhance this effect, which relieves feedback inhibition of upstream EGFR family kinases and resensitizes tumors to EGFR/HER2 blockade. In transgenic mouse models, HER2 and CDK4/6i collaborated to inhibit cell proliferation, control tumor growth *in vivo*, and delay tumor recurrence (44). Another study of Qingfei Wang and his colleagues showed less consistent results. In transgenic mouse models, the combination of anti-HER2 and CDK4/6i rapidly developed resistance. Two weeks of continuous anti-HER2/neu antibody plus palbociclib produced significant results: tumor regression, 52.74% average volume reduction, and significant inhibition of tumor cell proliferation, efficacy, and prolonged survival. Tumors treated with this combination, however,

TABLE 3 Clinical trials studying the application of CDK4/6 inhibitors in Patients with HER2+ brain metastasis.

Selected inclusion/Exclusion Criteria	Interventions	Primary End point
NCT04334330 (62) **A Phase II study to Evaluate the Efficacy of Palbociclib, Trastuzumab and Pyrotinib With Fulvestrant in ER/PR+ and HER2+ breast cancer patients with brain metastasis**		Status : Recruiting
Estimated Enrollment: 34 - Histologically confirmed ER/PR positive, HER2-positive metastatic breast cancer - Measurable disease in the brain, defined as at least 1 lesion measuring >= 10 mm on MRI at the time of registration - leptomeningeal disease or been treated with WBRT is not allowed	- palbociclib PO daily on days 1-21, combined with trastuzumab IV every three weeks, pyrotinib PO daily and fulvestrant IM every 4 weeks. Cycles repeat every 28 days - No specific drug dosage - Actual Study Start Date : December 4, 2020 Estimated Primary Completion Date : December 30, 2023	Current results: Objective response rate in the CNS: 28.6%, mPFS:10.6 months, The time to progression in the CNS was 8.5 months The median follow-up was 6.3 months duration:3 years
NCT02774681 **A Phase II Single Arm Study to evaluate the Efficacy of Palbociclib in Patients With Metastatic HER2-positive Breast Cancer With Brain Metastasis**		Status: Terminated Has Results
Estimated Enrollment: 12 - Histologically confirmed HER2-positive metastatic breast cancer - should not have received > 2 lines of chemotherapy for metastatic disease - Measurable disease in the brain, defined as at least 1 lesion measuring >= 5 mm on imaging at the time of registration - Any uncontrolled neurological symptom attributed to CNS metastasis or leptomeningeal disease or Previous treatment with Palbociclib is not allowed	- palbociclib PO daily on days 1-21. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity - trastuzumab IV over 30-90 minutes every 3 weeks - No specific drug dosage - Recruitment period: May 25, 2016- January 28, 2019	No RRR, Stable DiseaseCNS:6, Progressive Disease CNS:6 duration:3 years

RRR, Radiographic Response Rate in the CNS in Patients With HER2-positive Breast Cancer Who Have Brain Metastasis Treated With Palbociclib. No RRR was not calculated as the study did not met statistical analysis criteria due to study closing before total accrual was met.

rebounded and eventually developed resistance shortly after tumor regression. However, they discovered that switching to a combination immunotherapy containing Cabo, a potential MDSC/IMCs targeting inhibitor, could overcome resistance to the anti-HER2/neu antibody plus palbociclib (73).

In fact, these findings not only generated interest in the use of CDK4/6i in HER2+BC therapy, but they also demonstrated that the simultaneous treatment of HER2 targeted drugs and CDK4/6i is effective, and these two inhibitors may work well in combination.

6 Predictive biomarkers of sensitivity to CDK4/6 inhibitors

CDK4/6i combined with ET is the main therapeutic strategy for HR+/HER2- BC patients with metastasis. However, resistance to CDK4/6i leads to treatment failure and cancer progression. Treatment strategies for reducing CDK4/6 resistance have not yet been standardized, and reliable biomarkers of treatment response need to be identified, particularly in persons with HER2+ BC.

Raspé et al. (2017) found that CDK4 T172 phosphorylation was most closely connected to breast tumors cell line susceptibility to the particular CDK4/6 inhibitor PD0332991 (palbociclib). The primary rate-limiting step for CDK4 activation is CDK172-activated T4 phosphorylation, which binds cyclin D. In the study, gene expression profiles identified tumors that were less responsive to CDK4/6i. This response suggests that sub-population sensitivity studies to this agent may help guide its use in cases of HER2+ and basal-like tumors (74).

It was found that HER2-E tumor cells were sensitive to anti-HER2 therapy but did not die and acquired the luminal A phenotype. This is particularly important in HR+/HER2+ disease. This phenotype develops relatively quickly and leads to anti-HER2 resistance. Surprisingly, after exposure to the anti-HER2 pathway, palbociclib in combination with anti-HER2 therapy has been shown to be more effective. These results demonstrate the luminal A phenotype can serve as a biomarker of anti-HER2 remedy resistance and implies that developing a more lumen-like phenotype may make cells more susceptible to CDK4/6i. It's interesting to note that the HER2 targeted remedy boosted sensitivity to CDK4/6i by enhancing the luminal phenotype. Finally, discontinuing the *in vitro* HER2 targeted remedy or developing resistance to the anti-HER2 remedy causes the original HER2-E phenotype to return. Our findings encourage the development of treatment strategies using the CDK4/6i sub-type switching and maintaining the anti-HER2 remedy (75).

The findings of a different study point to the potential use of pRb as a biomarker to forecast CDK4/6i responsiveness in HER2 +BC. A correlation between the number of HER2 gene copies and pRb levels was observed in the 77 HER2+ cases that were investigated. This data suggests that the number of copies of the HER2 gene can be used to predict CDK4/6 activity, with more copies indicating higher CDK4/6 activity. In order to discover the best course of treatment, it might be necessary to take into account the drug dose related to the number of HER2 gene copies, if CDK4/6i is ever to be considered for a remedy for HER2+ BC (76).

7 Conclusions

HR+ BC has responded well to ET in combination with CDK4/6i. Still, research continues to search for more treatments.

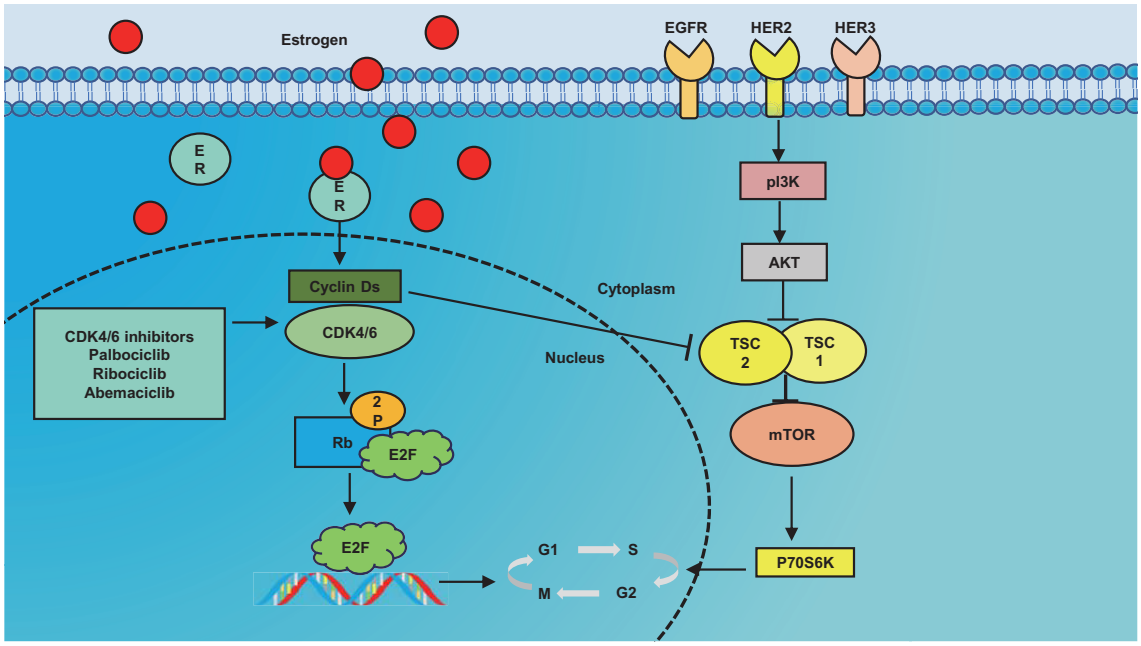

FIGURE 2
Simple association diagram between human epithelial growth factor receptor 2 (HER2) and estrogen receptor (ER) pathways. Description: Estrogen has the ability to enhance cell proliferation by increasing the levels of cyclin D1, CDK4/6 activity, and cyclin E/CDK2 levels. Additionally, HER2 can influence the PI 3 K/Akt/mTOR pathway to regulate cell proliferation. It is worth noting that these two pathways can be interconnected through TSC2. This implies that the D-CDK 4/6 pathway plays a crucial role in inhibiting the HER 2 pathway and serves as a fundamental principle for overcoming resistance to HER2 inhibitors. EGFR epidermal growth factor receptor, ER estrogen receptor, HER Human epidermal growth factor receptor, PI3K phosphoinositide 3-kinase, Akt kinase, mTOR mammalian target of rapamycin, CDK cyclin-dependent kinase, RB retinoblastoma-associated protein, E2F protein, p70S6K is members of the serine/threonine protein kinase family, TSC1 proteins and TSC2 proteins.

Preclinical research has been done on xenografts and HER2+ BC cell lines using CDK4/6i. Simultaneous targeting of HER2 and CDK, or DNA replication may be a suitable approach, but more clinical trials with larger sample sizes are essential for evaluating the benefits and drawbacks of CDK4/6i-based treatment regimens. At present, there are many effective targeted drugs for HER2+ BC, but their drug resistance often limits their clinical use.

Combining CDK4/6i with anti-HER2 therapy, such as trastuzumab and patuzumab, along with small-molecule tyrosine kinase inhibitors, has shown promise as a treatment modality. This regimen has demonstrated a higher survival benefit, with manageable adverse effects. Additionally, the combination of CDK4/6i and anti-HER2 targeting has been found to overcome anti-HER2 resistance, synergistically inhibiting cell proliferation, controlling tumor growth *in vivo*, and delaying tumor recurrence. However, it should be noted that this combination therapy can eventually lead to drug resistance. Nevertheless, studies suggest that combining it with certain immunotherapies may help overcome this resistance. Surprisingly, the CDK4/6i combined with anti-HER2 treatment has also shown good efficacy in treating HER2+ BM in BC. Therefore, for HER2+ BC patients who are unable or unwilling to undergo chemotherapy, the combination of CDK4/6i and anti-HER2 treatment could be a potential option, offering hope for extended survival.

In addition, if this combination therapy is a worthwhile option, more thorough clinicopathological characteristics and biomarkers of HER2+ BC sensitivity to CDK4/6i merit further investigation in pre-clinical research. And some clinical studies have even demonstrated the efficacy and safety of a three-drug regimen of CDK4/6i combined with endocrine therapy and anti-Her2 in HER2 + BC, an interesting chemotherapy-free combination. Our goal is to make better use of these novel targeted medications in the near future and give breast cancer patients more accurate and tailored care. Currently we are eagerly awaiting the outcomes of several trials of new CDK4/6i combinations.

Author contributions

CZ: Writing – original draft. FZ: Writing – original draft. JZ: Writing – original draft. YF: Writing – original draft. YCL: Writing – original draft. LL: Writing – original draft. JH: Writing – review & editing. GW: Writing – review & editing. HW: Writing – review & editing. XL: Writing – original draft. LX: Writing – original draft. JJ: Writing – original draft. CY: Writing – original draft. YH: Writing – original draft. YC: Writing – original draft. HZ: Writing – review & editing. YL: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Beijing Xisike Clinical Oncology Research Foundation (Xisike-Hengrui) (No. Y-HR2019-0436), the provincial key clinical Specialty Construction Project of Guizhou Province, and the National Key Clinical Specialty Construction project. The sponsors had no role in the design, analysis, and writing of the manuscript.

Acknowledgments

The authors acknowledge the support of the Foundation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 29 January 2024
DOI 10.3389/fonc.2024.1336696

OPEN ACCESS

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RECEIVED 11 November 2023
ACCEPTED 03 January 2024
PUBLISHED 29 January 2024

CITATION
Zheng H, Wu X, Guo L and Liu J (2024) MyD88 signaling pathways: role in breast cancer.
Front. Oncol. 14:1336696.
doi: 10.3389/fonc.2024.1336696

MyD88 signaling pathways: role in breast cancer

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MyD88 plays a central role in breast cancer, exerting a multitude of effects that carry substantial implications. Elevated MyD88 expression is closely associated with aggressive tumor characteristics, suggesting its potential as a valuable prognostic marker and therapeutic target. MyD88 exerts influence over several critical aspects of breast cancer, including metastasis, recurrence, drug resistance, and the regulation of cancer stem cell properties. Furthermore, MyD88 modulates the release of inflammatory and chemotactic factors, thereby shaping the tumor's immune microenvironment. Its role in immune response modulation underscores its potential in influencing the dynamic interplay between tumors and the immune system. MyD88 primarily exerts intricate effects on tumor progression through pathways such as Phosphoinositide 3-kinases/Protein kinase B (PI3K/Akt), Toll-like Receptor/Nuclear Factor Kappa B (TLR/NF-κB), and others. Nevertheless, in-depth research is essential to unveil the precise mechanisms underlying the diverse roles of MyD88 in breast cancer. The translation of these findings into clinical applications holds great promise for advancing precision medicine approaches for breast cancer patients, ultimately enhancing prognosis and enabling the development of more effective therapeutic strategies.

KEYWORDS

breast cancer, myeloid differentiation factor 88, epithelial-mesenchymal transition, tumor microenvironment, biomarker

Introduction

Breast cancer (BC) is one of the most prevalent malignant tumors in women (1). The global burden of breast cancer annually amounts to over 2.2 million diagnosed cases, resulting in over 600,000 fatalities (2). Encouragingly, in high-income countries, there has been a decline in breast cancer mortality, largely attributed to advancements in treatment modalities. However, there's a steady rise in breast cancer incidence, and it remains the

most common cause of cancer-related deaths in low-income countries (3). Clinically, specific subtypes of breast cancer are characterized by their histological appearance and the expression of hormone receptors and growth factors, namely estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) (4). Characterized by remarkable heterogeneity, breast cancer is primarily driven by factors such as metastasis, recurrence, and drug resistance, which contribute significantly to patient mortality (3).

Against this backdrop, Myeloid Differentiation Factor 88 (MyD88) assumes a crucial role in breast cancer. The MYD88 gene provides instructions for producing proteins involved in signaling within immune cells. Acting as an adapter, the MyD88 protein plays a crucial role in innate immunity by mediating cell activation through Toll-like receptors (TLRs). TLRs interact with adapter protein MyD88, initiating the activation of two key transcription factors: NF-κB, a dimeric protein responsible for expressing various inflammatory cytokines, chemokines, adhesion, and co-stimulatory molecules, triggering acute inflammation and stimulating the immune response. IRFs, a group of proteins responsible for expressing type I interferons, to establish the so-called antiviral state of cells (5). While MyD88 is renowned for the role in recognizing and responding to microbial pathogens in innate immunity (6, 7), it also plays a pivotal role in numerous non-immune processes, particularly within the context of tumor development. Currently, MyD88 is regarded as a critical signaling molecule with diverse roles in the development and progression of breast cancer. Engaging in various signaling pathways, MyD88 influences proliferation, migration, and invasion of breast cancer cells. Through cascading responses such as Phosphoinositide 3-kinases/Protein kinase B (PI3K/Akt), Wingless/Integrated (Wnt), Notch, Hedgehog, and Nuclear Factor kappa B (NF-κB), it orchestrates the epithelial-mesenchymal transition (EMT), conferring migratory and invasive traits upon tumor cells (8–15). By modulating signaling pathways governing the maintenance and functionality of breast cancer stem cells (BCSCs), MyD88 regulates tumor initiation, progression, recurrence, and therapy resistance (16). Activation of MyD88 intricately correlates with self-renewal, cytokine production and secretion, as well as expansion of tumor stem cells, thereby influencing tumor development and progression.

Through modulation of inflammatory responses and immune reactions, MyD88 impacts the immune microenvironment of breast cancer (17). It governs the effects of tumor cell-derived exosomes, stimulating the production of inflammatory factors and influencing the activity of macrophages, T cells, and NK cells. Moreover, the interaction of MyD88 with immune checkpoint therapy plays a pivotal role, significantly impacting breast cancer treatment outcomes. Notably, the expression level of MyD88 level closely associates with disease severity, prognosis, and staging of breast cancer (18). High MyD88 expression correlates intimately with clinical parameters like tumor size, lymph node metastasis status, and histological grade. Patients with high MyD88 expression tend to experience recurrences or metastases, whereas those with low expression exhibit better prognosis.

In this review, we provide a comprehensive overview of MyD88's roles in tumorigenesis, metastasis, drug resistance, the tumor microenvironment, and prognosis in breast cancer (Figure 1). We also explore potential avenues through which this understanding can drive future drug development. By delving deeper into the multifaceted impacts of MyD88 in breast cancer, we anticipate the emergence of novel strategies for the treatment and management of this disease.

The role of MyD88 in clinical disease assessment and prognosis

MyD88 assumes a pivotal role in the clinical evaluation and prognosis of breast cancer. A multitude of studies has revealed a profound association between MyD88 expression levels and the severity and prognostication of breast cancer (Table 1). Elevated MyD88 expression is correlated with larger tumor size, lymph node metastasis, and higher histological grades, suggesting MyD88's potential as a biomarker for appraising breast cancer's invasiveness and progression. The protein expression of MyD88 demonstrates a significant positive correlation with tumor size, stage, axillary lymph node metastasis, and distant metastasis (19). MyD88 protein expression is positively linked with axillary lymph node metastasis and histological grade. Both TLR4 and MyD88 protein expressions are positively correlated with breast cancer cell metastasis (18). Notably, MyD88 protein expression in breast cancer tissue surpasses that in adjacent non-cancerous tissue, and it is positively associated with axillary lymph node metastasis, histological grade, and distant metastasis (18). TLR4 and MyD88 protein expression levels are positively correlated with axillary lymph node metastasis and histological grade, and the co-expression of TLR4 and MyD88 is also positively correlated with breast cancer cell metastasis (18). The expression rate of MyD88 is notably higher in samples from patients with axillary lymph node metastasis (59%) compared to those without metastasis (25.6%). Similarly, the expression rate of MyD88 is higher in patients with stage III disease (65.6%) compared to those with stage I/II disease (44.1%) (18). The expression of MyD88 is intrinsically connected to patient prognosis, with higher MyD88 expression increasing the propensity for recurrence or metastasis. MyD88 expression levels inversely correlate with disease-free survival (DFS) and overall survival (OS), with lower MyD88 expression translating to better patient outcomes (21). A notable divergence in survival rates is evident between high and low MyD88 expression groups, as evidenced by Xiang's study (20). Kaplan-Meier survival curves underscore the significance of MyD88 expression in survival disparities between patients with high MyD88 expression and those with normal expression (20). Furthermore, MyD88's protein expression level exhibits a positive correlation with axillary lymph node metastasis and histological grade, while the co-expression of TLR4 and MyD88 is positively correlated with breast cancer cell metastasis (18).

MyD88, therefore, emerges as a potential diagnostic and therapeutic target in breast cancer. The assessment of MyD88 expression levels offers a means to comprehensively gauge breast cancer invasiveness and predict patient prognosis, thereby

10.3389/fonc.2024.1336696

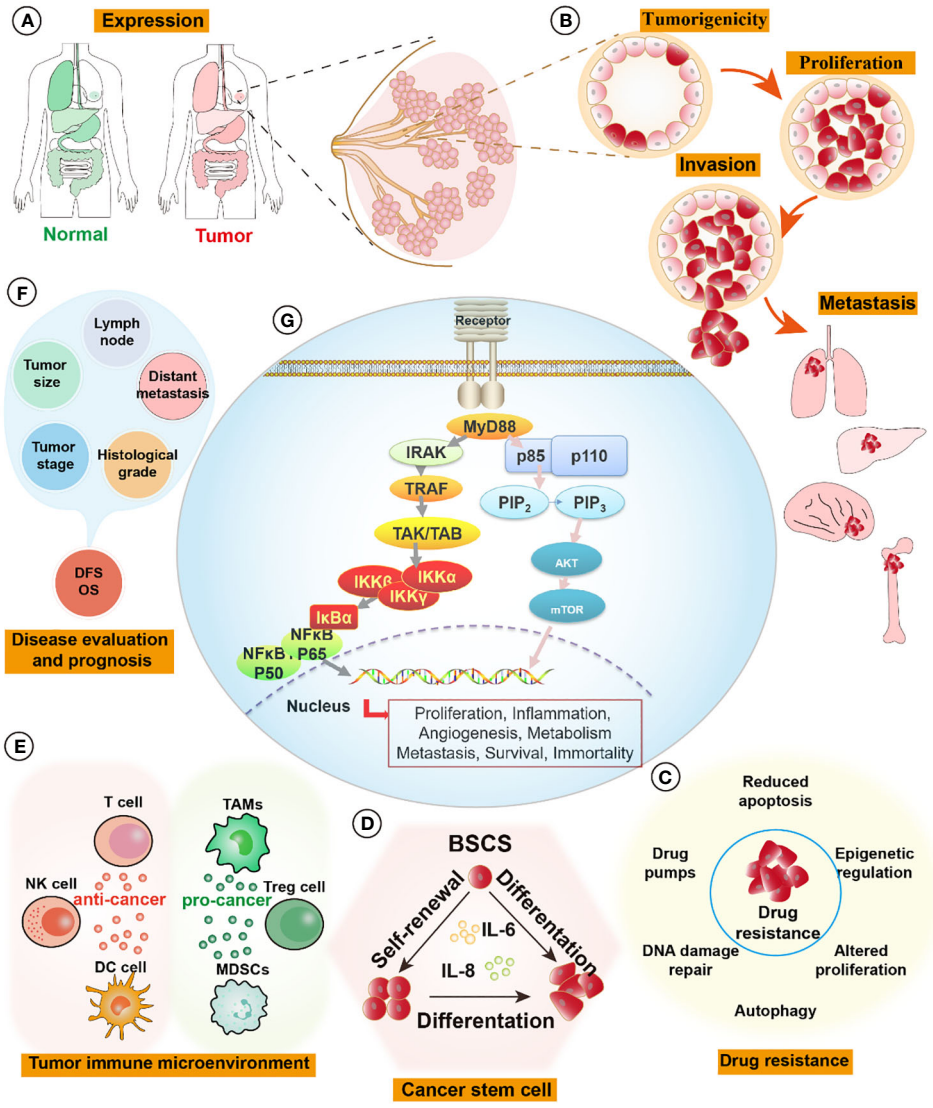

FIGURE 1
MyD88 signaling pathways in breast cancer. **(A)** The expression of MyD88 is elevated in tumor tissues compared to normal tissues, such as those found in breast, lung, liver, colon, and stomach organs. **(B)** Activation of the MyD88 pathway fosters the formation of cell spheroids and enhances tumor-forming capabilities. It influences tumor proliferation by activating cell cycle pathways and augments cell invasion and distant metastatic capabilities by activating relevant pathways like those associated with EMT signaling pathways. **(C)** MyD88 enhances tumor cell resistance to drugs by pumping drugs out, promoting DNA repair, inhibiting apoptosis, and activating cell survival pathways. **(D)** Upregulation of the MyD88 signaling pathway promotes self-renewal and differentiation of breast tumor stem cells, augmenting their survival capabilities. **(E)** In the tumor immune microenvironment, MyD88 exerts intricate effects. On one hand, it promotes tumor progression by increasing the infiltration and enrichment of tumor immunosuppressive cells (TAMs, Tregs, and MDSCs), thereby facilitating tumor growth and metastasis. On the other hand, it demonstrates anti-tumor effects by activating DC cells, NK cells, and tumor-killing T cells, initiating tumor-specific immunity and eliminating tumors. **(F)** The expression levels of MyD88 correlate significantly with breast tumor size, histological grading, lymph node involvement, tumor staging, distant metastasis, and overall prognosis. **(G)** MyD88 influences tumor proliferation, inflammation, angiogenesis, metabolism, metastasis, survival, and immortality through the Phosphoinositide 3-kinases/Protein kinase B (PI3K/Akt) and Toll-like Receptor/Nuclear Factor Kappa B (TLR/NF-κB) pathways. EMT, epithelial-mesenchymal transition; MDSCs, Myeloid-Derived Suppressor Cells, TAMs, Tumor-Associated Macrophages, Tregs, Regulatory T cells.

establishing a foundation for informed decisions in personalized treatment.

MyD88 expression in breast cancer

MyD88, a pivotal signaling molecule, has garnered attention for its dysregulated expression in various cancer types, including colorectal cancer (23), ovarian cancer (24), hepatocellular carcinoma (25), and pancreatic cancer (26), highlighting its role in tumor progression. In breast cancer, the expression of MyD88 varies within breast cancer cells, tumor tissues, and the surrounding microenvironment. While normal breast tissues typically maintain low MyD88 expression levels for physiological functions, breast cancer often exhibits significant upregulation. Studies indicate that adjacent normal tissues and benign breast tumors have lower

TABLE 1 MyD88 expression and clinical evaluation in breast cancer.

Technology	Results	Ref.
IHC	The expression of MyD88 in malignant tumors significantly surpasses that in adjacent normal tissue and benign tumors.	(18–20)
	Significant differences are observed in DFS and OS curves concerning MyD88 expression levels. Patients with lower MyD88 expression exhibit better DFS and OS compared to those with higher expression levels. Multifactorial analysis reveals MyD88 status as an independent risk factor for DFS and OS.	(20, 21)
	MyD88 expression correlates with ER and PR statuses, and patients with higher MyD88 expression experience more frequent recurrence or metastasis.	(21)
	No correlation has been found between MyD88 expression and ER or PR status.	(20)
	MyD88 expression levels relate to breast cancer TNM staging, being more detectable in stage III compared to stage I or II breast cancer patients.	(20)
WB	High expression of MyD88 significantly correlates with tumor size, tumor staging, axillary lymph node metastasis, and distant metastasis.	(19)
	The protein expression level of MyD88 in MDA-MB-231 cells is 1.6 times that of MCF-7 cells and 1.8 times that of MDA-Kb2 cells.	(18)
	High levels of MyD88 protein expression were confirmed in hormone-resistant breast cancer cell lines MDA-MB-231, MDA-MB-231HM, and MDA-MB-468, whereas such confirmation was not observed in MCF-7 and SKBR3 cell lines.	(21)
qRT-PCR	The expression of the MyD88 gene in breast cancer tissues is higher compared to adjacent normal tissues and benign tumors.	(19)
	In MDA-MB-231 cells, the gene expression level of MyD88 is 2.09 times higher than in MCF-7 cells.	(18)
	Zandi et al. assessed the baseline expression levels of MyD88 in different breast cancer cell lines—MCF7, SKBR3, MDA-MB-231, and BT-474—and observed the highest MyD88 mRNA level in MCF-7 cells.	(22)
IF	The average fluorescence intensity of MyD88 in MDA-MB-231 and MCF-7 cells was 0.136 and 0.05, respectively, there was no significant difference between the levels in MCF-7 and MDA-Kb2 cells	(18)

DFS, Disease-Free Survival; IF, Immunofluorescence; IHC: Immunohistochemistry; OS, Overall Survival; qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction; WB, Western Blotting.

MyD88 expression, whereas breast cancer demonstrates heightened and pronounced expression (19).

Differential MyD88 expression may also exist among distinct molecular subtypes of breast cancer. Zandi et al. conducted baseline expression level assessments of MyD88 in various breast cancer cell lines, including MCF7, SKBR3, MDA-MB-231, and BT-474, and found that MyD88 mRNA level was highest in MCF-7 cells (22, 27). However, another study revealed that within the MDA-MB-231 cell line, both the mRNA and protein expression levels of MyD88 were notably elevated in comparison to MCF-7 and MDA-Kb2 cells (which stably express an androgen- and glucocorticoid-responsive reporter) (18). Furthermore, there is evidence of a connection between MyD88 and tumor cell resistance. Ma et al. confirmed elevated levels of MyD88 protein in hormone-resistant breast cancer cell lines, MDA-MB-231, MDA-MB-231HM, and MDA-MB-468, compared to MCF-7 and SKBR3 cells (18). MyD88 and TLR4 expression and other clinical and pathological parameters. The expression of MyD88 was associated with ER and PR status ($p<0.001$). TLR4 expression was associated with PR status ($p=0.015$) (21). In another study, it was demonstrated that there is no correlation between the expression of MyD88 and the status of ER or PR (20). X. Chen et al. found that heightened MyD88 expression is linked to increased malignancy and unfavorable prognosis in breast cancer. Elevated levels of MyD88 expression show a significant correlation with tumor size, staging, axillary lymph node metastasis, and distant metastasis (19).

In summary, MyD88 expression in breast cancer exhibits variability. Understanding the expression patterns of MyD88 contributes to a better comprehension of its roles in breast cancer initiation, progression, and treatment. This knowledge provides essential insights into its potential as a therapeutic target.

Effect of MyD88 on tumorigenicity

The MyD88 protein exerts a profound influence on cancer cell tumorigenesis and the initiation of tumor growth. Serving as a crucial signaling adaptor protein, the TLR/MyD88 pathway plays a pivotal role in tumorigenicity. Activation of the MyD88 pathway promotes tumor formation, while MyD88 knockdown results in reduced clonogenicity in primary ER^{neg} tumors (14). Inhibiting MyD88 leads to decreased clonogenicity, and MyD88 shRNAs result in negative selection in the development of *in vivo* xenograft tumors derived from ER^{neg} breast cancer cells. Depletion of MyD88 in MCF-7 cells markedly inhibits tumor growth in nude mice, with tumor volumes generated from MyD88-deficient MCF-7 cells approximately half the size of those from control cells (20). TLR2, an upstream signaling molecule of MyD88, depletion impedes the tumor-initiating capacity of cells (28). For instance, TLR2-depleted spherical mammospheres cells only gave rise to tumor growth in 5 out of 14 mice, in contrast to control cells, which developed rapidly growing tumors in all mice (28). Additionally, $Tlr2^{-/-}$MMTV–Wnt1 ($Tlr2^{-/-}$Wnt1) mice exhibit markedly reduced tumor formation compared with that of their $Tlr2^{+/+}$MMTV–Wnt1 ($Tlr2^{+/+}$Wnt1) littermates, with median tumor-free days of 269 and 137, respectively (14). TLR2 neutralizing antibodies also block the growth of two independent ER^{neg} breast cancer xenografts *in vivo*. Short hairpin RNA (shRNA)-mediated knockdown of TLR2 in two breast cell lines (MDA-MB-468 and MDA-MB-231) similarly decreased clonogenic outgrowth (14). Moreover, suppression of constitutive downstream NF-κB activity in human breast cancer cell lines leads to reduced tumorigenicity (29). Furthermore, inhibiting NF-κB activity in human breast cancer cell lines can induce cell apoptosis (30) or diminish tumor-forming capability (31). Collectively, these findings underscore the

significance of the TLR2/MyD88/NF-κB pathway in tumorigenicity. These observations imply that the TLR2/MyD88/NF-κB pathway is implicated in *de novo* mammary tumor formation.

Effect of MyD88 on breast cancer proliferation

Cell proliferation is a central driver of breast cancer development and progression (32). In breast cancer, MyD88 is recognized as a key regulator with a significant impact on cell proliferation. Our previous studies have demonstrated that lipopolysaccharide (LPS) activates the MyD88 signaling pathway, promoting the *in vitro* proliferation of MCF-7 and MDA-MB-231 cells, as well as enhancing growth in xenograft mouse models *in vivo*. In our preliminary studies, a novel compound (TJ-M2010) was synthesized, drawing inspiration from the MyD88 molecular structure. This compound was engineered to specifically bind to the TIR domain of MyD88, aiming to disrupt the dimerization process of MyD88 (11). The application of TJ-M2010 hinders MyD88/NF-κB signaling transduction, resulting in the suppression of breast cancer cell proliferation mentioned above (33). Liu et al. employed a small-molecule compound known as 4210, which obstructs MyD88 dimerization (34), Their findings suggested that 4210 inhibition of MyD88 might induce nonspecific cell death in MDA-MB-231 cells (35). Another study demonstrated that treatment using the MyD88 inhibitor (ST2825) resulted in reduced growth of murine mammary carcinomas 4T1, 168, EMT6, and SM1, as well as the human breast cancer cell line MDA-MB-231 (36). In another study, a TLR3 ligand notably increased breast cancer cell proliferation, as indicated by the upregulation of cyclin D1 expression. However, the introduction of a MyD88 inhibitor disrupted the signal transduction of the TLR3-MyD88-NF-κB (p65)-IL6-Cyclin D1 pathway, leading to reduced breast cancer cell proliferation (37). Holleman et al. also confirmed that reduced MyD88 expression significantly inhibited MCF-7 cell proliferation (38). Similarly, the silencing of the MyD88 gene resulted in an increased G2 phase cell population in MCF-7 cells (20). NF-κB, a canonical downstream signaling molecule of TLR/MyD88, stimulates the transcription of the cyclin D1 promoter, thereby promoting cell growth (39). Additionally, caffeic acid phenethyl ester inhibited MDA-MB-231 cell proliferation by suppressing the TLR4/MyD88/NF-κB signaling pathway (40). Furthermore, MyD88 can affect breast cancer proliferation through the PI3K/AKT signaling pathway. The interaction between MyD88 and p85 enhances tumorigenic capabilities through the PI3K/Akt pathway (15). The MyD88 inhibitor TJ-M2010 interferes with the MyD88/PI3K/GSK-3β axis, consequently restraining breast cancer cell proliferation (33).

In conclusion, MyD88 plays a pivotal role in regulating breast cancer cell proliferation through the activation of multiple key signaling pathways. This highlights MyD88 as a significant potential therapeutic target, and inhibiting its function may contribute to restraining the growth and dissemination of breast cancer cells. However, further research is needed to gain deeper insights into the regulatory mechanisms of MyD88 in breast cancer cell proliferation, with the goal of developing more precise and effective treatment strategies.

The effect of MyD88 on metastasis

Metastasis represents a pivotal stage in cancer progression, involving the spread of cancer cells from the primary tumor site to distant organs or tissues, where secondary tumors form (41). MyD88 is a molecular orchestrator with multifaceted implications for cellular processes central to cancer metastasis. Multiple studies have underscored the substantial involvement of MyD88 in tumor metastasis. For example, ectopic expression of MyD88 has been shown to enhance the invasion of hepatocellular carcinoma cells, whereas MyD88 inhibition has led to reduced lung metastasis and portal vein tumor thrombosis (15, 25, 42). MyD88 also plays a critical role in breast cancer metastasis. K. Wu et al. found that MyD88 expression was increased in highly invasive BC cell lines compared to low invasive BC cell lines, suggesting that MyD88 could be a potential therapeutic target for the metastasis of BC (18). Research has indicated that the heightened downstream NF-κB activity resulting from MyD88 elevation leads to increased expression of invasion-associated proteases (43, 44). Moreover, NF-κB contributes to the induction and maintenance of epithelial-mesenchymal transition (EMT) in Ras-transformed breast epithelial cells, mediating invasive and metastatic tumor phenotypes. Substantial evidence supports the role of NF-κB in late-stage breast tumor development and metastasis (45). In MDA-MB-231 cells, the downregulation of MyD88 influences NF-κB nuclear translocation, resulting in a diminished invasion capacity of tumor cells (36). Additionally, by knocking down MyD88, the migration speed of MCF-7 cells is reduced, and their ability to traverse Matrigel is impaired. In another study, tumors with reduced Myd88 levels displayed reduced growth and metastasis (46). MiR-4317 inhibits the proliferation, migration, and invasion of breast cancer cells by targeting MyD88 (47). Advanced glycation end products (AGEs) can promote breast cancer cell migration and invasion through the activation of the RAGE/TLR4/MyD88 signaling cascade (48). Berberine significantly inhibits the TLR9-MyD88-NF-κB pathway, reversing breast cancer metastasis (49). LPS enhances invasiveness and metastatic potential of breast cancer cells by upregulating the MyD88-BLT2-NF-κB signaling cascade (50). MCF-7 cell lines, after 48 hours of incubation with 0.5 μM morphine, exhibit downregulated TLR4/MyD88/NF-κB pathways, resulting in decreased migration (51, 52). TLR4 silencing reduces the expression of MyD88 and MMP9, significantly inhibiting cell migration and invasion (53). Furthermore, a TLR4 antagonist blocks TLR4 and MyD88 expression, as well as cell invasion and migration (54).

EMT, which refers to the transformation of epithelial cells into mesenchymal-like cells, conferring migratory and invasive properties (55), is a pivotal process in cancer. Various cascading reactions relevant to cancer have been established as critical regulatory signals of EMT (56, 57). The MyD88/NF-κB signaling

pathway plays a crucial role in Snail-mediated EMT (58). EpRas cells are oncogenic and completely polarized Ha-Ras-transformed EpH4 mammary epithelial cells that undergo EMT, leading to the generation of mesenchyme-like cells (EpRasXT cells). Blocking the MyD88/NF-κB pathway eliminates the metastatic capability of mammary epithelial cells in murine models. Additionally, the reversal of NF-κB activity in EpRasXT cells nullifies the EMT process (45). Curcumin inhibits LPS-induced EMT in MDA-MB-231 and MCF-7 cells through the TLR4/MyD88/NF-κB/Snail signaling pathway (59, 60). The MyD88 inhibitor TJ-M2010-2 reverses TGF-β1-induced HK-2 cell EMT (61, 62). Similarly, the MyD88 inhibitor TJ-M2010-2 suppresses the proliferation, migration, and invasion of breast cancer cells by intervening in the MyD88/GSK-3β and MyD88/NF-κB signaling pathways (33).

In conclusion, MyD88 promotes cancer cell migration, invasion, and the establishment of secondary tumors in distant organs through diverse pathways, significantly impacting breast cancer metastasis. A comprehensive understanding of MyD88's mechanisms in metastasis aids in devising effective strategies to inhibit cancer spread, thereby enhancing patient prognosis.

The effect of MyD88 on drug resistance

Drug resistance poses a formidable challenge in cancer therapy, frequently resulting in reduced treatment effectiveness and disease relapse (63). MyD88 exerts a significant impact on drug resistance in breast cancer cells and is recognized as a key player associated with paclitaxel (PTX) resistance. Xiang et al. demonstrated the reversal of PTX resistance in breast cancer cells by targeting the miRNA-149-5p/MyD88 axis using ursolic acid (UA) (8). The PI3K/Akt pathway, a pivotal signaling route, becomes activated in PTX-resistant breast cancer (64). The observed heightened sensitivity of MCF-7 cells to paclitaxel following MyD88 downregulation implies its impact on inhibiting NF-κB activation, thereby blocking the PI3K/Akt signaling pathway (20). Elevated MyD88 levels in MDA-MB-231/PTX-resistant cells correlated with enhanced resistance, but MyD88 silencing increased PTX sensitivity (8). Activation of Akt may be linked to reduced paclitaxel-induced apoptosis (20), and MyD88 knockdown significantly suppressed Akt pathway activation in 231/PTX cells (8). The MyD88-regulated PI3K/Akt pathway appeared to mediate paclitaxel resistance in breast cancer cells by modulating Bax/Bcl-2 expression (8). Overexpression of MyD88 protein was associated with acquired paclitaxel resistance in triple-negative breast cancer (TNBC) cells. Dimethoxy-substituted compounds significantly reduced MyD88 overexpression in $TLR4^+$ MDA-MB-231 cells and enhanced PTX activity (65). *In vitro* and *in vivo* experiments reversed paclitaxel resistance by inhibiting the target gene MyD88 (66).

Furthermore, the MyD88/NF-κB signaling pathway plays a central role in drug resistance in breast cancer. Downregulation of MyD88 increased sensitivity to paclitaxel in MCF-7 cells by suppressing NF-κB activation and blocking the PI3K/Akt pathway (20). The NF-κB/Notch1 regulatory loop contributes to the maintenance of breast cancer stem cells and drug resistance (16). Additionally, the NF-κB pathway promotes the transcription of ABCC1/MRP1 and ABCG2/BCRP transporters (67). This pro-survival signaling pathway becomes more active in response to radiotherapy, chemotherapy, and trastuzumab in breast cancer cells (68–70). Consequently, the MyD88/NF-κB pathway is involved in mediating drug resistance mechanisms in tumor cells. Despite substantial evidence supporting the regulatory role of MyD88 in drug resistance, some studies have yielded contrasting results, such as LPS pre-treatment sensitizing MyD88-positive cells to docetaxel cytotoxicity. This suggests that pathways induced by LPS stimulation could serve as attractive targets for overcoming therapy-resistant diseases (71).

A comprehensive understanding of MyD88's role in drug resistance is crucial for devising strategies to overcome resistance and enhance the effectiveness of cancer treatment. Targeting MyD88 or its associated signaling pathways might offer a potential approach to increase cancer cell sensitivity to treatment and improve therapeutic outcomes. Further research is required to elucidate the potential mechanisms underlying MyD88-mediated drug resistance in breast cancer and other cancer types, as well as to explore therapeutic intervention methods.

The effect of MyD88 on cancer stem cells

Breast cancer stem cells (BCSCs) constitute a small subset of tumor cells endowed with the unique capacity for self-renewal and differentiation into diverse cell types (72). These cells wield a pivotal role in tumor initiation, progression, recurrence, and resistance to therapy.

MyD88 has been revealed as a regulator of the characteristics and behavior of BCSCs through diverse mechanisms, actively participating in signaling pathways that govern the maintenance and functions of BCSCs. For instance, Conti et al. observed that the stimulation of TLR2 with ligands like Pam3CSK4, LTA, and PGN-SA significantly enhances mammosphere formation. In contrast, the action of the MyD88 homodimerization-inhibitory peptide markedly hinders the formation of spherical mammospheres (28). Silencing the TLR2/MyD88 signaling pathway also reduces mammosphere generation in both mouse (4T1) and human (MDA-MB-231, MCF7) mammary cells (28). The upregulation of the MyD88/NF-κB signaling pathway acts as a pro-survival route implicated in the self-renewal of BCSCs (16). The NF-κB protein p65 directly binds to the Gli1 promoter, enhancing its transcription (73). Additionally, the MyD88/NF-κB signaling pathway governs the expression of various cytokines, notably IL-6 and IL-8, which are intricately linked to tumor progression and the survival of BCSCs (74, 75). Extracellular IL-6 induces malignant characteristics in ductal breast cancer stem/progenitor cells (74), while the overexpression of IL-8 promotes stemness, stromal traits, acquisition of resistance, and the recruitment of immunosuppressive cells that foster tumor growth in the tumor microenvironment (75). Novel therapies targeting IL-8 receptor signaling may serve as a

means to halt tumor progression. Machilin D reduces the secretion of IL-6/IL-8 and inhibits sphere growth (76).

In conclusion, MyD88 plays a significant role in regulating breast cancer stem cell characteristics and functions. A more profound understanding of the mechanisms through which MyD88 influences BCSCs holds the potential for the development of targeted therapies against these aggressive tumor-initiating cells. This offers both theoretical and practical implications for enhancing breast cancer treatment outcomes. Further research is warranted to comprehensively elucidate the mechanisms of MyD88 in BCSCs and its potential value as a therapeutic target.

The influence of MyD88 on the tumor immune microenvironment

The influence of MyD88 on the tumor immune microenvironment is of great significance in the context of breast cancer progression. The tumor microenvironment is a complex ecosystem comprising tumor cells and their immediate surroundings, exercising a profound impact on tumor development (77–79). Through the modulation of inflammatory responses and immune reactions, MyD88 orchestrates changes within the tumor immune microenvironment. Exosomes released by tumor cells can activate the TLR/MyD88/NF-κB signaling pathway in macrophages, prompting the release of pro-inflammatory cytokines (such as IL-6, TNF-α, GCSF, and CCL2) (80). Within breast cancer stem cells, the HMGB1/TLR2/MyD88 axis governs NF-κB activation, IL-6 and TGF-β production, and the activation of STAT3 and Smad3. IL-6, stimulated through STAT3, fosters malignant properties, expanding the breast cancer stem cell population (81). The TGF-β signaling pathway contributes to the maintenance, survival, and enhanced migratory capacity of the tumor stem cell population (82, 83). TNF-α promotes the expression of carcinogenic CCL5 variants in breast cancer cells, instigating tumor cell detachment and dissemination (57). Activation of the TLR2/MyD88/NF-κB pathway elevates the autocrine secretion of VEGF and MMP9 in MDA-MB-231 cells, recognized as pivotal factors for breast cancer cell invasion and adhesion (27). The inhibition of NF-κB significantly reduces the invasiveness of MDA-MB-231 cells (84, 85). NF-κB inhibition profoundly diminishes the invasiveness of MDA-MB-231 cells (86, 87).

The MyD88 pathway also mediates immune responses that impact breast cancer progression. For example, Astragalus polysaccharide (APS) may regulate host immunity and exert anti-tumor effects by activating the TLR4-mediated MyD88-dependent signaling pathway (17). A Arazyme activates TLR4-MyD88-TRIF, which reduces primary and metastatic tumor development in the 4T1 murine breast cancer model, thus extending survival (88). Inhibition of proprotein convertases 1/3 steers NR8383 macrophages toward an M1 activation phenotype mainly via TLR4/MyD88 signaling, leading to the secretion of factors that attract immature T helper lymphocytes and promote cytotoxic responses (89). Polysaccharide peptide (PSP) acts by upregulating the TLR4-TIRAP/MAL-MyD88 signaling pathway in peripheral blood mononuclear cells (PBMCs) from breast cancer patients, inducing the secretion of IL-12, IL-6, and TNF-α, thus serving as an immune adjuvant (90). Activation of the TLR5/MyD88/NME3/NFκB signaling pathway enhances host immunity, enhances the clearance of tumor xenografts, and potentially augments the effectiveness of immunotherapy, prolonging survival in breast cancer patients (91). Stimulation of TLR8 ligands through the MyD88/IRAK4/TRAF6/p38 signaling pathway reverses the suppressive function of $CD4^+$ $CD25^+$ Treg cells, eliminating the inhibitory effect of BTIL31 γδ1 Treg cells on T cell proliferation and function, as well as dendritic cell maturation and function (92).

Indeed, various immune effects have been observed in other cancers as well. Our previous research demonstrated that TJ-M2010-2 successfully mitigated inflammation induced by AOM/DSS, thereby altering the tumor microenvironment and reducing the incidence of colorectal cancer from 100% to 0% (11). MyD88 plays a critical role in tumor-derived exosome-mediated MDSC expansion and subsequent tumor metastasis (93). Furthermore, Pixatimod (PG545), a novel clinical-stage immune modulator, amplifies T cell infiltration when combined with anti-PD-1 therapy through the MyD88-dependent TLR9 pathway. Simultaneously, it inhibits the infiltration of tumor-associated macrophages (TAMs) and activates dendritic cells (DCs), thereby stimulating natural killer (NK) cells (94). IL-33 has the capacity to promote the differentiation and maturation of DC cells via the MyD88 pathway. This results in upregulated tumor immunity in $CD8^+$T cells and NK cells while inhibiting the proliferation of lung cancer cells (95).

The multifaceted influence of MyD88 on the tumor immune microenvironment involves the orchestration of multiple cytokines and intricate signaling pathways. A comprehensive comprehension of the role of MyD88 in shaping the tumor microenvironment greatly enhances our understanding of breast cancer development mechanisms, and it offers a strong theoretical foundation for the development of innovative therapeutic strategies. It is imperative that further research endeavors are undertaken to elucidate the intricate mechanisms through which MyD88 exerts its influence on the tumor immune microenvironment and to explore its potential applications in the development of novel treatments.

Conclusions and prospects

MyD88 assumes multifaceted, pivotal roles in the landscape of breast cancer, influencing diverse aspects such as tumor development, drug resistance, stem cell properties, and the intricacies of the immune microenvironment. A comprehensive understanding of the mechanisms underpinning MyD88's actions in breast cancer not only unravels the molecular complexities governing tumor progression but also unveils fresh perspectives and potential targets for shaping breast cancer treatment strategies. MyD88 emerges as a valuable asset in prognosis assessment, offering guidance for therapeutic choices and the development of innovative treatment modalities. Nonetheless, there exists a need for

further research to unveil the precise workings of MyD88 and translate its applications into clinical practice, with the ultimate aim of achieving more precise and personalized approaches to managing breast cancer.

Author contributions

HZ: Conceptualization, Project administration, Writing – original draft. XW: Funding acquisition, Project administration, Resources, Writing – original draft. LG: Investigation, Methodology, Writing – original draft. JL: Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The National Natural Science Foundation of China (Grant No. 81802895), the Natural Science Foundation of Hubei Province, China (Grant No.2023AFB493), and the Health and Family Planning Commission of Wuhan Municipality (Grant No.WX17Q16) awarded to JL. The Research Projects of Biomedical Center of Hubei Cancer Hospital (2022SWZX09) awarded to XW.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 09 February 2024
DOI 10.3389/fonc.2024.1341927

OPEN ACCESS

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RECEIVED 21 November 2023
ACCEPTED 17 January 2024
PUBLISHED 09 February 2024

CITATION
Li Y, Gao L, Chao Y, Lan T, Zhang J, Li R, Zhang Z, Li S, Lian J, Wang Z and Chen X (2024) Various interventions for cancer-related fatigue in patients with breast cancer: a systematic review and network meta-analysis. *Front. Oncol.* 14:1341927. doi: 10.3389/fonc.2024.1341927

Various interventions for cancer-related fatigue in patients with breast cancer: a systematic review and network meta-analysis

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Purpose: To investigate the effects of various intervention approaches on cancer-related fatigue (CRF) in patients with breast cancer.

Method: Computer searches were conducted on PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (VIP), and Wanfang databases from their establishment to June 2023. Selection was made using inclusion and exclusion criteria, and 77 articles were included to compare the effects of 12 interventions on patients with breast cancer.

Results: Seventy-seven studies with 12 various interventions were examined. The network findings indicated that cognitive behavioral therapy (CBT) (SMD, -1.56; 95%CI, -3.08~-0.04), Chinese traditional exercises (CTE) (SMD, -0.85; 95%CI, -1.34~-0.36), aerobic exercise (AE) (SMD, -0.77; 95%CI, -1.09~-0.45), multimodal exercise (ME) (SMD, -0.75; 95%CI, -1.26~-0.25), music interventions (MI) (SMD, -0.74; 95%CI, -1.45~-0.03), and yoga (YG) (SMD, -0.44; 95%CI, -0.83 to -0.06) can reduce CRF more than the control group (CG). For relaxation exercises (RE) (MD, -6.69; 95%CI, -9.81~-3.57), MI (MD, -5.45; 95%CI, -7.98~-2.92), AE (MD, -4.34; 95%CI, -5.90~-2.78), ME (MD, -3.47; 95%CI, -4.95~-1.99), YG (MD, -2.07; 95%CI, -3.56~-0.57), and mindfulness training (MD, -1.68; 95%CI, -2.91~-0.46), PSQI improvement was superior to CG. In addition, for CTE (MD, 11.39; 95%CI, 4.11-18.66), YG (MD, 11.28; 95%CI, 1.63-20.93), and AE (MD, 9.34; 95%CI, 0.26~18.42), Functional Assessment of Cancer Therapy-Breast improvement was superior to CG.

Conclusion: Cognitive behavioral therapy (CBT) is the most effective measure for alleviating CRF in patients with breast cancer and Relaxation exercises (RE) is the

most effective measure for improving sleep quality. In addition, Chinese traditional exercises (CTE) is the best measure for enhancing quality of life. Additional randomized controlled trials (RCTs) are expected to further investigate the efficacy and mechanisms of these interventions.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42023471574.

KEYWORDS

breast cancer, CFR, network meta-analysis, cancer, systematic review

Introduction

Breast cancer is the most common cancer in women. The American Cancer Society reports a yearly increase of 0.5% in the incidence of breast cancer in women. The projection for 2022 estimates approximately 287,850 new cases of breast cancer in women in the United States, accounting for 31% of all new cancer diagnoses in women (1). In recent years, the survival rate of patients has improved due to the emergence of neoadjuvant therapy. However, survivors face a series of physical and mental problems, such as premature menopause, body image disorder, fatigue, and depression (2–4). Patients with breast cancer commonly experience cancer-related fatigue (CRF) as one of the most common symptoms (5). Before undergoing anticancer treatment, women with breast cancer may have already experienced fatigue. The occurrence of CRF is closely connected to the factors inherent to the primary tumor, which may be associated with the abnormal expression of certain substances released by the cancer cells in the patient, such as IL1, IL6, and TNF-a interferon. The severity of fatigue is proportional to the amount of interleukin released by tumor cells into the blood (6). When starting treatment, between 60% and 90% of women with breast cancer may experience fatigue (7). Severe fatigue is experienced by approximately a quarter of breast cancer survivors (8). An increase in the burden on patients' families and caregivers can be caused by CRF. In addition, the time it takes for patients to return to work early after cancer treatment may be prolonged by CRF (9–13).

In recent years, there has been a growing interest in investigating CRF for breast cancer. The effects of aerobic exercise (AE), resistance exercise, relaxation training, yoga (YG), music, and other intervention methods on CRF in patients with breast cancer have been investigated in previous studies. Traditional meta-analyses have also demonstrated the effectiveness of YG and resistance training (RT) in reducing CRF in patients with breast cancer (14, 15). Olsson et al. offered a comprehensive overview of the effects of rehabilitation interventions and discovered that CRF was positively affected by exercise and YG (16). Health-related quality of life in breast cancer survivors can be significantly impaired by the occurrence of CRF. Practical exercise training can enhance mitochondrial function and plasticity in patients, thereby improving the occurrence of CRF (17–19). However, evidence-based recommendations regarding the most effective type of intervention for improving CRF in patients with breast cancer are still lacking. Therefore, it is crucial to identify a suitable intervention for reducing CRF among complex interventions in patients with breast cancer.

Network meta-analysis (NMA), also called meta-analysis of mixed treatment comparisons or multiple treatment comparisons (20), offers a method to compare the size of the impact of various intervention types on CRF in patients with breast cancer by estimating direct and indirect comparisons. Although two previously published NMA studies were identified (21, 22), the study only reported on the effects of various exercise interventions, and no further studies were conducted on other intervention types. Consequently, this study aims to conduct an NMA on relevant randomized controlled trials (RCTs) to compare the effects of various interventions on CRF in patients with breast cancer. The findings of this study are crucial for developing clinical practice guidelines recommending the best intervention to improve the outcome of CRF in patients with breast cancer.

Methods

This NMA was designed based on the guidelines for Preferred Reporting Items of Systematic Review and Network Meta-Analysis (23), which are registered in the PROSPERO database (CRD42023471574).

Search strategies

Searches for RCT-related studies on CRF in breast cancer, published up to July 2023, were conducted using databases such as PubMed, Web of Science, Embase, Cochrane Library, China National Knowledge Infrastructure, and Wanfang. The search involved a combination of subject and free words. The search strategy can be found in Additional Document 1 (Appendix 1).

Study selection

In this study, YL and LG were selected as independent reviewers to screen the titles and abstracts of the retrieved literature using search strategies to identify literature that met the inclusion criteria. In case of disagreement, checks and discussions were performed by XA C to reach a consensus. Duplicate data were de-duplicated using EndNote software (24). A full-text assessment of potentially eligible studies was conducted based on the inclusion and exclusion criteria. Any differences between the reviewers were resolved through discussion, and EndNote software was used to manage this phase.

Inclusion criteria

Studies that met the following criteria were included (1): Study type: RCT (2). Studies that included adult patients (18 years or older) diagnosed with breast cancer that were not limited to cancer stage and current treatment options for breast cancer (3); Interventions: AE, RT, Chinese traditional exercises (CTE), other exercise (OE), multimodal exercise (ME), YG, stretching exercise (STE), music interventions (MI), cognitive behavioral therapy (CBT), mindfulness training (MT), and relaxation exercises (RE); and (4) Outcomes: at least one outcome measure. The primary outcome measure was CRF assessed using the Functional Assessment of Cancer Therapy (FACT)-Fatigue Scale, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQC30), Piper Fatigue Scale (PFS), Schwartz Cancer Fatigue Scale (SCFS), and Multidimensional Fatigue Inventory (25). The secondary outcomes were sleep quality versus quality of life as measured using the Pittsburgh Sleep Quality Index (PSQI) and Functional Assessment of Cancer Therapy-Breast (FACT-B). Each intervention is defined in Additional Document 1 (Appendix 2). Each outcome measure is defined in Additional Document 1 (Appendix 3).

Exclusion criteria

(1) Patients with severe complications (2). Studies with outcomes that did not align with the design of this study (3). Studies with data that could not be integrated, such as incorrect or incomplete information.

Data extraction

The reviewers independently extracted the following data: first author, publication year, country, sample size, body mass index (BMI), age, weight, height, weight, intervention, tumor stage, intervention time, intervention frequency, and outcome indicators. Data are presented as mean ± standard deviation.

Risk of bias assessment

Two reviewers (LG and ZR Z) independently assessed the risk of bias, and a third reviewer adjudicated using Cochrane collaboration tools, such as sequence generation, assignment hiding, blinding, incomplete outcome data, non-selective outcome reporting, and other sources of bias (26). Each criterion was judged to have a low, unclear, or high risk of bias (27).

Data analysis

The "Netmeta" package (28) in R-4.2.1 software (29) was used for NMA. Network plots were generated using the STATA 15.1 "network plot" feature to describe and present various forms of motion. Nodes were used to represent various interventions, and edges were used to depict favorable intervention comparisons. Inconsistencies between direct and indirect comparisons were evaluated using the node segmentation method (30). Combined estimates and 95% confidence intervals (95% CI) were computed using random effects network element analysis. In studies where the same unit of measurement was of interest, the mean difference (MD) was considered a treatment effect when analyzing the results or evaluating the standardized mean difference (SMD). Different exercise treatments were compared using a pairwise randomized effects meta-analysis. The heterogeneity of all pair-to-pair comparisons was evaluated using the I^2 statistic, and publication bias was evaluated using the p-value of Egger's test. Publication bias and secondary study effects, analyzed by the results of more than a dozen reported studies, were identified using funnel plots.

Results

Literature selection

After removing duplicates, 4006 records were retrieved, and 3624 papers were discarded. The full text of the remaining 382 records was analyzed, and 305 cases did not satisfy the inclusion criteria: inconsistent intervention measures (172), inconsistent outcome indicators (31), data deficiency (9), and duplicate study (8). In the end, 77 (32–108) studies were included. Figure 1 shows the research flowchart. Articles from the full-text evaluation and the reasons for their exclusion are provided in Additional Document 1 (Appendix 8).

Study and participant characteristics

Studies comparing the effects of 12 various interventions on patients with breast cancer, published between 2001 and 2022, were included. The intervention durations ranged from 1 week to 12 months, and a total of 5,254 patients were reported in the included studies. Among these studies, 62 reported CFR, 23 reported PSQI, and 17 reported FACT-B. The participants had an average age of 18-73 years, an average BMI of 21.06 ± 2.26-72.3 ± 13.1, an average height of 145.64 ± 24.07-170.2 ± 5.4 cm, and an average weight of 54.74± 6.66-74.3 ± 17.0 Kg.

Table 1 shows the characteristics of the studies and participants. The risk of bias assessment for each study is

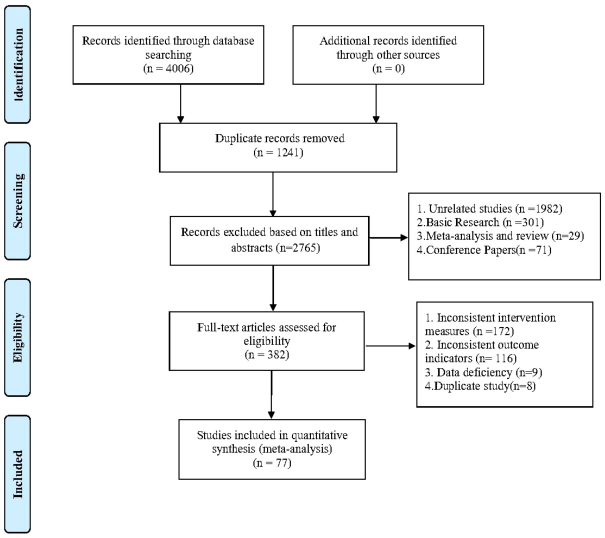

FIGURE 1
Flow of trials through the review.

presented in Additional Document 1 (Appendix 4), and Figure 2 presents the aggregated data.

Outcomes

CRF

A total of 62 (32–94) studies, involving 5385 participants, assessed CRF. In the NMA, 12 interventions were included (Figure 3A): AE, CG, RT, RE, CTE, OE, ME, YG, SE, MI, CBT, and MT. Superior CFR improvement compared with CG was observed for CBT (SMD, -1.56; 95%CI, -3.08~-0.04), CTE (SMD, -0.85; 95%CI, -1.34~-0.36), AE (SMD, -0.77; 95%CI, -1.09~-0.45), ME (SMD, -0.75; 95%CI, -1.26~-0.25), MI (SMD, -0.74; 95%CI, -1.45~-0.03), and YG (SMD, -0.44; 95%CI, -0.83 to -0.06) (Figure 4A). Comparison of adjusted funnel plots did not provide evidence of significant publication bias, as confirmed by Egger's test (P = 0.085) (Additional Document 1: Appendix 5.1). Heterogeneity, intransitivity, and inconsistencies in network meta-analyses were also evaluated (Additional Reference 1: Appendix 6.1). Furthermore, direct comparisons of the CRF were assessed. (Additional Reference 1: Appendix 7.1).

Sleep quality

In 23 (37, 42, 50, 55, 59, 67, 69, 71, 72, 78, 79, 82, 83, 86–89, 92, 94–98) studies, PSQI was assessed in 2334 participants. Eleven interventions were included in the NMA (Figure 3B): RE, MI, AE, ME, YG, MT, SE, CTE, OE, and CG. PSQI improvement was superior to YG for RE (MD, -4.62; 95%CI, -8.08~-1.16), MI (MD, -3.38; 95%CI, -6.33~-0.44), and AE (MD, -2.27; 95%CI, -4.43~-0.11). In addition, PSQI improvement was superior to MT for RE (MD, -5.01; 95%CI, -8.36~-1.66), MI (MD, -3.77; 95%CI, -6.58~-0.95), and AE (MD, -2.66; 95%CI, -4.64~-0.67). RE (MD, -6.14; 95%CI, -10.19~-2.09), MI (MD, -4.90; 95%CI, -8.52~-1.28), and AE (MD, -3.79; 95%CI, -6.81~-0.77) demonstrated superior PSQI improvement compared with SE. Furthermore, PSQI improvement was superior to CTE for RE (MD, -6.29; 95%CI, -9.60~-2.98), MI (MD, -5.05; 95%CI, -7.82~-2.28), AE (MD, -3.94; 95%CI, -5.86~-2.02), and ME (MD, -3.08; 95% CI, -4.93~-1.22). RE (MD, -6.69; 95%CI, -10.85~-2.52), MI (MD, -5.45; 95%CI, -9.20~-1.70), AE (MD, -4.34; 95%CI, -7.51~-1.16), and ME (MD, -3.47; 95%CI, -6.61~-0.34) demonstrated superior PSQI improvement compared to OE. Additionally, RE (MD, -6.69; 95%CI, -9.81~-3.57), MI (MD, -5.45; 95%CI, -7.98~-2.92), AE (MD, -4.34; 95%CI, -5.90~-2.78), ME (MD, -3.47; 95%CI, -4.95~-1.99), YG (MD, -2.07; 95%CI, -3.56~-0.57), and MT (MD, -1.68; 95%CI, -2.91~-0.46) demonstrated superior PSQI improvement compared to CG (Figure 4). Comparison of the adjusted funnel plot did not provide evidence of significant publication bias, as confirmed by Egger's test (*P* = 0.744) (Additional document 1: Appendix 5.2). Heterogeneity, inaccessibility, and inconsistencies in the network meta-analyses were evaluated (Additional Reference 1: Appendix 6). In addition, direct comparisons of the PSQI scores were evaluated. (Additional Reference 1: Appendix 7.2).

Quality of life

A total of 17 (41, 42, 53, 59, 77, 93, 96, 99–108) studies evaluated FACT-B in 1372 participants. Seven interventions were included in the NMA (Figure 3C): CG, AE, ME, CBT, YG, SE, and OE. CTE (MD, 11.39; 95%CI, 4.11-18.66), YG (MD, 11.28; 95%CI, 1.63-20.93), and AE (MD, 9.34; 95%CI, 0.26~18.42) demonstrated superior FACT-B improvement compared with CG. (Figure 4C). The comparison of the adjusted funnel plots did not provide evidence of significant publication bias, as confirmed by Egger's test (*P* = 0.365) (Additional document 1: Appendix 5.3). Heterogeneity, inaccessibility, and inconsistencies in network meta-analyses were also evaluated (Additional Reference 1: Appendix 6). In addition, direct comparisons of FACT-B were assessed (Additional Reference 1: Appendix 7.3).

Discussion

Breast cancer has the highest cancer incidence in women worldwide, and the survival rate of patients with breast cancer has been increasing. A range of side effects are often experienced by survivors of breast cancer, with CRF being a common side effect (109).

Several factors, including tumor stage, radiotherapy, chemotherapy, surgery, hormone therapy, radiation therapy dose, tumor burden, and the combination of these therapies, contribute to the development of CRF. Patients receiving cyclophosphamide, fluorouracil, adriamycin, or docetaxel experience more severe CRF than patients receiving paclitaxel alone (8, 110). An increase in the incidence of fatigue from 10% to 29% after treatment was observed in a cross-sectional study (111).

Furthermore, a patient's CRF can be affected by factors such as family dysfunction, social support system, occupation, and hope level. Patients experiencing family dysfunction not only confront the physical pain induced by the disease and radiotherapy and

TABLE 1 General characteristics of all included studies.

Name	Years	Country	Group	Age	BMI	Height (cm)	Weight (kg)	Tumor staging	Sample size	Intervention time	Intervention frequency	Outcomes
Gokal (32)	2016	UK	AE/CG	52.08 ± 11.7/ 52.36 ± 8.9	27.20 ± 4.82/ 28.25 ± 5.83	NA	NA	I-III	25/25	12weeks	5 times a week, >20min/times	FACT-F
Pinto (33)	2005	USA	AE/CG	53.14 ± 9.70	27.01 ± 4.65/ 28.26 ± 5.33	NA	NA	0-II	43/43	12weeks	> 30 minutes, 5 days a week	Linear analog scale for fatigue)
Mock (34)	2005	USA	AE/CG	51.3± 8.9/ 51.6 ± 9.7	25.5± 4.0 25.8± 5.1	NA	NA	0–III	54/54	6weeks	> 60 minutes per week	PFS
Husebø (35)	2014	Norway	AE/CG	50.8 ± 9.7/ 53.6 ± 8.8	NA/NA	NA/NA	69.0 ± 11.6/ 72.0 ± 15.7	I–III	33/34	15 weeks	30 minutes/day	SCFS-6
Mock (36)	2001	USA	AE/CG	48.64 ± 10.69/ 27.95 ± 5.94	23.86 ± 3.94/ 27.95 ± 5.94	145.64 ± 24.07/ 168.59 ± 35.10	65.54 ± 2.47/ 65.18 ± 2.68	I-IIIa	28/22	NA	> 90 minutes per week	PFS
Wang (37)	2011	USA	AE/CG	48.40 ± 10.15/ 52.3 ± 8.84	NA/NA	156.86± 5.04/ 156.74 ± 4.45	55.68 ± 6.91/ 54.74 ± 6.66	I-II	30/32	6weeks	3-5 times a week	FACT-F/PSQI
Schmidt (38)	2015	Germany	RT/RE	52.2 ± 9.9/ 53.3 ± 10.2	25.7 ± 4.6/ 26.3 ± 4.9	NA/NA	NA/NA	0–III	49/46	12weeks	Twice a week	EORTC QLQ30-Fatigue
Steindorf (39)	2014	Germany	RT/RE	55.2 ± 9.5/ 56.4 ± 8.7	26.9 ± 5.4/ 27.6 ± 4.8	NA/NA	NA/NA	0–III	80/80	12weeks	2 times/week	EORTC QLQ30
Han (40)	2019	China	CTE/CG	46.39 ± 5.79/45.52 ± 6.50	NA/NA	NA/NA	NA/NA	I-III期	23/21	12weeks	2 times/day, 5 days/week	PFS-R
Yang (41)	2022	China	CTE/CG	NA/NA	NA/NA	NA/NA	NA/NA	I-III	43/43	12weeks	20 minutes/time, 2 times/day, 5 days/week	PFS-R/ FACT-B
Hui (42)	2022	China	CTE/CG	69.51 ± 5.73	NA/NA	NA/NA	NA/NA	I-III	49/49	6 months	2 times/day, 20 minutes/time	CFS/PSQI/ FACT-B

(Continued)

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TABLE 1 Continued

Name	Years	Country	Group	Age	BMI	Height (cm)	Weight (kg)	Tumor staging	Sample size	Intervention time	Intervention frequency	Outcomes
Xie (43)	2022	China	OE/AE	18~50	22.05± 2.67/ 22.07 ± 3.08	NA/NA	NA/NA	I-IV	45/45	Four cycles of chemotherapy	3 ~ 4 times/week, 30 ~ 40 min/time	CFS
Xu (44)	2012	China	AE/CG	47.3±12.8	NA	NA	NA	NA	39/39	8weeks	20- 30 minutes/time	RPFS
Hao (45)	2013	China	AE/CG	46 ± 11.13/48 ± 11.32	NA	NA	63.74 ± 8.52/ 60.29 ± 8.26	I-III	28/28	15weeks	For the first 3 weeks, 3 times a week, 15min each time, then increase by 5min every 3 weeks, and reach 35min in 13-15 weeks.	RPFS
Mijwel S (46)	2018	Sweden	AE/CG	54.4± 10.3/ 52.6 ± 10.2	NA	165.3 ± 6.6/ 166.4 ± 7.0	67.7 ± 13.0/ 69.1± 11.0	I-III	70/60	16weeks	Twice a week	CRF
Cohen (47)	2021	USA	ME/ AE/RE	59.71 ± 6.99/58.56 ± 10.41/ 53.62 ± 8.03	28.21± 5.39/ 26.04 ± 3.91/ 27.93 ± 6.35	158.2 ± 40.65/ 140.2 ± 23.07/ 162.08 ± 38.90	64.00 ± 1.93/ 63.46 ± 2.78/ 64.58 ± 2.64	I-III	13/14/13	NA	90min, three times/week.	PFS
Courneya (48)	2007	Canada	AE/ RT/CG	49/ 49.5/49	26.7 ± 5.6/ 26.1 ± 5.5/ 27.1 ± 5.4	NA	69.4 ± 13.3/ 69.7 ± 14.4/ 72.6 ± 15.2	I-III	78/82/82	17weeks	Three times/week	FACT-F
Moadel (49)	2007	USA	YG/CG	55.11 ± 10.07/ 54.23 ± 9,81	NA	NA	NA	I-IV	108/56	12weeks	12sessions/week, 90minutes/session	FACIT-F
Bower (50)	2012	USA	YG/CG	54.4 ± 5.7/ 53.3 ± 4.9	NA	NA	NA	0-II	16/15	12weeks	Twice/week, 90 minutes/time	FSI/PSQI
Vadiraja (51)	2017	India	YG/CG	50.54 ± 8.53	NA	NA	NA	NA	33/31	3 months	NA	FSI
Vardar (52)	2015	Turkey	ME/AE	49.89 ± 4.65/47.38 ± 7.57	29.16 ± 5.74/ 29.27 ± 5.92	NA	NA	I-II	19/21	6weeks	30 minutes/day, 3 days/week	EORTC QOL-C30 -Fatigue

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TABLE 1 Continued

Name	Years	Country	Group	Age	BMI	Height (cm)	Weight (kg)	Tumor staging	Sample size	Intervention time	Intervention frequency	Outcomes
Cramer (53)	2015	Germany	YG/CG	48.3 ± 4.8/ 50.0 ± 6.7	NA/NA	169.9 ± 7.3/ 170.2 ± 5.4	69.8 ± 11.9/ 74.3 ± 17.0	I-III	19/21	12weeks	90min/week	FACIT-F/ FACT -B
Vadiraja (54)	2009	India.	YG/CG	NA	NA	NA	NA	II-III	44/44	6weeks	> 3 times/week	EORTC QoL C30 Fatigue
Chaoul (55)	2018	USA	YG/ SE/CG	49.5 ± 9.8/ 50.4 ± 10.3/ 49 ± 10.1	NA	NA	NA	I–III	74/68/85	12weeks	75-90min/time	BFI/PSQI
Lötzke (56)	2016	YG/CG	YG/CG	51.0 ± 11.0/ 51.4 ± 11.1	NA	NA	NA	NA	45/47	12weeks	60 minutes/week	EORTC QLQ-C30 Fatigue
Banasik (57)	2011	USA	YG/CG	63.33 ± 6.9/ 62.4 ± 7.3	NA	NA	NA	II-IV	9/9	8weeks	90 minutes/time	Fatigue Likert Scale
Strunk (58)	2018	Germany	OE/CG	54.2 ± 7.8/ 51.5 ± 8.4	NA	NA	NA	NA	30/21	24weeks	Twice/week, 90 minutes/time	QLQ-C30 Fatigue
Danhauer (59)	2009	USA	YG/CG	54.3 ± 9.6 /57.2 10.2	NA	NA	NA	Ductal carcinoma *in situ* -IV	13/14	10weeks	75 minutes/10 weeks	FACT-F/PSQI/ FACT-B
Jong (60)	2018	Netherlands	YG/CG	51 ± 8/ 51 ± 7.3	NA	NA	NA	I-III	47/36	12weeks	Once a week	EORTC QLQ-C30, Fatigue
Wang (61)	2014	China	YG/CG	18~60	NA	NA	NA	NA	40/42	4 months	4 times/week, 1 time/day, 50min/time	CFS
Zeng (62)	2017	China	MI/YG/ CG/ME	NA	NA	NA	NA	NA	20/24/ 23/22	4 months	Once/two days, 30 min/once, once/two days, 40 min/ once, NA, once/two days, 40 min/once	CFS
Xiang (63)	2017	China	MI/YG/ ME/CG	18~60	NA	NA	NA	NA	20/22/ 24/23	16weeks	Once every 2 days, 30 min/every 2 days, 40min/every 2 days, 40min/NA each time	CFS
Yang (64)	2020	China	ME/CG	49.17 ± 13.24/ 49.24 ± 12.09	NA	NA	NA	NA	79/83	1 month	2-5 times/week	PFS-R

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TABLE 1 Continued

Name	Years	Country	Group	Age	BMI	Height (cm)	Weight (kg)	Tumor staging	Sample size	Intervention time	Intervention frequency	Outcomes
Liu (65)	2018	China	AE/CG	55.2± 2.3/ 51.2± 3.2	NA	NA	NA	NA	30/30	5weeks	The first 4 weeks, 15 min/day, from the fifth week after 30 min, > 3 times/week	PFS-R
Li (66)	2019	China	AE/CG	48.0± 11.0/47.0 ± 10.0	NA	NA	NA	NA	46/46	1weeks	NA	PFS-R
Liu (67)	2015	China	AE/CG	18-62	NA	NA	NA	NA	34/35	8weeks	15 min/day for the first 4 weeks, 30 min/day for the last 4 weeks, > 3 times/week.	CRF/PSQI
Yu (68)	2020	China	AE/CG	44.01 ± 2.11/ 44.25 ± 2.24	NA	NA	NA	NA	44/44	6weeks	30min/each time, 4 times/week,	PFS-R
Chang (69)	2016	China	ME/CG	42.59 ± 6.37	NA	NA	NA	I=IV	51/49	18weeks	ME: 2-3times/day,	PSQI
Yu (70)	2021	China	YG/CG	38.8 ± 10.9/ 39.5 ± 10.3	NA	NA	NA	II-III	59/59	8weeks	Twice/day, 3 ~ 5 times/week	CFS
Yang (71)	2022	China	AE/CG	45. 56 ± 2. 37/ 45. 32 ± 2. 18	NA	NA	NA	NA	32/32	During chemotherapy	30 min/day, > 3 times/week	PFS-R/PSQI
Zhang (72)	2020	China	MI/CG	48.4 ± 8.19/ 45.48 ± 5.64	NA	NA	NA	I-IV	40/40	3 months	15 to 30 min/time	PFS-R/PSQI
Bolam (73)	2019	Sweden	RT/ CG/AE	52.7 ± 10.3 /24.6 ± 4.8/ 24.8 ± 4.4	25.1 ± 4.3/ 24.6 ± 4.8/ 24.8 ± 4.4	NA	NA	I–IIIa	74/60	6weeks	60 minutes/time, twice a week	CRF
Park (74)	2020	Japan	ME/CG	53.21 ± 8.4/54.19 ± 9.27	NA	NA	NA	0-III	35/36	8weeks	20-45 min/day	BFI
Paulo (75)	2018	Brazil	MI/SE	63.2 ± 7.1/ 66.6 ± 9.6	66.9 ± 10.3/ 72.3 ± 13.1	154.1 ± 6.7/ 153.1 ± 4.5	NA	I-III	18/15	9 months	45 minutes, 3 times a week/2 times a week.	EORTC QLQ-BR23 Fatigue

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TABLE 1 Continued

Name	Years	Country	Group	Age	BMI	Height (cm)	Weight (kg)	Tumor staging	Sample size	Intervention time	Intervention frequency	Outcomes
Wang (76)	2022	China	CTE/CG	NA	22.15 ± 3.33/ 21.06 ± 2.26	NA	NA	NA	10/10	16weeks	3 times/week, 40min/each time	PFS-R
Wei (77)	2022	China	CTE/CG	40-75/ 40-75	22.86 ± 2.55/ 23.26 ± 2.56	NA	NA	I~III	35/35	12weeks	5 times/week, 30min/each time	MFSI-SF/ FACT-B
Schad (78)	2013	Germany	AE/CG	61.7± 9.4/ 59.3 ± 11.0	NA	NA	NA	I-III	30/30	6weeks	One hour per week	CFS/PSQI
Liao (79)	2022	China	CTE/CG	53.12± 7.02/54.63 ± 8.44	22.14± 2.67/ 23.37 ± 3.92	NA	NA	I–III	33/35	12weeks	2 times/week, 90 minutes/week	EORTC QLQ-C30-Fatigue/PSQI
Boing (80)	2017	Brazil	AE/CG	54.1 ± 7.6	NA	NA	NA	NA	8/11	12weeks	twice a week, 60 minutes/time	PFS-R
Naraphong (81)	2014	Thailand	ME/CG	46.36± 9.37/47.17 ± 6.87	NA	NA	NA	I–IIIa	11/12	10weeks	3-5 days/week	CRF
Irwin (82)	2017	USA	CTE/ CBT	59.6 ± 7.9/ 60.0 ± 9.3	25.6 ± 4.5/ 26.2 ± 5.9	NA	NA	NA	38/42	3 months	120 times per week	PSQI/MFSI-SF
Chen (83)	2013	China	CTE/CG	45.3 ± 6.3/ 44.7 ± 9.7	NA	NA	NA	0-III	49/46	3 months	40 minutes, 5 times a week	BFI/PSQI
Huang (84)	2016	China	CTE/CG	NA	NA	NA	NA	NA	31/33	12 weeks	30 minutes/time	EFS
Cao (85)	2016	China	MT/CG	35.45 ± 9.21/36.12 ± 9.67	NA	NA	NA	I-III	100/ 100	8weeks	45min/days	CFS
Jiang (86)	2019	China	ME/CG	43.48 ± 9.72/42.63 ± 9.56	NA	NA	NA	I-III	58/50	4weeks	2 times/day, 20min/each time	PSQI/CFS
Fan (87)	2021	China	MT/CG	46.32 ± 5.69 /45.26 ± 5.42	NA	NA	NA	I-III	90/90	5weeks	2 ~ 3 h/time	PFS-R/PSQI

(Continued)

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TABLE 1 Continued

Name	Years	Country	Group	Age	BMI	Height (cm)	Weight (kg)	Tumor staging	Sample size	Intervention time	Intervention frequency	Outcomes
Wang (88)	2021	China	MT/CG	49. 15 ± 8. 39/51. 00 ± 9. 94	NA	NA	NA	I-III	41/43	6weeks	Once a week, 30 ~ 60min/time	CFS/PSQI
Bower (89)	2015	USA	MT/CG	46.1/47.7	NA	NA	NA	0-III	39/32	6weeks	6 times a week	FSI/PSQI
Reich (90)	2014	USA	MT/CG	58.0 ± 10.3/ 58.2 ± 9.5	NA	NA	NA	0- III	17/24	6weeks	15-45 min/day	Fatigue
Rahmani (91)	2015	Iran	MT/CG	43.25± 3.07/44.08 ± 3.28	NA	NA	NA	l-III	12/12	8weeks	Once/week, 2 hours/time	FSS
Lengacher (92)	2016	USA	MT/CG	56.5 ± 10.2/57.6 ± 9.2	NA	NA	NA	0-III	167/155	6weeks	6 days/week	FSI/PSQI
Daley (93)	2007	Daley	AE/CG	51.6 ± 8.8/50.6 ± 8.7	28.5 ± 4.4/27.6 ± 4.1	NA/NA	77.2 ± 12.1/73.9 ± 11.3	NA	34/38	8weeks	3 times/week	RPFS/FACT-B
Wang (94)	2017	China	CTE/CG	50.5	NA	NA	NA	I-III	45/41	3 months	20 minutes/time	CFS/PSQI
Li (95)	2019	China	YG/CG	47.5 ± 8.2/46.7 ± 9.5	NA	NA	NA	NA	45/45	2 months	60 min/time, 3 times/week	PSQI
Xiong (96)	2019	China	ME/CG	51.8 ± 4.8/ 51.5 ± 4.6	NA	NA	NA	NA	49/49	During chemotherapy	2 times/daily. 3 times/week	PSQI/FACT-B
Yuan (97)	2020	China	RE/AE	47.87± 11.94/ 45.13 ± 10.77	24.02± 2.11/ 24.07 ± 2.52	NA	NA	I-III	47/47	12weeks	RE: It was performed once every 1d, 15 min/time AE:3times/week, 15-40 minutes/time	PSQI
Zhuang (98)	2021	China	CTE/CG	35-50	NA	NA	NA	NA	70/70	12weeks	The first week 10min/time, 3 times/week, the second week extended to 10-20min/time, 4 times/week; Time in the third week 10-20min/time, 5 times/week.	PSQI
Liu (99)	2022	China	YG/CG	NA	24.71 ± 3.69/ 23.69 ± 3.16	NA	NA	I-II	61/62	8weeks	90 minutes/week	FACT-B

(Continued)

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TABLE 1 Continued

Name	Years	Country	Group	Age	BMI	Height (cm)	Weight (kg)	Tumor staging	Sample size	Intervention time	Intervention frequency	Outcomes
Stan (100)	2016	USA	YG/SE	61.4 ± 7.0/ 63.0± 9.3	NA	NA	NA	0-II	18/16	12weeks	> 3 times/week	FACT-B
Rogers (101)	2015	USA	AE/CG	54.9 ± 9.3/ 53.9 ± 7.7	NA	NA	NA	I-III	105/112	3 months	> 3 times/week	FACT-B
Dieli (102)	2018	USA	ME/CG	NA	BMI≥25.0	NA	NA	0-III	50/50	16weeks	> 3 times/week	FACT-B
Jin (103)	2017	China	YG/CG	55~73	NA	NA	NA	NA	50/50	16weeks	3 times/week, 1 h/each time	FACT-B
Du (104)	2019	China	AE/CG	50.1 ± 3.84/50.8 ± 3.00	23.17 ± 3.01/ 23.93 ± 2.79	NA	NA	I-III	40/40	4weeks	3 times/day, 20 minutes/time	FACT-B
Li (105)	2017	China	CTE/CG	47.31 ± 9.85/45.43 ± 10.94	NA	NA	NA	0-III	31/30	3 months	Once/day, 5day/week	FACT-B
Odynets (106)	2019	Ukraine	OE/ YG/CTE	59.40 ± 1.24/59.10 ± 1.37	NA	NA	NA	NA	45/30	12 months	3 times/week, 60 minutes/day	FACT-B
Fong (107)	2013	China	CG/CTE	58.3 ± 10.1/53.8 ± 4.2	NA	155.5 ± 4.3/156.7 ± 6.0	50.4 ± 7.4/55.6 ± 8.8	NA	12/16	6 months	3 times/week, 60 minutes/time	FACT-B
Loh (108)	2014	Malaya	AE/AE	18-65	NA	NA	NA	I-II	32/31	8 weeks	Twice a day/twice a week	FACT-B

Tai Chi (TC), Yoga (YG), Music interventions (MI), Aerobic exercise (AE), Relaxation training (RE), Cognitive behavioral therapy (CBT), Mindfulness training (MT), Sling exercise (SE), Qigong (QG), Baduanjin exercise (BE), Stretching exercise (STE), Resistance training (RT), Other exercise (OE), Chinese traditional exercises (CTE), CTE (TC, QG, BE), Multimodal exercise (ME), Control group (CG).
NA, Not Applicable.

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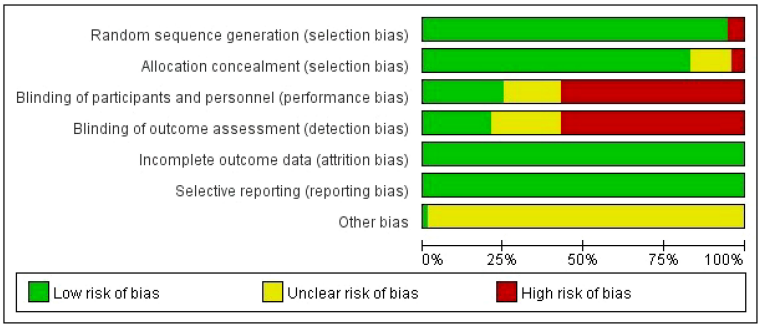

FIGURE 2
Percentage of studies examining the efficacy of interventions in patients with breast cancer with low, unclear, and high risk of bias for each feature of the Cochrane Risk of Bias Tool.

chemotherapy but also endure the pressure arising from the family disorder, contributing to an increase in fatigue level (112). The onset of breast cancer and subsequent lifelong treatment impose medical burdens on patients, leading to emotional and role changes among some patient's family members. This exacerbates the psychological burden and contributes to increased CRF (112). Sørensen HL et al. (113) discovered a close relationship between social support and physical and mental fatigue experienced by patients with breast cancer, with a stronger correlation observed for mental fatigue. Patients lacking social support face difficulties in receiving adequate support and assistance and struggle to effectively express sad emotions, leading to increased pressure on patients and a consequent worsening of fatigue. Yao Li et al. (114). found that farmers' lower fatigue levels may be attributed to their low educational level, limited avenues for acquiring tumor-related knowledge, and higher expectations regarding disease prognosis. Furthermore, farmers may shoulder less social responsibility than patients with higher education, experiencing less social pressure, and exhibiting less noticeable fatigue. CRF has a tendency to persist and can result in dysfunction, reduced quality of life, and the emergence of negative emotions. Patients with breast cancer experience pain from CRF before or during treatment. Currently, a significant number of patients with breast cancer experience CRF, emphasizing the urgent need for its management.

Currently, numerous studies are focusing on improving CRF in breast cancer. This study conducted a literature search from 2001 to 2022, yielding 77 articles. Twelve various interventions (Aerobic exercise, Resistance training, Chinese traditional exercises, Other exercise, Multimodal exercise, Yoga, Stretching exercise, Music interventions, Cognitive behavioral therapy, Mindfulness training, Relaxation exercises, Control group) were analyzed to investigate their impact on patients with breast cancer and determine which intervention can effectively enhance CRF, alleviate depression, and improve quality of life in these patients.

The findings of this study indicate that CBT, CTE, AE, ME, MI, and YG were more effective than CG in improving CRF in patients with breast cancer. CBT can alter patients' thinking, beliefs, and behaviors, correcting erroneous cognition and eliminating negative emotions through psychological treatment (115). Cognitive behavioral therapy can reduce CRF in patients with cancer through cognitive therapy, behavioral therapy, and psychological intervention. Clinical practice guidelines have recommended Cognitive behavioral therapy to reduce CRF in adults (116). The findings of this study are consistent with those of the traditional meta (117). In this study, we referred to Tai Chi, Qigong, and Baduanjin as Chinese traditional exercises. It was found that Chinese traditional exercises significantly improved CRF in patients with breast cancer compared to Control group. Tai Chi can regulate the nervous regions of the downstream stress response pathway by regulating the neuroendocrine system, including the autonomic nervous system and the hypothalamic-pituitary-adrenal axis. This regulation impacts the production of inflammatory factors (31, 118), leading to the downregulation of inflammatory factors, improvement in the inflammatory environment of patients' tumors, and alleviation of CRF. Furthermore, Tai Chi is typically performed in groups, fostering a strong sense of community and social support among participating patients. They can share difficult everyday experiences and feel comfortable facing similar adversities. Additionally, social support can play a crucial role in buffering patients' stress (119). Social support can enhance patients' self-management ability, coping ability, and quality of life, including CRF (120). CTE regulates the respiratory system, enhances physiological function, promotes metabolism, and improves physical fitness, which can improve CRF in patients with breast cancer to a certain extent.

A relatively severe symptom burden caused by cancer diagnosis and treatment leads to patients being prone to sleep disorders (121). Sleep disorders affect more than 50% of patients with cancer, with some experiencing persistent and recurring sleep disorders within one year of completing treatment. Prolonged sleep disorders exacerbate patients' pain, fatigue, psychological pain, and other symptoms, significantly affecting their quality of life and prognosis (122, 123).

This study discovered that Relaxation exercises, Music interventions, Aerobic exercise, and Multimodal exercise were more likely to improve the sleep quality of patients with breast cancer than Chinese traditional exercises, Other exercise, and Control group. Relaxation exercises regulates the function disturbed by tension stimulation, promotes muscle relaxation, reduces the arousal level of the cerebral cortex, and facilitates falling asleep through

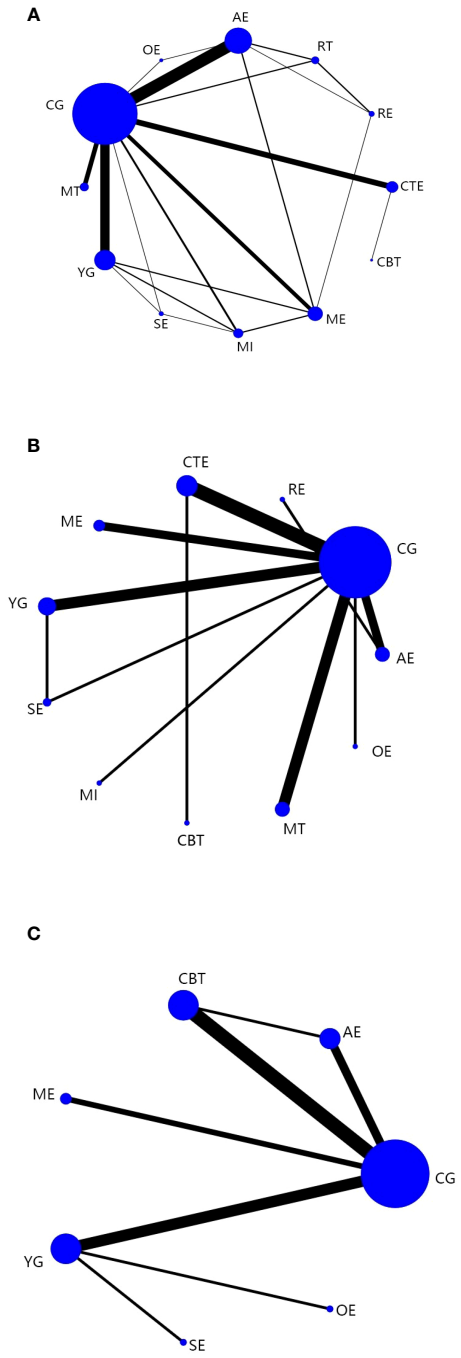

FIGURE 3
Network plots: Tai chi (TC), Yoga (YG), Music interventions (MI), Aerobic exercise (AE), Relaxation training (RE), Cognitive behavioral therapy (CBT), Mindfulness training (MT), Sling exercise (SE), Qigong (QG), Baduanjin Exercise (BE), Stretching exercise (STE), Resistance training (RT), Other exercise (OE), Chinese traditional exercises (CTE), CTE (TC, QG, BE), Multimodal exercise (ME), Control group (CG). **(A)** is network plots of CRF. **(B)** is network plots of Sleep quality. **(C)** is network plots of Quality of Life. The size of the nodes represents the number of times the exercise appears in any comparison of that treatment, and the width of the edges represents the total sample size in the comparisons it connects.

consciously repeated exercises of muscle tension and relaxation. Music interventions can affect the release of morphine peptides and other substances in the body and slow down negative emotions associated with sleep disorders, such as anxiety and depression, to improve sleep quality (117). Furthermore, Music interventions can regulate the body and mind of patients, inducing relaxation and guiding them into a relaxed and happy state, which contributes positively to stabilizing emotions and relieving pain. Relaxation exercises and Music interventions are also cost-effective and easy to practice. Using Relaxation exercises is recommended to improve sleep in patients with breast cancer and those experiencing sleep disorders. Moreover, this study observed that Chinese traditional exercises, Yoga, and Aerobic exercise were more effective than Control group in improving patients' quality of life.

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A

CBT	-0.71 (-2.15,0.73)										
-0.71 (-2.15,0.73)	CTE										**-0.85 (-1.34, -0.36)**
-0.71 (-2.55,1.13)	0.00 (-1.15,1.15)	OE	-0.80 (-2.23,0.63)								-0.10 (-1.57,1.37)
-0.79 (-2.35,0.76)	-0.08 (-0.67,0.50)	-0.09 (-1.13,0.95)	AE	0.14 (-0.94,1.23)		0.41 (-1.15,1.97)		-0.05 (-1.04,0.94)			**-0.82 (-1.16, -0.49)**
-0.81 (-2.41,0.79)	-0.10 (-0.80,0.60)	-0.10 (-1.25,1.04)	-0.02 (-0.58,0.54)	ME	-0.19 (-1.24,0.86)	-0.60 (-2.17,0.97)			-0.12 (-1.16,0.93)		**-0.69 (-1.29, -0.09)**
-0.82 (-2.50,0.85)	-0.11 (-0.98,0.75)	-0.12 (-1.37,1.14)	-0.03 (-0.80,0.74)	-0.01 (-0.81,0.79)	MI				0.07 (-0.98,1.13)	-1.04 (-2.58,0.51)	-0.74 (-1.59,0.11)
-1.01 (-2.81,0.79)	-0.30 (-1.38,0.79)	-0.30 (-1.70,1.10)	-0.21 (-1.18,0.75)	-0.20 (-1.22,0.83)	-0.19 (-1.37,1.00)	RE		-0.04 (-1.04,0.96)			
-1.08 (-2.69,0.54)	-0.37 (-1.10,0.37)	-0.37 (-1.55,0.81)	-0.28 (-0.92,0.35)	-0.27 (-1.01,0.48)	-0.25 (-1.15,0.65)	-0.07 (-1.18,1.05)	MT				-0.49 (-1.04,0.06)
-1.11 (-2.82,0.60)	-0.40 (-1.33,0.52)	-0.41 (-1.69,0.87)	-0.32 (-1.10,0.46)	-0.30 (-1.19,0.59)	-0.29 (-1.34,0.76)	-0.10 (-0.95,0.74)	-0.04 (-1.00,0.92)	RT			-0.08 (-1.07,0.91)
-1.12 (-2.69,0.45)	-0.41 (-1.03,0.22)	-0.41 (-1.52,0.70)	-0.32 (-0.82,0.17)	-0.31 (-0.91,0.29)	-0.30 (-1.05,0.45)	-0.11 (-1.15,0.92)	-0.04 (-0.72,0.63)	-0.01 (-0.88,0.86)	YG	-0.21 (-1.61,1.20)	**-0.41 (-0.81, -0.02)**
-1.63 (-3.45,0.19)	-0.92 (-2.04,0.20)	-0.92 (-2.37,0.52)	-0.83 (-1.89,0.22)	-0.82 (-1.92,0.28)	-0.81 (-1.86,0.24)	-0.62 (-2.01,0.77)	-0.55 (-1.70,0.59)	-0.52 (-1.79,0.76)	-0.51 (-1.53,0.51)	SE	0.08 (-1.32,1.48)
-1.56 (-3.08, -0.04)	**-0.85 (-1.34, -0.36)**	-0.86 (-1.90,0.18)	**-0.77 (-1.09, -0.45)**	**-0.75 (-1.26, -0.25)**	**-0.74 (-1.45, -0.03)**	-0.55 (-1.52,0.41)	-0.49 (-1.04,0.06)	-0.45 (-1.23,0.33)	**-0.44 (-0.83, -0.06)**	0.07 (-0.94,1.07)	CG

B

RE		-2.35 (-5.05,0.35)							
-1.24 (-5.26,2.78)	MI								**-5.45 (-7.98,-2.92)**
-2.35 (-5.05,0.35)	-1.11 (-4.09,1.86)	AE							**-4.34 (-5.90,-2.78)**
-3.21 (-6.67,0.24)	-1.98 (-4.91,0.96)	-0.86 (-3.01,1.29)	ME						**-3.47 (-4.95,-1.99)**
-4.62 (-8.08,-1.16)	**-3.38 (-6.33,-0.44)**	**-2.27 (-4.43,-0.11)**	-1.41 (-3.51,0.70)	YG		-0.90 (-3.72,1.92)			**-2.07 (-3.56,-0.57)**
-5.01 (-8.36,-1.66)	**-3.77 (-6.58,-0.95)**	**-2.66 (-4.64,-0.67)**	-1.79 (-3.71,0.13)	-0.39 (-2.32,1.55)	MT		-0.90 (-3.39,1.59)		**-1.80 (-3.16,-0.43)**
-6.14 (-10.19,-2.09)	**-4.90 (-8.52,-1.28)**	**-3.79 (-6.81,-0.77)**	-2.92 (-5.90,0.06)	-1.52 (-4.09,1.06)	-1.13 (-3.99,1.73)	SE			0.10 (-2.75,2.95)
-6.29 (-9.60,-2.98)	**-5.05 (-7.82,-2.28)**	**-3.94 (-5.86,-2.02)**	**-3.08 (-4.93,-1.22)**	-1.67 (-3.54,0.20)	-1.28 (-2.76,0.19)	-0.15 (-2.97,2.67)	CTE		-0.31 (-1.52,0.91)
-6.69 (-10.85,-2.52)	**-5.45 (-9.20,-1.70)**	**-4.34 (-7.51,-1.16)**	**-3.47 (-6.61,-0.34)**	-2.07 (-5.21,1.08)	-1.68 (-4.70,1.34)	-0.55 (-4.33,3.23)	-0.40 (-3.38,2.58)	OE	-0.00 (-2.76,2.76)
-6.69 (-9.81,-3.57)	**-5.45 (-7.98,-2.92)**	**-4.34 (-5.90,-2.78)**	**-3.47 (-4.95,-1.99)**	**-2.07 (-3.56,-0.57)**	**-1.68 (-2.91,-0.46)**	-0.55 (-3.14,2.04)	-0.40 (-1.52,0.72)	-0.00 (-2.76,2.76)	CG

C

CTE				-5.80 (-25.09,13.49)		**12.64 (4.92,20.36)**
0.10 (-11.98,12.19)	YG		1.50 (-16.45,19.45)		2.28 (-14.36,18.92)	**11.28 (1.63,20.93)**
0.59 (-13.55,14.73)	0.48 (-15.01,15.98)	ME				10.80 (-1.33,22.92)
1.60 (-20.03,23.24)	1.50 (-16.45,19.45)	1.02 (-22.70,24.73)	STE			
2.05 (-8.56,12.66)	1.94 (-11.31,15.19)	1.46 (-13.69,16.61)	0.44 (-21.87,22.75)	AE		7.19 (-2.90,17.28)
2.38 (-18.18,22.95)	2.28 (-14.36,18.92)	1.80 (-20.94,24.54)	0.78 (-23.70,25.26)	0.34 (-20.93,21.61)	OE	
11.39 (4.11,18.66)	**11.28 (1.63,20.93)**	10.80 (-1.33,22.92)	9.78 (-10.60,30.16)	**9.34 (0.26,18.42)**	9.00 (-10.24,28.24)	CG

FIGURE 4
League tables of outcome analyses: Tai chi (TC), Yoga (YG), Music interventions (MI), Aerobic exercise (AE), Relaxation training (RE), Cognitive behavioral therapy (CBT), Mindfulness training (MT), Sling exercise (SE), Qigong (QG), Baduanjin Exercise (BE), Stretching exercise (STE), Resistance training (RT), Other exercise (OE), Chinese traditional exercises (CTE), CTE (TC, QG, BE), Multimodal exercise (ME), Control group (CG). **(A)** is the results of CRF's network meta-analysis. **(B)** is the results of Sleep quality's the network meta-analysis. **(C)** is the results of Quality of Life's the network meta-analysis. Data are mean differences and 95% credibility intervals for continuous data.

Study strengths and limitations

This review has several advantages. First, mesh meta-analysis was used to directly and indirectly compare various interventions. Notably, more accurate interventions were included and meticulously classified into 12 various interventions, with each being defined. The effects of various intervention methods on CRF, PSQI, and quality of life were examined, along with the investigation of additional intervention measures. The findings of this study can be used as an optimal reference.

However, certain limitations exist in this study. First, the duration, intensity, and frequency of the interventions were not considered. Second, the implementation quality of the blind approach in the included literature is not high, and the outcome indicators are all subjective, lacking objective indicators. A description of the biological parameters should be added. Third,

only Chinese–English literature was included, which may have resulted in heterogeneity. Fourth, all the studies were small sample studies; therefore, it is recommended to conduct future studies with large samples. Finally, in this study, the effects of tumor stage, patient treatment, patient's psychological status, and patient's family situation on CRF were not considered, which will have a particular impact on the results of this study.

Conclusion

Evidence from systematic reviews and meta-analyses strongly recommends CBT for improving CRF in patients with breast cancer. RE and CTE are recommended to enhance the quality of sleep in patients with breast cancer. This study includes limited results, and it is recommended that future investigations include more studies to further validate the findings and select appropriate interventions based on the circumstances of patients with breast cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

YL: Writing – original draft, Writing – review & editing. LG: Data curation, Writing – original draft. RL: Data curation, Writing – original draft. JZ: Data curation, Software, Writing – original draft. ZZ: Data curation, Writing – original draft. SL: Writing – original draft, Data curation. XC: Supervision, Writing – original draft, Writing – review & editing. YC: Writing – review & editing, Supervision, Resources. TL: Writing – review & editing, Resources. JL: Writing – review & editing, Resources. ZW: Writing – review & editing, Supervision, Resources.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1341927/full#supplementary-material

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frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 15 February 2024
DOI 10.3389/fimmu.2024.1287824

OPEN ACCESS

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RECEIVED 02 September 2023
ACCEPTED 12 January 2024
PUBLISHED 15 February 2024

CITATION
Alaluf E, Shalamov MM and Sonnenblick A (2024) Update on current and new potential immunotherapies in breast cancer, from bench to bedside.
Front. Immunol. 15:1287824.
doi: 10.3389/fimmu.2024.1287824

Update on current and new potential immunotherapies in breast cancer, from bench to bedside

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Impressive advances have been seen in cancer immunotherapy during the last years. Although breast cancer (BC) has been long considered as non-immunogenic, immunotherapy for the treatment of BC is now emerging as a new promising therapeutic approach with considerable potential. This is supported by a plethora of completed and ongoing preclinical and clinical studies in various types of immunotherapies. However, a significant gap between clinical oncology and basic cancer research impairs the understanding of cancer immunology and immunotherapy, hampering cancer therapy research and development. To exploit the accumulating available data in an optimal way, both fundamental mechanisms at play in BC immunotherapy and its clinical pitfalls must be integrated. Then, clinical trials must be critically designed with appropriate combinations of conventional and immunotherapeutic strategies. While there is room for major improvement, this updated review details the immunotherapeutic tools available to date, from bench to bedside, in the hope that this will lead to rethinking and optimizing standards of care for BC patients.

KEYWORDS

breast cancer, immunotherapies, immunooncology, cancer treament, immune escape mechanisms

Introduction

In the last years, there have been many advances and optimization in the treatment of breast cancer (BC). However, despite such progress, resistance to therapy and disease relapse remain important challenges in the management of BC in a considerable proportion of patients. Specifically, impressive advances have been seen in cancer immunotherapy during the last decade. Cancer immunotherapy exploits the host's immune system to eradicate tumor cells. Although BC has long been considered a non-immunogenic process,

immunotherapy for the treatment of BC is emerging as a new therapeutic approach with considerable potential, supported by a plethora of completed and currently ongoing preclinical and clinical studies in various types of BC immunotherapies. Tumor-infiltrating lymphocytes (TILs) are more commonly found in Human epidermal growth factor receptor 2 (HER-2)-positive BC and triple negative BC (TNBC), where the median percentages are 15% and 20%, respectively (1). However, 10% of TILs are also found in hormone receptor (HR)-positive BC (1). TILs can specifically target tumor cells following activation by antigen presenting cells (APC) via tumor antigen peptide presentation to human leukocyte antigen (HLA) molecules. TILs are associated with a better prognosis in TNBC (2) and node-positive TNBC (1). In HER2-positive BC, the presence of TILs showed contradictory data regarding trastuzumab therapy benefit (3, 4). TILs have also been associated with a higher probability of pathological complete response (pCR) in neoadjuvant settings (5, 6). Likewise, in HR-positive BC, CD8+ T-cell infiltration has been associated with survival (7), although this is currently under debate since contradictory results have been found for this BC subtype in neoadjuvant (8, 9) and adjuvant (10) settings. This led to suggest that the tumor-eradicating properties of TILs are an efficient part of the antitumor immune response and could therefore be exploited as immunotherapy to improve the clinical outcome of BC patients. γδ T-cells and natural killer (NK) cells have also been associated with a better prognosis in all BC subtypes (11, 12). Many targets are constantly being discovered on antitumor lymphoid cells, such as immune checkpoints. In addition, other immune cells of the tumor microenvironment (TME) contribute positively or negatively to the antitumor immune response and are currently a topic of intense preclinical and clinical research, such as tumor-infiltrating myeloid cells (13). Herein, we summarize the current and new potential immunotherapeutic strategies showing promising results in the emerging field of BC immunotherapy. We provide a basis for reflection on the available immunotherapeutic tools to date in the hope that this will lead to rethinking and optimizing standards of care for BC patients.

Directed monoclonal antibodies

HER2 is overexpressed in 15-20% of BC and correlates with higher grade, aggressive phenotype, and poor clinical outcome. Immunotherapies in the form of monoclonal antibodies specifically binding to HER-2 receptor, added to chemotherapy, are the cornerstone for HER-2-overexpressing BC therapy and have led to significant improvements in HER2-positive BC prognosis compared to previous chemotherapy regimens. Trastuzumab has been approved for the treatment of HER2-positive BC patients for approximately the past 20 years and acts through several mechanisms of action. It suppresses the HER2 intracellular signaling pathway by binding to the transmembrane HER2 receptor, which is followed by its internalization, degradation, and downregulation of PI3K pathway. In addition, trastuzumab activates both the innate and adaptive immune systems. Indeed, this monoclonal antibody enhances antibody-dependent cellular cytotoxicity (14), antibody-dependent cellular phagocytosis and macrophage activation (15), Fc-mediated immune priming by dendritic cells (DCs) (16), effector HER-2-specific T cell response (17) and memory T-cell response (16) (Figure 1). These mechanisms seem to be critical for the induction of a pCR after neoadjuvant therapy in HER2-positive BC patients (18). Pertuzumab is a dual HER2/HER3 monoclonal antibody approved in combination with trastuzumab and taxane-based chemotherapy for first-line therapy in HER2-positive metastatic BC and in adjuvant and neoadjuvant settings. It works by blocking HER2 heterodimerization and may act by promoting an antitumor immune response (19), although data regarding its mechanisms of action and its synergism with trastuzumab is still limited. To improve the efficacy of trastuzumab, the immunogenic properties of trastuzumab may be exploited in association with other strategies. For example, margetuximab was approved in combination with chemotherapy for third-line therapy in metastatic HER2-positive BC disease. Margetuximab is a monoclonal antibody similar to trastuzumab, whose modified Fc fragment has a much greater affinity for its activating Fcγ receptors and a decreased affinity for its inhibitory Fcγ receptors on tumor-infiltrating NK cells and macrophages. This way margetuximab promotes antibody-dependent cellular cytotoxicity and phagocytosis processes against tumor cells. This may explain the influence of Fcγ receptor polymorphism on overall survival of BC patients treated with margetuximab compared to trastuzumab (20). In light of the success of anti-HER2 therapies in HER2-positive BC, one might wonder why other monoclonal antibodies targeting tumor antigens other than HER2 have not been developed for other BC subtypes. Such monoclonal antibodies, synergizing with the potential antitumoral properties of the TME, might be particularly efficient in BC tumors wherein myeloid cells are abundant.

Antibody-drug conjugates

Antibody-drug conjugates (ADC), a new emerging class of antineoplastic agents with a high therapeutic index and impressive clinical efficacy, display both immune mechanisms of action, like those of naked directed monoclonal antibodies, combined with the targeted delivery of chemotherapy directly to antigen-expressing tumor cells (21). They are therefore known as "biological missiles". Recently, other mechanisms of action have been suggested to contribute to both their antitumor activity and adverse events (such as thrombocytopenia). For example, the release of chemotherapy into the TME may lead to the recruitment of particularly immunosuppressive, protumoral and tissue repairing myeloid cells. ADC may be taken up by macrophages through Fcγ receptors, leading to myeloid cell depletion or modulation of the activation state (22). Chemotherapy may also deplete regulatory T cells by diffusing into the TME through a bystander effect. Chemotherapy may also further promote NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) or regulate tumor antigen presentation by DCs (Figure 2). Trastuzumab emtansine is an ADC of trastuzumab covalently linked to the cytotoxic agent emtansine (DM1/maytansinoid). It is approved for HER2-positive BC

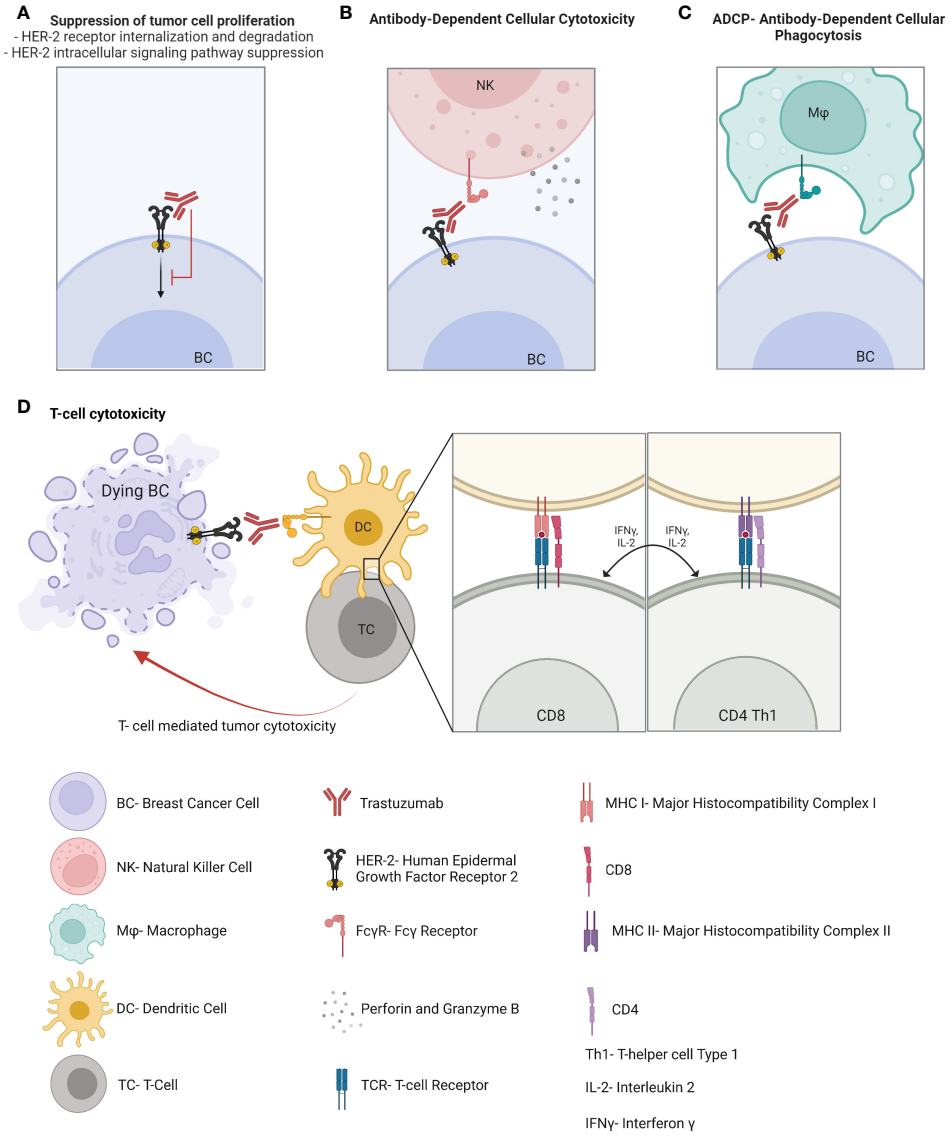

FIGURE 1
Mechanisms of action of monoclonal antibodies on the antitumor immune response.

patients in the metastatic setting and in the adjuvant setting for HER2-positive BC patients with residual invasive disease following neoadjuvant trastuzumab and chemotherapy. Trastuzumab-deruxtecan is the following ADC of trastuzumab linked to the topoisomerase 1 inhibitor deruxtecan (DXt/camptothecin). While TDM-1 has a non-cleavable linker and an average of 3.5 molecules of payloads per antibody, trastuzumab deruxtecan displays a cleavable linker with 8 molecules of a different payload. These differences could affect their antitumor mechanisms of action, such as the bystander effect and cellular toxicity. The efficacy and safety of trastusumab deruxtecan was compared with trastuzumab emtansine in the DESTINY-BREAST03 phase 3 randomized clinical trial, showing a lower risk of disease progression or death (23). Trastuzumab deruxtecan received accelerated approval in 2019 and has now become the new standard of care for second-line therapy in HER2-positive BC patients who have received a prior anti-HER2 based regimen either in the metastatic setting, or in the neoadjuvant or adjuvant setting and have developed disease recurrence during or within 6 months of completing therapy. In addition, it is approved for locally advanced or metastatic HER2-low (IHC 1+ or IHC 2+/FISH-) BC patients who have received a prior chemotherapy in the metastatic setting or developed disease recurrence during or within six months of completing adjuvant chemotherapy. Moreover, trastuzumab deruxtecan is currently compared, when used with or without pertuzumab, to the standard of care which is taxane, pertuzumab and trastuzumab as first-line treatment in the DESTINY-BREAST09 phase 3 clinical trial. Trastuzumab deruxtecan in association with tucatinib is also currently studied in metastatic BC patients, including with active brain metastasis, in the HER2-CLIMB-04 phase 2 clinical trial. Another ADC, sacituzumab govitecan, targets the human trophoblast cell-surface antigen 2 (Trop-2), which is highly expressed in BC, and is coupled with a high drug-to-antibody ratio to SN-38, the active metabolite of irinotecan. This leads to the delivery of high concentrations of the chemotherapy to the tumor cells by intracellular

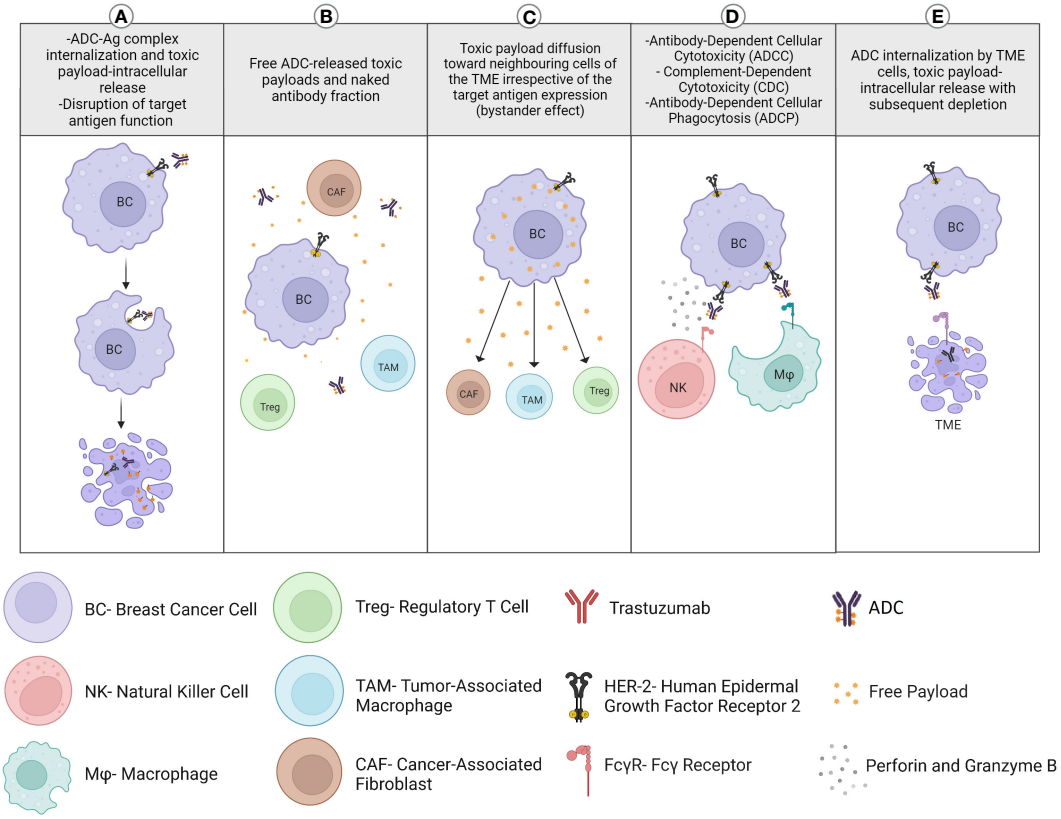

FIGURE 2
Mechanisms of action of antibody-drug conjugates (ADC) on the antitumor immune response.

uptake of SN-38, thereby also allowing the cells of the TME to be eradicated by SN-38 which is released extracellularly from the tumor cells through a bystander effect. This antitumor effect may also be mediated by a significant antibody-dependent cellular cytotoxicity effect against the Trop-2-positive tumor cells (24). Sacituzumab govitecan received accelerated approval in 2020 for the treatment of refractory metastatic TNBC following at least two prior chemotherapies, by showing promising results for this notoriously difficult-to-treat group of patients (25). Recently, it has demonstrated extended progression-free survival (PFS) and overall survival (OS), as well as greater health-related quality of life benefits than chemotherapy, and moved to second-line therapy of TNBC (at least one in the metastatic setting) (25). It may also represent a new option for endocrine-resistant hormone receptor-positive/HER2-negative metastatic BC, since it has recently shown a longer PFS and a statistically significant OS compared to standard chemotherapy (capecitabine, eribulin, vinorelbine or gemcitabine) after CDK4/6 inhibitors and 2 to 4 previous lines of chemotherapy (26). The indication of sacituzumab govitecan is currently investigated in case of residual disease after neoadjuvant chemotherapy.

Immune checkpoint inhibitors

During the last decade, the emergence of immune checkpoint inhibitors (ICI) has revolutionized the field of cancer therapies, especially in advanced or metastatic cancers where they have shown unprecedented and durable efficacy. They are approved in many different cancer types such as lung cancer, melanoma, renal cell carcinoma, and in any high microsatellite instability or mismatch repair deficiency. Used alone or in combination with other ICI or chemotherapies, they represent a staggering proportion of the ongoing clinical trials in oncology. In all cancer types, the most widely studied immunotherapeutic agents to date are ICI blocking cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), programmed cell death receptor-1 (PD-1) and programmed cell death ligand-1 (PD-L1). While PD-1 is mainly expressed on TILs, PD-L1 is expressed on both cancer cells and tumor-infiltrating immune cells. Although the impact of ICI on the immune response remains to be fully elucidated, the PD-1/PD-L1 immune inhibitory axis is thought to be upregulated in the TME and to impair the effector stage of the antitumor immune response (27).

PD-1 inhibitors

Nivolumab and pembrolizumab are human monoclonal antibodies that block PD-1 and therefore the interaction of PD-1 with its ligand PD-L1, preventing T-cell suppression.

In TNBC, although pembrolizumab showed antitumor activity in the phase 1b KEYNOTE-012 and the phase 2 KEYNOTE-086 trials, the KEYNOTE-119 phase 3 trial comparing pembrolizumab with

chemotherapy did not show significant improvement in OS in the second or third-line treatment of patients with metastatic TNBC (28). However, in the KEYNOTE-355 phase 3 study, pembrolizumab combined with chemotherapy significantly improved PFS compared with chemotherapy alone in patients with advanced or metastatic PD-L1-positive TNBC (CPS≥10) (29). Moreover, the follow-up of the patients with CPS of 10 or more showed a significantly longer OS with no new safety signals identified when pembrolizumab was added to chemotherapy (30). In addition, among patients with previously untreated stage II or III TNBC, the rate of pCR at definitive surgery was higher in pembrolizumab plus neoadjuvant chemotherapy compared with placebo plus chemotherapy in the KEYNOTE-522 phase 3 trial, along with disease-free survival (DFS) (31). Based on these results, the FDA approved in 2021 pembrolizumab for high-risk, early-stage, TNBC, in combination with chemotherapy as neoadjuvant treatment, and then continued as a single agent as adjuvant treatment. The FDA also granted accelerated approval in 2020 to pembrolizumab in combination with chemotherapy for patients with locally advanced or metastatic TNBC whose tumors express PD-L1. Other treatments with the combination of pembrolizumab and eribulin showed promise for patients with metastatic TNBC, with efficacy that seems greater than reports of either drug alone, according to the KEYNOTE-150 phase 1b/2 study (NCT02513472) (32). A pilot study comparing nivolumab with capecitabine and with combination therapy as adjuvant therapy after residual disease following neoadjuvant chemotherapy is under investigation (NCT03487666).

In HER2-positive BC, the combination of pembrolizumab plus trastuzumab demonstrated a tolerable safety profile, activity and durable clinical benefit, in advanced HER-2-positive, trastuzumab-resistant, PD-L1-positive BC disease (33).

In HR-positive BC patients, fewer clinical trials have been performed so far. In a phase 1b study, pembrolizumab was well tolerated with modest but durable partial response in certain patients with previously treated, advanced, PD-L1-positive HR-positive HER2-negative BC (34). In women with early-stage, high-risk, HR-positive HER2-negative BC, an ongoing phase 2 trial in the neoadjuvant setting with pembrolizumab in association with standard chemotherapy showed improved pCR (35). Since CDK4/6 inhibitors induce, in addition to a tumor cell cycle arrest, an enhanced antitumor immune response (36), they may be used in synergy with ICI to increase tumor immunogenicity (NCT02648477). Other studies currently assessing PD-1 inhibitors in different settings are summarized in Table 1.

PD-L1 inhibitors

Atezolizumab, durvalumab and avelumab are human monoclonal antibodies that block PD-L1 and therefore PD-1/PD-L1 interaction, T-cell activation and proliferation.

In TNBC, the association of durvalumab with nab-paclitaxel and doxorubicin-cyclophosphamide neoadjuvant chemotherapy suggested a high rate of pCR in a phase 1/2 trial (43), which was higher in PD-L1-positive and TILs-high than PD-L1-negative patients (44). This has not been observed in the phase 3 trial comparing the addition of atezolizumab to carboplatin and nab-paclitaxel with the chemotherapy regimen alone in a neoadjuvant setting (40). However, the IMpassion130 phase 3 study demonstrated a prolonged PFS with atezolizumab plus nab-paclitaxel in metastatic TNBC patients (37) and a clinically meaningful OS benefit in previously untreated PD-L1-positive patients, compared with placebo plus nab-paclitaxel (38). Following this clinical trial, nab-paclitaxel with atezolizumab received accelerated approval in March 2019 for first-line treatment of locally advanced or metastatic TNBC whose tumors express PD-L1 (>=1%). However, this has been withdrawn after IMpassion131 clinical trial results showing that atezolizumab and paclitaxel under the same settings did not show any improvement of PFS or OS versus paclitaxel alone (39).

The addition of atezolizumab to TDM-1 did not show any improvement and was associated with more adverse events in previously treated HER2-positive locally advanced or metastatic BC patients who received prior trastuzumab and taxane based therapies. However, a benefit in terms of PFS in favor of the combination has been observed in the PD-L1-positive subgroup of patients (45). Further study is required in subpopulations of patients.

Further results from various clinical trials investigating anti-PD-L1 treatments in TNBC, HER2-positive or HR-positive BC patients are summarized in Table 1 (41, 46).

CTLA-4 inhibitors

CTLA-4 on TIL surface mediates T-cell suppression by binding to CD80 and CD86 (expressed on the surface of antigen-presenting cells), therefore competing with the co-stimulatory receptor CD28 on the cell surface of T cells (47). Ipilimumab is a human monoclonal antibody that targets CTLA-4, thus preventing its inhibitory effect on T-cell activation. Ipilimumab is under investigation in various settings in BC which are summarized in Table 1 (42).

Optimizing trial design

Major clinical trials, such Keynote-119 trial assessing pembrolizumab monotherapy, failed to improve OS versus single-drug chemotherapy per investigator's choice after first-line metastatic TNBC. This underscores the inefficacy of PD-1 inhibitors alone and suggests the association with chemotherapies for the next trials. Indeed, various chemotherapies (such as anthracyclines or taxanes) result in tumor cell death and debris which induce immunogenic cell death. Immunogenic cell death is mediated by damage-associated molecular patterns (DAMPs), promoting tumor phagocytosis and antigen presentation, and may facilitate the induction of a robust antitumor immune response by ICI. In contrast, Keynote-355 showed improvement of PFS and OS in PD-L1-positive metastatic TNBC when chemotherapies including platinum or taxanes were associated with PD-1 inhibitors. This suggests the selection of such chemotherapies combined with ICI for further trials. On an early setting, Keynote-522 showed improved pCR when neoadjuvant and adjuvant pembrolizumab was combined with neoadjuvant carboplatin-

TABLE 1 Published and ongoing clinical trials related to ICI.

TNBC	Setting	Regimens	Results	Safety data	references
KEYNOTE-119 phase 3	Metastatic	pembro 200mg 1x/3 weeks vs investigator's choice chemo (capecitabine, eribulin, gemcitabine, or vinorelbine)	OS ≈	Anemia and leupopenia ↘ with pembro vs chemo. AE of grade≥3 in 20% of both groups	(28)
KEYNOTE-355 phase 3	Metastatic	Chemo (nab-paclitaxel, paclitaxel, or gemcitabine/ carboplatin)+pembro 200mg 1x/3 weeks vs placebo	↗ PFS (≥CPS 10) ↗ OS (≥CPS 10): 23 vs 16.1 months	68.1% of AE of grade≥3 in pembro +chemo vs 66.9% in chemo	(29, 30)
KEYNOTE-522 phase 3	Early (neoadjuvant and adjuvant)	Chemo (paclitaxel+carboplatin)+4 cycles of neoadjuvant pembro 200mg 1x/3 weeks vs placebo. Additional four cycles of pembro vs placebo and then doxorubicin +cyclophosphamide or epirubicin+cyclophosphamide for both groups. Adjuvant pembro vs placebo.	↗ DFS ↗ pCR: 64.8% vs 51.2%	78% of AE of grade≥3 in pembro+chemo vs 73% in chemo	(31)
KEYNOTE-150 phase 1b/2	Metastatic	Pembro 200mg d1+eribulin 1.4mg/m² d1, d8/21	↗ ORR in PD-L1+ tumors: 28.4% vs 17.3%	26.3% of neutropenia of grade≥3	(32)
IMpassion130 phase 3	Metastatic	Nab-paclitaxel 100mg/m² d1,8,15/28+atezolizumab 840mg d1, d15 vs placebo	↗ PFS: 7.2 vs 5.5 months in PD-L1+ tumors: 7.5 vs 5 months OS ≈ Withdrawn due to lack of benefit.	48.7% of AE of grade≥3 in the atezo group vs 42.2% in placebo, 57,3% of potential immune -related AE vs 41.8%, respectively	(37, 38)
IMpassion131 Phase 3	Metastatic	Paclitaxel 90mg/m² d1,8,15/28+atezolizumab 840mg d1 and d15 vs placebo	PFS ≈ OS ≈	53% of AE of grade≥3 in atezo group vs 46% in placebo. Higher incidence of low-grade hypo and hyperthyroidism in atezo group	(39)
Pilot study	Early (residual disease)	adjuvant capecitabine 1250mg/m² bid d1-d14/21 or nivolumab 360mg 1x/3 weeks or both	Immunoscore change?	?	NCT03487666
Phase 2	Metastatic	carboplatin+nivolumab 360mg 1x/3 weeks vs placebo	PFS?	?	NCT03414684
Phase 3	Metastatic	Chemotherapies+pembrolizumab 200mg 1x/3 weeks vs placebo	PFS? OS?	?	NCT02819518
Phase 1/2	Metastatic	Niraparib up to 300mg/day d1-21+pembrolizumab 200mg d1/21	DLT? ORR?	?	NCT02657889
IMpassion030 Phase 3	Early (adjuvant)	paclitaxel 80mg/m² 1x/week for 12 weeks followed by dose-dense doxorubicin 60mg/m² or epirubicin 90mg/ m²+cyclophosphamide 600mg/m² 1x/2weeks for 4 doses +atezolizumab 840 mg 1x/2 weeks for 10 doses and then maintenance 1200 mg 1x/3weeks to complete 1 year vs placebo	iDFS?	?	NCT03498716
NeoTRIP Phase 3	Early (neoadjuvant)	Neoadjuvant carboplatin+nab-paclitaxel 125mg/m² d1 and d8+atezolizumab 1200mg 1x/3weeks for 8 cycles vs placebo, followed by adjuvant anthracyclines for 4 cycles	pCR ≈ EFS?	Liver transaminase abnormalities in atezo group	NCT02620280 (40)
IMpasision031 phase 3	Early (neoadjuvant)	Nab-paclitaxel 125mg/m² 1x/week+atezolizumab 840mg 1x/2weeks for 12 weeks followed by atezolizumab 840mg +doxorubicin 60mg/m²+cyclophosphamide 600mg/m² 1x/2weeks for 4 doses followed by adjuvant atezolizumab 1200mg 1x/3weeks for 11 doses vs placebo	↗ pCR in all-randomized population and PD-L1+ status	30% of AE of grade≥3 in atezo group vs 18% in placebo, such as febrile neutropenia, pneumonia and pyrexia	NCT03197935 (41)

(Continued)

TABLE 1 Continued

TNBC	Setting	Regimens	Results	Safety data	references
Phase 3	Early relapsing metastatic	Chemotherapy (capecitabine or gemcitabine/carboplatin) and atezolizumab 1200mg 1x/3weeks vs placebo	OS?	?	NCT03371017
Phase 3	Early (adjuvant)	Adjuvant avelumab 10mg/kg 1x/2 weeks vs placebo	DFS? OS?	?	NCT02926196
Phase 2	Stage III (neoadjuvant)	Neoadjuvant ipilimumab 1mg/kg 6 weekly for 2 doses +nivo 240mg every 2 weeks for 6 weeks+weekly paclitaxel 80mg/m² for 12 weeks+postoperative nivolumab 480 mg 4-weekly for further 9 months	pCR 37.5% in PD-L1+ and 23% in PD-L1-, 57.6% of ORR	?	ongoing (42)
GeparNuevo Phase 2	Early (neoadjuvant)	Nab-paclitaxel 125mg/m² 1x/week+durvalumab 1.5g 1x/4 weeks vs placebo followed by doxorubicin 60mg/m²+cyclophosphamide 600mg/m²+durvalumab vs placebo	pCR ≈ ↗iDFS 85.6% in durva group vs 77.2% in placebo ↗DDFS 91.7% in durva group vs 78.4% in placebo ↗OS 95.2% in durva group vs 83.5% in placebo		NCT02685059
HER2+ BC	**Setting**	**Regimens**	**Results**	**Safety data**	**references**
PANACEA Phase 1b/2	Metastatic	Trastuzumab 6mg/kg+pembro 200mg 1x/3 weeks vs placebo	OR of 15% in PD-L1+ tumors, 0% in PD-L1- tumors	29% of AE of grade≥3. Immune-related AE in 19% such as thyroid dysfunction, pneumonitis and autoimmune hepatitis.	(33)
KATE2 Phase 2	Metastatic	TDM-1 3.6mg/kg+atezolizumab 1200mg 1x/3 weeks vs placebo	PFS ≈ In PD-L1+ tumors: PFS 8.5 months in atezo group vs 4.1 months in placebo	19% of AE of grade≥3 in atezo group vs 3% in placebo	(43)
Phase 2	Early (neoadjuvant)	Neoadjuvant pembro 200mg+trastuzumab 6mg/kg +pertuzumab 420mg/kg 1x/3 weeks	pCR?	?	NCT03988036
Phase 2	Metastatic	5 administrations of pembro 200mg 1x/3 weeks or VRP-HER2 vaccine 4x10EE8 IU 1x/2 weeks for 3 injections or both of them	Anti-HER2 TILs and antibodies?	?	NCT03632941
Phase 3	Metastatic	Paclitaxel 1x/week or docetaxel 1x/3 weeks+ trastuzumab +pertuzumab+atezolizumab 1x/3 weeks vs placebo for 2 years	PFS? OS?	?	NCT03199885
IMpassion050 Phase 3	Early	Neoadjuvant doxorubicin 60mg/m²+cyclophosphamide 600mg/m²+atezolizumab 840 mg 1x/2 weeks vs placebo followed by paclitaxel 80mg/m²1x/week for 12 weeks +trastuzumab 6mg/kg+pertuzumab 420mg 1x/3weeks. Adjuvant to complete up to 1 year HER2-target therapy +atezolizumab 1200mg 1x/3 weeks vs placebo	pCR ≈ DFS? OS?	Neoadjuvant: 47.3% of AE of grade≥3 in atezo group vs 42.2% in placebo Adjuvant: 13.4% of AE of grade≥3 in atezo group vs 9.8% in placebo	NCT03726879 (44)
Phase 2a	Metastatic	Atezolizumab+paclitaxel+trastuzumab+pertuzumab	Antitumor activity (RECIST)?	?	NCT03125928
Phase 1b	Metastatic	Durvalumab+trastuzumab 1x/3 weeks	Recommended phase 2 dose?	?	NCT02649686

(Continued)

TABLE 1 Continued

Luminal	Setting	Regimens	Results	Safety data	references
I-SPY2 Phase 2	Early (neoadjuvant)	neoadjuvant paclitaxel 80 mg/m²+pembrolizumab 200mg 1x/3 weeks vs placebo followed by doxorubicin 60 mg/m²+cyclophosphamide 600 mg/m². Standard-of-care adjuvant therapy.	pCR 30% vs 13% for HR +HER2- and 60% vs 22% for TNBC	immune-related AE such as thyroid dysfunction (1,4% ≥3) and adrenal insufficiency (7,2% of ≥3)	NCT01042379 (35)
Phase 2	Metastatic	Pembrolizumab 1x/3 weeks for 24 months+aromatase inhibitor po QD	ORR? OS?	?	NCT02648477
Phase 2	Early (residual disease)	Adjuvant pembrolizumab 1x/3 weeks up to 24 months +hormone therapy	DFS? OS	?	NCT02971748
KEYNOTE-756 phase 3	Early (neoadjuvant)	Neoadjuvant pembrolizumab 200mg 1x/3 weeks vs placebo+paclitaxel 80mg/m² 1x/week followed by doxorubicin 60mg/m² or epirubicin 100mg/m²+cyclophosphamide 600mg/m² 1x/2 weeks. Adjuvant pembrolizumab 200mg 1x/3 weeks vs placebo +hormone therapy	pCR? EFS?	?	NCT03725059
Phase 3	Metastatic	pembrolizumab 200mg 1x/3 weeks vs placebo +chemotherapy (paclitaxel, nab-paclitaxel, liposomal doxorubicin or capecitabine)	PFS? OS?	?	NCT04895358
Phase 2	Early (neoadjuvant)	Tremelimumab 3mg/kg+exemestane 25mg daily followed by durvalumab 20mg/kg 1x/4weeks+exemestane 25mg daily	CD8+ T cells? pCR?	?	NCT02997995
Phase 2	Metastatic	Atezolizumab 840mg 1x/2 weeks+cobimetinib daily	ORR?	?	NCT03566485
Phase 2b	Metastatic	Chemotherapy (pegylated liposomal doxorubicin 20mg/m² 1x/2 weeks+cyclophosphamide 50mg daily first 2 weeks in each 4 week cycle)+ibilimumab 1mg 1x/6 weeks and nivolumab 240mg 1x/2 weeks vs placebo	PFS?	?	NCT03409198
Phase 2	Metastatic (hypermutated)	Nivolumab 1x/2 weeks+ibilimumab 1x/6 weeks	ORR?	?	NCT03789110
Phase 2	Metastatic	sacituzumab govitecan 10mg/kg 2x/3 weeks +pembrolizumab 1x/week vs placebo	PFS? ORR? OS?	?	NCT04448886
Phase 3	Early	neoadjuvant chemotherapy (paclitaxel followed by anthracycline+cyclophosphamide) and adjuvant hormonotherapy of investigator's choice+neoadjuvant and adjuvant nivolumab vs placebo	pCR?	?	NCT04109066

paclitaxel followed by anthracycline-based chemotherapy. The IMPassion031 trial combining atezolizumab to nab-paclitaxel followed by atezolizumab with anthracycline-based chemotherapy also significantly increased pCR rate. This further suggests the synergy of ICI with anthracyclines or taxanes. The Impassion130 trial showed increased PFS when atezolizumab was added to nab-paclitaxel chemotherapy in metastatic previously untreated PD-L1-positive TNBC. The trial received accelerated FDA approval which was later withdrawn due to lack of benefit. Accumulating data suggests that immune checkpoint inhibitor antitumor activity is mediated by a non-specific FcγR-mediated modulation of the TME, and not only by the blockade of PD-1/PD-L1 axis on TILs. For example, anti-CTLA4 antibodies may activate FcγR-mediated elimination of intratumoral regulatory T cells (48) and anti-PD-L1 antibodies may activate FcγR-mediated reprogramming of tumor-infiltrating myeloid and NK cells (49). This highlights the role of the Fc domain of therapeutic ICI antibodies in their antitumor activity. The albumin found in nab-paclitaxel (albumin-bound paclitaxel, Abraxane) may prevent the non-specific binding of the ICI antibodies (through Fcγ receptors). Indeed, "blocking solutions", such as bovine serum albumin, are commonly used in laboratories to prevent such non-specific antibody bindings. This might explain the lack of antitumor activity of ICI associated with nab-paclitaxel and suggest replacing nab-paclitaxel by other chemotherapies in the next clinical trials. The involvement of nab-paclitaxel in parallel with atezolizumab in a neoadjuvant setting, such as in the neoTRIP trial, also showed disappointing results up to now,

regarding pCR in TNBC patients. In the IMPassion131 trial, adding atezolizumab to paclitaxel did not significantly improve PFS, even in the PD-L1-positive TNBC patients. It should be noted that, according to *post hoc* analyses, most patients were administered corticosteroid premedication throughout paclitaxel therapy, which might alter immunotherapy efficacy. Nevertheless, although the Kaplan-Meier curves remained overlapping for the first 7 months, the trend began to diverge thereafter, favoring the atezolizumab arm and thus, suggesting that the impact of ICI may begin later on, and therefore the need for a longer follow-up.

Regarding HER2-positive BC, HER2-directed monoclonal antibodies, such as trastuzumab, already significantly improved HER2-positive BC prognosis and are probably potent tools for next-generation immunotherapies. Indeed, these antibodies have multiple mechanisms of action, as described above. Therefore, the addition of ICI to trastuzumab/pertuzumab seems to be a promising combination that can at the same time target the tumor cells (through antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis by anti-HER2 therapy) and trigger myeloid and lymphoid compartment activation (through anti-HER2 therapy and immune checkpoint inhibitor). Associated chemotherapies may further promote tumor immunogenicity. For example, in a metastatic setting, the phase 1b/2 PANACEA trial showed encouraging results, although associated usual chemotherapies were unfortunately missing and should be investigated. ADC are expected to deliver a toxic payload specifically to antigen-expressing-tumor cells. However, ADC have also been clinically shown to be effective in low antigen expression, whereas trastuzumab is not effective. This highlights other mechanisms of action of ADC which might be antigen-independent, as described above. While these mechanisms of action are not yet clear today, combination therapies including ADC are even more difficult to suggest. In phase 2 KATE2 trial, investigating TDM-1 combined with atezolizumab, there is a trend in increased PFS in favor of atezolizumab only in the PD-L1-positive tumors. Adding chemotherapy to ADC and ICI might facilitate the inflammatory response. On the other hand, chemotherapy might suppress immune cells from the TME and by this way alter this inflammatory response. Clinical trials combining ADC with chemotherapy and/or ICI are ongoing (NCT04538742)(NCT04556773) and will first have to assess the adverse events of such combinations. In early HER2-positive BC disease, phase 3 IMpassion050 trial has investigated the association of atezolizumab to anthracycline-based chemotherapy followed by paclitaxel, trastuzumab and pertuzumab in a neoadjuvant setting. Although the primary endpoint has not been reached (pCR was not improved), the neoadjuvant period was short. We should certainly wait a bit longer to observe the known long-term impact of immunotherapy by checking EFS – which is a secondary end point of this study. Indeed, following the administration of the treatments, the mature dendritic cells migrate to the draining lymphoid organs, activate antitumor tumor T cells, which proliferate and go back to the tumor site to kill specifically the cancer cells. The common concept of pCR, which is associated with an important prognosis factor after chemotherapy, might therefore be very different in an immunotherapy setting and we are not sure if pCR is a proper primary endpoint to choose. To optimize the trial design, we would suggest adding atezolizumab to taxanes (with or without platins) and HER2 blockade (monotherapy or dual therapy) neoadjuvant sequence, and not to the anthracycline-based neoadjuvant chemotherapy sequence. Indeed, this may allow the combination of chemotherapy, anti-HER2 blockade and ICI in parallel, whose mechanisms of actions might synergize.

In luminal BC, fewer trials involving immunotherapies have been performed so far. While the first trials were disappointing, many results of ongoing trials are awaited. In high-risk early-stage luminal HER2-negative BC, the pCR rate increased from 13% to 30% in the phase 2 adaptively randomized I-SPY2 trial by adding pembrolizumab to neoadjuvant paclitaxel chemotherapy followed by anthracycline-based chemotherapy. This was associated with a high EFS rate of 93% at 3 years. This chemo-immunotherapy combination is similar to those that are effective in the other BC subtypes, and might succeed in the randomized phase 3 Keynote 756. In metastatic luminal BC, results are encouraging as well (50). While a previous trial adding pembrolizumab to eribulin did not improve PFS or OS in luminal metastatic BC patients pretreated with 2 or more lines of hormonal therapy (51), there are many other combination therapies to investigate. For example, combinations of ICI with aromatase inhibitors and CDK 4/6 inhibitors might synergize. Indeed, CDK 4/6 inhibitors induce the presentation of tumor antigens on dendritic cells, stimulate cytotoxic T cells and suppress regulatory T cells activity (52). The impact of hormone therapies on the antitumor immune response is less clear, but aromatase inhibitors might improve CD8 T cell/regulatory T cell ratio (53).

New immune checkpoint targets

Immune checkpoint discovery has increased interest in other recently discovered inhibitory and stimulatory immune checkpoint pathways and gave rise to new clinical trials with many other potential immune checkpoint targets (Figure 3). For example, Lymphocyte Activation Gene-3 (LAG-3) signaling pathway is mainly expressed on lymphocytes where it inhibits T-cell activation, proliferation and cytokine production (54). The combination of eftilagimod α (a monoclonal antibody inhibiting LAG-3) with paclitaxel showed clinical benefit in 90% of metastatic BC patients at 6 months, supporting the future development of this agent in combined first-line regimens (55). Ongoing clinical studies investigate its safety and efficacy alone, associated with other immunotherapies, or through a bispecific antibody targeting ICI and LAG-3 simultaneously (NCT02614833)(NCT03742349)(NCT03849469). T-cell Immunoglobulin and Mucin domain-containing protein 3 (TIM-3) contains multiple co-inhibitory receptors expressed on different types of immune cells (56). TIM-3 antagonistic monoclonal antibodies are currently being clinically investigated in advanced tumors including BC, alone or with a co-blockade with other immunotherapies or chemotherapies (NCT02817633)(NCT04370704)(NCT05287113)(NCT03446040). T-cell Immunoreceptor with Ig and ITIM domains (TIGIT) is restricted to lymphocytes where it exerts its immunosuppressive effect by competing with costimulatory signals such as CD226, like the CD28/CTLA-4 pathway (57). Monoclonal antibodies targeting TIGIT undergo early-stage clinical trials as monotherapy or in combination with current ICI in patients with locally advanced or metastatic malignancies including BC

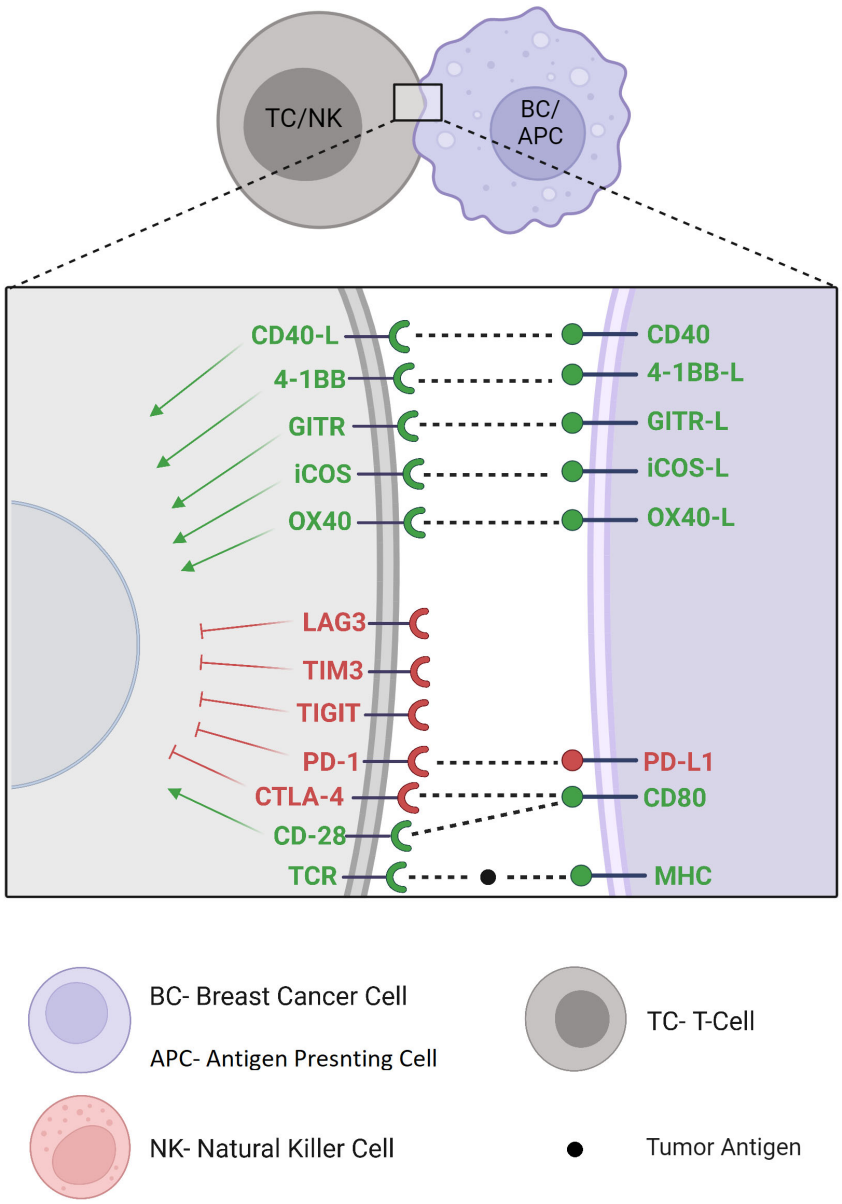

FIGURE 3
New immune checkpoint targets.

(NCT03628677). V-domain Immunoglobulin Suppressor of T-cell Activation (VISTA) is expressed on myeloid cells, regulatory T cells, and on a lesser extent, on BC tumor cells (58). Preclinical trials of VISTA monoclonal antibody-mediated blockade showed remarkable protective antitumor effects (59). CA-170 is an orally available dual small molecule inhibitor of VISTA and PD-L1 being examined in patients with advanced tumors such as BC (NCT02812875). B7 Homolog 3 protein (B7-H3) displays complex immunomodulatory activity in innate and adaptive immunity with costimulatory as well as inhibitory functions and still requires further elucidation. Its receptor has not yet been identified (60). The blockade of B7-H3 is being investigated in early-stage clinical trials in various cancers including BC (NCT03729596)(NCT04145622)(NCT03406949). OX40 and OX40 ligands are co-stimulatory molecules expressed on different types of immune cells whose interaction leads to T-cell proliferation and decreased regulatory T cells. Although this axis seems to play a controversial role in the antitumor response, many oncological clinical trials are focusing on agonistic antibodies with potential antitumor activity (61) (NCT03971409)(NCT02410512). Inducible CO-Stimulator of T cells (ICOS) is a costimulatory molecule induced by T-cell activation, leading to secondary stimulatory signals. Besides its expression on antitumor effector TILs, ICOS is also expressed on regulatory T cells from the TME, on which it confers an immunosuppressive activity. Since ICOS does not induce a cytotoxic immune response independently (62), clinical investigation of monoclonal antibodies for potential synergistic effects in association with other immunotherapies in advanced malignancies, including BC, are currently conducted (NCT02904226, NCT03447314,

NCT03829501). Glucocorticoid-Induced TNFR-Related gene (GITR) activation promotes effector T-cell activity and inhibits tumor-infiltrating regulatory T-cell function. GITR agonism alone does not seem to be sufficient to induce a significant clinical response to therapy. However, there is a rationale for reinvigoration TILs exhaustion through combination with PD-1 blockade (63), which is currently under clinical investigation in advanced malignancies including BC (NCT02628574)(NCT03126110). 4-1BB is a powerful costimulatory signal whose agonistic stimulation on CD8+ T-cells enhances survival, function and memory differentiation in preclinical models (64). Anti-4-1BB agonist monoclonal antibodies are under clinical investigation in combination with ICI in patients with locally advanced or metastatic TNBC (NCT02554812)(NCT03971409) and HER2-positive BC (NCT03414658). A bispecific antibody targeting HER2 and 4-1BB has also shown encouraging data of safety and clinical benefit in phase 1 clinical trial (65) (NCT03330561)(NCT03650348). In addition, 4-1BB upregulation through Fcγ receptor stimulation on NK cells (66) may suggest a synergy between 4-1BB agonism and anti-HER2 blockade/anti-HER2 ADC, which is also currently investigated (NCT03364348).

CD40 is mainly expressed on antigen-presenting cells, where ligation by CD40 ligand results in antigen-presenting cell activation and therefore priming and activation of effector T cells (67). Agonist monoclonal antibodies are under investigation, as monotherapy or in combination, in patients with advanced malignancies including BC (NCT03329950)(NCT05029999).

Adoptive cellular therapy

Despite their antitumor reactivity, TILs, if they are not suppressed within the TME, are often exhausted, contributing to tumor immune escape mechanisms. Autologous TILs can be isolated from tumor specimens, expanded, and activated *in vitro*, and reinfused into the patient, alone or in combination with interleukins. In TCR-engineered lymphocytes and CAR T cell therapy, the cells are isolated from a patient's peripheral blood through leukapheresis, and genetically modified to express either a T cell receptor (TCR) or a chimeric antigen receptor (CAR) (Figure 4). Unlike its success in haematological malignancies with FDA's approval of tisagenlecleucel and axicabtagene ciloleucel, adoptive cell therapy in solid tumors is associated with major obstacles. This includes the identification of appropriate specific tumor antigen targets and the presence of a strong immunosuppressive TME. However, numerous adoptive cellular strategies have recently been developed to fight solid tumors and are under investigation in preclinical studies and clinical trials, including in BC patients. Once expressed in T cells and accompanied by costimulatory domains (such as 4-1BB or CD28), CAR T cells display antigen-specific recognition, activation, proliferation and cytotoxic function, independent of MHC presentation, which is a major advantage compared to TCR therapy (see hereunder) (68). Several targets have been proposed to date, such as mesothelin (69) (NCT02792114)(NCT02414269), c-Met (70), CEA (NCT04348643), EPCAM (NCT02915445), CD70 (NCT02830724), or MUC1 (NCT04020575)(NCT04025216). Clinical trials have also been started with an infusion of CAR T-cells targeting HER2 (NCT04511871)(NCT04430595)(NCT03740256), including intraventricular administration in patients with brain metastases (NCT03696030). CAR-T cells could also be designed to recognize a universal motif such as an Fc portion of immunoglobulins (71), allowing a potential antitumor activity in synergy with antibody treatments such as trastuzumab. Besides, other molecules demonstrated *in vitro* and *in vivo* antitumor activity against BC, such as Natural killer group 2, member D (NKG2D) (72) or Receptor tyrosine kinase-like orphan receptor 1 (ROR1) (73). CAR-T cells targeting these molecules will probably be clinically investigated soon as well.

Although CAR T-cell therapy has attracted major interest, TCR therapy can target both surface and intracellular proteins whose peptides are presented onto MHC molecules (Figure 4). As a result, TCRs could target more tumor antigens and be more tumor-specific (74). In a metastatic HER2-overexpressing BC patient, an adoptive transfer of autologous HER2-specific cytotoxic T cells has been shown to lead to accumulated T cells in the bone marrow along with a loss of bone marrow-residing disseminated tumor cells (75). The adoptive transfer of allogeneic T cells in metastatic BC patients suggested their contribution to a transient early tumor response (76). TCR T-cell therapy is currently being evaluated in patients with metastatic or locally recurrent and unresectable disease including BC through tumor-associated antigen-specific cytotoxic T lymphocyte infusion (NCT03412877)(NCT03093350).

In addition, several clinical studies investigating TIL therapy in BC have been performed. A phase 2 clinical trial demonstrated a case of adoptive transfer of autologous mutant-protein-specific TILs in association with pembrolizumab and interleukin-2 that led to the complete and durable regression of metastatic disease in an HR-positive BC patient who failed to respond to multiple previous lines of therapy (77). Clinical trials are currently investigating the transfer of autologous TILs in patients with pretreated metastatic BC (NCT01174121)(NCT04111510).

Lastly, a growing interest in CAR-NK cell immunotherapy (78) has demonstrated promising preclinical studies in TNBC. In the future, personalized immunopeptidomic profiling of the tumors may allow us to identify new potential therapeutic target antigens (79). Next-generation engineering CAR-T cells may also target several aspects of the TME to treat solid tumors such as the tumor stroma and vasculature as opposed to tumor cells alone, as it has recently been suggested *in vivo* (80). In addition, next-generation CAR-T cells endowed with bispecific CAR dual specificity targeting multiple tumor antigens have been shown to potentiate the antitumor activity in tumor models and to minimize parallel reactivity against normal tissues harboring single antigen, which could present a novel approach focusing CAR-T cells to tumor cells (81).

Bispecific antibodies

With FDA's approval of blinatumomab in the treatment of B-cell malignancies, recent efforts have focused on the extension of multifunctional bispecific antibodies to BC immunotherapy. Most bispecific antibodies for cancer immunotherapy consist of two arms. One arm binds tumor-associated antigens on cancer cells.

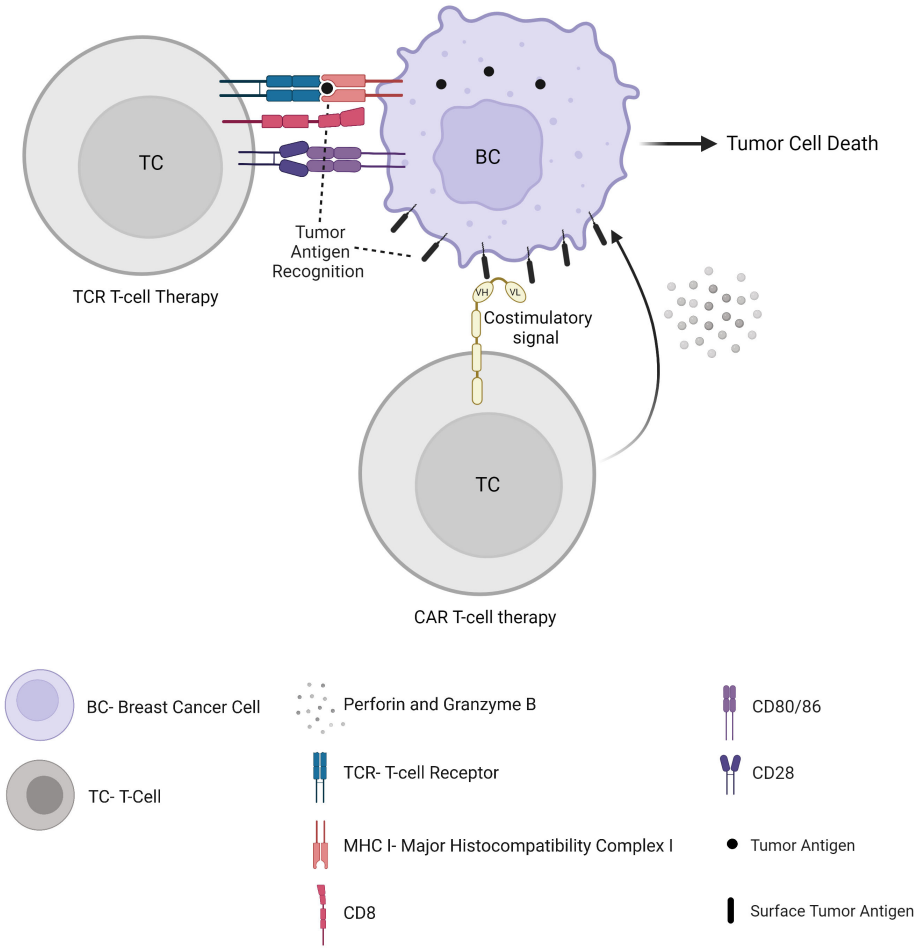

FIGURE 4
Adoptive cellular therapy.

The other arm binds effector immune cells, with selective binding to activate Fcγ receptors, resulting in the formation of a tri-cell complex and specific elimination of tumor cells (Figure 5). This elimination is independent of TCR specificity, co-stimulatory signals, or peptide antigen presentation, representing a major advantage of bispecific antibodies in cancer immunotherapy. Compared to trastuzumab, it has been shown that these trifunctional antibodies mediate the elimination of tumor cells expressing HER2/neu at low levels, which was associated with a Th1-based cytokine release (82) and a potent antitumor activity (83). Ertumaxomab which targets CD3 and HER2 simultaneously has shown encouraging results in phase 1 clinical trials, with an antitumor response seen in 30-59% of metastatic BC patients, especially in HER2-positive BC patients (84). This provides a strong rationale for further studies involving unlimited combinations of bi- or tri-specific antibodies in the therapeutic strategies against BC. Consecutively, new phase 1 and 2 clinical trials evaluate the efficacy of bi- or tri-specific antibodies in locally advanced or metastatic BC patients, through for example HER2/CD3 bispecific target (NCT03448042), NK cell/T-cell/HER2 tri-specific target (NCT04143711), HER2/PD-1 bi-specific target (NCT04162327), or two non-overlapping domains of HER2 target (NCT04224272). A multitude of other bispecific antibodies are designed to simultaneously target molecules on the tumor cells and/or the TME, such as different immunosuppressive pathways (85) or cell-cell adhesion molecules overexpressed on BC tumor cells (86).

Therapeutic peptide and protein-based cancer vaccines

In the age of cancer immunotherapy we see a renewed interest in harnessing cancer-targeting vaccines for therapeutic purposes. Such vaccines can be preventive or therapeutic. Several preventive vaccinations are FDA-approved, such as HPV-related genital/head and neck cancers, and HBV-related hepatocellular carcinoma. Among the FDA-approved therapeutic cancer vaccines today are the Bacillus Calmette-Guérin (BCG) for early-stage bladder cancer and Sipuleucel-T (Provenge) for the treatment of metastatic castrate-resistant prostate cancer. No vaccine has been approved for clinical use in BC to date. However, a rising interest for the development of peptide vaccines has been seen in recent years. For example, the most

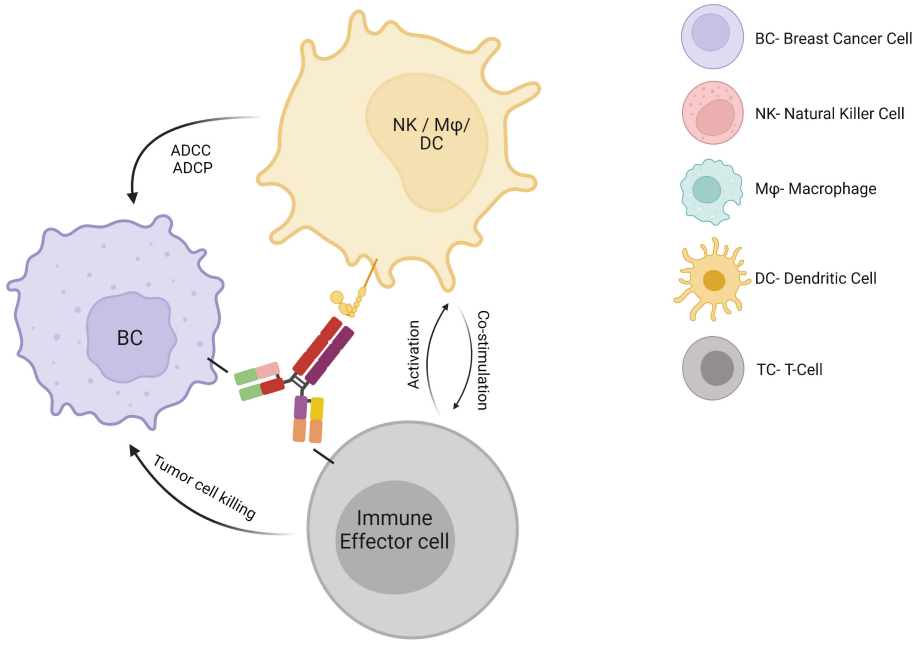

FIGURE 5
Specific elimination of tumor cells by bispecific antibody.

studied cancer vaccine which has been successful to date is the E75 HER2 peptide vaccine, also known as Nelipepimut-S when combined with Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF). E75 is a small peptide derived from the HER2 receptor which is expected to bind HLA molecules and therefore activate cytotoxic effector T cells (Figure 6). This vaccine demonstrated in high-risk HLA-A2 BC patients a recurrence rates of 5.6% in vaccinated patients compared to 14.2% in control unvaccinated participants at 20 months follow-up (87). However, the observed difference could not be repeated in later analyses, possibly due to a lack of immune re-boost. Alternatively, one could speculate that the vaccine could be more efficient with trastuzumab or with an associated myeloid-compartment targeting strategy. Consequently, a phase 1/2 clinical trial assessed the administration of nelipepimut-S and GM-CSF in high risk BC patients in the adjuvant setting, with booster inoculations every 6 months until trial completion at 5 years. This trial demonstrated prevention of disease recurrence at 94.6% in optimally vaccinated patients versus 80.2% in the control group, with minimal toxicities (88). Unfortunately, the phase 3 study was discontinued due to futility (89). However, the addition of trastuzumab to nelipepimut-S and GM-CSF has shown a DFS of 92.6% compared with 71.9% for trastuzumab and GM-CSF group in a phase 2b clinical trial in patients with TNBC (90), with no additional overall or cardiac toxicity compared with trastuzumab alone. Together, this suggests a synergy between trastuzumab and the HER2 peptide vaccine and highlights the importance of selecting the most relevant arms when designing a clinical trial. In addition, it highlights the fact that most BC patients are defined as HER2-negative tumors following fluorescence *in situ* hybridization (FISH) amplification methods, though they display some HER2 expression by immunohistochemistry (IHC) and therefore might respond to anti-HER2 therapies in some combination therapies. In HER2-positive BC patients, a possible synergistic immunologic effect of nelipepimut-S and trastuzumab is currently being investigated (NCT02297698). A meta-analysis of randomized clinical trials suggested significant benefits to vaccination over control, though the high heterogeneity of the patients treated in the involved trials led to unclear final results (91). Moreover, the trials generally show low toxicity of therapeutic vaccination. Vaccination thus remains an attractive strategy that seems to promote both effector immune response and protective memory immunity potentially controlling tumor relapse. Preventive BC vaccines are therefore also under investigation in patients in remission to prevent or delay relapse (NCT02780401)(NCT03384914). Additional HER2-derived peptide cancer vaccines have demonstrated beneficial impacts on BC antitumor immunity and clinical outcome, such as GP2 and AE37 (92), folate receptor α peptide vaccine (93) (NCT03012100), sialyl-Tn (sTn) conjugated to keyhole limpet hemocyanin (KLH) (94), and oxidized mannan-MUC-1 vaccine (95). STn-KLH vaccine (an epitope found among others on MUC1 that activates estrogen receptor-α function), given concurrently with endocrine therapy, offered a robust antibody response to the vaccine and an OS advantage to metastatic BC patients in a retrospective blinded review involving 1028 women (96). This further justifies prospective randomized trials combining anti-MUC1 vaccine with endocrine therapies. Unlike peptide-based cancer vaccines, protein-based cancer vaccines have not been explored to the same extent up till now. However, clinical studies have suggested safety, immunogenicity, long-term survival and a few high grade adverse events of protein-based cancer vaccines in patients with HER2-overexpressing BC refractory to trastuzumab (97). They are under clinical investigation in BC patients undergoing neoadjuvant

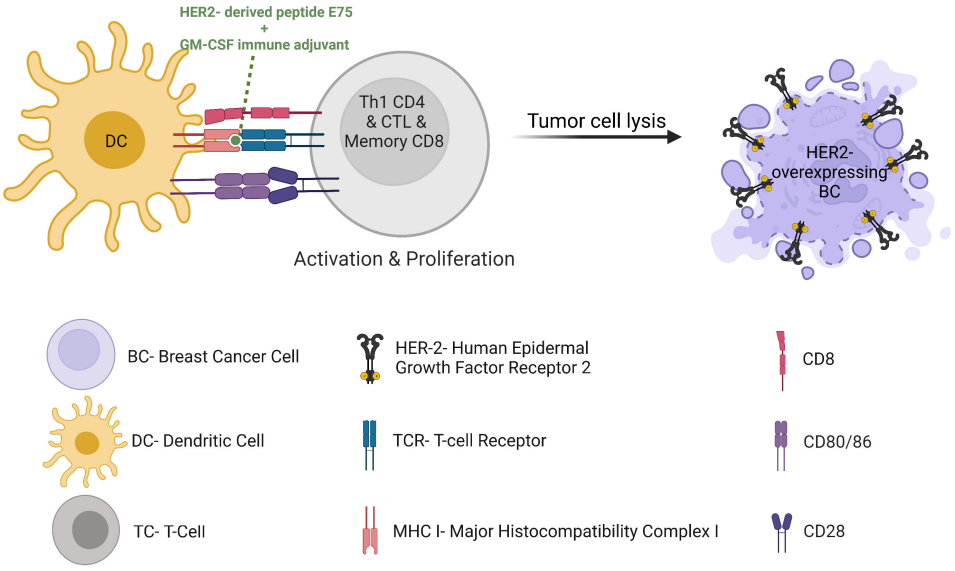

FIGURE 6
HER2-positive breast cancer vaccine.

endocrine therapy (NCT02204098) and in combination with ICI (NCT03632941). Thanks to the next-generation sequencing of tumor mutations and epitope-prediction strategies, new patient-specific neoantigen-based cancer vaccine will probably allow inducing highly specific antitumor immune responses (NCT02316457).

Autologous tumor cells

Personalized vaccines targeting patient-specific mutated neo-antigens, through autologous tumor cells, alone or pulsed on dendritic cells, are new potential strategies (98). Autologous tumor cell-based cancer vaccines, obtained from tumor cell isolation, may overcome the difficulties of selection of an appropriate tumor-associated antigen. These tumor cells harbor a complete and individualized repertoire of tumor-associated antigens, therefore potentially triggering a polyclonal T-cell response against various tumor cells (99) (Figure 7). Several translational and clinical trials undergone in BC patients have suggested that autologous tumor cell-based vaccines may be effective and safe, even among patients with depressed immunity (100). BC cell lines can also be used and have shown the induction of a humoral and T cell-mediated immune response alone or in combination with low-dose chemotherapy or costimulatory molecules, although the clinical results showed limited success. This is possibly because the cell lines do not express the antigen repertoire of the tumor because of inter- and intra-tumoral heterogeneity among cancer patients (101). This obstacle may be overcome by an autologous tumor cell-based vaccine, which may also minimize tumor immune escape through antigen loss observed in clinical trials (102). In this line, autologous BC tumor cells harvested from stage II-III and metastatic BC patients, irradiated and reinfused, led to encouraging results (103). Vaccination using irradiated, genetically modified GM-CSF-secreting tumor cells has shown to induce an enhanced antitumor immunity in a phase 1/2 clinical trial alone or in association with chemotherapy in metastatic BC patients (104). In an additional clinical trial, an irradiated BC cell line, endowed with antigen-presenting cell activity and in association with interferon-α, showed in heavily pretreated advanced BC patients an objective tumor regression in parallel with a decrease in circulating cancer-associated cells, with no serious adverse events (105). Unfortunately, clinical data about autologous tumor vaccination in BC is still limited to date, despite its promising antitumor immune potential.

B cell-based vaccines

Trastuzumab's success led to enthusiasm for B cell-based vaccines, which induce an endogenous active and specific humoral B cell-immune response (antibodies with antitumor activity) from the patient's own B cells. The antibodies secreted in the patients are similar to those of the drug itself, and may offer a promising alternative to antibody administration. Indeed, trastuzumab antibody yield seems to be a major challenge for large-scale production. Trastuzumab is usually produced in Chinese Hamster Ovary Cells in incubators of 80 up to 12 000 liters, which makes a year of treatment per person very expensive. HER-2 B-cell peptide vaccine already demonstrated a robust production of anti-HER2 antibodies in patients with advanced or metastatic HER2-positive cancer of the stomach or the gastroesophageal junction. Besides, there is a correlation between the levels of anti-HER2 antibodies and the clinical response to therapy. In addition, the early data indicated a benefit in OS (hazard ratio of 0.418) with no added toxicity, in association with standard-of-care chemotherapy, compared to chemotherapy alone (106). The combination of two peptide B-cell epitope vaccines, representing trastuzumab and pertuzumab binding sites, showed safety and

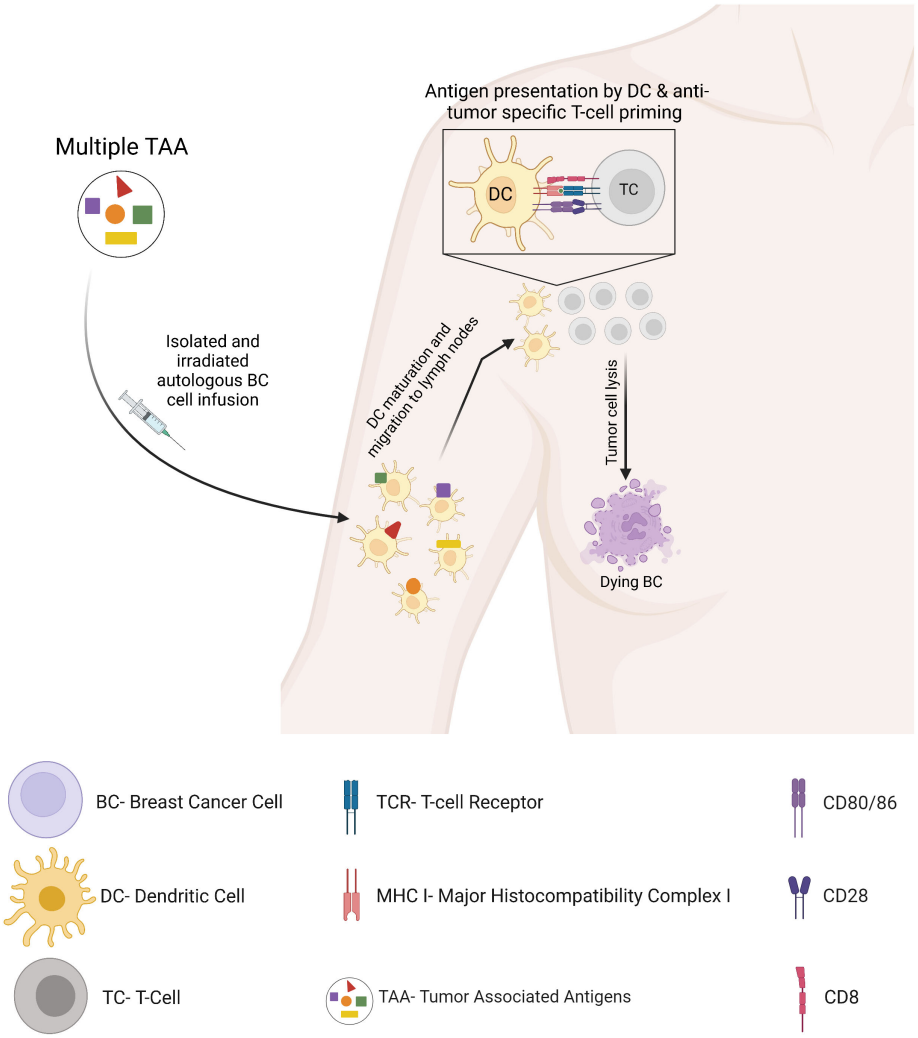

FIGURE 7
Autologous tumor cell vaccine.

antitumor activity performed in patients with advanced solid tumors including BC (107). Other clinical trials are ongoing (NCT01376505). The next generation of clinical studies will have to involve the combination of vaccines with ICI, since preclinical data suggest synergistic mechanisms of action. Indeed, IFNγ secreted by cytotoxic antitumor T cells upregulates PD-1/PD-L1 axis-mediated immune suppression. The addition of ICI to vaccine administration may block the induced immunosuppressive feedback and allow the induction of robust antitumor immunity (108).

Dendritic cell-based vaccines and TLR agonists

Whole-cell dendritic cell (DC) vaccines are tumor-specific DC infusion, mainly isolated from patients' peripheral blood monocytic cells, exogenously matured and expanded using various cytokines, and loaded with tumor lysate or antigens (Figure 8). This approach has shown therapeutic potential with limited toxicities and is extensively investigated in clinical trials. For example, DC loaded with tumor antigens, in association with chemotherapy, have been shown to significantly increase PFS and OS in metastatic BC patients compared to chemotherapy alone over a 10-year follow-up (109). Another clinical study showed a prolonged PFS from 31% to 76.9% with DC vaccine in ER/PR double-negative stage II/III BC patients (110). In addition, the DC vaccine added to neoadjuvant chemotherapy increased the pCR rate to 28.9% compared to 9.09% in the control group with neoadjuvant chemotherapy alone (111). Remarkably, these results were observed in the PD-L1-negative population, and may probably be explained by a poorly immunosuppressed TME where the vaccine can trigger an appropriate antitumor activity. This supports again the hypothesis that the combination of ICI to cancer vaccine might provide a synergistic antitumor activity. Lapuleucel-T, consisting of an adoptive transfer of autologous antigen-presenting cells activated *in vitro* with a recombinant fusion protein comprising HER-2

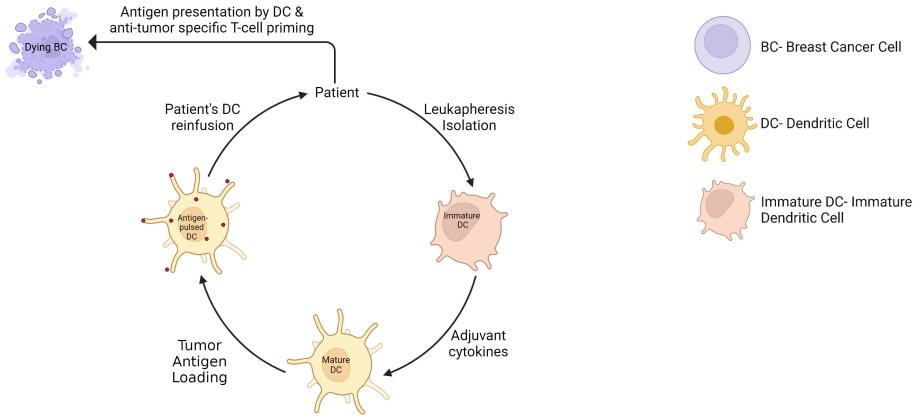

FIGURE 8
Dendritic cell vaccine therapy.

sequences, demonstrated safety and significant antitumor immune response in patients with HER-2-overexpressing metastatic BC, associated with clinical response or disease stabilization in some patients (112). Another example is the vaccination with p53 peptide-pulsed DCs, which showed disease stabilization of advanced BC patients, and a correlation between p53 expression of tumor cells and the induction of a p53-specific T cell response (113). Other clinical trials are currently investigating DC vaccine in BC patients, such as HER2-pulsed DC vaccine (NCT02063724) an multiepitope DC vaccine (NCT00266110), and are also investigated in a neoadjuvant setting (NCT02018458)(NCT02061423) (NCT03387553).

Human DC cells express Toll-Like receptors (TLRs) which play a key role in recognizing signals from the microenvironment and adapting accordingly. The DCs can be targeted and activated through TLR agonists which have shown promise in preclinical and clinical cancer studies, enhancing antitumor T-cell response. Several clinical trials are currently evaluating TLR agonists. For example, topical TLR-7 agonist imiquimod, in association with nab-paclitaxel, has shown an immune-mediated disease regression in treatment-refractory BC chest wall metastases in phase 2 clinical trial (114). Systemic TLR7 agonists are also under development to activate the antitumor immune response in patients with advanced cancer (115). Therapeutic cancer vaccination using TLR3 agonists, such as PolyICLC, are also currently under investigation in patients with advanced cancer including BC (NCT02643303). Systemic DC expansion is also being tested in patients with cancers including metastatic BC, through vaccination with polyICLC in association with Flt3L (NCT03789097).

DNA/mRNA-based cancer vaccines

The favorable clinical experience observed with COVID-19 vaccine may help facilitate research and development in the field of DNA/mRNA-based cancer vaccines. These vaccines are mainly viral replicon particles, or completely synthetic lipid nanoparticles, and contain the genetic information coding for tumor-specific antigens or tumor-associated antigens. Upon administration, the DNA is transcribed into mRNA, which is then translated to synthesize peptides and proteins within the ribosomes. This leads to the presentation of the peptides onto HLA, ultimately activating highly specific cytotoxic and memory T cells against tumor, possibly for a longer period of time compared to peptide or protein-based cancer vaccines. A phase 1 clinical trial investigating a naked plasmid DNA vaccine in metastatic BC patients is currently ongoing (NCT02204098) and preliminary evidence suggested an increased specific CD8+ T-cell proliferation and cytotoxicity along with an improved PFS rate (116). Another phase 1 clinical trial of 66 BC patients with advanced HER2-positive disease investigated the administration of a plasmid DNA coding for HER2 molecule, associated with GM-CSF as an adjuvant, for 3 immunizations. This study demonstrated the induction of an anti-HER2 immunity in most patients, persisting after the end of the vaccinations, and safety with 10-year postvaccine toxicity assessments (117). Importantly, the authors underscore the fact that high anti-HER2 immunity is associated with favorable clinical outcomes after trastuzumab therapy. In line with these results, a randomized phase 2 trial is currently in progress (117). Moreover, in TNBC patients with residual disease after neoadjuvant chemotherapy, another phase 1 clinical trial has indicated that DNA vaccine induced neoantigen-specific immune response in 88.8% of the patients along with a PFD of 87.5% in the vaccinated patients, compared to 49% in historical controls (118). Adverse events were mainly injection site reactions and other limited toxicities. The administration of a mRNA-based vaccine encoding a portion of HER2 (VRP HER2), with or without an additional anti-HER2 targeted therapy, also demonstrated stable diseases and partial response in a phase 1 clinical trial with advanced HER2-positive BC patients (119). In this study, PFS correlated with perforin expression by memory T cells. Other clinical trials investigating DNA/mRNA-based vaccine immunotherapies are ongoing, for example in advanced and metastatic BC patients (NCT02157051), with concurrent ICI (NCT03632941), or in non-metastatic, node-positive BC patients who are in remission (NCT02780401).

γδ T cells

In contrast to conventional αβ T cells, γδ T cells are a distinct little subset of T cells, which typically recognize and kill malignant cells rapidly and independently of HLA restriction. This highlights the antitumor impact that γδ T cells may play in the next generations of cancer immunotherapies, for example through adoptive transfers of allogeneic cells from healthy donors rather than autologous ones. In addition, γδ T cells contribute also indirectly to the antitumor immune response by communicating with other tumor-infiltrating immune cells (120). Vγ9Vδ2 T-cell therapy has demonstrated safety and higher survival of late-stage lung or liver cancer patients (121). In BC, although γδ T cells have been suggested to display a degree of functional plasticity with possible opposing effects on the growth of breast tumors (122), the antitumor potential of these cells makes them an encouraging immunotherapeutic tool to exploit as well. Indeed, γδ T cells were correlated with an improved pCR rate following neoadjuvant therapy, and an improved DFS and OS (11). In addition, the antitumor activity demonstrated by the use of bisphosphonates in some BC clinical trials may be at least partly due to γδ T cell activation (123). Furthermore, BC-infiltrating γδ T cells have been suggested to be involved in the efficacy of trastuzumab, through a mechanism of ADCC (124). γδ T cells have also been associated with remission in TNBC patients (125), although conflicting observations have been reported regarding a potential suppressive function of γδ T cells on αβ T cells (126).

For example, a phase 1 clinical trial was conducted in which the bisphosphonate zoledronate, a Vγ9Vδ2 T-cell agonist, plus low-dose interleukin-2 (IL-2), were administered to terminal advanced metastatic BC patients. The treatment was well tolerated by all patients. In addition, the patients who showed a robust peripheral population of Vγ9Vδ2 T cells had declining CA15.3 levels and displayed partial remission and stable disease, compared to patients who failed to sustain Vγ9Vδ2 T cells (127). Infusions and other agonists of Vγ9Vδ2 T cells are investigated in clinical trials (128) (NCT03183206)(NCT02781805) as monotherapy or in combination with an ICI (NCT04243499). Furthermore, we expect that adoptive transfers of engineered T cells expressing antitumor γδ TCR will also be investigated in BC patients in the upcoming years (129). Although most clinical results have not shown encouraging results thus far, these attractive cells must be better understood and will then certainly show us their hidden secrets, hopefully to the advantage of BC immunotherapy.

Tumor-associated macrophages

Macrophages are among the most abundant immune cells within the TME, where they play a key role in the antitumor immune response through pro-inflammatory macrophage activation, tumor cell phagocytosis and antigen presentation. Clinically approved cancer therapies such as trastuzumab exert at least partially their effects through these mechanisms (130), while pre-clinical studies attempt to optimize these mechanisms (131). Experimental mouse models and clinical studies demonstrated that macrophages are educated by the TME and generally adopt protumoral and immunosuppressive functions, in contrast to their tumoricidal role following *in vitro* activation (132). A meta-analysis of BC patients demonstrated that Tumor-Associated Macrophages (TAMs) were significantly correlated with an aggressive phenotype, metastasis and poor clinical prognosis (133), lack of pCR, resistance to chemotherapy (134) and tamoxifen (135). Furthermore, the protumoral effect of TAMs is often further reinforced after cancer treatments, through macrophage recruitment and polarization, limiting the efficacy of chemotherapy and radiotherapy and promoting early tumor recurrence (136). Clinical trials investigating TAMs-targeting strategies (Figure 9) suggest potential impact in the treatment of cancer.

Blocking SIRPα on the surface of TAMs and DCs, which blocks its interaction with CD47 on the surface of tumor cells and their subsequent "don't eat me" signal, restores the phagocytosis of tumor cells. Clinical trials suggested safety and therapeutic potential (137) in several cancer patients. Furthermore, the concomitant blockade of the CD47/SIRPα axis with tumor-targeting monoclonal antibodies such as trastuzumab may provide a synergistic phagocytic antitumor activity. In this way, the preliminary antitumor activity of this association has been demonstrated in rituximab-refractory non-Hodgkin lymphoma patients and in solid tumors (138, 139). In BC, no clinical trial has assessed such a combination up to now. However, CD47 gene expression has been found to limit the therapeutic activity of trastuzumab in HER2-positive BC patients (15). Other "don't eat me" anti-phagocytic signals include PD-L1, or recently highlighted CD24, which has been recently suggested as a new therapeutic target for BC immunotherapy (140). Other potential myeloid immunotherapeutic strategies may be the blockade of myeloid cell recruitment to the TME, differentiation into TAMs or proliferation within the TME. For example, CCR2-CCL2 axis contributes to metastatic BC progression and early relapse through mechanisms such as myeloid cell recruitment or TAM polarization (141). Another example is the colony-stimulating factor 1 (CSF1)/colony-stimulating factor 1 receptor (CSF-1R) axis, which is a key regulator of myeloid cell differentiation and chemotaxis, and which has been associated with BC progression and mortality (142). However, several treatments targeting these signaling pathways have been investigated in early phase clinical trials and have had disappointing results in the clinic to date, in part due to compensatory feedback mechanisms with no long-term benefit in solid tumors or metastatic cancers. Depletion of TAM could also be a valuable approach to facilitate the antitumor immune response in BC. For example, trabectidin (Yondelis) is effective at killing TAMs in addition to cancer cells (143). Therefore, its antitumor activity should be investigated in some BC subtypes with a high proportion of TAM, such as HR-positive BC (11), where TAM have been associated with worse survival (144). Remarkably, trabectidin has already shown a manageable safety profile and up to 56% of stable disease in several clinical trials with advanced or metastatic BC patients (145). In heavily pretreated metastatic HER2-positive or TNBC patients, partial response occurred in 12% of HER2-positive BC patients (146). In addition, zoledronic acid, which is commonly

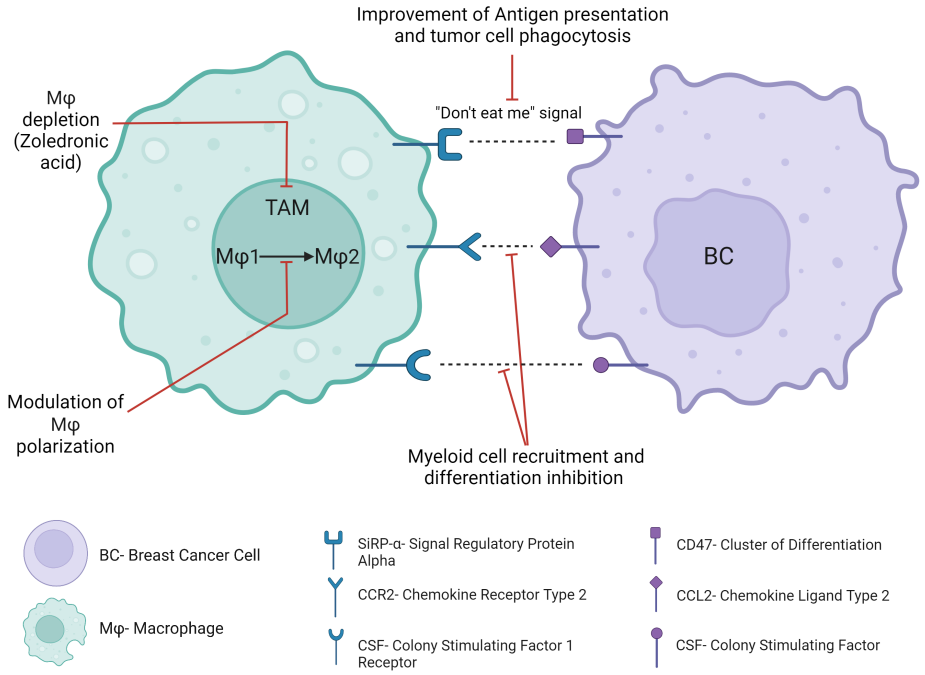

FIGURE 9
Antitumor strategies targeting tumor-associated macrophages.

used in bone metastatic patients, showed extra-squeletal beneficial effects such as the prevention of distant relapse in BC patients after menopause (147), likely through TAMs depletion (148). One of the limitations of such myeloid immunotherapeutic strategies is the targeting of macrophages as a whole population, without taking into consideration the functional properties of subpopulations of macrophages. Indeed, some macrophage subtypes have for example been correlated with less advanced stages, less aggressive tumors (149) and better survival (150) in BC patients, while other subtypes have been suggested to be an independent prognostic marker for longer DFS and OS (151). In this context, another strategy aims at targeting a specific so-called "M2-like" subpopulation of macrophages or switching TAM polarization toward an antitumor phenotype, rather than depleting macrophages indiscriminately (152). Modulators of macrophage phenotype are tested in clinical trials in patients with solid tumors including BC patients, in combination with ICI (NCT02637531).

Radiotherapeutics

Radiotherapeutics is based on a theranostic strategy that combines a whole-body non-invasive mapping of the cancer disease (through a targeted radioactive drug) and the delivery of a targeted therapy to the cancer cells (through a second radioactive drug). Although evidence is still limited, radiotherapeutics is emerging as a superior approach when compared to usual 18F-FDG PET-CT approach in detecting primary and metastatic BC disease. In addition, radiotherapeutics may help select patients who will benefit from therapy, and can become a new efficient targeted therapeutic option in metastatic setting. For example, the radiolabelling of trastuzumab with zirconium-89 or lutetium-177 has been suggested as specific radioimmunotherapy for HER2-positive BC patients (153, 154). Clinical trials assessing Her2 expression detection and anti-HER2 radionuclide therapy are currently ongoing in BC patients (NCT04674722) (NCT04467515). Prostate-specific membrane antigen (PSMA) is specifically expressed in tumor-associated vasculature of solid tumors such as BC, suggesting that it may be targeted as a new anti-angiogenic therapy. Progressive TNBC patients are currently being recruited to assess the concordance between lesions observed on Ga-PSMA PET-CT and 18F-FDG PET-CT and evaluate the feasibility of lutetium-177 PSMA therapy (NCT06059469). Another example is the Fibroblast Activation Protein (FAP)-targeted radionuclide therapy such as lutetium-177-FAPi which targets FAP-expressing Cancer-Associated Fibroblasts (CAFs), stroma cells from the TME endowed mainly with protumoral and immunosuppressive properties. Several studies suggested feasibility, safety, detection of metastases in specific areas (such as in the brain), and reduction of pain in metastatic BC (155, 156). Many other therapeutic isotopes and immunosuppressive targets within the TME might provide new radiotherapeutic and palliative tools for metastatic BC patients.

Oncolytic viruses

Oncolytic viruses have been produced within the last decade. They are engineered to (or they preferentially) target tumor cells. Then, viruses replicate specifically in tumor cells, and stimulate

antitumor immunity, ultimately killing tumor cells. This is mediated by the release of damage-associated molecular patterns and pathogen-associated molecular patterns, along with tumor-specific or tumor-associated antigens presentation. Furthermore, the oncolytic viruses induce immunogenic tumor cell death. Talimogene laherparepvec, a genetically modified herpes simplex oncolytic virus, has been approved by the FDA for the local treatment of metastatic melanoma with unresectable cutaneous lesions. In BC, many preclinical studies suggest antitumor effects of oncolytic virotherapy and its synergistic effects with chemotherapy.

The oncolytic herpes virus HF10 has been injected in BC patients with recurrent BC and suggested higher tumor-infiltrating CD8+ T cells, although the number of patients involved in the study was limited (157). Given the limited efficacy observed in single virotherapy to date, combination drug approaches including virotherapy are being further evaluated in BC patients. For example, a phase 2 randomized study of paclitaxel alone or in combination with oncolytic reovirus demonstrated, in 74 previously treated metastatic BC patients, a significantly longer OS when the combination treatment was administered (158). In another study, SD were observed in metastatic TNBC patients treated by virotherapy associated with low-dose cyclophosphamide (159). Oncolytic virotherapy is currently being investigated in advanced and metastatic BC patients in association with chemotherapy (NCT01656538)(NCT02630368), chemotherapy and ICI (NCT02630368)(NCT02977156) (NCT04215146). The sequence of the therapies over time might also be important in the efficacy of combinatorial therapeutic strategies. For example, preliminary oncolytic virotherapy has been suggested to sensitize BC to following chemo- or immunotherapy, since it induces a preexisting non-exhausted antitumor immunity (160). In this way, clinical trials are now recruiting BC patients for oncolytic virotherapy in association with neoadjuvant chemotherapy (NCT02779855)(NCT03564782), or with radiation therapy followed by pembrolizumab (NCT03004183). In addition, new immunotherapeutic strategies, such as oncolytic virotherapy coding for localized trastuzumab monoclonal antibody production (161), may provide synergistic antitumor effects. Non-replicating virus strategies have also been investigated. One strategy consists of the delivery of a gene that converts a drug into a cytotoxic drug. For example, a phase 1 trial used a local injection of a retrovirus encoding the human cytochrome P450 gene in BC metastatic cutaneous nodules in association with oral administration of the prodrug cyclophosphamide. The trial suggested safety and antitumor efficacy (162).

Cytokine-based immunotherapy

Cytokines are major and pleiotropic regulators of the immune response. In the era of cancer immunotherapy, a renewed interest in the properties of cytokines has led to an increased number of clinical trials assessing their safety and efficacy. In BC, since cytokine-based immunotherapy has shown limited efficacy up to now, cytokines in association with other immunotherapies are currently being investigated to increase their efficacy. For example, it has been hypothesized that interferon-α (IFN-α), which upregulates tumor antigen presentation on tumor cells, might synergize with a cancer vaccine. Therefore, IFN-α has been administered in association with the CEA vaccine in thirty-three CEA-expressing cancer patients including BC. The administration of IFN-α induced a significantly increased OS compared to the vaccine alone (163). Interferon-γ (IFN-γ) is another cytokine which plays a crucial role in tumor cell cytotoxicity, and has also been suggested to be used as an adjuvant for immunotherapy. Hence, clinical trials are for example currently studying the association of IFN-γ with paclitaxel, trastuzumab and pertuzumab in HER-2-positive BC patients (NCT03112590), or with ICI in TNBC (NCT02614456). TGF-β is a cytokine which contributes to the immune suppression of the TME and has been associated with resistance to cancer immunotherapy. Its blockade during radiotherapy in metastatic BC patients has shown a higher median OS (164). Other major pro-inflammatory cytokines, such as IL-12 or IL-15, stimulate among others the production of IFN-γ from CD8+ T cells and induce the differentiation of CD4+ T cells into Th1. IL-12 or IFNγ, in combination with ICI and chemotherapy, may synergize efficiently. Indeed, these cytokines play key roles in the crosstalk between myeloid and lymphoid cells and in cellular cytotoxicity. This is currently under clinical investigation (NCT03567720)(NCT03112590). However, the severe IL-12-mediated toxicity restricted its use in clinical trials. IL-15 is currently investigated in association with ICI in patients with refractory cancers such as BC (NCT03388632). Finally, IL-2 plays a crucial role by stimulating, among others, the proliferation and the cytotoxicity of CD8+ T cells, but also the proliferation of regulatory T cells, which are major suppressors of the antitumor immune response. To overcome these limitations, engineered IL-2 and new inhibitors of regulatory T cell activity are under investigation. ICI have been suggested to inhibit regulatory T cell activity, and are therefore investigated in association with IL-2 in advanced cancers including BC (NCT05086692). The properties of cytokines make them a promising additional tool in cancer immunotherapy and will probably help facilitate the antitumor immune response, once they will be better understood and exploited.

Immunometabolic targets

Accumulating evidence indicate a metabolic competition for consumption of glucose, amino acids and fatty acids between the tumor cells and the tumor-infiltrating immune cells. These molecules are essential for immune cell survival and activity, modulating in this way the antitumor immune response. This led to the concept of metabolic reprogramming. For example, enzymes such as arginase-1 (Arg-1) or indoleamine 2,3-dioxygenase (IDO-1), which catabolize arginine and tryptophan respectively, seem to be mainly involved in immunosuppressive pathways. Therefore, they have been targeted in BC preclinical models, demonstrating synergistic antitumor effects in association with chemotherapy (165, 166) and ICI (167). There are still a few immunometabolic targets investigated in BC clinical trials to date. A randomized clinical trial with HER2-negative metastatic BC patients failed to show improved PFS when IDO-1 inhibitor was added to chemotherapy (168). In

contrast, IDO-1 inhibitor in association with p53-DC vaccine has been shown to increase the IFN ɣ-producing CD8 and the IL-2-producing CD4 T cell response in phase 1/2 study of metastatic BC patients, although it did not increase the objective response rate (169). Arginase inhibition, alone or in combination with ICI, is currently being evaluated in phase 1 study in advanced or metastatic cancer patients (NCT02903914). Accumulating evidence suggests that metabolic changes in adipose tissue are also associated with immunological dysregulations in BC (170). Fasting, or anti-hyperglycemic agents such as metformin, may modulate the antitumor immune response, improve the response to chemo- and immunotherapies, and reduce side effects (171). While fasting has been suggested to reduce the risk of BC recurrence (172), fasting (NCT05023967) and other metabolic health patterns (NCT05432856) are currently being clinically investigated. In addition, in most preclinical studies, hypoxia within the TME, and its associated VEGF induction, also contributes to tumor immune escape mechanisms and tumor progression. Moreover, hypoxia generally increases along with tumor progression, further promoting its deleterious effects (173). Furthermore, hypoxia increases extracellular levels of adenosine in the TME. Adenosine binds to its receptors on immune cells and further contributes to the establishment of an immunosuppressive TME (174). Rapidly proliferating malignant cells generate also high amount of lactate, a by-product of tumoral aerobic glycolysis. Lactate contributes to acidosis, stimulates angiogenesis, acts as cancer cell metabolic fuel, exerts deleterious effects on tumor-infiltrating immune cells and has been suggested to predict response to immunotherapy (175). Unfortunately, hypoxia-inducible factor 1-α (HIF-1α) and lactate metabolism inhibitors have mainly been investigated at a basic research level up to now or have only occasionally been clinically assessed. Adenosine has emerged as a key negative regulator of antitumor immunity through the CD39-CD73-A2AR pathway (176). Several inhibitors of this pathway are currently being evaluated in early phase clinical trials alone or in combination with ICI or chemotherapy in patients with advanced solid tumors including BC patients (NCT02740985)(NCT02503774) (NCT02754141), with trastuzumab (NCT05143970) or in combination with radiotherapy (NCT03875573). Nevertheless, metabolic reprogramming is emerging and require further preclinical and clinical investigations before leading to safe and efficient immunotherapeutic adjuvants for BC immunotherapies.

Discussion

It is well established that the TME plays a crucial role in cancer outcomes and response to therapy. Indeed, it provides a supportive but also active protumoral and immunosuppressive framework for tumor progression and dissemination. On the other hand, evidence demonstrating how to harness the immune system in favor of strong antitumor immunity is overflowing. To improve research and development in BC immunotherapy, clinical trials must be critically designed, considering both molecular and cellular mechanisms at play and its clinical pitfalls.

The next generation of cancer immunotherapy will probably involve combination immunotherapies. Indeed, the current approach of cancer immunotherapy mainly focuses on the T-cell compartment, allowing one to speculate that additional complementary immunotherapeutic strategies targeting different immune compartments could lead to synergistic therapeutic approaches. More specifically, numerous clinical trials assess immunotherapeutic combinations that mechanistically regulate redundant pathways (such as the combination of two different ICI for example). Instead, targeting different complementary pathways could trigger a stronger antitumor immune response. Specifically, the combination of myeloid and lymphoid "immune checkpoints" should be investigated further. Lymphoid immune checkpoints on T cells are widely studied. In contrast, myeloid cells (such as macrophages and dendritic cells) and their potential associated therapeutic targets are clinically less well-known. However, the myeloid cells are still key modulators of the adaptive immune system. Indeed, they can cooperate with TILs to develop a strong and long-lasting antitumor immune response together. Unfortunately, the tumor modulates its TME. Instead of promoting an inflammatory response, many preclinical studies demonstrated that most tumor-associated myeloid cells strongly suppress TILs. Therefore, there has been an explosive growth of clinical trials targeting tumor-infiltrating myeloid cells worldwide, most of them still at an early phase (177, 178). However, targeting only the lymphoid or the myeloid compartment of the TME may not adequately restore the antitumor immune response. We believe that restoring a positive crosstalk between tumor-infiltrating myeloid and lymphoid immune cells may optimize BC immunotherapy.

Distinct macrophages and dendritic cells have been suggested to predict the response to ICI immunotherapy in human BC (179, 180) and other solid tumors (181, 182). An antitumor vaccine has demonstrated long-term tumor control in a HER2-positive BC model but only when combined with anti-PD-1 treatment. This has led to the investigation of this therapeutic combination in a phase 2 clinical trial (NCT03632941). Furthermore, a first-in-human phase 1 trial supports further clinical investigation of evorpacept, a protein that promotes tumor phagocytosis by dendritic cells and macrophages, combined with pembrolizumab, in patients with solid tumors (139). Lastly, eganelisib, a potential first-in-class tumor macrophage-targeting agent (NCT02637531), is already showing PFS benefit in metastatic TNBC patients in addition to atezolizumab and nab-paclitaxel in an ongoing phase 2 MARIO-3 trial (NCT03961698).

Moreover, compensatory mechanisms are very often at play in the TME, further suggesting the need for combination immunotherapies. For example, several studies suggested an increased immunosuppressive microenvironment after CAR T cell therapy (102). CAR T-cells infusion without targeting the TME in parallel, which suppresses TILs, might seem senseless. New complementary strategies aiming at improving T-cell trafficking into/proliferation within the TME, such as myeloid cell depletion, may improve CAR T-cell efficacy. In addition, tumor PD-L1 upregulation occurs in response to IFNγ release by effector immune cells, leading to subsequent immune suppression, a process known as

adaptive immune resistance, where tumor cells protect themselves from immune attack (108). Therefore, the addition of ICI to other types of immunotherapies, such as cancer vaccines for example, may restrain induced immunosuppressive feedback. Moreover, combinations of conventional therapies and immunotherapies should be investigated. Indeed, although radiotherapy and chemotherapy can result in immunogenic cell death (183), they can also limit their own therapeutic effects. For example, conventional cytotoxic drugs and vascular-targeting agents induce tumor cells to produce macrophage recruitment factors (136), while macrophages can also be recruited and polarized during radiotherapy treatment (184). This promotes tumor tissue repair and early tumor recurrence, which might be thwarted by myeloid-targeting immunotherapeutic strategies. Other myeloid and lymphoid-based treatment combinations involving, in addition to an ICI, a CD40 agonist (NCT03424005) or an antitumor vaccine (NCT03632941), may synergize by activating dendritic cells. An additional rational combination might be the concomitant blockade of the CD47/SIRPα axis (or other anti-phagocytic signal blockade) with trastuzumab, which might provide a synergistic phagocytic antitumor activity. In accordance with this hypothesis, CD47 gene expression has been found to limit the therapeutic activity of trastuzumab in HER2-positive BC patients (15). In addition, some treatments such as corticosteroids, mainly used for symptomatic purposes in oncology, should be administered carefully, since their impact on the antitumor immune response is still not well understood (185). The sequence of immunotherapies over time might also impact their efficacy. Indeed, the TME involves a complex dynamic network of immune cells interacting with each other, displaying changes in their activation state over time, and in this way affecting tumor progression and response to therapy. This concept is underestimated in trials evaluating heavily pretreated metastatic BC patients and excluding earlier settings. Furthermore, the dose and schedule of therapies play an important role in their activities. For example, Gonadotropin-Releasing Hormone (GnRH) agonists achieve castration because of continuous pituitary stimulation in contrast to the physiologic pulsatile fashion. Another example is cyclophosphamide, which has cytotoxic and immunosuppressive effects at high dosage, but displays immunostimulatory and antiangiogenic effects at a daily lower dose. New trial designs involving metronomic chemotherapies in association with ICI should be done in search of the optimal antitumor activity (NCT03971045). In a near future, new technologies such as machine learning will probably be a tool of an inestimable value to help us better understand immune cell subpopulation activities and interactions under therapies, and suggest efficient combinations of therapies.

Predictive biomarkers of response to immunotherapy are sorely lacking today and must be developed. Indeed, PD-1 and PD-L1 expression as biomarkers are not reliable enough, probably because the response to therapy is much more complex than that of those sole molecules. While TILs are considered in clinical practice as a promise of good prognosis (186, 187) and response to therapy, they consist of many subpopulations of lymphocytes exerting various and sometimes opposite immune functions (188). For example, cytotoxic CD8+ T cells and CD4+ Th1 T cells are associated with BC survival. On the other hand, potent immunosuppressive TILs such as regulatory T cells are associated with poor BC clinical outcome. As seen previously, different tumor-infiltrating myeloid cells can also exert critical opposite functions around the tumor cells and in metastatic niches. Based on the characterization of various tumor-infiltrating immune cell subpopulations and their correlations with clinical outcomes, a cytotoxic/regulatory immunogenic ratio might be conceived and used as a new predictive biomarker of response to therapy in future biopsies.

To conclude, immunotherapy using ICI has shown unprecedented and long-term efficacy in the treatment of cancer patients with various advanced solid tumors in the last decade, and is currently used in many oncology treatments and clinical trials (189). It has become one of the pillars of cancer treatment, but its success is currently still the visible tip of the iceberg. Indeed, countless efforts have been made to develop cancer immunotherapies and multiple new promising therapies are likely to emerge. Meanwhile, many challenges are still to be overcome, and the next decade will see the fruitfulness of BC immunotherapy investigations, including appropriate synergistic combinations with standards of care.

Author contributions

EA: Writing – original draft, Writing – review & editing. MS: Writing – review & editing. AS: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors thank the Fonds Joseph Wybran (Brussels) for their support. Figures were created using an institutional license for BioRender.com.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 05 March 2024
DOI 10.3389/fonc.2024.1337579

OPEN ACCESS

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RECEIVED 13 November 2023
ACCEPTED 19 February 2024
PUBLISHED 05 March 2024

CITATION
Wang Y, Bu N, Luan X-f, Song Q-q, Ma B-F, Hao W, Yan J-j, Wang L, Zheng X-l and Maimaitiyiming Y (2024) Harnessing the potential of long non-coding RNAs in breast cancer: from etiology to treatment resistance and clinical applications.
Front. Oncol. 14:1337579.
doi: 10.3389/fonc.2024.1337579

Harnessing the potential of long non-coding RNAs in breast cancer: from etiology to treatment resistance and clinical applications

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Breast cancer (BC) is the most common malignancy among women and a leading cause of cancer-related deaths of females worldwide. It is a complex and molecularly heterogeneous disease, with various subtypes that require different treatment strategies. Despite advances in high-resolution single-cell and multinomial technologies, distant metastasis and therapeutic resistance remain major challenges for BC treatment. Long non-coding RNAs (lncRNAs) are non-coding RNAs with more than 200 nucleotides in length. They act as competing endogenous RNAs (ceRNAs) to regulate post-transcriptional gene stability and modulate protein-protein, protein-DNA, and protein-RNA interactions to regulate various biological processes. Emerging evidence suggests that lncRNAs play essential roles in human cancers, including BC. In this review, we focus on the roles and mechanisms of lncRNAs in BC progression, metastasis, and treatment resistance, and discuss their potential value as therapeutic targets. Specifically, we summarize how lncRNAs are involved in the initiation and progression of BC, as well as their roles in metastasis and the development of therapeutic resistance. We also recapitulate the potential of lncRNAs as diagnostic biomarkers and discuss their potential use in personalized medicine. Finally, we provide lncRNA-based strategies to promote the prognosis of breast cancer patients in clinical settings, including the development of novel lncRNA-targeted therapies.

KEYWORDS

breast cancer, metastasis, therapy resistance, long non-coding RNA (LncRNA), competitive endogenous RNA (ceRNA), liquid biopsy

1 Introduction

Breast cancer (BC) may develop due to a variety of factors, including genetic mutations, lifestyle choices, and environmental exposures, with its incidence further influenced by various demographic and socioeconomic elements (1). Despite significant progress in cancer research frontier, BC remains a serious public health issue across the globe. According to latest statistics of GLOBOCAN, an estimated 2.3 million cases of BC were newly diagnosed from 185 countries, accounting for 11.7% of total cancer cases worldwide (1). BC is now recognized as the leading cause of cancer-related mortality in women, with 684,996 deaths reported in 2020. Predominantly attributed to rapid advancements in diagnostic technologies and an increase in the use of mammographic screening, BC has now surpassed lung cancer to become the most commonly diagnosed cancer in women globally. Importantly, it also ranks as the second most common malignancy worldwide, following closely behind lung cancer (1). While less common, BC does occur in men, making up approximately 1% of all BC instances globally (2). Hence, incessant research efforts are crucial to alleviate the significant public health, societal and economic burden posed by BC.

The incidence of BC is governed by a confluence of factors - genetic, epigenetic, and environmental, among others (3). In light of this, BC emerges as a multifaceted disease marked by vast heterogeneity in pathology, genomic alterations, gene expression profiles, and the tumor microenvironment (TME). BC is classified into different biological subtypes, with each subtype displaying unique pathological characteristics and diverse clinical outcomes (4). Indeed, while there have been substantial advancements in diagnostic techniques and treatment strategies over the past decade, the prognosis for BC patients remains unsatisfactory. Survival rates for BC are highly dependent on the stage at which the disease is identified, with early detection correlating to a higher survival advantage (5). Now, it is evident that metastasis accounts for the greatest proportion of BC-related mortality (6). Even though it is detected at an early stage, a notable percentage of women may see their disease evolve into a more aggressive subtype after undergoing initial therapy. This change is likely due to the molecular heterogeneity of BC. Molecular heterogeneity pertains to the genetic variation found within tumor cells, which can result in worse disease progression and resistance to treatment. This is why there is an ongoing focus on creating personalized and targeted therapies.

According to the St. Gallen guidelines, breast cancer (BC) is categorized into four subtypes (7). This categorization is based on the expression status of specific molecular biomarkers including the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 labeling index, which is a marker of cell proliferation. The four subtypes of BC are Luminal A, Luminal B, HER2-enriched, and Basal-like subtypes (Figure 1). The Basal-like subtype is also known as triple-negative breast cancer (TNBC) due to the absence of ER, PR, and HER2 receptors, which makes this subtype particularly challenging to treat. With high metastatic properties and a lower rate of early detection, TNBC poses a significant challenge to treatment (8). Hence, improving survival rates in breast cancer patients, particularly those with the aggressive triple-negative subtype, necessitates the identification of novel molecular biomarkers. These biomarkers are key in assessing the risk of metastasis and treatment response, and additionally, there is a critical need to develop innovative therapies tailored towards tackling this formidable disease.

Rapidly evolving imaging techniques have prominently emerged as the primary tool for early diagnosis of breast cancer, supported by considerable clinical evidence suggesting their effectiveness in reducing breast cancer mortality (9). Further advancements in machine learning-based digital imaging techniques have notably enhanced diagnostic accuracy (10). However, their widespread application is curtailed by high costs and lack of specificity, rendering them unsuitable for early phase breast cancer detection (11). Similarly, conventional tumor diagnostic markers such as carcinoembryonic antigen (CEA), CA 15-3, and CA 125 prove impractical in detecting early phase breast cancer due to their low sensitivity.

Over the past few decades, there has been a continuous pursuit of diagnostic, prognostic, and predictive biomarkers to enhance breast cancer (BC) management. Numerous large molecules, such as DNA (APC, $RARb_2$) (12), proteins (HER2,P53) (13), autoantibodies(MUC1) (14), and ncRNAs (non-coding RNAs), have been utilized as biomarkers for diagnosis. These biomarkers can be detected from serum samples, other body fluids, or tissues of tumor patients. Liquid biopsy, a more accessible, cost-effective, and repeatable sampling process, serves as a valuable source for such biomarkers, avoiding tumor heterogeneity issues (15). Long non-coding RNAs (lncRNAs) are non-protein-coding transcripts exceeding 200 nucleotides in length (16). Researchers have discovered abnormal lncRNA expression in both BC cell lines and tissues (17), revealing their potential role in breast cancer initiation and development. In this review, we synthesize the existing literature on lncRNAs' roles in various aspects of BC and possible underlying mechanisms. This analysis offers novel insights into the diagnosis, prognosis, and prediction of BC, paving the way for improved management and treatment strategies.

Abbreviations: BC, breast cancer; ncRNA, non-coding RNA; lncRNA, long non-coding RNA; ceRNA, competitive endogenous RNA; TME, tumor microenvironment; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; CEA, carcinoembryonic antigen; EVs, extracellular vesicles; ILVs, intraluminal vesicles; MVBs, multivesicular bodies; PRC2, Polycomb Repressive Complex 2; CSCs, cancer stem cells; EMT, epithelial-mesenchymal transition; TICs, tumor-initiating cells; BCSCs, breast cancer stem cells; RT, radiotherapy.

2 Overview of ncRNAs

The advent of sequencing technologies has drastically revolutionized our understanding of the human genome. We now know that approximately 75% of the human genome is transcribed into RNA, yet only a small fraction— about 3%— translates into protein-coding mRNAs (18). The largest and most critical family of

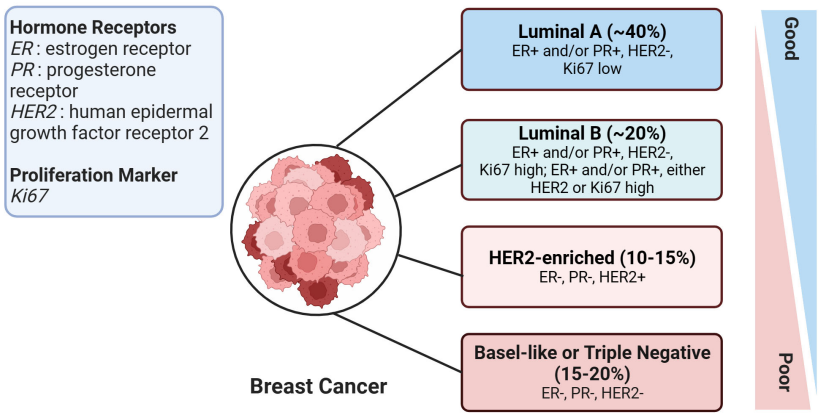

FIGURE 1
An overview of human breast cancer subtypes. This figure illustrates the various subtypes of breast cancer, with the approximate proportion (%) of each subtype among all breast cancer cases provided in brackets. Prognosis severity increases from top to bottom, signifying that the subtypes at the bottom are associated with worse prognoses. The figure was created in BioRender.com.

RNAs, non-coding RNAs (ncRNAs), do not code for any proteins. Despite their lack of protein-coding potential, ncRNAs fulfill vital roles in numerous pathophysiological processes, particularly in cancer. NcRNAs can be categorized into two main groups based on their size: long non-coding RNAs (lncRNAs) and small non-coding RNAs (sncRNAs). The latter is a collection of ncRNAs shorter than 200 nucleotides and includes microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs), and small nuclear RNAs (snRNAs) (19). In contrast, lncRNAs, as the name suggests, are longer than 200 nucleotides. Circular RNAs (circRNAs) constitute the third subgroup of ncRNAs. These distinguished by their covalent, close-loop (circular) single-stranded structures (20). Among these, lncRNAs represent the most intricate and challenging class of ncRNAs. These RNAs offer novel routes for research, potentially leading to new diagnoses, treatments, and understandings of diseases like cancer.

2.1 Classification of lncRNAs

LncRNAs were initially discovered as mRNA-like transcripts that possess both RNA- and protein-like functions. Generally, they lack significant open reading frames (ORFs) and are not translated into proteins, except for a few micropeptide-encoding lncRNAs (21). To date, over 27,000 lncRNAs have been annotated and the number continues to grow, while the functions of a large number of lncRNAs remain unexplored (22). Similar to mRNAs, most lncRNAs are transcribed by RNA polymerase II and are subjected to a series of processes including 5'-capping, splicing, and poly-adenylation at the 3' end (23). LncRNA is the most complex type of ncRNA, with no uniform standard for classification. They can be categorized by length, genomic location, and mechanism of action (24). Based on their genomic localization, they can be classified as intronic, intergenic, sense, antisense, and enhancer lncRNAs. According to their mechanisms of action, they are categorized into four types: guide, scaffold, decoy, and signaling lncRNAs (25). Guide lncRNAs can bind to transcription factors and direct them to specific targets. Scaffold lncRNAs act as a "central platform" that binds different effector molecules simultaneously, like a "scaffold," to integrate various signal pathways. Silencing guide and scaffold lncRNAs can result in altered localization or even loss of function of effector molecules. Decoy lncRNAs interact with target transcriptional regulators and block downstream signals. Signaling lncRNAs regulate downstream gene transcription without protein translation (26).

LncRNAs play a crucial role in the regulation of the genome and have a high level of tissue specificity, suggesting their integral role in maintaining cellular functions (21). They are involved in numerous biological processes, such as transcription, splicing, and translation. Notably, they can participate in chromatin remodeling and epigenetic regulation (16). Additionally, lncRNAs have been found to be dysregulated in various types of cancers, indicating that their abnormal expression or function could contribute to cancer development or progression (17). Thus, they hold promise as biomarkers for early detection, prognosis, and potential therapeutic targets in cancer treatment.

2.2 Subcellular localization and biological functions of lncRNAs

Though originally characterized as "transcriptional noise" without biological functionality, the biological activity and influence of lncRNAs on various pathophysiological processes have garnered increasing attention. The function of lncRNAs largely depends on their subcellular localization (23). These molecules are known to localize in both the nucleus and the cytoplasm (27). The majority of lncRNAs are found in the nucleus, the site of their biogenesis and processing, where they perform their functions. Nuclear lncRNAs are involved in gene regulation at both the epigenetic and transcriptional levels. They

can bind directly to DNA or transcription factors and assist in the regulation of chromatin structure (28). Other lncRNAs require export to the cytoplasm where they target mRNAs, miRNAs, and proteins to regulate gene expression post-transcriptionally and translationally (29). For instance, lncRNAs can operate as "miRNA sponges" or as competitive endogenous RNA (ceRNA). These lncRNAs competitively bind with miRNAs, which allows them to indirectly control the expression of target genes at the post-transcriptional level. Moreover, recent research has begun to explore the subcellular localization of lncRNAs, shedding light on their presence in specific organelles such as mitochondria and the endoplasmic reticulum (ER) (30).

LncRNAs play crucial roles in various biological processes, including embryonic development, organogenesis, immune function, stem cell differentiation, and pluripotency, by regulating gene expression and protein translation (31). Notably, numerous lncRNAs display dysregulation in a variety of malignancies, with the up- or down-regulation of these lncRNAs either promoting or inhibiting tumor progression. Broadly speaking, lncRNAs can be divided into two categories: oncogenic and tumor-suppressive (32). Oncogenic lncRNAs, which typically exhibit overexpression in tumor tissues compared to healthy samples, play a role in promoting tumorigenesis. As a result, inhibiting these lncRNAs presents a promising potential anticancer strategy. Conversely, lncRNAs with tumor-suppressive properties are generally downregulated in cancerous tissues. Increasing the levels of these lncRNAs could serve as an approach for combating the disease. Moreover, lncRNAs have been found to contribute to tumor metastasis and therapeutic resistance (33). This makes them invaluable subjects of study in cancer research, as many could be developed into novel biomarkers for the diagnosis, progression, invasion, metastasis, and prognosis of cancer, or even as targets for therapeutic intervention.

2.3 Exosomal lncRNAs

Exosomes are tiny, nano-sized extracellular vesicles (EVs) that can be found in various human body fluids like blood, urine, and saliva (34). These exosomes can be secreted by all types of cells, and their formation begins when the plasma membrane invaginates to create intraluminal vesicles (ILVs), which then mature into multivesicular bodies (MVBs). These MVBs either fuse with the plasma membrane to release exosomes into the extracellular space or they get degraded in lysosomes (35). The contents of these exosomes can vary greatly, depending on the specific tissues and organs. They may contain nucleic acids (like mRNAs and ncRNAs), proteins, or even synthetic drugs, demonstrating that exosomes play a critical part in mediating communication between cells. The application of this knowledge extends further into cancer research, where it has been found that exosomes secreted by tumor cells contain tumor-specific long non-coding RNAs (lncRNAs), which reveal the original cellular pathophysiological state (36). These exosomal lncRNAs play a crucial role as regulators in the development of cancer. They are involved in various processes including the growth, proliferation, metastasis of cancer cells, promotion of angiogenesis, drug resistance, and immunomodulation, among other functions.

Indeed, changes in exosomal lncRNAs have been observed in various tumor types, suggesting that they can potentially serve as biomarkers for cancer diagnosis and prognosis (37). One of the advantages of exosomal lncRNAs is their high stability, which can be attributed to the protection provided by their lipid bilayers. This makes them more suitable candidates for developing biomarkers compared to other types of molecules. Moreover, unlike traditional *in-situ* biopsies, exosomes can be isolated from easily accessible body fluids like blood and urine, without the need for more invasive procedures. This makes the process of monitoring exosomes in body fluids much more convenient, feasible, and less invasive for patients (38). As a result, the utilization of exosomal lncRNAs as biomarkers holds great promise in advancing cancer detection and management.

2.4 LncRNAs-miRNAs-mRNAs network

Micro RNAs (miRNAs) are small non-coding RNAs with lengths ranging from 17 to 25 nucleotides (39). Generally, miRNAs bind to the target mRNAs at their 3'untranslated regions (3'UTRs), subsequently inhibiting translation (40). A single miRNA can target multiple mRNA molecules, and a specific mRNA can be targeted by multiple miRNAs simultaneously. Like lncRNAs, aberrantly expressed miRNAs have been found in various cancer types, including BC (41). The mechanism of lncRNAs-mediated bioprocess regulation often involves miRNAs. In fact, lncRNAs interact with miRNAs through multiple mechanisms to regulate gene expression. Together, they form the lncRNAs-miRNAs-mRNAs axis. In other words, lncRNAs can act as "miRNA sponges" by competitively binding with miRNAs, indirectly regulating the expression of mRNAs (42, 43). These competing endogenous RNAs (ceRNAs) networks are extensively present in all aspects of breast cancer and are not listed separately in this article (Figure 2).

3 LncRNAs in breast cancer

Advancements in targeted therapy, immunotherapy, and innovative combination treatments have significantly enhanced the survival rate of patients with BC (44, 45). Nonetheless, BC still remains the leading cause of cancer-related mortality among women due to its high rates of recurrence, metastasis, and therapeutic resistance. One important reason for this is the complex and molecularly heterogeneous nature of BC. Until now, accumulating studies have demonstrated that lncRNAs play important roles in the progression, metastasis, and even treatment response of BC. Dysregulation of lncRNAs can disrupt normal transcription, leading to abnormal gene expression and ultimately tumor progression through various mechanisms (46). In both cancer and other diseases, a multitude of physiological and pathological processes are intricately linked to lncRNAs. Current studies have shown that the expression of lncRNAs in BC cells and

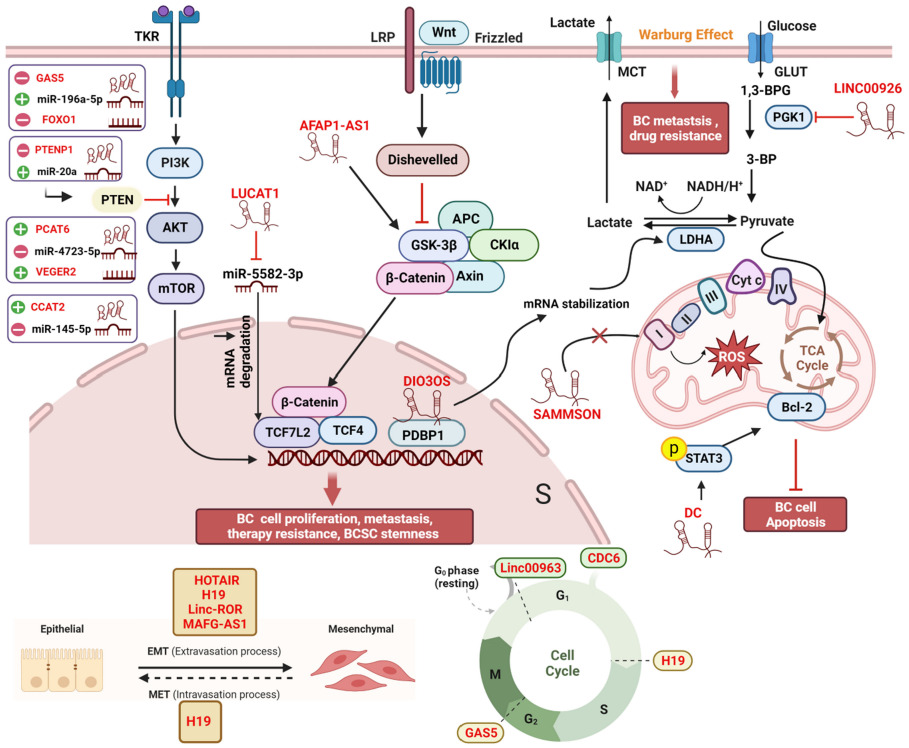

FIGURE 2
Comprehensive regulatory network of lncRNAs in breast cancer cells. Within the cellular environment, a complex regulatory network involving lncRNAs, their target genes, miRNAs, and interacting proteins is established. This network encompasses diverse signaling pathways, including Wnt/β-catenin, PI3K/AKT, and MAPK/ERK, coordinating various biological processes such as EMT, autophagy, the Warburg effect, oxidative phosphorylation, cell cycle arrest, angiogenesis, and treatment response in breast cancer patients. The lncRNA-miRNA-mRNA axis and ceRNA networks play pivotal roles in modulating gene expression during cancer development and progression, offering potential targets for therapeutic interventions and biomarker discovery. All lncRNAs are colored in red. "-" represents oncogene; "+" represents tumor suppressor; "⊥" represents inhibition; "↑" represents promotion. The figure was created in BioRender.com.

tissues differs significantly from that in normal cells and tissues (47). A large number of lncRNAs have been shown to be involved in tumor progression, metastasis, as well as treatment resistance (Figure 2). Based on their functions and expression patterns in BC, lncRNAs can be classified as tumor suppressor genes or oncogenes, which will be discussed in detail below.

3.1 LncRNAs and BC progression

A large number of studies have provided evidence that lncRNAs are involved in BC progression, making them potential targets for biomarker design and discovery of novel anticancer drugs. In the following sections, we summarize several particular lncRNAs which play important roles in BC.

3.1.1 Hotair

HOTAIR (HOX Transcript Antisense Intergenic RNA) is a long non-coding RNA (lncRNA) composed of 2158 nucleotides. It has the distinction of being the first lncRNA identified in correlation with a poor prognosis in breast cancer (BC). HOTAIR is considered an oncogenic lncRNA, and its overexpression has been reported in nearly all solid tumors (48). Notably, it is observed in both the cytoplasm and the nucleus. In terms of its mode of operation, HOTAIR acts as a "scaffold" that binds to and recruits the Polycomb Repressive Complex 2 (PRC2) to its target genes. Here, the histone methylase activity of PRC2 represses gene transcription (49). HOTAIR's influence on BC is wide-ranging, contributing to the progression of BC, metastasis, and therapeutic resistance. Its overexpression has been identified in BC tissues and cells (50). In one study, Shi et al. found that HOTAIR enhances the proliferation, invasion, and migration of triple-negative BC cells through the HOTAIR/miR-203/CAV1 axis (51). Another study indicated that a complex of HBXIP, HOTAIR, and LSD1—where HOTAIR serves as a scaffold—can activate the pro-oncogenic transcription factor c-Myc, amplifying the growth of BC cells both *in vitro* and *in vivo* (52). Additionally, HOTAIR has been found to regulate the proliferation, migration, apoptosis, and invasion of MCF-7 cells by modulating the p53/Akt/JNK signaling pathway (53).

3.1.2 H19

H19, the first identified long non-coding RNA (lncRNA) with riboregulatory function, is one of the most extensively studied lncRNAs in cancer (54). It is overexpressed in numerous solid tumors, including breast cancer (BC). H19 has been reported to bind with E2F1, a critical factor in the G1/S transition of the cell cycle. This association is linked to increased proliferation of BC cells as well as the progression to a more aggressive phenotype (55). As a

miRNA sponge, H19 encourages BC cell proliferation, along with promoting invasiveness and migration. Conversely, the silencing of H19 can induce cell cycle arrest and apoptosis by regulating miR-138 and SOX4 (56).

3.1.3 Other oncogenic lncRNAs

HOTAIR and H19 are classic and widely studied oncogenic lncRNAs in BC. In addition to these, numerous other lncRNAs have been identified to play oncogenic roles in BC progression. For instance, lncRNA CDC6 (cell division cycle 6) has been positively correlated with BC stages. Overexpression of CDC6 deregulates the G1 phase of the cell cycle, promoting BC cell migration. Concurrently, CDC6 acts as a molecular sponge for miR-215, further enhancing BC cell proliferation (57). NEAT1 (nuclear paraspeckle assembly transcript 1) is a structural component of nuclear paraspeckles (58). Although predominantly found in the nucleus, NEAT1 can be translocated to the cytoplasm, a process mediated by Pinin. In the cytoplasm, NEAT1 serves as a "scaffold" for the PGK1/PGAM1/ENO1 complex, promoting the penultimate step of glycolysis via substrate channeling (59). Accelerated glycolysis, also known as the Warburg effect, is a key metabolic change in cancer. Consequently, NEAT1 promotes tumor initiation, growth, and metastasis. GATA3 (GATA Binding Protein 3) is a transcription factor that regulates cell differentiation and acts as a tumor suppressor in BC progression. GATA3-AS1, the antisense RNA1 of GATA3, has been found to promote TNBC cell proliferation and migration by facilitating GATA3 degradation through ubiquitination (60). Additional lncRNAs related to BC initiation and progression are listed in Table 1.

TABLE 1 List of lncRNAs involved in BC progression.

LncRNA	Cellular localization	Subject	Mechanism of action	Biological functions
Oncogenic: up-regulated in BC cells and tissues				
ROR (61)	Nucleus	MCF-7	Acts as "decoy" of MLL1 to promote H3K4 methylation and increase TIMP3	Promotes BC cell proliferation and invasion, inhibits apoptosis
MAFG-AS1 (62)	Cytoplasm	MCF-7	MAFG-AS1/miR-150-5p/MYB axis	Enhances the BC cell viability and inhibits apoptosis
UCA1 (63)	Cytoplasm	MCF-7, MDA-MB-231	Up regulates PTP1B by sequestering miR-206	Promotes cell proliferation and colony formation *in vitro*
TDRKH-AS1 (64)	Not described	MCF-7, MDA-MB-231	miR-134-5p/CREB1 axis	Promotes BC cell proliferation and invasion
lncSNHG3 (65)	cytoplasm	T47D, MDA-MB-231, MDA-MB-468 etc	increasing CSNK2A1 expression level	Promotes malignant progression of breast cancer
AL133467.1 (66)	Not described	MCF-7, MDA-MB-231	Not described	Impedes BC cells' proliferation and migration
Antitumor lncRNAs: down-regulated in BC cells and tissues.				
RP11-551L14.4 (67)	Not described	T47D, BT474	miR-4472	Inhibits cell proliferation, colony formation and attenuates cell cycle
MEG3 (68)	Cytoplasm	MDA-MB-231, MCF-7	MEG3/ miR-141-3p /RBMS3 axis	Inhibits xenograft growth, promotes apoptosis
EGOT (69)	Not described	BT549	Hedgehog pathway	Inhibits cell viability and migration
MEG3 (70)	nucleus and cytoplasm	MCF-7 and BT-474	miR-330/CNN1 axis	decreases cells in S stage and promotes apoptosis
HCG11 (71)	Not described	MCF7 and BT474	SRSF1/β-catenin axis	suppresses cell proliferation
LacRNA (72)	Cytoplasm	MDA-MB-231, BT549, T47D	stabilizes PHB2 and represses MYC targets	suppresses breast cancer metastasis
Dual-effect lncRNAs				
DANCR (ANCR) (73)	Cytoplasm	MDA-MB-231 MDA-MB-468	Recruits EZH2 to inhibit the transcription of SOCS3	Promotes cell viability and migration *in vitro*, as well as xenograft growth *in vivo*
DANCR (ANCR) (74)	Cytoplasm	MDA-MB-231, MCF-7	Promotes EZH2 degradation	Inhibits cell migration and invasion

3.1.4 Antitumor lncRNAs

There are several lncRNAs with diverse roles in the progression of BC. Among them, GAS5 (Growth Arrest Specific 5), a tumor suppressor gene, was first isolated from mouse NIH3 cells. It has been observed that the presence of GAS5 is diminished in TNBC tissues, linked to an aggressive disease phenotype. Conversely, the overexpression of GAS5 within TNBC cells considerably promotes apoptosis (programmed cell death) in cancer cells while also inhibiting cell division (75). From a mechanistic standpoint, GAS5 functions as a ceRNA, countering miRNA-196a-5p, thus negating the protumor effects of the miRNA-196a-5p/FOXO1/PI3K/AKT pathway. GAS5's anticancer effects involve multiple interactions with various miRNAs and proteins to encourage the apoptosis of BC cells via several pathway (76). PTCSC3 (Papillary Thyroid Carcinoma Susceptibility Candidate 3), found to be a tumor suppressor in numerous cancers, may serve as an upstream inhibitor of H19, regulating cell proliferation in TNBC cells. While it's common for lncRNAs to modulate miRNAs, mRNAs, and chromatin, the regulation of one lncRNA by another is a seldom-witnessed phenomenon (77). PDCD4 (Programmed Cell Death 4) is a well-documented tumor suppressor gene. Its NATs (Natural Antisense Transcripts), known as PDAD4-AS1, can enhance the expression of PDCD4 through stabilizing PDCD4 RNA. Both PDCD4 and PDAD-AS1 negatively regulate BC cell proliferation by inhibiting cell cycle progression (78).

3.1.5 Dual-effects lncRNAs

The above-mentioned lncRNAs have been shown to have either a promoting or inhibitory effect on the progression of BC. However, in certain circumstances, specific lncRNAs exhibit dual effects on BC progression. A single lncRNA can have opposite effects on the same subtype of BC or conflicting effects on different subtypes. For instance, MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) has been identified as an oncogenic lncRNA that promotes the progression and metastasis of BC (79). In contrast, Kim et al. discovered that deficiency of MALAT1 induces BC metastasis, which can be reversed by the exogenous supplementation of MALAT1. This was observed in genetically engineered mouse models and xenograft models (80). Interestingly, another study found no differences in serum MALAT1 levels between BC patients and healthy controls (81). PTENP1 (phosphatase and tensin homolog pseudogene 1) is a pseudogene of the tumor suppressor PTEN, with a highly homologous region upstream of PTEN's 3'-UTR. PTENP1 represses cell proliferation and promotes apoptosis. Gao et al. discovered that both PTENP1 and PTEN are downregulated in ER-positive cell lines MCF-7 and T47D. Overexpression of PTENP1 suppresses BC progression, while knockdown of PTENP1 enhances malignant behavior in these BC cells (82). Mechanistically, PTENP1 acts as an antitumor lncRNA by sponging miR-20a and regulating BC progression through the PTEN/PI3K/AKT pathway. Similar results were obtained in a previous study (83). However, another article mentions that upregulation of PTENP1 decreased PTEN gene expression in ER-positive MCF-7 and T47D cells and accelerated MCF-7 tumor growth *in vivo*. On the other hand, PTENP1 upregulation increased PTEN transcript levels and inhibited the growth rate of ER-negative MDA-MB-231 cells, suggesting that PTENP1 influences BC growth depending on the ER status (84).

XIST (X inactive-specific transcript), a key initiator of X chromosome inactivation in female mammals, has been recognized to play important roles in tumor progression regulation. Several studies have shown that XIST is downregulated and acts as an anti-cancer factor in BC. Knockdown of XIST promotes proliferation of MCF-7 cells and ovarian cancer cells. Mechanistically, XIST competes with miR-101 to upregulate C/EBP and KLF6 expression, which inhibits macrophage polarization toward the M2 phenotype, thereby suppressing BC cell proliferation and migration (85). Conversely, Zhao et al. found that XIST expression is upregulated in BC tissues and cell lines, and XIST knockdown significantly represses cell proliferation, migration, invasion, and anti-apoptotic activities in BC cells (86). Mechanistically, XIST acts as a sponge for miR-125b-5p, thereby upregulating the expression of the BC promoter NLRC5.

3.2 LncRNAs and BC metastasis

Metastasis, the most devastating stage of cancer progression, is responsible for the majority of cancer-related deaths. Many cancers, including BC, tend to metastasize preferentially to specific organs, a phenomenon known as organotropism (87). BC tends to metastasize to the brain, bones, lungs, and liver (87). Despite its significance, the process of metastasis is not fully understood, which has hindered the development of early predictive methods and effective treatment options for metastatic BC patients. To gain the ability to survive and metastasize, cancer cells often undergo a series of changes, including genetic and epigenetic alterations, as well as metabolic reprogramming (88). Research has indicated that cancer stem cells (CSCs), epithelial-mesenchymal transition (EMT), and autophagy are the three main mechanisms driving tumor metastasis (89). Within BC, there exists a small subpopulation of cells known as tumor-initiating cells (TICs) or breast cancer stem cells (BCSCs), which have the ability to generate daughter BCSCs (90). These daughter BCSCs possess the capability for unlimited proliferation through self-renewal and differentiation into BC cells. Only BCSCs have the potential to form recurrent or metastatic tumors (91). EMT is a dynamic process in which epithelial cells lose their polarity and intercellular cohesion, transforming into migratory mesenchymal cells. Although EMT is reversible, it provides cancer cells with increased motility and migration capabilities by breaking down intercellular bonds in the epithelial cells (92). EMT plays a crucial role in cancer progression and metastasis. Furthermore, EMT is closely intertwined with CSCs; for example, BCSCs can derive from human mammary epithelial cells through induction of EMT (93). Autophagy is a self-degradative process that can have a dual role in tumorigenesis. In the early stages of tumorigenesis, it exhibits anticancer effects, while in later stages, it contributes to tumor cell proliferation and survival, playing a fundamental role in tumor maintenance (42).

Interestingly, numerous lncRNAs have been identified to be involved in the aforementioned three mechanisms. For instance, HOTAIR is known to play a key role in the invasion, proliferation, colony formation, and self-renewal capacity of BCSCs by regulating SOX2 and NF-kB (94, 95). MiR-7, a metastasis-suppressing miRNA, inhibits both SETDB1 and the cellular EMT process in BCSCs. In MDA-MB231 cells and BC patients, HOTAIR functions by inhibiting miR-7 (96). Furthermore, HOTAIR can regulate autophagy, which is critical for BC cell survival, through its interactions with matrix metalloproteins (97). The functions and mechanisms of lncRNAs involved in these three mechanisms of tumor metastasis are summarized in Table 2.

TABLE 2 Role and mechanism of lncRNAs involved in three metastasis axes of BC.

LncRNA	Role and mechanism in		
	BCSCs	EMT	Autophagy
H19	Sponges let-7 and increases the expression of LIN28 to promote BCSCs maintenance (98)	Sponges miR-200b/c and let-7b differently, modulates the reversible shifts between epithelial and mesenchymal states (99)	Promotes autophagy and inhibits EMT via H19/Let-7/LIN28 pathway (100)
ROPM	Comprise ROPM/PLA2G16/lipid metabolism axis to maintain BCSCs properties (101)	Not investigated	Not investigated
ROR	Increases stemness of BCSCs, actives Wnt/β-catenin pathway (102)	Prevents the degradation of miR-205, induces EMT (103)	Inhibits Gem-induced autophagy by decreasing miR-34a (104)
MAFG-AS1	Not investigated	Regulates the EMT of BC *in vivo* by targeting the miR-150-5p/MYB axis (62)	Inhibits autophagy by sponging miR-3612 to elevate FKBP4 (105).
LUCAT1	Increases stemness of BCSCs via competitively binding miR-5582-3p with TCF7L2 and activating the Wnt/β-catenin pathway (106)	Not investigated	Not investigated
LINC00511	Targets miR-185-3p/E2F1 as ceRNA to enhance Nanog expression and facilitate BC cells stemness (107)	Not investigated	Not investigated

In addition to the three mechanisms mentioned above, there are other ways in which lncRNAs contribute to metastasis. For example, a novel lncRNA called LINC02273 forms a complex with hnRNPL and activates the oncogene AGR2, thus promoting BC metastasis (108). Cancer cells often undergo metabolic changes, such as increased glucose uptake and glycolysis, to satisfy the energy requirements for their malignant behavior (109). LINC00926 retards BC metastasis by inhibiting the PGK1-mediated Warburg effect, thereby reducing glucose uptake and lactate production (110). Hypoxia is also a hallmark of the tumor microenvironment (TME). In the hypoxic TME, the activation of the HIF (hypoxia-inducible factor) pathway promotes tumor progression and metastasis (111). For instance, HIF-2-induced lncRNA RAB11B-AS1 enhances angiogenic factors VEGFA and ANGPTL4 in hypoxic BC cells, leading to angiogenesis and metastasis (112). Additionally, lncRNA PCAT6 facilitates TNBC metastasis by sponging miR-4723-5p and binding to USP14, resulting in enhanced stability of VEGFR2 protein and activation of the Akt/mTOR pathway (113).

3.3 LncRNAs and therapeutic resistance of BC

In recent years, significant progress has been made in the development of various therapies for the management of BC. These therapies include (i) surgery, (ii) chemotherapy (especially for TNBC patients), (iii) trastuzumab (a HER2-specific monoclonal antibody for HER2-positive patients), (iv) endocrine therapy (e.g., tamoxifen) for ER-positive patients, (v) radiation therapy, and (vi) immunotherapy (114). Unfortunately, the emergence of treatment resistance often leads to metastasis and recurrence of BC, rendering it an incredibly challenging disease to treat. Extensive research has been conducted to understand the mechanisms underlying treatment resistance. The prevailing view is that BC is a stem cell disease, with BC stem cells (BCSCs) being the critical cells responsible for chemo-resistance and radio-resistance during BC therapy (115). Furthermore, the emerging field of lncRNA research has shown that lncRNAs play an essential role in treatment resistance through various molecular pathways, including increasing drug efflux, suppressing apoptosis, promoting BCSCs stemness, and acting as ceRNAs. As a result, lncRNAs have the potential to serve as biomarkers and promising targets to overcome drug/radiation resistance in BC patients.

3.3.1 LncRNAs and chemotherapy resistance

Among the different treatment options for BC, chemotherapy is the most widely used in clinical settings as it can improve patients' survival rates. Chemotherapy drugs commonly used for BC treatment include taxanes (paclitaxel and docetaxel), anthracyclines (doxorubicin and epirubicin), platinum drugs, and 5-Fluorouracil (5-FU). However, chemotherapy resistance remains a significant challenge in breast cancer treatment. Cancer cells become resistant to chemotherapy drugs, causing cancer growth and spread. Several factors contribute to chemotherapy resistance in breast cancer, such as tumor heterogeneity, genetic mutations, the tumor microenvironment, upregulated drug efflux pumps, metabolic reprogramming, and epigenetic changes, among others. For more details on the lncRNAs involved in chemotherapy resistance, please refer to Table 3.

TABLE 3 Roles and Mechanisms of lncRNAs in BC chemotherapy.

Drug	LncRNA	Role	Mechanism
Paclitaxel	FTH1P3 (116)	Inducing	FTH1P3 acts as a sponge of miR-206 and increases expression of ABCB1 to trigger paclitaxel resistance
	LINC00160 (117)	Inducing	LINC00160 up-regulates TFF3 by recruited C/EBPα to promote paclitaxel resistance of MCF-7 cells
	MAPT-AS1 (118)	Inducing	MAPT-AS1 upregulates MAPT and its protein TAU, which competes against paclitaxel at the microtubules
	EGOT (119)	Blocking	EGOT enhances autophagosome accumulation by increasing ITPR1 expression, thereby sensitizes BC cells to paclitaxel
Docetaxel	EPB41L4A-AS2 (120)	Blocking	EPB41L4A-AS2 promotes docetaxel sensitivity by activating ABCB1
	H19 (121)	Inducing	H19 targets and sustains PARP-1 activity to mediate the resistance of BC cells and patients
Doxorubicin	MALATI (79)	Inducing	MALAT1 targets miR-570-3P to decrease sensitivity of BC cells to doxorubicin
	SAMMSON (122)	Inducing	SAMMSON increases glycolysis and decreases mitochondrial respiration, leading to doxorubicin resistance
	SNHG10 (123)	Blocking	SNHG10 up-regulates miR-302b via promoting methylation, and enhances doxorubicin sensitivity of TNBC cells
Epirubicin	lnc005620 (124)	Inducing	lnc005620 upregulates ITGB1 and decreases the effects of epirubicin
	NONHSAT101069 (125)	Inducing	NONHSAT101069 promotes epirubicin resistance via NONHSAT101069/miR-129-5p/Twist1 axis in BC cells
Cisplatin	DANCR (126)	Inducing	DANCR upregulates KLF5 and induces the cisplatin resistance in TNBC patients by inhibiting p27
	HULC (127)	Inducing	HULC upregulates IGF1R and increases the expression of tumor stem cell markers to enhance cisplatin resistance
5-fluorouracil	CCAT2 (128)	Inducing	CCAT2 activates mTOR pathway to trigger 5-Fu resistance
	SNORD3A (129)	Blocking	Sponges miR185-5p and upregulates UMPS to increase 5-FU sensitivity

3.3.2 LncRNAs and tamoxifen resistance

Estrogen receptor-positive (ER-positive) BC accounts for 75% of all BC cases, and ER therapy is crucial to inhibit estrogen-dependent tumor growth (130). ER therapy is the first-line adjuvant therapy for ER-positive BC patients and has been shown to reduce the recurrence and mortality whether chemotherapy is given concurrently (131). Aromatase, a rate-limiting enzyme that converts androgen to estrogen, is a vital target for aromatase inhibitors (AIs) such as tamoxifen, letrozole, and anastrozole. However, AI drug resistance persists in clinical practice, leading to tumor recurrence and metastasis (132). Numerous lncRNAs have been identified to be involved in AI drug resistance. Of particular importance is tamoxifen, one of the most commonly used AI drugs. For example, lncRNA DIO3OS interacts with PTBP1 to upregulate LDHA mRNA stability, activating glycolytic metabolism in tamoxifen-resistant BC cells and promoting ER-independent cell proliferation both *in vitro* and *in vivo* (133). Additionally, DILA1 upregulates the oncoprotein Cyclin D1 by inhibiting its phosphorylation and subsequent degradation. The upregulation of Cyclin D1 promotes BC cell proliferation and leads to tamoxifen resistance in both *in vivo* and *in vitro* settings (134). Furthermore, HOTAIR is highly expressed in tamoxifen-resistant breast cancer patients compared to newly diagnosed patients before tamoxifen treatment. The upregulation of HOTAIR activates the ER transcriptional program, resulting in increased BC cell proliferation and tamoxifen resistance (135). Overexpression of H19 in BC cells and tamoxifen-resistant BC cells activates autophagy through the H19/SAHH/DNMT3B axis, which contributes to tamoxifen resistance in BC cells (136). For more lncRNAs involved in tamoxifen resistance, please refer to Table 4.

3.3.3 LncRNAs and trastuzumab resistance

In the past, HER2-positive breast cancer patients often had a poor prognosis. However, the discovery of trastuzumab, a recombinant humanized monoclonal antibody that targets the extracellular domain of HER2, has significantly improved the outcomes for these patients (149). Although other anti-HER2 agents like pertuzumab and lapatinib have been developed, trastuzumab remains the gold standard treatment. Unfortunately, the effectiveness of trastuzumab is limited by the emergence of drug resistance (150). Recent research has identified several long non-coding RNAs (lncRNAs) that are closely associated with trastuzumab resistance, including SNHG14, ATB, and AGAP2-AS1 (Table 5).

TABLE 4 Role and Mechanisms of lncRNAs in BC tamoxifen resistance.

lncRNA	Role	Mechanism
SNHG6 (137)	Inducing	SNHG6 decrease tamoxifen sensitivity of BC cells by inhibiting miR-101and inducing EMT
LINP1 (138)	Inducing	LINP1 downregulates ER protein and attenuates estrogen response to trigger tamoxifen resistance
DSCAM-AS1 (139)	Inducing	DSCAM-AS1 promotes propagation of tamoxifen-resistant BC cells and inhibits apoptosis
HNF1A-AS1 (140)	Inducing	HNF1A-AS1 sponges miR-363 to promote SERTAD3 expression, stimulating tamoxifen resistance of BC cells
CCAT2 (141)	Inducing	CCAT2 sponges miR-145-5p, and activates PI3K/AKT/mTOR signaling pathway to promote tamoxifen resistance
BNAT1 (142)	Inducing	BNAT1 activates ERα signaling in tamoxifen resistant BC cells
BDNF-AS (143)	Inducing	BDNF-AS acts as scaffold of RNH1/TRIM21, abolishes RNH1-regulated mTOR mRNA decay to activate mTOR pathway
ATXN8OS (144)	Inducing	ATXN8OS activates VASP via sponge miR16-5p and promotes BC cell migration and metastasis
DC (145)	Inducing	DC promotes phosphorylation of STAT3 and upregulates Bcl-2 and Bcl-xL to reduce tamoxifen-induced apoptosis
LINC00894-002 (146)	Blocking	LINC00894-002 upregulates miR-200a-3p and miR-1b-1p, consequently inhibits TGF-β and oncogenic ZEB1
Uc.57 (147)	Blocking	Uc.57 inhibits BCL11A and its downstream PI3K/AKT and MAPK pathways
ADAMTS9-AS2 (148)	Blocking	ADAMTS9-AS2 upregulates PTEN by sponging miRNA-130a-5p and improves BC cells' sensitivity to tamoxifen

TABLE 5 Role and Mechanisms of lncRNAs in BC trastuzumab resistance.

lncRNA	Role	Mechanism
AGAP2-AS1 (151)	Inducing	AGAP2-AS1 upregulates CPT1 and induces FAO to cause resistance
SNHG7 (152)	Inducing	SNHG7 inhibits miR-186 to promote proliferation, apoptosis resistance, migration and EMT of BC cells
ATB (153)	Inducing	ATB sponges miR-200c, upregulates ZEB1 and ZNF-217 and thereby induces EMT to promote trastuzumab resistance
ZNF649-AS1 (154)	Inducing	ZNF649-AS1 binds to PTBP1 and promotes ATG5 transcription to induce autophagy and trastuzumab resistance
SNHG14 (155)	Inducing	SNHG14 promotes H3K27 acetylation and increases PABPC1, leading to activation of NRF2 pathways and BC cell survival
GAS5 (156)	Blocking	GAS5 downregulates miR-21, increases G2/M cell cycle arrest and DNA damage to increase BC cells radiosensitivity

3.3.4 LncRNAs and radiotherapy resistance

Radiotherapy (RT) is commonly used as an adjuvant treatment after surgery for various types of breast cancer, including TNBC, metastatic BC, and advanced BC, as it has shown great benefits in reducing recurrence (157). The success of radiotherapy depends on the radiosensitivity of the tumor, which is influenced by factors such as cancer stem cells (CSCs), the tumor microenvironment, DNA repair, and gene expression (158). Unfortunately, some breast tumors develop resistance to radiation, leading to treatment failure and recurrence. Understanding the mechanisms of radiation resistance is crucial for improving the efficacy of radiotherapy. Numerous studies have found associations between specific lncRNAs and radiation resistance in breast cancer. For example, LINC00963 has been found to be upregulated in breast cancer tissues and correlated with aggressive tumor characteristics. Knockdown of LINC00963 has been shown to enhance DNA damage and oxidative stress, making breast cancer cells more sensitive to radiation (159). LINC00963 achieves this through its interactions with miR-324-3P, which normally inhibits the expression of ACK1, a driver of tumor progression. Further information on lncRNAs implicated in radiation resistance is summarized in Table 6. These findings highlight the importance of lncRNAs in mediating resistance to trastuzumab and radiotherapy in breast cancer. Further research is necessary to elucidate the underlying mechanisms and identify potential therapeutic targets to overcome drug and radiation resistance in breast cancer patients.

3.4 Circulating lncRNAs as biomarkers of BC

Accurate tumor biomarkers play a crucial role in diagnosing and predicting the prognosis for patients. Continuing efforts are

TABLE 6 Role and Mechanisms of lncRNAs in BC radiation resistance.

lncRNAs	Effect	Mechanism
DUXAP8 (160)	Inducing	DUXP8 activates the PI3K/AKT/mTOR and inhibits E-cadherin and RHOB via interaction with EZH2 to enhance the radiation resistance
HOTAIR (161)	Inducing	HOTAIR acts as a sponge of miR-449-5p, upregulates the expression of HSPA1A to enhance BC cells radiation resistance
HOTAIR (162)	Inducing	HOTAIR increases radiation resistance by inhibiting HOXD10 and the PI3K/AKT-BAD signaling pathway in BC cells
FGD5-AS1 (163)	Inducing	FGD5-AS1 acts as a sponge of miR-497-5p, up-regulates the expression of MACC1 to enhance BC cells radiation resistance
AFAP1-AS1 (164)	Inducing	AFAP1-AS1 activates the Wnt/β-catenin pathway and them induces radiation resistance of TNBC
GAS5 (156)	Blocking	GAS5 sensitizes BC cells to radiation by inhibiting DNA repair and sponging miR-21
LINC00963-FOSB (165)	Inducing	mediating transcriptional activation of UBE3C to induce ubiquitination-dependent protein degradation of TP73

being made to identify new biomarkers with high sensitivity and specificity. These biomarkers are valuable for evaluating tumor stage, metastasis risk, treatment response, and the development of new therapies (166). Circulating nucleic acids, including circulating RNAs such as lncRNAs, miRNAs, and piRNAs, have emerged as a promising class of potential biomarkers for improving tumor diagnosis. Compared to circulating DNAs, circulating RNAs offer higher specificity and sensitivity, garnering significant research attention (167). This review predominantly focuses on circulating lncRNAs as biomarkers for breast cancer, given their stability and abundance in the bloodstream, making them reliable cancer biomarkers (168). As indicated by previous findings on changes in breast cancer cells and tissues, there is a growing focus on circulating lncRNAs. Encouragingly, certain circulating lncRNAs can reflect cancer status, and changes in these lncRNAs are correlated with the degree of tumor progression and clinical features. Furthermore, some of these lncRNAs can identify specific subtypes of breast cancer, while a few others may serve as potential therapeutic agents or treatment targets (168).

For instance, HOTAIR has been found to be significantly elevated in the serum of breast cancer patients compared to healthy individuals, suggesting its potential as a diagnostic biomarker (169). One study indicated that HOTAIR exhibits a stronger diagnostic capability for breast cancer than CEA and CA 15-3, given its association with ER, Her-2, and lymph node metastasis (170). Notably, these researchers have observed a significant decrease in HOTAIR expression levels post-surgery. However, some studies have suggested the existence of potential technical errors in previous experimental results, warranting further investigation to determine whether HOTAIR could be adopted as a prognostic marker (171). Therefore, further studies are needed to assess whether HOTAIR could be adopted as a potential prognostic marker.

Another example is HISLA (HIF-1α-stabilizing long noncoding RNA), which can be transmitted by extracellular vesicles from tumor-associated macrophages to breast cancer cells (172). HISLA is overexpressed in both breast cancer tissues and patient serum and is significantly associated with advanced grade, histological grade, distant metastasis, and poor survival. Moreover, serum levels of HISLA were observed to decrease significantly after surgery, suggesting its potential as a biomarker for diagnosing and prognosticating breast cancer (173). Similarly, H19 is released into the plasma from tumor cells upon breast cancer initiation, leading to an upregulation of plasma H19 levels. Consistently, plasma H19 levels were found to decrease significantly after surgery, and they have been significantly correlated with ER, PR, Her-2, and lymph node metastasis, indicating the potential use of H19 as a diagnostic and monitoring biomarker for breast cancer (174). In addition, a group of researchers detected the serum levels of lncRNAs PVT1, HOTAIR, NEAT1, and MALAT1 from Egyptian breast cancer and fibroadenoma patients, as well as healthy donors, and found that serum PVT1, HOTAIR, and NEAT1 could serve as potential biomarkers for breast cancer. Specifically, HOTAIR and NEAT1 demonstrated feasibility in differentiating between breast cancer and fibroadenoma (81). Moreover, El-Ashmawy et al. identified the upregulation of lncRNAs FAM83H-AS1 and ATB in the serum of breast cancer patients, with ATB exhibiting superior diagnostic accuracy compared to CA 15-3, a well-established serum protein marker (175). Conversely, FAM83H-AS1 demonstrated prognostic rather than diagnostic value, showing a significant association with tumor lymph node metastasis and tumor size (175). A brief overview of circulating lncRNAs with potential applications as biomarkers for breast cancer is presented in Figure 3 (176–181). Some reported lncRNAs may exhibit relatively low specificity or sensitivity when used individually, making it feasible to combine several lncRNAs or use them in conjunction with traditional markers.

4 Therapeutic strategies against lncRNAs in breast cancer

In the ongoing battle against breast cancer, the significance of lncRNAs has come to the forefront of cancer research. Given their crucial role in the etiology and progression of breast cancer, as well as the development of resistance to current therapies, lncRNAs present an enticing target for novel treatments (182). Recent advances have led to the development of various strategies targeting lncRNAs, utilizing antisense oligonucleotides (ASOs), small molecules, and natural compounds.

ASOs represent one of the spearheads in the quest for lncRNA-targeted interventions. These synthetic molecules are engineered to be complementary to specific RNA sequences, allowing them to bind and neutralize lncRNAs through varied mechanisms. Among the mechanisms is the induction of RNase H-dependent degradation of the lncRNA, reducing its oncogenic influence in tumor cells (183). The potential of ASOs is illustrated by their effectiveness against HOTAIR and MALAT1, lncRNAs implicated in breast cancer metastasis and oncogenesis (184, 185). By subduing these lncRNAs, ASOs have demonstrated a capacity to inhibit cancer stem cell properties and reduce metastasis in preclinical models.

Small molecules like quercetin have also emerged as a viable approach to modulate lncRNAs. Quercetin, a widely available dietary flavonoid, has been found to diminish the expression of lncRNA MALAT1 in breast cancer cell lines (186). Through such downregulation, it exerts antitumor effects, likely by disrupting the complex interplay between key cellular pathways, p53, and miRNAs that revolve around MALAT1. This provides a compelling case for the therapeutic repurposing of naturally occurring compounds with regulatory effects on lncRNAs.

Additionally, natural products such as curcumin, recognized for its myriad health benefits, have been shown to regulate the expression of certain lncRNAs. A noteworthy example includes

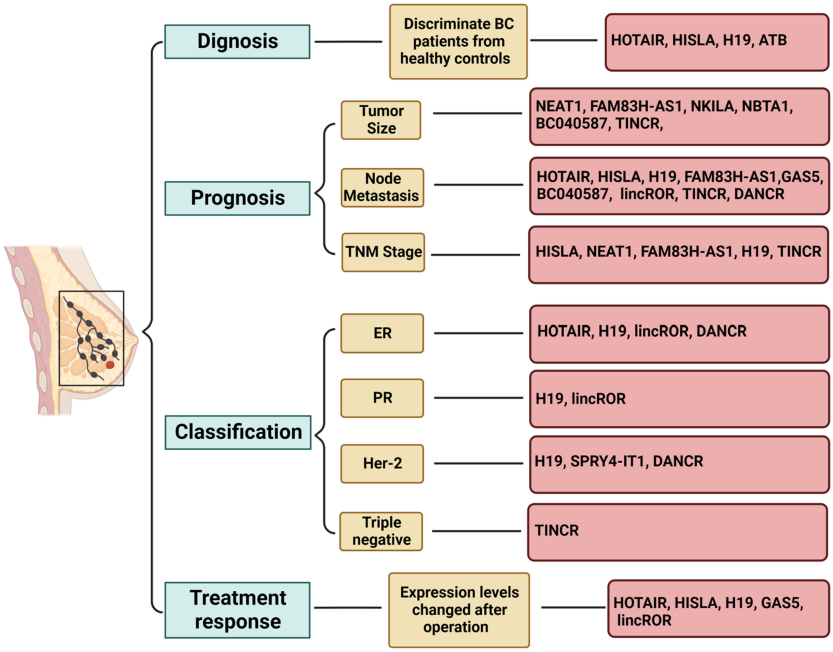

FIGURE 3
Role of circulating lncRNAs as potential biomarkers in breast cancer. Long non-coding RNAs (lncRNAs) have demonstrated potential as biomarkers in various aspects of breast cancer, including diagnosis, prognosis, subtype classification, and treatment response. Utilizing a combination of multiple lncRNAs may offer a viable strategy for leveraging lncRNA-based biomarkers in clinical applications. The figure was generated using BioRender.com.

its ability to downregulate lncRNA H19, an action that can suppress the resistance of breast cancer cells to tamoxifen (187). Such findings underscore the potential therapeutic role of natural products in influencing lncRNA expression and combating drug resistance, critical hurdles in current treatment paradigms.

Collectively, the innovative application of ASOs, small molecules, peptides, and natural products holds immense promise for the future of breast cancer therapy, specifically through targeting lncRNAs. These emerging strategies may provide a breakthrough in addressing the challenges BC presents, including drug resistance, tumor recurrence, and metastasis, thereby improving outcomes for patients worldwide. As research progresses, the integration of these treatments into the clinical setting could transform the landscape of breast cancer management, offering hope for more effective and personalized therapeutic options.

5 Discussion and conclusion

Breast cancer (BC) remains a leading cause of cancer-related deaths in women, with different subtypes exhibiting distinct molecular characteristics and treatment responses. While significant progress has been made in improving the prognosis of BC patients through established treatments such as radical surgery and adjuvant therapy, a considerable number of patients still succumb to metastasis or resistance to these treatments. Therefore, there is a continuous need to explore novel therapeutic targets and molecular mechanisms.

The initiation and progression of breast cancer are influenced by a combination of genetic, epigenetic, and non-genetic factors. Previous research has primarily focused on genetic abnormalities and classic epigenetic factors such as histone and DNA modifications (188–191). Recently, ncRNAs, particularly lncRNAs, have emerged as crucial epigenetic regulators in cancer research (28). LncRNAs, which are longer than 200 nucleotides and have limited protein coding potential, play various biological roles in BC development through multiple mechanisms. Dysregulated lncRNAs have been closely associated with BC cell growth, apoptosis, invasion, EMT, autophagy, and therapeutic resistance, contributing to BC progression. Therefore, these lncRNAs hold promise as predictive biomarkers or therapeutic targets for BC patients.

In addition to downstream regulation, the upstream regulatory mechanism of lncRNAs has garnered significant research attention. One such mechanism involves N6-methyladenosine (m^6A), the most frequent posttranscriptional modification found on mRNA, which has also been discovered on lncRNAs (192, 193). The interaction between m^6A readers and lncRNAs, as well as the reciprocal regulation of m^6A modifiers by lncRNAs, plays a role in BC development. For instance, the oncogenic lncRNA MIR210HG is induced by the m^6A reader IGF2BP1 in an m^6A-dependent manner to promote BC progression and metastasis (194). Another oncogenic lncRNA LNC942 bound to METTL14 (an m^6A writer) directly and promoted m^6A methylation and stabilization of CXCR4 and CYP1B1, promoting BC cells proliferation and inhibiting apoptosis (195). Collectively, m^6A/

lncRNAs axis enriches BC regulatory network and provides novel targets for BC prevention and management.

Interestingly, some lncRNAs have been found to encode short peptides that possess functional roles in cancer. For example, the lncRNA HOXB-AS3 encodes a conserved 53-amino acid peptide that suppresses colon cancer growth (196). Similarly, some peptides encoded by lncRNAs are involved in BC initiation and progression. For instance, the lncRNA LINC00908 contains a small open reading frame (ORF) encoding a 60-amino acid polypeptide named ASRPS, which acts as a small regulatory peptide of STAT3 (197). Downregulation of ASRPS was associated with poor clinical outcome, indicating that ASRPS is a potential antitumor peptide. Mechanistically, ASRPS directly binds to STAT3, inhibiting its phosphorylation and downstream VEGF expression, thereby suppressing TNBC angiogenesis (197). Additionally, the lncRNA CTD-2256P15.2 encodes a micropeptide called PACMP that regulates BC progression, multiple drug resistance, and ionizing radiation resistance by maintaining CtIP abundance and promoting PARP-1-dependent poly (ADP-ribosyl)ation (PARylation) through direct binding to DNA damage-induced poly (ADP-ribose) chains (198).

In summary, a substantial number of lncRNAs have been identified as oncogenic in BC, with their overexpression associated with aggressive disease and poor patient survival. Conversely, certain lncRNAs exhibit tumor-suppressive effects and are frequently downregulated in BC. Hence, targeting oncogenic lncRNAs while activating antitumor lncRNAs can provide unique opportunities in the battle against BC. Advances in multiomics sequencing technologies and bioinformatics tools are anticipated to uncover an increasing number of lncRNAs involved in BC progression, expanding the repertoire of diagnostic and prognostic biomarkers. With deeper understanding of the role of lncRNAs in BC initiation and progression, oncogenic lncRNAs may be developed as potential therapeutic targets for BC treatment. However, it is important to note that most studies investigating lncRNAs and BC are still at the preclinical stage, and further research is required to elucidate the underlying mechanisms. Future studies should focus on identifying specific and sensitive lncRNAs for early diagnosis, risk stratification, prognosis monitoring, and personalized treatment of BC.

Author contributions

YW: Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. BN: Data curation, Investigation, Methodology, Software, Validation, Writing – original draft. XL: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing. QS: Investigation, Methodology, Software, Validation, Writing – original draft. B-FM: Investigation, Software, Visualization, Writing – original draft. WH: Investigation, Validation, Visualization, Writing – review & editing. JY: Investigation, Methodology, Visualization, Writing – original draft. LW: Conceptualization, Methodology, Visualization, Writing – review & editing. XZ: Methodology, Software, Visualization, Writing – review & editing. YM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants from the State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia Fund (SKL-HIDCA-2023-JY6), the National Natural Science Foundation of China (82000155), the Natural Science Foundation of Zhejiang Province (LY23H160017), and the China Postdoctoral Science Foundation (No. 2021M702877).

Acknowledgments

We would like to express our sincere gratitude to our colleagues and researchers for their invaluable insights, thought-provoking discussions, and constructive feedback. We would also like to apologize to fellow researchers whose remarkable work could not be referenced due to space constraints.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 07 March 2024
DOI 10.3389/fonc.2024.1360975

OPEN ACCESS

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RECEIVED 24 December 2023
ACCEPTED 16 February 2024
PUBLISHED 07 March 2024

CITATION
Sang B, Fan Y, Wang X, Dong L, Gong Y, Zou W, Zhao G and He J (2024) The prognostic value of absolute lymphocyte count and neutrophil-to-lymphocyte ratio for patients with metastatic breast cancer: a systematic review and meta-analysis. *Front. Oncol.* 14:1360975. doi: 10.3389/fonc.2024.1360975

The prognostic value of absolute lymphocyte count and neutrophil-to-lymphocyte ratio for patients with metastatic breast cancer: a systematic review and meta-analysis

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Background: Neutrophil-to-lymphocyte ratio (NLR) is considered a potential prognostic marker in early breast cancer. However, the prognosis of absolute lymphocyte count (ALC) and NLR in metastatic breast cancer (MBC) has been reported in a few studies, and conclusions are still conflicting. This present manuscript aims to provide further solid evidence regarding the prognostic values of ALC and NLR in MBC patients.

Method: Eligible studies that reported the associations between ALC or NLR and MBC were included by searching relative electronic databases. Overall survival (OS) and progression-free survival (PFS) were used as outcome measures. The hazard ratio (HR) values and 95% confidence interval (CI) of the outcome measures were collected as effect sizes, and further analysis and discussion were conducted according to the pooled HR, subgroup analysis, publication bias, and interstudy heterogeneity.

Results: Twenty-nine studies comprising 3,973 patients with MBC were included. According to our findings, lower ALC was significantly associated with poorer prognosis of OS (HR = 0.57, 95% CI 0.48 to 0.68) and PFS (HR = 0.68, 95% CI 0.58 to 0.79), and greater NLR was associated with poorer OS (HR = 1.50, 95% CI 1.35 to 1.67) and PFS (HR = 1.82, 95% CI 1.42 to 2.35). Furthermore, the prognostic values of ALC and NLR in MBC were also observed in the subgroup analyses regarding cutoff values and ethnicities.

Conclusion: Low ALC and elevated NLR were observed to be significantly associated with adverse OS and PFS in MBC, indicating that ALC and NLR may act as potential prognostic biomarkers of MBC patients. Meanwhile, our results

will also provide some novel evidence and research clues for the selection and development of clinical treatment strategies for MBC patients.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42021224114.

KEYWORDS

ALC, NLR, MBC, prognostic marker, meta-analysis

1 Background

Breast cancer has become the leading cause of morbidity and mortality in women worldwide (1). Most patients with early-stage breast cancer have a good prognosis, but metastatic breast cancer (MBC) is generally regarded as an incurable disease (2, 3). At present, distant metastasis and multiorgan metastasis remain a great challenge for disease recurrence and long-term survival in patients with MBC (4). It was estimated that the median overall survival (OS) of MBC patients was approximately 3 years, with a 5-year survival rate of approximately 25%, and patients with lung metastasis and bone metastasis were even less than 20% (4, 5). However, the underlying mechanisms of distant metastasis and colonization of breast cancer have not been elucidated (6). Furthermore, although multiple modalities or drug options are available in clinics, such as primary surgery and CDK4/6 inhibitors in combination with endocrine and dual anti-inhibitors, the prognosis of these patients is still unsatisfactory (7–10). Therefore, exploring appropriate prognostic biomarkers is of great clinical significance for improving prognosis and monitoring treatment and their response.

As a heterogeneous disease, the occurrence and development of MBC are closely related to inflammatory factors (11). In recent years, it has been found that absolute lymphocyte count (ALC) and neutrophil and platelet count may affect the progression of MBC (12). Pretreatment neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) and other immune and inflammatory biomarkers have been reported to be independent predictors of breast cancer prognosis (13–15). It was observed that these easy-to-obtain, non-invasive, and individualized peripheral blood biomarkers are of great prognostic value in numerous tumors including breast cancer, and increased NLR and PLR and decreased ALC may be associated with poor prognosis (16, 17). However, several previous meta-analyses only focus on breast cancer, and thus, there is still great uncertainty for the values of these potential prognostic biomarkers in MBC (2). Furthermore, although the ALC and NLR have been studied as prognostic markers of MBC in recent years, their prognostic values have not been determined uniformly, and different viewpoints still exist (18–20). Nevertheless, to the best of our knowledge, there is still a lack of relevant meta-analysis to provide relatively systematic and solid evidence regarding the prognostic value of ALC and NLR in MBC.

ALC and NLR, as major peripheral blood biomarkers that influence tumorigenesis and development, are expected to play a key role in the selection of drug therapies for patients with MBC in the future. Therefore, this study conducted a comprehensive and detailed meta-analysis of the effects of ALC and NLR on OS and progression-free survival (PFS) in patients with MBC and a subgroup discussion of factors affecting the assessment of their prognostic values, hoping to provide a useful reference for the long-term survival and improvement of the quality of life of patients.

2 Method

2.1 Search strategies

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was strictly followed, and our study protocol was registered at the PROSPERO website (https://www.crd.york.ac.uk/PROSPERO/) before the start of the study. The identifier of the systematic review registration was PROSPERO CRD42021224114. Based on the prognostic correlation of ALC and NLR with MBC, the following keywords were formulated as search terms: “Metastatic breast cancer,” “Metastatic breast Neoplasms,” “Absolute lymphocyte count,” “Baseline lymphocyte count,” “ALC,” “neutrophil-to-lymphocyte ratio,” and “NLR.” The detailed search strategies are provided in Supplementary Table 1. A comprehensive literature search of relevant English language studies published up to April 2023 was conducted through online medical databases such as PubMed, EMBASE, the Cochrane Library, and Web of Science. In order to avoid omission of literature that met the inclusion criteria, the literature searches and backward searches were conducted independently based on the same strategy by the two researchers to collect eligible literature as detailed and complete as possible.

2.2 Inclusion and exclusion criteria

According to the pre-established inclusion and exclusion criteria, the title, abstract, and full-text article were screened. No restrictions were placed on the enrolled studies, including region,

race, MBC subtype, age, and treatment regimen. When the results differed between the two researchers, a discussion with a third researcher was conducted and a final decision was made.

The inclusion criteria for the articles were as follows: 1) the study subjects are MBC patients; 2) the study evaluates the prognostic value of ALC or NLR in patients with MBC; 3) the study reports the results of the OS or PFS or provides available data to calculate the hazard ratio (HR) and 95% confidence interval (CI) of the OS or PFS; and 4) when the data are published repeatedly, the most recent study with the most detailed information will be selected.

The exclusion criteria for the articles were as follows: 1) reviews, case reports, comments, and conference abstracts are excluded; 2) studies about animals or cells are also excluded; and 3) studies with incomplete data are not considered.

2.3 Data extraction

After the two researchers independently completed the literature selection based on pre-designed criteria, the Excel and EndNote software were used to manage and extract the needed information. The OS and PFS, the primary survival outcomes, were extracted in the form of HR and 95% CI. Other detailed information was also extracted as follows: name of the first author, year of publication, country, study type, age of the patients, treatment, follow-up period, tumor subtype, number of metastatic sites, and cutoff values of ALC and NLR. If more than two or more sample sets appeared in the same study, the more complete and detailed data would been adopted. Further analysis is carried out when all researchers confirm the correctness of the data.

2.4 Quality assessment

The qualities of all the included studies were objectively assessed by two researchers using the standard Newcastle–Ottawa scale (NOS), which is composed of the following three quality indicators: outcome assessment, comparability, and selection. Each individual study was scored from 0 to 9 based on these parameters. The higher the score, the better the quality of the literature (21).

2.5 Statistical analysis

The pooled HR values were statistically analyzed using the Cochrane Collaboration's Review Manager (version 5.3) and visually presented by forest plots. When the HRs and 95% CIs cannot be extracted from the table in the articles, these were indirectly acquired using the Engauge Digitizer (version 4.1) from the Kaplan–Meier graph (22). The random-effects model was used to evaluate the prognostic value of ALC and NLR in MBC patients with remarkable heterogeneity, and the fixed-effects model was adopted. Heterogeneity between studies was calculated through the Cochran Q and I^2 statistics, and the source of heterogeneity was estimated by subgroup analysis according to region, sample size, cutoff values, tumor subtype, and therapeutic strategies. At the same time, publication bias and sensitivity analysis of the main outcomes were also performed when applicable by using the Stata software (version 15.0) (23).

3 Results

3.1 Characteristics of the included studies

A total of 579 articles were retrieved from four databases, consisting of 147 from PubMed, 32 from the Cochrane Library, 274 from EMBASE, and 126 from the Web of Science. After reviewing all the literature, 351 articles were included through screening of the title, abstract, and full text. Finally, 29 articles were included in this meta-analysis after strict screening (18–20, 24–49) (Figure 1). The information of all the included literature was collected and pooled, and the basic characteristics are described in Table 1. Twenty-nine studies were from nine countries, 28 of which were published within the last 5 years, and the average age of the included 3,952 patients was 51–63 years. Most of the patients have visceral metastasis and two-organ metastasis. Only 14 studies analyzed the prognostic value of ALC and 26 studies discussed the effects of NLR on MBC patients. The primary cutoff values of 1,500 and 3 were used in most of the studies. Detailed information is shown in Table 2. All NOS scores of the included studies were 8–9, suggesting high quality according to the quality assessment.

3.2 Correlation between ALC and OS in MBC patients

A total of 1,584 MBC patients from 12 studies were enrolled in this present meta-analysis to assess the correlation between ALC and OS. The pooled analysis of all studies demonstrated that low ALC was significantly associated with poor OS (fixed-effects model, HR = 0.57, 95% CI = 0.48 to 0.68, $p < 0.01$) with low heterogeneity (I^2 = 14%, p = 0.31, Figure 2).

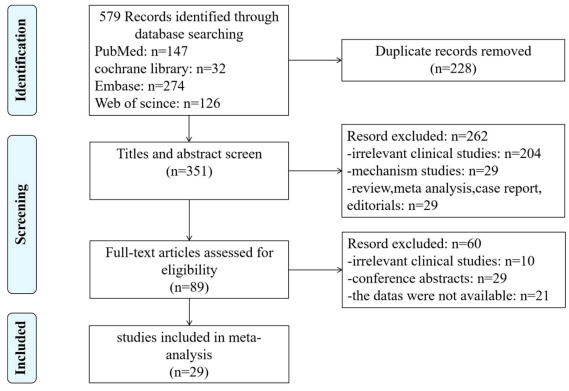

FIGURE 1
Selection process of the studies included.

10.3389/fonc.2024.1360975

TABLE 1 Characteristics of the included studies.

Study	Year	Country	Study type	Sample size	Ages (years)	Treatment	Endpoint	NOS scores
Sawa	2022	Japan	Single center	243	58 (22–90)	Hormonal therapy Chemotherapy	OS	9
Jimbo	2022	Japan	Single center	108	56 (32–86)	Endocrine therapy Chemotherapy	OS	8
Emile	2022	France	Single center	114	51 (30–75)	CDK4/6 inhibitor plus Endocrine therapy	OS and PFS	8
Inoue	2022	Japan	Single center	131	NR	Chemotherapy Endocrine therapy Anti-HER2 therapy	OS	9
Shikanai	2022	Japan	Single center	107	58 (35–87)	CDK4/6 inhibitor CDK4/6 inhibitor plus Endocrine therapy	PFS	8
Xiang	2022	China	Single center	94	51 (46–62)	Chemotherapy	OS	9
Takamizawa	2022	Japan	Single center	91	NR	Eribulin Capecitabine	OS and PFS	9
Shao	2022	China	Single center	129	51 (25–82)	Anti-HER2 therapy T-DM1 TKI	OS and PFS	9
Yilmaz	2022	Turkey	Single center	101	56 (26–88)	CDK4/6 inhibitor plus Endocrine therapy	OS and PFS	8
Koyama	2021	Japan	Single center	120	61 (20–82)	Eribulin Adjuvant or Neoadjuvant Chemotherapy	OS and PFS	8
Nakamoto	2021	Japan	Single center	94	62 (37–83)	Eribulin	OS	8
Morisaki	2021	Japan	Single center	88	NR	Eribulin	OS and PFS	8
Oba	2021	Japan	Single center	60	58.6 ± 11.9	Eribulin	OS and PFS	8
Miyagawa	2020	Japan	Multicenter	179	NR	Bevacizumab plus Paclitaxel	OS and PFS	8
Gerratana	2020	Italy	Single center	396	NR	NR	OS	9
Sata	2020	Japan	Single center	74	NR	Eribulin Adjuvant or Neoadjuvant Chemotherapy	OS and PFS	7
Myojin	2020	Japan	Single center	104	59 (38–82)	Eribulin Anti-HER2 therapy	PFS	8
de la Cruz-Ku	2020	Peru	Single center	118	NR	Chemotherapy Others	OS	9
Liu	2020	China	Single center	176	56	Chemotherapy Endocrine therapy Targeted therapy Others	OS and PFS	9
Ueno	2020	Japan	Single center	125	57	Eribulin	OS and PFS	8
De Giorgi	2019	USA	Single center	280	NR	Systemic treatment	OS	8
Che	2019	China	Single center	68	51 (27–74)	Anti-HER2 therapy Chemotherapy	OS and PFS	9

(Continued)

TABLE 1 Continued

Study	Year	Country	Study type	Sample size	Ages (years)	Treatment	Endpoint	NOS scores
Ivars Rubio	2019	Spain	Single center	263	59 (19–95)	Chemotherapy Endocrine therapy Chemotherapy plus Biological agents Anti-HER2 therapy Anti-VEGF therapy	OS and PFS	9
Imamura	2019	Japan	Multicenter	53	NR	T-DM1	OS and PFS	8
Blanchette	2018	Canada	Single center	154	56 (47–63)	Anti-HER2 therapy	OS	8
Takuwa	2018	Japan	Single center	171	59 (31–92)	Multidisciplinary therapy	OS	9
Miyagawa	2018	Japan	Single center	59	63 (34–83)	Eribulin Others	OS and PFS	8
Vernieri	2018	Italy	Single center	57	56 (33.7–78.9)	Chemotherapy	OS and PFS	8
De Giorgi	2012	USA	Single center	195	54 (24–84)	Systemic treatment	OS and PFS	8

Models	Cutoff	Follow-up (months)	Tumor subtype (100%)	Visceral metastasis (%)	Number of metastatic sites (%)
ALC and NLR	ALC: 1,500; NLR: 3	26.4 (0.1–192.1)	NR	159 (65.4)	NR
ALC and NLR	ALC: 1,629; NLR: 1.99	NR	NR	NR	NR
ALC	ALC: 1,500	NR	$HR^+/HER2^-$	NR	NR
NLR	NLR: 2.52	59 (6–151)	NR	NR	≥2; 12 (9.2)
ALC and NLR	ALC:1,505; NLR: 2.5	NR	$ER^+/HER2^-$	67 (62.6)	NR
NLR	NLR: 2.285	30 (2–109)	NR	NR	NR
ALC and NLR	ALC: 1,500; NLR: 3	19.1	NR	NR	NR
NLR	NLR: 3	21.0 (2.0–46.0)	$HER2^+$	89 (69.0)	>2; 41 (31.8)
NLR	NLR: 2.19	NR	$HR^+/HER2^-$	32 (31.7)	≥2; 49 (48.5)
ALC and NLR	ALC: 1,285; NLR: 3.3	15.2 (0.9–65.9)	$HER2^-$	100 (83.3)	≥2; 58 (48.3)
ALC and NLR	ALC: 1,500; NLR: 3	NR	$HER2^-$	77 (81.9)	≥3; 75 (79.8)
ALC	ALC: 1,500	15.9 (1.7–75.6)	NR	64 (72.7)	NR
NLR	NLR: 2.32	13.7 (1.63–54.17)	NR	48 (80.0)	NR
ALC and NLR	ALC: 1,500; NLR: 3	NR	NR	144 (80.4)	NR
NLR	NLR: 2	53	NR	NR	≥2; 183 (46.2)
ALC and NLR	ALC: 1,500; NLR: 3	NR	NR	34 (45.9)	NR
ALC and NLR	ALC: 1236; NLR: 3.3	NR	NR	75 (72.1)	NR
NLR	NLR: 2.5	24	Triple negative	NR	≥2; 60 (50.8)

(Continued)

TABLE 1 Continued

Models	Cutoff	Follow-up (months)	Tumor subtype (100%)	Visceral metastasis (%)	Number of metastatic sites (%)
NLR	NLR: 2.085	25.4	NR	77 (43.8)	NR
ALC and NLR	ALC: 1,500; NLR: 5	NR	NR	91 (72.8)	NR
NLR	NLR: 3	NR	NR	NR	NR
ALC and NLR	ALC: 1,000; NLR: 3	26.5 (2.28–97.2)	$HER2^+$	NR	≥2; 44 (64.7)
NLR	NLR: 2.32	44.9 (6–107)	NR	65 (24.7)	≥2; 111 (42.2)
NLR	NLR: 2.56	NR	$HER2^+$	37 (69.8)	≥3; 26 (49.1)
NLR	NLR: 3.18	NR	$HER2^+$	NR	≥2; 70 (45.8)
NLR	NLR: 1.9	44 (0–271)	NR	120 (70.2)	≥2; 119 (69.6)
NLR	NLR: 3	NR	NR	44 (74.6)	NR
NLR	NLR: 2.5	NR	Triple negative	37 (64.9)	>2; 21 (36.8)
ALC	ALC: 1,000	NR	NR	NR	NR

OS, overall survival; PFS, progression-free survival; NOS, Newcastle–Ottawa scale; NR, not reported; ALC, absolute lymphocyte count; NLR, neutrophil to lymphocyte ratio; T-DM1, trastuzumab emtansine; TKI, tyrosine kinase inhibitor.

TABLE 2 Subgroup analyses of ALC for OS and PFS.

Subgroups	Independent cohorts	HR (95% CI) (H/L*)	*p*-value	Heterogeneity	
				I^2, %	*p*-value
Overall survival	**12**				
Cutoff value					
>1,500	1	0.13 [0.02, 0.72]	0.02	–	–
1,500	8	0.59 [0.48, 0.73]	<0.00001	0	0.56
<1,500	3	0.56 [0.42, 0.76]	0.0002	49	0.14
Region					
Asia	10	0.60 [0.49, 0.72]	<0.00001	23	0.23
America	1	0.45 [0.27, 0.73]	0.001	–	–
Europe	1	0.57 [0.33, 0.97]	0.04	–	–
Sample size					
≥100	7	0.59 [0.49, 0.72]	<0.00001	25	0.24
<100	5	0.49 [0.33, 0.72]	0.0003	0	0.41
Treatment					
Eribulin	4	0.64 [0.44, 0.93]	0.15	43	0.02
Chemotherapy	1	0.57 [0.28, 1.18]	0.13	–	–
CDK4/6 inhibitor plus Endocrine therapy	1	0.57 [0.33, 0.97]	0.04	–	–
Bevacizumab plus Paclitaxel	1	0.53 [0.35, 0.80]	0.003	–	–
Tumor subtype					
$HER2^+$	1	0.33 [0.13, 0.81]	0.02	–	–
$HER2^-$	2	0.51 [0.20, 1.26]	0.06	72	0.14

(Continued)

TABLE 2 Continued

Subgroups	Independent cohorts	HR (95% CI) (H/L*)	*p*-value	Heterogeneity	
				I^2, %	*p*-value
Tumor subtype					
$HR^+/HER2^-$	1	0.57 [0.33, 0.97]	0.04	–	–
Progression-free survival	**10**				
Cutoff value					
>1,500	1	0.75 [0.29, 1.93]	0.55	–	–
1,500	5	0.65 [0.52, 0.82]	0.0003	0	0.67
<1,500	4	0.69 [0.56, 0.86]	0.0007	76	0.006
Region					
Asia	8	0.74 [0.61, 0.89]	0.001	44	0.08
America	1	0.55 [0.38, 0.79]	0.001	–	–
Europe	1	0.59 [0.38, 0.89]	0.01	–	–
Sample size					
≥100	7	0.70 [0.59, 0.82]	<0.00001	18	0.3
<100	3	0.59 [0.41, 0.85]	0.005	72	0.03
Treatment					
Eribulin	2	0.73 [0.49, 1.08]	0.2	39	0.12
Chemotherapy	1	0.68 [0.38, 1.22]	0.19	–	–
CDK4/6 inhibitor plus Endocrine therapy	1	0.59 [0.38, 0.89]	0.01	–	–
Bevacizumab plus Paclitaxel	1	0.69 [0.43, 1.11]	0.12	–	–
Tumor subtype					
$HER2^+$	1	0.26 [0.12, 0.55]	0.0005	–	–
$HER2^-$	1	1.02 [0.71, 1.48]	0.91	–	–
$HR^+/HER2^-$	1	0.59 [0.38, 0.89]	0.01	–	–
$ER^+/HER2^-$	1	0.75 [0.29, 1.93]	0.55	–	–

No statistical results were in line.
HR, hazard ratio; CI, confidence interval; H, high; L, low.

3.3 Correlation between ALC and PFS in MBC patients

A total of 10 studies involving 1,262 MBC patients were incorporated to estimate the connection between ALC and PFS. The combined analysis of all the studies indicated that a lower ALC level was related to a shorter PFS (fixed-effects model, HR = 0.68, 95% CI = 0.58 to 0.79, $p < 0.01$) with low heterogeneity ($I^2 = 40\%$, $p = 0.09$, Figure 3).

3.4 Correlation between NLR and OS in MBC patients

Twenty-three studies with 3,276 MBC patients appraised the association between NLR and OS. Nine studies showed that NLR may be a potential prognostic biomarker for OS, but no remarkable correlation between NLR and OS was observed in 14 publications. However, according to the pooled analysis, it was demonstrated that high NLR values were obviously associated with poor OS (fixed-effects model, HR = 1.50, 95% CI = 1.35 to 1.67, $p < 0.01$) with low heterogeneity ($I^2 = 5\%$, $p = 0.40$, Figure 4).

3.5 Correlation between NLR and PFS in MBC patients

Fourteen studies with 1638 MBC patients evaluated the relationship between the NLR and PFS. The pooled outcome showed that higher NLR was markedly connected with adverse PFS (random-effects model, HR = 1.82, 95% CI = 1.42 to 2.35, $p < 0.01$) with high heterogeneity ($I^2 = 80\%$, $p = 0.0002$, Figure 5).

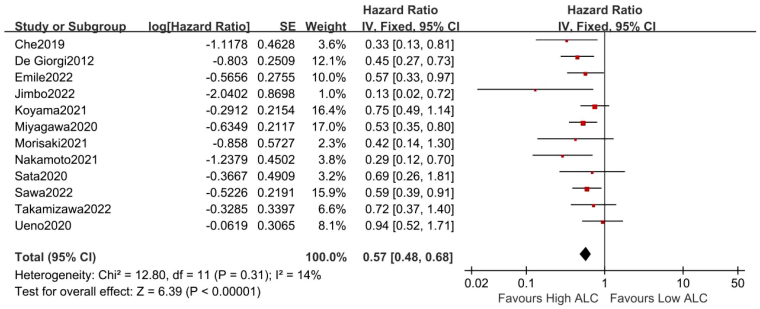

FIGURE 2
Forest plot of ALC for OS.

3.6 Subgroup analyses for the relationship between ALC and OS/PFS

Based on the extracted data and influencing factors, subgroup analysis was performed on the study region, cutoff value, sample size, treatment, and tumor subtype. The effects of ALC on the OS and PFS of patients with MBC among different subgroups are shown in Table 2.

It was found that the significant relationship between ALC and OS was not affected by the cutoff value, region, and sample size, suggesting that ALC might be a promising biomarker for OS in MBC patients. Moreover, the high ALC was significantly associated with better OS in MBC patients given CDK4/6 inhibitor plus endocrine therapy (HR = 0.57, 95% CI = 0.33 to 0.97, p = 0.04) and bevacizumab plus paclitaxel therapy (HR = 0.53, 95% CI = 0.35 to 0.80, p = 0.003), and the association between ALC and OS was also observed in MBC patients with $HER2^+$ (p = 0.02) and HR^+/$HER2^-$ (p = 0.04), but since only one study was involved, further high-quality research with a large sample is required in the future.

As for the subgroup analysis of the relationship between ALC and PFS, although no relationship between ALC and PFS was observed in one study with an ALC cutoff value of >1,500, the remaining results of the ALC cutoff value of ≤1,500, region, and sample size further indicated that high ALC was associated with better PFS. Likewise, the subgroup analysis regarding treatment and tumor subtype involved extremely limited studies, and therefore, a larger number of studies with bigger sample sizes need to be further explored.

3.7 Subgroup analyses for the relationship between NLR and OS/PFS

As shown in Table 3, the subgroup analyses based on the cutoff value of NLR and sample sizes also demonstrated the promising prognostic value of NLR in OS. Furthermore, it was observed that higher NLR was associated with worse PFS in patients treated with bevacizumab plus paclitaxel (HR = 2.03, 95% CI = 1.27 to 3.24, p = 0.003) and T-DMI (HR = 3.69, 95% CI = 1.62 to 8.41, p = 0.002). Additionally, similar results were found in MBC patients with ER^+/$HER2^-$ (HR = 3.23, 95% CI = 1.23 to 8.44, p = 0.02) and triple-negative breast cancer (HR = 2.65, 95% CI = 1.37 to 5.16, p = 0.004). However, some non-significant results were presented in certain subgroup analyses on treatment and tumor subtype, which may account for the limited number of studies, and further solid evidence is required based on high-quality studies with large samples.

3.8 Sensitivity analysis and publication bias

Further sensitivity analysis was performed and presented in Figure 6. It was indicated that the pooled HRs and 95% CIs did not alter significantly, suggesting that these results were robust.

The publication bias of the included studies was evaluated, and the corresponding results are presented in Supplementary Figures 1, 2. No potential publication bias was observed regarding the results of the association between ALC and OS/PFS (p > 0.05) and between

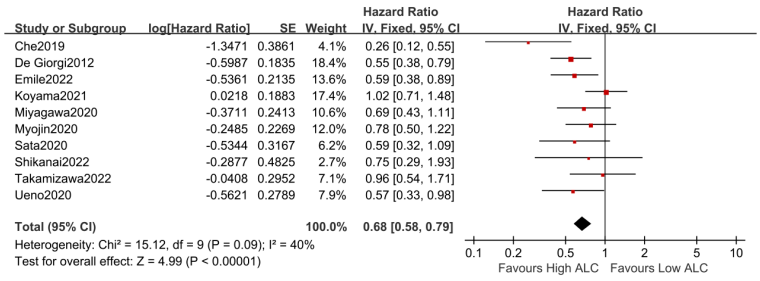

FIGURE 3
Forest plot of ALC for PFS.

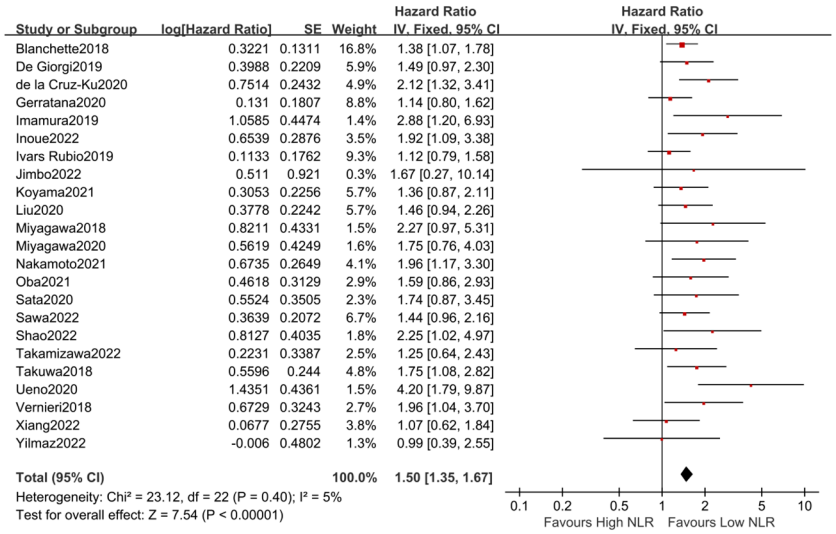

FIGURE 4
Forest plot of NLR for OS.

NLR and OS ($p > 0.05$). However, as for the NLR and PFS, the funnel plot was obviously asymmetric and $p < 0.001$, indicating that potential publication bias probably existed (Figures 7A, B).

4 Discussion

With the increasing incidence and mortality rate of breast cancer, more and more studies are devoted to investigating certain available and highly sensitive biomarkers of diagnosis and prognosis for early screening and prognosis monitoring of patients. If the simple and accessible, non-invasive biomarker in peripheral blood samples can be found to monitor the response to treatment based on the baseline level before treatment, the timely adjustment of the dose of treatment and the combination of drugs may be able to reduce the suffering and financial pressure of the patients. ALC and NLR were previously reported for different cancer types as possible prognostic indicators, but these were only retrospective data and were globally inconclusive according to their heterogeneity. Furthermore, it still remains uncertain as to what extent systemic inflammatory markers are directly involved in immune response, which may require further investigation by conducting additional studies. As the first systematic review and meta-analysis to investigate the prognostic value of ALC and NLR in MBC patients, we found that the low ALC and high NLR were significantly associated with poor prognosis of patients with MBC, particularly in Asian populations, suggesting that these two biochemical markers may act as promising biomarkers for prognosis in human MBC.

As a prognostic marker, NLR has attracted the attention of many researchers in the treatment of early breast cancer and other tumors. A systematic review and meta-analysis of the complete pathological response to neoadjuvant therapy in breast cancer investigated the prognostic value of NLR (50). Although the overall results showed that lower NLR was associated with higher complete pathological response, it did not reach statistical significance in a 5-year disease-free survival (DFS). Furthermore, Xue et al. also failed to confirm the prognostic value of NLR in DFS

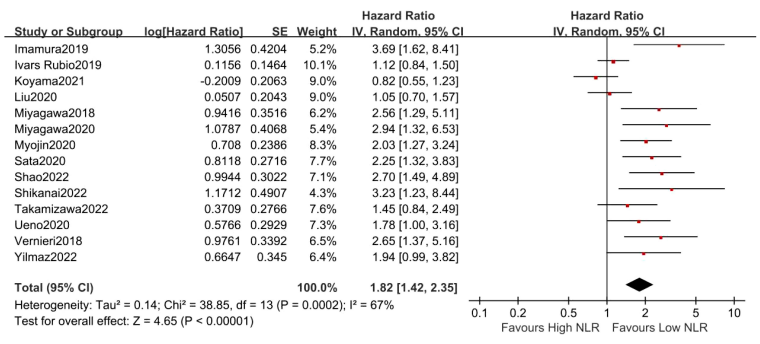

FIGURE 5
Forest plot of NLR for PFS.

10.3389/fonc.2024.1360975

TABLE 3 Subgroup analyses of NLR for OS and PFS.

Subgroups	Independent cohorts	HR (95% CI) (H/L*)	*p*-value	Heterogeneity	
				I^2, %	*p*-value
Overall survival	**23**				
Cutoff value					
>3	3	1.47 [1.19, 1.83]	0.0004	67	0.05
3	8	1.63 [1.33, 2.01]	<0.00001	0	0.89
<3	12	1.44 [1.24, 1.68]	<0.00001	16	0.29
Region					
Asia	17	1.62 [1.40, 1.88]	<0.00001	0	0.61
America	3	1.51 [1.24, 1.85]	<0.00001	17	0.3
Europe	3	1.21 [0.96, 1.53]	0.1	20	0.28
Sample size					
≥100	15	1.46 [1.29, 1.64]	<0.00001	13	0.3
<100	8	1.66 [1.32, 2.08]	<0.00001	0	0.54
Treatment					
Eribulin	4	1.86 [1.35, 2.55]	0.16	42	0.0001
Chemotherapy	3	1.17 [0.65, 2.12]	0.12	53	0.60
CDK4/6 inhibitor plus Endocrine therapy	1	0.99 [0.39, 2.55]	0.99	–	–
Bevacizumab plus Paclitaxel	1	1.75 [0.76, 4.03]	0.19	–	–
T-DMI	1	2.88 [1.20, 6.93]	0.02	–	–
Anti-HER2 therapy	1	1.38 [1.07, 1.78]	0.01	–	–
Tumor subtype					
$HER2^+$	3	1.52 [1.20, 1.92]	0.17	44	0.0005
$HER2^-$	2	1.58 [1.13, 2.22]	0.29	11	0.007
$HR^+/HER2^-$	1	0.99 [0.39, 2.55]	0.99	–	–
Triple negative	2	2.06 [1.41, 3.02]	0.85	0	0.0002
Progression-free survival	**14**				
Cutoff value					
>3	3	1.41 [0.77, 2.59]	0.27	79	0.008
3	5	2.20 [1.68, 2.90]	<0.00001	0	0.49
<3	6	1.81 [1.19, 2.77]	0.006	70	0.0005
Region					
Asia	12	1.88 [1.42, 2.50]	<0.00001	64	0.001
Europe	2	1.63 [0.71, 3.77]	0.25	82	0.02
Sample size					
≥100	9	1.61 [1.19, 2.20]	0.002	69	0.001
<100	5	2.24 [1.67, 3.00]	<0.00001	7	0.37
Treatment					
Eribulin	2	1.60 [1.08, 2.37]	0.61	0	0.02

(Continued)

TABLE 3 Continued

Subgroups	Independent cohorts	HR (95% CI) (H/L*)	*p*-value	Heterogeneity	
				I^2, %	*p*-value
Treatment					
Chemotherapy	2	1.79 [0.80, 4.03]	0.11	62	0.16
CDK4/6 inhibitor plus Endocrine therapy	1	1.94 [0.99, 3.82]	0.05	–	–
Bevacizumab plus Paclitaxel	1	2.03 [1.27, 3.24]	0.003	–	–
T-DMI	1	3.69 [1.62, 8.41]	0.002	–	–
Tumor subtype					
$HER2^+$	2	3.01 [1.86, 4.86]	0.55	0	<0.00001
$HER2^-$	1	0.82 [0.55, 1.23]	0.33	–	–
$HR^+/HER2^-$	1	1.94 [0.99, 3.82]	0.05	–	–
$ER^+/HER2^-$	1	3.23 [1.23, 8.44]	0.02	–	–
Triple negative	1	2.65 [1.37, 5.16]	0.004	–	–

No statistical results were in line.
HR, hazard ratio; CI, confidence interval; H, high; L, low; T-DMI, trastuzumab emtansine.

and OS after neoadjuvant therapy in breast cancer patients, which may be related to the reason that only three studies reporting OS and five studies reporting DFS were included, and the included studies were highly heterogeneous (51). Interestingly, in recent studies, Zhou et al. reported that high NLR was significantly associated with poor prognosis of OS and DFS in patients treated with neoadjuvant therapy (52). Therefore, whether high NLR can be used as a biomarker of poor prognosis on perioperative treatment for early-stage breast cancer still needs further study. Other studies conducted by Cupp et al. (53) and Templeton et al. (54) investigated the prognostic value of NLR in patients with early breast cancer and other cancers and also reached a similar discrepant conclusion. However, these previous studies did not perform detailed analysis on the relationship between NLR and complex MBC. The prognostic value of NLR on MBC was mentioned by Guo et al. (17) in a subgroup analysis, but only three relevant studies were included. Therefore, the prognostic effects of NLR in MBC still need to be further explored by studies with larger sample sizes and a high level of evidence. Thus, we conducted a systematic review and meta-analysis comprehensively studying the relationship between NLR and MBC.

In addition, more evidence indicated that the prognostic value of ALC is mainly presented in the treatment of inflammatory diseases (55–57). There are few studies on the prognostic value of ALC in cancer, most of which are single clinical studies. It was found that lymphopenia increased the risk of death in lung cancer patients treated with chemotherapy and immunosuppressive therapy, and low baseline lymphocyte count was a risk factor for poor survival (58). Feng et al. also reported similar results regarding the roles of ALC in diffuse large B-cell lymphoma (59). Although

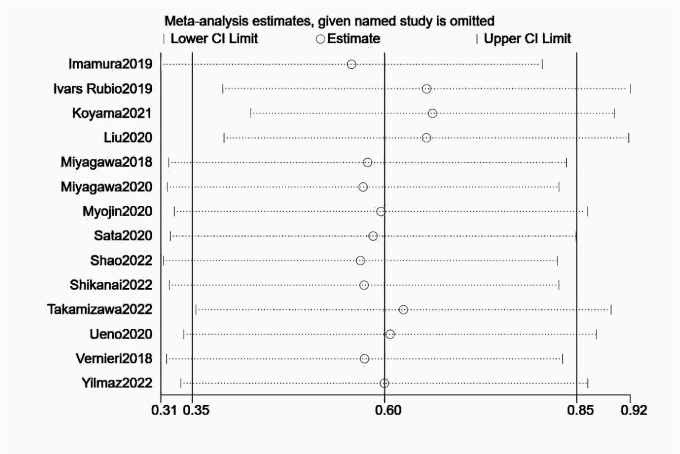

FIGURE 6
Sensitivity analyses of NLR for PFS.

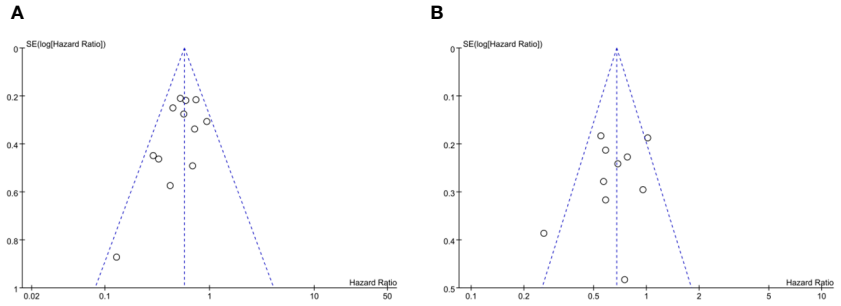

FIGURE 7
Funnel plot **(A)** and Egger's test **(B)** of NLR for PFS.

the types of cancer in the two studies differed from those in the present study, the conclusions concerning low ALC and poor prognosis were consistent. The relationship between ALC and the prognosis of MBC has received much attention and controversy in recent years. However, the prognostic role of ALC in MBC that can provide a reference for patients in clinical practice still needs to be verified. Fortunately, our present study supplies further evidence for this vacancy.

The conclusion that NLR and ALC are associated with the prognosis of MBC is mostly based on clinical evidence, and the mechanisms between them still remain unclear. Some studies believed that inflammatory processes play a significant role in supporting or inhibiting tumor progression and metastasis, and the changes in inflammatory cells are related to the occurrence and development of tumors (60). As an important indicator of inflammatory immunity, the number of neutrophils and lymphocytes can affect the proliferation, angiogenesis, and distant metastasis of tumor cells by secreting related cytokines and chemokines (54). IL-1β (61), IL-6 (62), IL-10 (63), etc. not only lead to epigenetic modifications (methylation of DNA) but also promote the activation of epithelial–mesenchymal transition (EMT), tumor cell homing, and positive feedback amplification of the protumorigenic inflammatory loop between tumor and resident cells (64). Just like this, the individual or mutual ratio of inflammatory immune cells such as neutrophils, lymphocytes, and monocytes has aroused the strong interest of researchers and is considered an important index to evaluate the prognosis of patients with inflammation or tumor (65). Previous studies have explored them as a poor prognostic factor affecting breast cancer patients, but the exact mechanism is still under study.

In our study, all of the included studies were of high quality, and the description of the statistical analysis and results was relatively cautious and objective. The advantages and disadvantages of other similar studies and the trend of current research were discussed objectively, and the conclusions were considered to be stable and reliable in agreement with the findings of this study. This is the first systematic review and meta-analysis to evaluate the relationship between ALC/NLR and the prognosis of MBC, which confirms the prognostic value of ALC/NLR in the treatment of MBC and provides further reference for the clinical research and clinical application of ALC and NLR in the future.

Admittedly, although the process of this systematic review and meta-analysis was strictly controlled, there were still some limitations. First, at the phase of raw data extraction, some studies only reported univariate hazard ratios, which may affect the pooled results of effect sizes and thus cause an overestimation of the conclusions. Second, the prognosis of patients with MBC may be affected by complex and multiple factors, such as cancer subtypes, cancer stages, stage of tumor progress, metastatic organs or numbers, detection methods, treatment options, and regions. The heterogeneous effects of these factors should be further studied when applicable. However, the lack of original data or the limited number of studies makes it impossible to perform subgroup analysis or obtain more valid and robust results based on the subgroup analyses. Third, all studies were comparable based on the baseline characteristics, but the included patients may have multisystem invasion and other underlying inflammatory diseases, which may also affect the level of ALC and NLR. Fourth, statistical heterogeneity was observed in the results on the prognostic value of NLR in PFS, so the clinical application of this part of the conclusion should be considered with more caution. Meanwhile, in future studies, investigators should fully take these limitations into consideration.

In conclusion, low ALC and high NLR were significantly associated with poor OS and PFS according to our results, indicating that ALC and NLR might be potential prognostic markers for patients with MBC. The application of these two simple and accessible, non-invasive and individualized prognostic indicators may provide a better reference on the choice of treatment for patients with MBC in the future. Meanwhile, some high-quality clinical studies with large samples are required to further validate these findings in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

BS: Writing – original draft, Writing – review & editing, Formal analysis, Methodology, Software, Supervision. YF: Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. XW: Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. LD: Data curation, Writing – original draft. YG: Data curation, Writing – original draft. WZ: Supervision, Writing – review & editing. GZ: Supervision, Writing – review & editing. JH: Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The study was supported by the Construction of research platform for early clinical trials of innovative drugs from Yunnan Province Science and Technology Department (No. 202302AA310007).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1360975/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 12 March 2024
DOI 10.3389/fonc.2024.1332783

OPEN ACCESS

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RECEIVED 03 November 2023
ACCEPTED 23 February 2024
PUBLISHED 12 March 2024

CITATION
Zhang J, Li L, Zhang L, Zhe X, Tang M, Lei X and Zhang X (2024) Meta-analysis of dynamic contrast enhancement and diffusion-weighted MRI for differentiation of benign from malignant non-mass enhancement breast lesions.
Front. Oncol. 14:1332783.
doi: 10.3389/fonc.2024.1332783

Meta-analysis of dynamic contrast enhancement and diffusion-weighted MRI for differentiation of benign from malignant non-mass enhancement breast lesions

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Purpose: The objective of this study was to conduct a meta-analysis comparing the diagnostic efficacy of models based on diffusion-weighted imaging (DWI)-MRI, dynamic contrast enhancement (DCE)-MRI, and combination models (DCE and DWI) in distinguishing benign from malignant non-mass enhancement (NME) breast lesions.

Materials and methods: PubMed, Embase, and Cochrane Library were searched, from inception to January 30, 2023, for studies that used DCE or DWI-MRI for the prediction of NME breast cancer patients. A bivariate random-effects model was used to calculate the meta-analytic sensitivity, specificity, and area under the curve (AUC) of the DCE, DWI, and combination models. Subgroup analysis and meta-regression analysis were performed to find the source of heterogeneity.

Results: Of the 838 articles screened, 18 were eligible for analysis (13 on DCE, five on DWI, and four studies reporting the diagnostic accuracy of both DCE and DWI). The funnel plot showed no publication bias ($p > 0.5$). The pooled sensitivity and specificity and the AUC of the DCE, DWI, and combination models were 0.58, 0.72, and 0.70, respectively; 0.84, 0.69, and 0.84, respectively; and 0.88, 0.79, 0.90, respectively. The meta-analysis found no evidence of a threshold effect and significant heterogeneity among trials in terms of DCE sensitivity and specificity, as well as DWI specificity alone ($I^2 > 75\%$). The meta-regression revealed that different diagnostic criteria contributed to the DCE study's heterogeneity ($p < 0.05$). Different reference criteria significantly influenced the heterogeneity of the DWI model ($p < 0.05$). Subgroup analysis revealed that clustered ring enhancement (CRE) had the highest pooled specificity (0.92) among other DCE features. The apparent diffusion coefficient (ADC) with a mean threshold $<1.3 \times 10^{-3}$ mm^2/s had a slightly higher sensitivity of 0.86 compared to 0.82 with an ADC of $\geq 1.3 \times 10^{-3}$ mm^2/s.

Conclusion: The combination model (DCE and DWI) outperformed DCE or DWI alone in identifying benign and malignant NME lesions. The DCE-CRE feature was the most specific test for ruling in NME cancers.

KEYWORDS

non-mass enhancement lesions, meta-analysis, breast cancer, dynamic contrast enhancement, diffusion-weighted imaging

Introduction

According to the Breast Imaging-Reporting and Data System (BI-RADS) MRI vocabulary, "non-mass enhancement (NME)" refers to distribution and internal enhancement that do not meet the requirements for a mass after injecting a contrast medium (1, 2). However, there is a lack of characteristic manifestations of breast NME lesions, and the overlap of various features of non-mass breast cancer (BCa) with benign breast lesions, like fibrocystic and inflammatory alterations, as well as focal adenosis, can be found in malignant lesions such as lobular carcinoma, diffuse invasive BCa, invasive ductal carcinoma, ductal carcinoma *in situ* (DCIS), and, on rare occasions, specific forms of BCa (3, 4).

The descriptors of BI-RADS for NME are limited to morphological enhancement and distribution patterns (5). It can be difficult to distinguish between benign and malignant NME using breast MRI for BI-RADS diagnosis (6). Because MRI-guided biopsies are rarely used, it is common to perform unnecessary or delayed surgery.

Currently, most institutions prefer dynamic contrast enhancement (DCE) MRI to diagnose NME. DCE can reveal morphological features (focal, linear, and segmental), enhancement characteristics (homogeneous, heterogeneous, clumped, and clustered ring enhancement), and kinetic patterns, which are valuable for distinguishing benign NME lesions from malignant NME tumors (6).

The internal enhancing characteristics of NME lesions provide less information about malignancy than do those of masses. NME is the most common presentation for DCIS and non-palpable invasive malignancies (7, 8), despite the prevalence of NME lesions on DCE-MRI being significantly lower than that of masses (76% versus 13%). High-risk lesions, benign disorders such as fibrocystic disease, and hormone alterations have also been connected to NME (9, 10). Consequently, NME cancers present a challenge for breast DCE-MRI.

Diffusion-weighted imaging (DWI) is one functional imaging technique that is gaining popularity as a means of increasing accuracy; it may also prove to be a useful tool for the diagnosis and treatment of individuals with NME lesions. DWI is becoming a routine technique for breast MRI, despite the fact that the apparent diffusion coefficient (ADC) value is not included as a classification indicator in the BI-RADS 2013. Several studies have demonstrated the effectiveness of DWI in the detection of NME lesions (11, 12). DWI with ADC mapping is now advised in conjunction with DCE-MRI within a clinical multiparametric (mp) MRI strategy (13–15). It significantly enhances specificity in distinguishing between benign and malignant breast tumors, avoiding unnecessary breast biopsies (11, 12, 16, 17).

Shao et al. investigated the diagnostic utility of the DCE in NME lesions approximately 10 years ago (18). Many studies have now demonstrated the efficacy of DWI in the diagnosis and treatment of NME breast cancers. They discovered that DWI provided similar but superior diagnostic information to DCE and that DWI could be combined with DCE to improve accuracy (11, 19). It is suspected that mpMRI and DWI are not as effective for distinguishing benign from malignant NME lesions (14). A detailed systematic review would be useful for analyzing the vast amount of information now available.

Therefore, we conducted a meta-analysis to examine the diagnostic efficacy of models based on the DWI, DCE, and combination models (DCE and DWI) in detecting NME cancer.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses—Diagnostic Test Accuracy (PRISMA-DTA) statement (20) was followed in this meta-analysis. The Cochrane Collaboration Diagnostic Test Accuracy Working Group (21) was used to conduct this meta-analysis.

Method for searching the literature

A comprehensive search for published research up to January 30, 2023, was conducted utilizing the PubMed, Embase, and Cochrane Library databases. The search terms were ("Nonmass" OR "Non-mass-like" OR "Non-Mass" OR "non-mass enhancement") AND ("MR imaging" OR "MRI" OR "magnetic resonance imaging" OR "MR") and "breast". The search was limited to original studies written in English or Chinese and published on paper. Furthermore, the references of the included articles were manually searched.

Study selection

Two researchers (L.**. and J.**. with 8 and 6 years of breast MRI experience, respectively) individually assessed the whole texts of any papers that might be qualified after screening the titles and abstracts of the papers that were retrieved. All disagreements regarding potential eligible papers were resolved by a third researcher (L.C.**. with 10 years of experience in breast MRI).

The inclusion criteria were as follows: a) patients: patients with NME lesions; b) index test: breast MRI was used as a diagnostic test for NME lesions; c) comparison: pathological and/or clinical follow-up results; d) outcomes: diagnostic accuracy for differentiating NME benign lesions from cancers; and e) study design: retrospective or prospective trials. We excluded studies without absolute numbers of true positive (TP), false positive (FP), true negative (TN), and false negative (FN) cases; and letters to the editor, case reports, conference abstracts, review articles, meta-analyses, and animal experiments.

Data extraction

The data extracted from each study were the study characteristics (first author; publication year; country; study design; reference standard; sample size; cancer prevalence; percentage of DCIS in all cancers; subtype of malignant lesions; data source; diagnostic criteria; sample inclusion time; TP, FP, FN, and TN numbers), patient characteristics (age, gender, menopausal status, and number of total non-mass-like lesions), and imaging characteristics (MRI pulse sequences, b value, magnetic field strength, position of the patients, and slice thickness). If mean results were reported in the case of multiple reviewers, the results were used for the analyses; if not, the results of the more experienced reader were used (22). The two authors (L.**. and J.**.) extracted the data independently. Inconsistencies between the two researchers were re-evaluated by a third researcher (L.C.**.).

Study quality assessment

Two authors (L.**. and J.**.) independently evaluated the quality of the included studies using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) criteria (23). The final results were based on a consensus discussion.

Statistical analysis

Statistical analyses were performed using R 4.1.2. Meta-analyses were carried out using the bivariate random-effects model (24). Pooled sensitivity and specificity were calculated using 2 × 2 contingency tables. Similarly, the areas under the curve (AUCs) of summary receiver operating characteristic (ROC) curves were estimated. Threshold effects were assessed by determining if the ROC curve followed a "shoulder-arm" distribution. The I^2 statistic, which ranges from 0% to 100%, was used to assess the heterogeneity of study results. An I^2 of more than 75% implies significant heterogeneity between groups (25).

Subgroup analyses and meta-regression were employed to investigate the effects of the heterogeneity-causing factors. Subgroup analyses were performed for studies with different DCE patterns of MR distribution: heterogeneous, clumped, and clustered ring enhancement (CRE), washout, plateau and plateau or washout time-signal intensity curve (TIC), and the different cut-offs of ADC values.

The meta-regression included the following covariates: a) predesign (prospective *vs.* retrospective), b) publication year (before *vs.* after 2013), c) diagnostic criteria [Internal enhancement pattern (IEP) *vs.* others; ADC values $\geq 1.3 \times 10^{-3}$ or $<1.3 \times 10^{-3}$ mm^2/s], d) MR magnet strength (1.5 T *vs.* 3.0 T), e) reference (histopathology *vs.* histopathology or follow-up), f) cancer prevalence ≥50% (yes *vs.* no), g) max b value ≥800 s/mm^2 *vs.* < 800 s/mm^2 or not reported, and h) race (Caucasian *vs.* Asian).

Analysis of publication bias

Visual examination of funnel plots was used to evaluate potential publication bias (26). A *p*-value of less than 0.05 was considered statistically significant.

Clinical utility

The clinical value was tested using a Fagan plot, which yielded the posttest probability (*p* post) of NME when the pretest probabilities (*p* pre, suspicion of NME) for the DCE, DWI alone, and combination models were computed (27).

Results

Literature search

The systematic literature search yielded 838 articles. After removing duplicates, 649 articles were excluded after title screening and abstract review. A total of 106 full-text articles were assessed for eligibility, of which 88 papers were excluded. Finally, 18 studies were included in the meta-analysis based on the diagnostic criteria since they reported sufficient quantitative data. Figure 1 presents the flowchart of the study selection process. Among the 18 included studies, 13 reported on the diagnostic accuracy of DCE-MRI (5, 11, 13, 19, 28–36), nine evaluated DWI-MRI (11, 12, 14–17, 19, 30, 31), and four reported the diagnostic accuracy of both DCE and DWI (11, 12, 14, 19).

Study and patient characteristics

Table 1 shows the baseline characteristics of the included studies. The subjects were all female. Thirteen articles described 38 datasets that used the DCE sequence. Ten studies reported 10 datasets evaluating segmental features as the index test (5, 11, 13, 28–32, 34, 35). Four datasets assessed heterogeneous characteristics

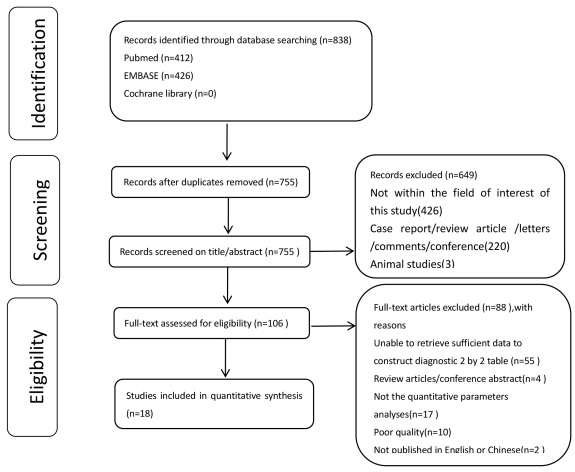

FIGURE 1
The flowchart of the study selection process.

(5, 13, 32, 33), whereas six datasets used clumped characteristics (5, 11, 30–33). Five datasets employed the CRE characteristic (5, 29–31, 36), five datasets were analyzed based on the DCE washout TIC pattern (11, 13, 30–32), and five datasets were examined using the plateau TIC (11, 13, 30–32). Furthermore, six datasets used the washout/plateau TIC (11, 13, 19, 30–32), while four datasets used DCE combined with DWI as the index test (11, 12, 14, 19). Twenty-nine studies (5, 12, 15–17, 28–32, 34, 36) utilized 1.5-T MRI, five studies (13, 14, 19, 33, 35) used 3.0-T MRI, and one study used 1.5-T and/or 3.0-T MRI (26). Half of the included studies were from Asian countries (5, 11, 12, 16, 19, 30, 33–35). To diagnose NME lesions, seven studies utilized pathology or clinical diagnostic criteria follow-up (5, 12, 17, 28, 31, 32, 35), while 11 studies used pathology as a reference standard (11, 13–16, 19, 29, 30, 33, 34, 36).

Quality assessment

The results of the QUADAS-2 assessment are compiled in Figures 2A, B. Of the studies, 11.1% (2/18) in the patient selection domain were scored as "unclear" (12, 34). Two studies did not report consecutive patients. Index test result blinding when interpreting the reference standard was unclear in four studies (13, 16, 19, 28). Index test result blinding when interpreting the reference standard was unclear in three studies (5, 33, 35). Three investigations did not report whether the threshold was pre-specified (5, 17, 28). Because there was inconsistent histopathological analysis used as the reference standard for all included cases, two studies were classified as having unclear risk of flow and timing bias domain (5, 35).

Meta-analysis

Pooled sensitivity and specificity analysis of DCE-MRI, DWI-MR, and combination models

For DCE-MRI (38 datasets in 13 studies), sensitivity and specificity varied considerably across individual studies (0.33–0.96

TABLE 1A The baseline characteristics of the included studies.

Author (year of publication)	Country	Magnet field strength, manufacturer	Reference	Data source	b value (s/mm^2)	Study design	Diagnostic criteria	Enhanced scan sequence and direction	Slice thickness	Position of the patients
Akiko Shimauchi 2015 (5)	Japan	1.5 T, Siemens	Histopathology or follow-up	Single institution	–	Retrospective	IEP; distribution; IEP and distribution	3D T1 fs VIBE, coronal	0.9 mm	–
Andréa Alves Maciel Di Ninno 2021 (28)	Brazil	1.5 T, Siemens/GE	Histopathology and follow-up	Bi-center	–	Prospective	Distribution	3D fs T1 fast spoiled gradient-echo, sagittal	–	Prone
Eduardo de Faria Castro Fleury 2022 (29)	Brazil	1.5 T, Siemens	Histopathology	Single institution	–	Retrospective	IEP; distribution	fs T1-GE, sagittal	–	Prone
Fatma Zeinhom Moukhtar 2014 (15)	Egypt	1.5 T, GE	Histopathology	Single institution	750	Retrospective	ADC threshold 1.35	3D VIBRANT, axial	1.2 mm	Prone
Gang Liu 2022 (30)	China	1.5 T, Philips	Histopathology	Single institution	1,000	Retrospective	IEP; distribution; TIC; ADC threshold 1.3	3D T1-FFE, coronal	2 mm	Prone
Hale aydIn 2019 (31)	Turkey	1.5 T, GE	Histopathology or follow-up	Single institution	–	Retrospective	IEP; distribution; diffusion restriction; TIC; IEP and distribution	2D T1 fs GE, axial	2.8 mm	Prone

(Continued)

TABLE 1A Continued

Author (year of publication)	Country	Magnet field strength, manufacturer	Reference	Data source	b value (s/mm^2)	Study design	Diagnostic criteria	Enhanced scan sequence and direction	Slice thickness	Position of the patients
Hidetake Yabuuchi 2010 (11)	Japan	1.5 T, Philips	Histopathology	Single institution	–	Retrospective	IEP; distribution; TIC; ADC threshold; BI-RADS	3D T1-FFE with WATS, coronal	1 mm	Prone
Isabelle Thomassin-Naggara 2011 (32)	Canada	1.5 T, Philips	Histopathology or follow-up	Single institution	–	Retrospective	IEP; distribution; TIC	fs T1-GE, axial	1 mm/ 1.6 mm	Prone
Jiejie Zhou 2021 (33)	China	3.0 T, GE	Histopathology	Single institution	–	Retrospective	IEP; distribution; BI-RADS; IEP and distribution; DL; ML	3D VIBRANT	1.2 mm	–
K. Pinker 2013 (13)	Austria	3.0 T, Siemens	Histopathology	Single institution	–	Prospective	IEP; distribution; TIC	3D T1-FLASH, coronal; T1-VIBE	–	Prone
Keiichi Sotome 2007 (34)	Japan	1.5 T, GE	Histopathology	Single institution	–	Retrospective	Distribution	2D fs T1-SPGR, axial	5 mm	–
Lijun Wang 2022 (35)	China	3.0 T, GE	Histopathology or follow-up	Single institution	–	Retrospective	DL; distribution; BI-RADS	–	–	Prone
Liuquan Cheng 2013 (16)	China	1.5 T, GE	Histopathology	Single institution	1,000	Retrospective	ADC threshold 1.35	3D VIBRANT, axial	1.2 mm	Prone
Magdalena Lunkiewicz 2020 (36)	Switzerland	1.5 T, Siemens/3.0 T, Siemens	Histopathology	Two institutions	–	Retrospective	IEP; distribution	T1 fat-saturated, axial	1 mm	Prone
Maria Adele Marino 2022 (14)	USA/Italy	3.0 T, Siemens	Histopathology	Single institution	850	Retrospective	IEP; distribution; TIC; ADC threshold 1.305; DCE; DCE+ADC	T1 VIBE, coronal	–	Prone
Sibel Kul 2014 (17)	Turkey	1.5 T, Siemens	Histopathology or follow-up	Single institution	1,000	Retrospective	ADC threshold 0.9	3D fs T1 FLASH	1.5 mm	Prone
Tsugumi Imamura 2010 (12)	Japan	1.5 T, Philips	Histopathology or follow-up	Single institution	1,000	Retrospective	IEP; distribution; TIC; ADC threshold 1.1	3D fs T1-FFE, coronal	2 mm	Supine
Xiaoping Yang 2020 (19)	China	3.0 T, Siemens	Histopathology	Single institution	800	Retrospective	Kinetic curve plateau or washout; ADC threshold 1.235	d T1-weighted gradient echo fast low-angle shot (FLASH) image with fat suppression (FS-CE-T1W-GRE)	1.6 mm	Prone

GE, gradient echo; FFE, fast field echo; VIBRANT, volume imaging for breast assessment; 3D fs T1-SPGR, coronal three-dimensional spoiled gradient-recalled echo pulse; THRIVE, T1-weighted high-resolution isotropic volume examination; FLASH, coronal T1-weighted turbo fast low-angle shot; VIBE T1-weighted volume-interpolated breath-hold examination; FSPGR, fast spoiled gradient-recalled echo.

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TABLE 1B The baseline characteristics of the included studies.

Author (year of publication)	Study design	Sample inclusion time	Age	Preme-nopaus-al	Cancer prevalence	DCIS	IDC with DCIS	IDC	ILC	Muci-nous carci-noma	Apo-crine carci-noma
Akiko Shimauchi 2015 (5)	Retrospective	2009.4–2010.12	49 (29–80)	–	57.3% (86/150)	59.3% (51/86)	–	25.6% (22/96)	3.49% (3/86)	2.33% (2/86)	–
Andréa Alves Maciel Di Ninno 2021 (28)	Prospective	2011.1–2015.7	–	–	61.5% (48/78)	57.9% (22/38)	21.1% (8/53)	15.8% (6/38)	2.6% (1/38)	–	–
Eduardo de Faria Castro Fleury 2022 (29)	Retrospective	2018.1–2021.7	49.3	–	6.25% (6/96)	83.3% (5/6)	–	16.7 (1/6)	–	–	–
Fatma Zeinhom Moukhtar 2014 (15)	Retrospective	2012.7–2013.5	–	–	69.2% (27/39)	77.8% (21/27)	–	22.2% (6/27)	–	–	–
Gang Liu 2022 (30)	Retrospective	2018.3–2021.3	-, (18–70)	–	47.5% (56/118)	57.1% (32/56)	–	42.9% (24/56)	–	–	–
Hale aydIn 2019 (31)	Retrospective	2015.1–2017.12	45.9 (18–79)	–	23.3% (30/129)	–	–	–	–	–	–
Hidetake Yabuuchi 2010 (11)	Retrospective	2006.4–2007.11	55.4 (33–82)	–	68.9% (31/45)	54.8% (17/31)	–	41.9% (13/31)	–	–	3.2% (1/31)
Isabelle Thomassin-Naggara 2011 (32)	Retrospective	2008.1–2009.6	51.4 (28–78)	43.2% (19/44)	47.7% (21/44)	27.3% (12/21)	19.0% (4/21)	6.8% (3/21)	4.5% (2/21)	–	–
Jiejie Zhou 2021 (33)	Retrospective	2017.1–2019.12	–	–	69.3% (104/150) 71.1% (32/45)	42.3% (44/104)	–	53.8% (56/104)	–	–	–
K. Pinker 2013 (13)	Prospective	2007.9–2011.9	–	–	47.2% (17/36)	4.8% (14/209)	–	51.7% (152/209)	9.2% (27/209)	2.4% (7/209)	–
Keiichi Sotome 2007 (34)	Retrospective	2003.5–2005.5	49.4 (24–75)	–	56.3% (18/32)	16.7% (3/18)	–	16.7% (3/18)	38.9% (7/18)	–	–
Lijun Wang 2022 (35)	Retrospective	2014.1–2015.9	48 (40–57)	–	32.2% (68/211)	–	–	–	–	–	–
Liuquan Cheng 2013 (16)	Retrospective	2009.7–2010.5	–	–	68.9% (42/61)	33.3% (14/42)	–	66.7% (28/42)	–	–	–
Magdalena Lunkiewicz 2020 (36)	Retrospective	2011.1–2017.5	52.6 (31.5–91)	–	26.4 (19/72)	31.6% (6/19)	5.3% (1/19)	26.3% (5/19)	15.8% (3/19)	–	–
Maria Adele Marino 2022 (14)	Retrospective	2007.9–2013.7	51.8 (26–76)	–	59.1% (39/66)	10.3% (4/39)	–	61.5% (24/39)	23.1% (9/39)	–	–
Sibel Kul 2014 (17)	Retrospective	2008.8–2012.11	–	–	38.4% (28/73)	–	–	–	–	–	–
Tsugumi Imamura 2010 (12)	Retrospective	2005.8–2006.9	51.5 (27–81)	–	59.26% (16/27)	18.8% (3/16)	62.5% (10/16)	1.23% (2/16)	–	–	–
Xiaoping Yang 2020 (19)	Retrospective	2014.1–2018.9	44.41 ± 10.64 and 45.56 ± 11.96	–	77.19% (281/364)	–	–	–	–	–	–

DCIS, ductal carcinoma *in situ*; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

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FIGURE 2
Methodological quality summary of all included studies by using QUADAS-2 **(A, B)**.

of sensitivity and 0.33–0.95 of specificity), with meta-analytic summary sensitivity and specificity 95% CI of 0.58 (0.50, 0.66) and 0.72 (0.64, 0.78), respectively (Table 2).

For DWI-MRI (nine datasets in nine studies), sensitivity and specificity demonstrated relatively small degrees of variation across individual studies (0.69–0.94 of sensitivity and 0.48–0.81 of specificity). The meta-analytic summary sensitivity and specificity were 0.84 (0.77, 0.89) and 0.69 (0.59, 0.78), respectively.

For DWI combined with DCE (four datasets in four studies), sensitivity and specificity demonstrated relatively small degrees of variation across individual studies (0.79–0.94 of sensitivity and 0.55–1 of specificity). The meta-analytic summary sensitivity and specificity were 0.88 (0.78, 0.94) and 0.79 (0.63, 0.89), respectively. Figure 3 depicts the hierarchical ROC curves of the region-based DCE-MRI, DWI-MRI, and combination model studies. The sensitivity and specificity ROC curves did not demonstrate a threshold effect.

Heterogeneity testing demonstrated that there was considerable heterogeneity among the studies for DCE and specificity of DWI alone ($I^2 > 75\%$). There was no significant heterogeneity ($I^2 < 50\%$) in DCE combined with DWI approaches. The forest plots of all the studies are shown in Figure 4, along with the I^2 score and pooled estimates for each modality.

Subgroup analysis

Based on the DCE and DWI of malignant and benign NME lesions, we performed a subgroup analysis. A subgroup analysis showed the DCE covariates, including segmental, heterogeneous, clumped, CRE, washout, plateau, and washout/plateau features. Distribution features produced the best diagnostic performance for

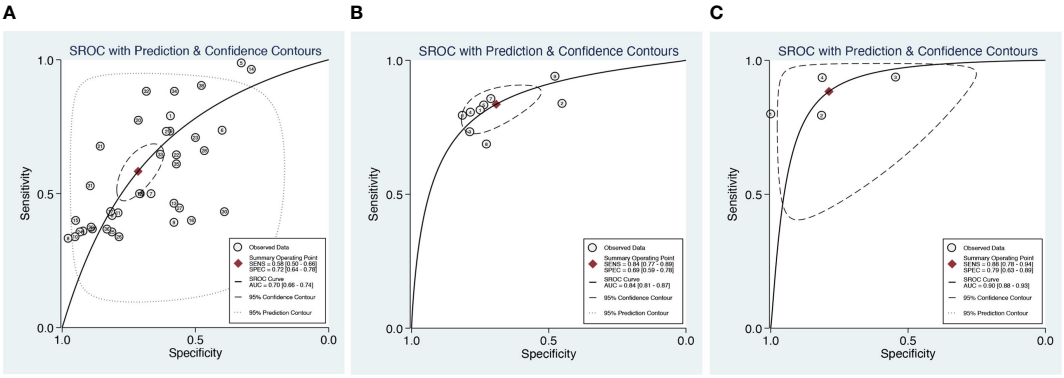

FIGURE 3
Summary receiver operating characteristic (SROC) curves of DCE **(A)**, DWI **(B)**, and combination model **(C)** with 95% confidence intervals.

separating malignant from benign NME lesions with an AUC 95% CI of 0.72 (0.68–0.75), as Table 3 illustrates. Additionally, washout/plateau had the highest pooled sensitivity of 0.84 (0.71, 0.92), while CRE had the highest pooled specificity of 0.92 (0.86, 0.96). DWI subgroup analysis showed that an ADC mean threshold $<1.3 \times 10^{-3}$ mm^2/s had a slightly higher sensitivity of 0.86 (0.74, 0.93) than 0.82 (0.75, 0.87) with ADC $\geq 1.3 \times 10^{-3}$ mm^2/s and similar specificity.

Meta-regression analysis

The DCE and DWI-MRI underwent meta-regression analysis to identify the source of heterogeneity. The meta-regression results are shown in Table 4. For the DCE model, the pooled specificity of studies diagnostic criteria with distribution or TICs 0.74 (0.66–0.83) was higher than that of studies with IEP as diagnostic criteria 0.68 (0.57–0.78). Asians had a higher pooled sensitivity of 0.66 (0.57–0.75) as compared to Caucasians, which had 0.45 (0.33–0.58).

For the DWI MRI, the specificity of studies using histopathology or follow-up as a reference standard with 0.76 (0.64–0.88) was higher than that of studies using histopathology as a reference with a specificity of 0.65 (0.53–0.77). Using the threshold of ADC $< 1.3 \times 10^{-3}$ mm^2/s, the sensitivity and specificity of 0.85 (0.77–0.92) and 0.71 (0.59–0.83), respectively, were higher than 0.82 (0.77–0.92) and 0.66 (0.51–0.81), respectively, with ADC $\geq 1.3 \times 10^{-3}$ mm^2/s as cut-off. Likewise, Asians had a higher pooled sensitivity of 0.86 (0.79–0.92) as compared to Caucasians, which had 0.80 (0.71–0.90). As the number of studies is limited, we did not evaluate meta-regression analysis for the combination model.

Publication bias

Deeks' funnel plot (Figure 5) of the DCE-MRI and DWI-MR demonstrated that there was no publication bias (p = 0.75 and 0.94, respectively).

Clinical utility

As illustrated in Figure 6, we computed the posttest probabilities on Fagan plots for the DWI, DCE alone, and DCE combined with DWI models. In the event of a positive pretest, using the DCE combined with DWI model would increase the posttest probability to 51 from 20% with a positive likelihood ratio (PLR) of 4, while in the event of a negative pretest, it would decrease to 4% with a negative likelihood ratio (NLR) of 0.15.

Discussion

In the present meta-analysis, we investigated the diagnostic value of the combination model (DCE and DWI), DCE alone, and DWI alone in NME breast lesions. We demonstrated that combining DCE and DWI may improve sensitivity and specificity when compared to either DCE or DWI alone. We first systematically assessed the ability of DWI to differentiate between malignant and benign NME lesions, and we discovered that DWI outperformed DCE in terms of diagnostic accuracy and had a comparatively high pooled sensitivity. The findings confirmed the possibility of DWI as a shorter and simpler protocol for breast MRI.

DCE-MRI is a vital technique for identifying NME lesions, as well as internal enhancement models and morphologic characteristics, and is the most important parameter (19). We found that DCE-MRI had moderate sensitivity and specificity of 0.58 and 0.72, respectively, which were similar to the 2013 meta-analysis of Shao et al. (18), who reported that the pooled weighted estimates of sensitivity and specificity were 0.5, and 0.8, respectively. One possible explanation for moderate sensitivity may be that there was no association between malignancy and some of the included DCE features, such as clumped pattern enhancement and homogeneity, distribution type, and clumped pattern combinations (31). Likewise, various studies have found no connection between homogeneous patterns and malignancy (37, 38). Therefore, we used subgroup analysis to find the valuable DCE features.

The subgroup results revealed that the washout/plateau TICs demonstrated the highest diagnostic sensitivity, while the CRE characteristic had the highest diagnostic specificity. CRE is the main feature of intraductal carcinoma of NME, accounting for 72.2% of all malignant lesions in this study, and five studies were not reported. The pathological basis of CRE in intraductal carcinoma is its abundant blood supply. After enhancement, the

TABLE 2 Results of pooled estimates and heterogeneity measures for MRI studies of the NME lesions.

	No. of studies	No. of data	No. of lesions	Sensitivity (95% CI)	Heterogeneity		Specificity (95% CI)	Heterogeneity		AUC	Heterogeneity	
					Cochran Q *p*-value	I^2 (%)		Cochran Q *p*-value	I^2 (%)		Cochran Q *p*-value	I^2 (%)
DCE	13	38	4,223	0.58 (0.50, 0.66)	<0.01	0.9069	0.72 (0.64, 0.78)	<0.01	0.9036	0.70 (0.66–0.74)	<0.01	0.9961
DWI	9	9	844	0.84 (0.77, 0.89)	<0.01	0.661	0.69 (0.59, 0.78)	<0.01	0.7674	0.84 (0.81–0.87)	<0.01	0.9299
Combination model	4	4	193	0.88 (0.78, 0.94)	0.12	0.4859	0.79 (0.63, 0.89)	<0.01	0.4926	0.90 (0.88–0.93)	0.258	0

NME, non-mass enhancement; DCE, dynamic contrast enhancement; DWI, diffusion-weighted imaging; AUC, area under the curve.

matrix around the catheter and the guide tube wall can be improved, and small ring enhancement can be demonstrated when perpendicular to the catheter section (31). When lesions exhibit CRE features, they should be diagnosed as malignant.

Research has shown that TIC is useful in the diagnosis of breast disease (11, 13, 19, 30–32). This finding may be attributed to the presence of varying degrees of arteriovenous shunts in breast tissue, as well as capillary hypertrophy and high endothelial permeability in malignant breast lesions (31, 32). Our findings indicate that plateau curves or washout TICs are highly sensitive but have low specificity in diagnosing NME BCa. Unfortunately, TICs are semi-quantitative analyses, and plateau curves might indicate either malignant or benign lesions, reducing TICs' diagnostic ability.

Furthermore, it is worth noting that the dynamic type is related to the pathological type of breast cancer. Dynamic curves can help distinguish between benign lesions and invasive NME BCa, but they cannot tell the difference between DCIS and benign lesions. Moreover, a washout curve could help differentiate DCIS from invasive NME lesions (31). We were unable to define the pathology type due to the small sample size, and more research is still needed to determine how curve type and pathological type are related.

DWI combined with the quantitative ADC value has been useful in the diagnosis of breast lesions (16, 19, 30). In this investigation, we discovered that DWI and ADC values can help diagnose malignant NME lesions. It appears to have increased sensitivity to NME BCa. However, we cannot recommend ADC thresholds for NME lesions because the ADC values of included studies ranged from 0.9 to 1.35×10^{-3} mm^2/s. Moreover, subgroup results indicated that ADC values with a mean threshold of $<1.3 \times 10^{-3}$ mm^2/s had slightly higher sensitivity than those with a threshold of $\geq 1.3 \times 10^{-3}$ mm^2/s. Similar to our results, previous studies have found an ADC mean threshold of $<1.3 \times 10^{-3}$ mm^2/s, which is considered a suspicious diffusion hindrance level by the EUSOBI DWI working group consensus (39). Unfortunately, more research was not conducted to determine the optimal individual threshold of ADC value for differentiating between benign and malignant NME lesions due to the small sample size and variability.

Our findings revealed that the DWI combined with DCE model outperformed the DCE- and DWI-alone models in discriminating between benign and malignant NME. This result highlights the importance of actively integrating DWI with the morphologic and functional data from DCE-MRI.

In our experience, these sequences often complement one another, thereby reducing erroneous readings. Moreover, breast DCE combined with DWI can provide unique morphological, functional, and molecular information about breast tumors, which may significantly improve the BCa diagnostic level. Additionally, DWI is fast and sensitive, but its spatial resolution is poor, and it cannot observe lesions comprehensively (16). Therefore, it is better to combine it with DCE, which has high spatial resolution.

However, the specificity and sensitivity of included studies range from one another, which can be explained by a variety of reasons such as variances in research group size, use of different BI-RADS lexicons resulting in distinct internal enhancement classifications, and changes in evaluation. Heterogeneity is common in meta-analyses. In our study, the different diagnostic criteria significantly influenced heterogeneity in

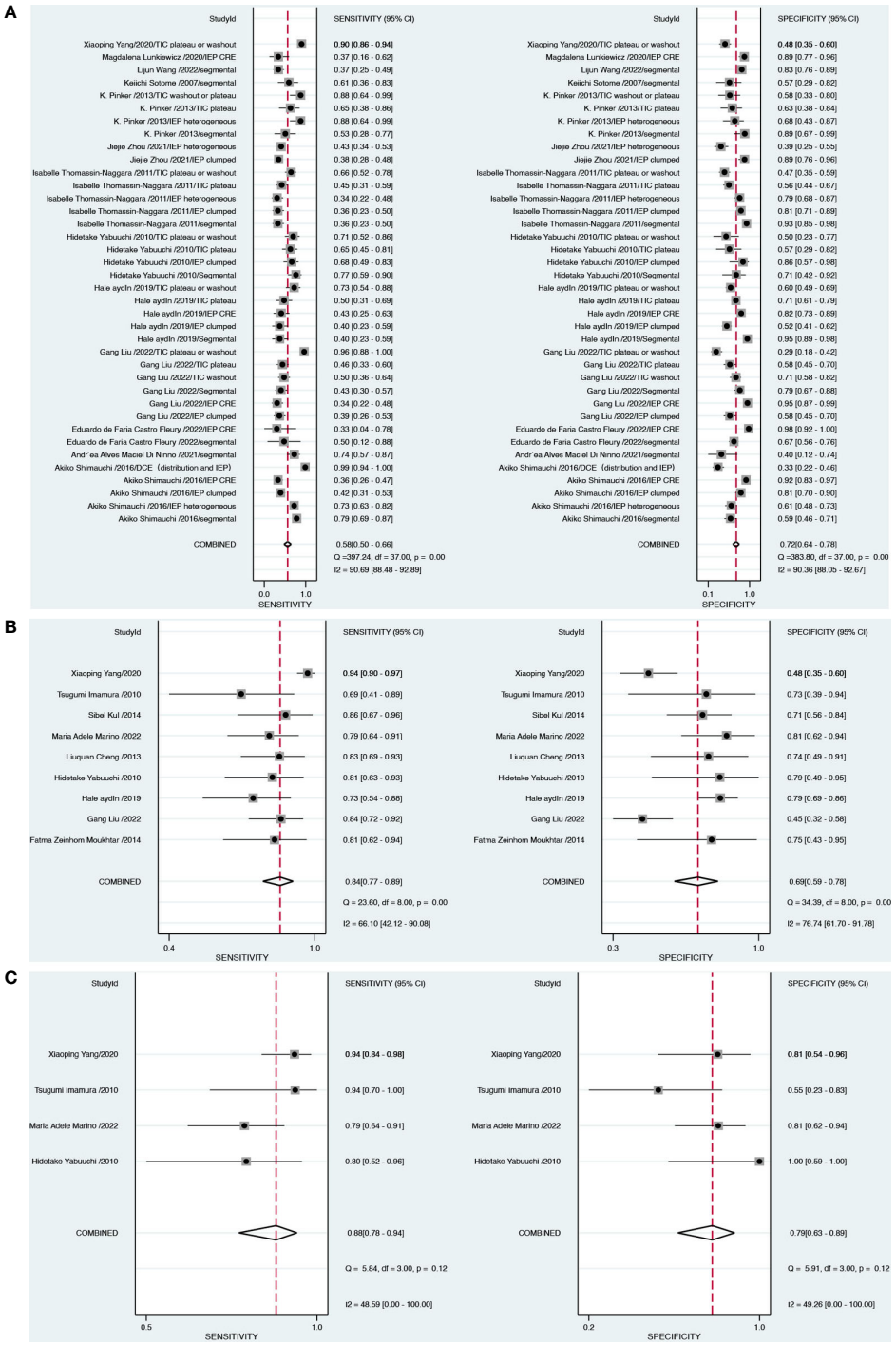

FIGURE 4
Forest plots demonstrate the pooled sensitivity and specificity of DCE **(A)**, DWI **(B)** and combination model **(C)**.

DCE-MRI data. The meta-regression analysis revealed that the DCE model had higher diagnostic sensitivity and specificity in segmental or TIC than in IEP. A previous review reported that IEP clumped pattern enhancement and homogeneity are common in benign lesions (31). As a result, the specificity of studies may be limited. Furthermore, study design can impact research quality and lead to heterogeneity.

In the DWI model, the different reference standard was the source of heterogeneity. There is higher sensitivity in reference to histopathology than histopathology or follow-up, but the specificity was limited. Because the accuracy of the diagnostic test is determined by comparing the results of the index test with those of the reference standard, comprehension of the reference standard may influence the interpretation of the index test findings. However, excluding some benign NME lesions that reference standards based on follow-up, this maybe lead to sample selection bias. In addition, different imaging parameters also create heterogeneity.

TABLE 3 Summary of subgroup analyses: breakdown of malignant and benign NME lesions.

Parameter	No. of studies	No. of data	No. of lesions	Sensitivity (95% CI)	Heterogeneity		Specificity (95% CI)	Heterogeneity		AUC	Heterogeneity	
					Cochran Q *p*-value	I^2 (%)		Cochran Q *p*-value	I^2 (%)		Cochran Q *p*-value	I^2 (%)
Distribution	10	10	996	0.56 (0.44, 0.68)	<0.01	0.8385	0.77 (0.66, 0.86)	<0.01	0.8698	0.72 (0.68–0.75)	<0.01	0.9732
Heterogeneous	4	4	467	0.60 (0.36, 0.80)	<0.01	0.9198	0.63 (0.46, 0.77)	<0.01	0.8517	0.65 (0.61–0.69)	<0.01	0.8929
Clumped	6	6	723	0.42 (0.35, 0.48)	0.06	0.5289	0.76 (0.62, 0.86)	<0.01	0.8901	0.49 (0.44–0.53)	0.004	0.7976
CRE	5	5	565	0.36 (0.30, 0.44)	0.94	0	0.92 (0.86, 0.96)	<0.01	0.7609	0.47 (0.43–0.52)	0.015	0.713
Washout	5	5	459	0.23 (0.12, 0.38)	<0.01	0.8208	0.89 (0.79, 0.94)	0.01	0.7388	0.66 (0.62–0.70)	<0.01	0.9089
Plateau	5	5	459	0.52 (0.44, 0.60)	0.29	0.1965	0.63 (0.55, 0.69)	0.3	0.1861	0.58 (0.54–0.63)	0.493	0
Washout/ plateau	6	6	745	0.84 (0.71, 0.92)	<0.01	0.8513	0.48 (0.39, 0.57)	0.01	0.6699	0.62 (0.58–0.67)	<0.01	0.9049
ADC cut-off ≥1.3	4	4	284	0.82 (0.75, 0.87)	0.94	0	0.68 (0.50, 0.82)	<0.01	0.7871	0.83 (0.80–0.86)	0.031	0.6409
ADC cut-off <1.3	4	4	431	0.86 (0.74, 0.93)	<0.01	0.8154	0.67 (0.52, 0.80)	0.03	0.6766	0.84 (0.80–0.87)	0.001	0.8288

NME, non-mass enhancement; AUC, area under the curve; CRE, clustered ring enhancement; ADC, apparent diffusion coefficient.

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TABLE 4 Univariable meta-regression evaluating the effect of confounding factors on sensitivity and specificity of DCE and ADC.

Parameter	Category	No. of data	Sensitivity (95% CI)	*p*	Specificity (95% CI)	*p*
DCE						
Predesign	Prospective	6	0.49 (0.09–0.89)	0.64	0.85 (0.67–1.00)	0.58
	Retrospective	41	0.59 (0.51–0.67)		0.70 (0.63–0.77)	
Publication year	After 2013	37	0.59 (0.50–0.68)	0.82	0.71 (0.63–0.79)	0.11
	Before 2013	10	0.56 (0.39–0.74)		0.73 (0.60–0.87)	
Diagnostic criteria	Single IEP	19	0.58 (0.45–0.70)	0.45	0.68 (0.57–0.78)	0.02
	Others	28	0.59 (0.48–0.70)		0.74 (0.66–0.83)	
MR magnet strength	Only 3.0 T	8	0.91 (0.75–1.00)	0.05	0.48 (0.01–0.96)	0.3
	Others	39	0.57 (0.49–0.65)		0.72 (0.65–0.79)	
Reference	Histopathology	26	0.59 (0.47–0.71)	0.59	0.72 (0.62–0.82)	0.09
	Histopathology or follow-up	21	0.58 (0.46–0.69)		0.71 (0.62–0.81)	
Cancer prevalence	≥50%	21	0.64 (0.53–0.75)	0.78	0.71 (0.61–0.81)	0.05
	<50%	26	0.53 (0.41–0.64)		0.72 (0.63–0.81)	
Race	Caucasian	21	0.45 (0.33–0.58)	0.02	0.76 (0.67–0.85)	0.31
	Asian	26	0.66 (0.57–0.75)		0.68 (0.58–0.77)	
ADC						
Publication year	After 2013	7	0.85 (0.79–0.91)	0.57	0.67 (0.57–0.77)	0.25
	Before 2013	2	0.77 (0.60–0.93)		0.77 (0.56–0.98)	
Diagnostic criteria	ADC cut-off ≥1.3 × 10^{-3} mm^2/s	4	0.82 (0.74–0.91)	0.01	0.66 (0.51–0.81)	0.18
	ADC cut-off <1.3 × 10^{-3} mm^2/s or not reported	5	0.85 (0.77–0.92)		0.71 (0.59–0.83)	
MR magnet strength	3.0 T	2	0.90 (0.84–0.96)	0.17	0.65 (0.45–0.86)	0.33
	1.5 T	7	0.80 (0.74–0.87)		0.70 (0.59–0.81)	
Reference	Histopathology	6	0.86 (0.80–0.91)	0.3	0.65 (0.53–0.77)	0.04
	Histopathology or follow-up	3	0.77 (0.65–0.90)		0.76 (0.64–0.88)	
Cancer prevalence	≥50%	6	0.85 (0.78–0.91)	0.12	0.73 (0.61–0.85)	0.69
	<50%	3	0.81 (0.70–0.92)		0.66 (0.50–0.82)	
Max b value	≥800 s/mm^2 *vs.*	7	0.85 (0.79–0.91)	0.42	0.66 (0.55–0.77)	0.07
	<800 s/mm^2 or not reported	2	0.78 (0.63–0.92)		0.77 (0.62–0.92)	
Race	Caucasian	4	0.80 (0.71–0.90)	0	0.77 (0.68–0.85)	0.86
	Asian	5	0.86 (0.79–0.92)		0.58 (0.47–0.70)	

DCE, dynamic contrast enhancement; ADC, apparent diffusion coefficient.

This is the first meta-analysis with adequate MRI data to evaluate DWI, DCE methods, and the current combination model for assessing NME lesions. Furthermore, Deek's funnel plot demonstrated the absence of published bias, implying that our findings are reliable. However, there are certain limitations. First, there was significant heterogeneity for both sensitivity and specificity. As a result, we conducted subgroup analysis and meta-regression analysis to investigate the sources of study heterogeneity, as described above. Second, there may have been interpretation bias because six of the included studies used reference criteria ranging from pathological analysis to surgical or biopsy findings to radiological follow-up. Third, the acquisition of various indicators is dependent on multiple imaging parameters (b value, Tesla), which resulted in some variation between investigations. Furthermore, differences in MR sequence input, ground truth, and other variables may influence the results, although this is considered a minor limitation, as most studies had similar source data. To address these limitations, we recommend further large-scale studies to unify the use of b values and DCE parameters in diagnosing NME lesions.

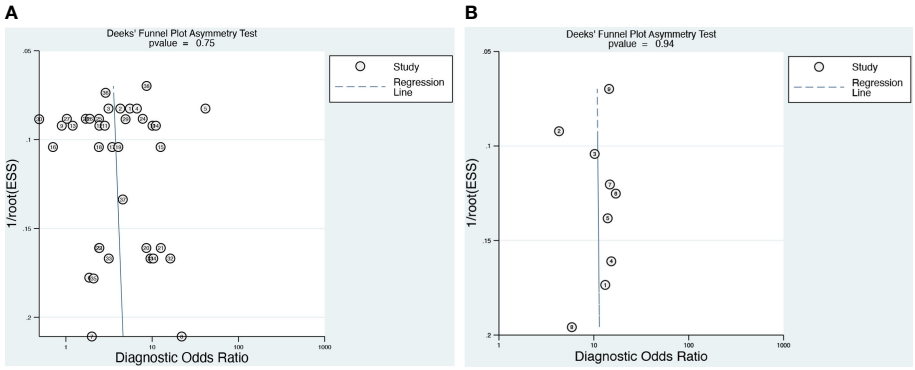

FIGURE 5
The funnel plot of publication bias for DCE **(A)** and DWI **(B)**.

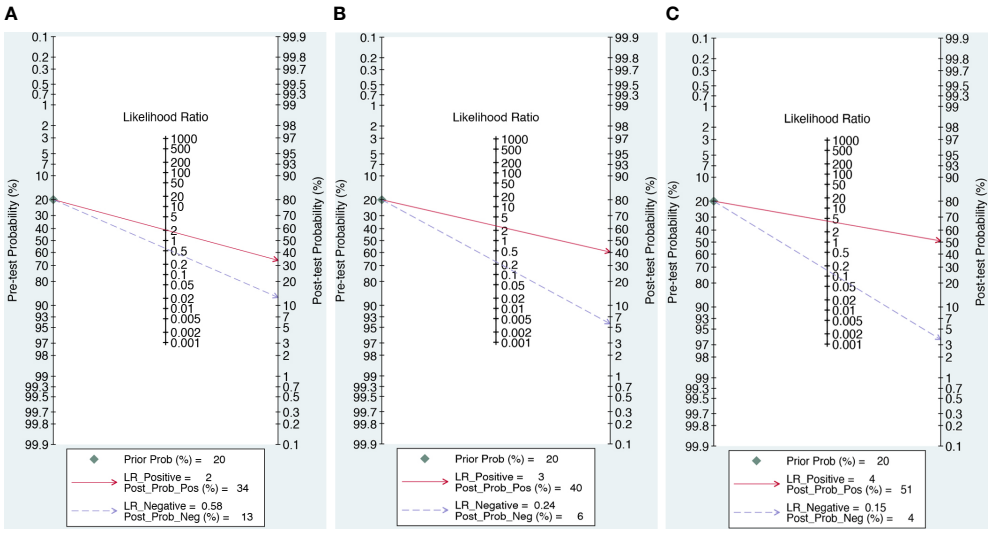

FIGURE 6
The posttest probabilities on Fagan plots for the DWI **(A)**, DWI **(B)** and combination model **(C)**.

In conclusion, our findings showed that the combination model (DCE and DWI) provided extremely good diagnostic performance in distinguishing between malignant and benign NME lesions. DWI has a substantially higher sensitivity for detecting NME lesions than DCE. The DCE-CRE feature was the most specific test for detecting NME cancers. Therefore, further study should combine DWI with DCE to test the accuracy of differentiating cancers from NME benign lesions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

JZ: Software, Writing – original draft. LL: Conceptualization, Investigation, Methodology, Writing – original draft. LZ: Methodology, Supervision, Writing – review & editing. XZ: Data curation, Investigation, Writing – original draft. MT: Formal Analysis, Validation, Writing – review & editing. XL: Methodology, Supervision, Writing – review & editing. XLZ: Formal Analysis, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 12 March 2024
DOI 10.3389/fimmu.2024.1302587

OPEN ACCESS

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RECEIVED 26 September 2023
ACCEPTED 16 February 2024
PUBLISHED 12 March 2024

CITATION
Akinsipe T, Mohamedelhassan R, Akinpelu A, Pondugula SR, Mistriotis P, Avila LA and Suryawanshi A (2024) Cellular interactions in tumor microenvironment during breast cancer progression: new frontiers and implications for novel therapeutics. *Front. Immunol.* 15:1302587. doi: 10.3389/fimmu.2024.1302587

Cellular interactions in tumor microenvironment during breast cancer progression: new frontiers and implications for novel therapeutics

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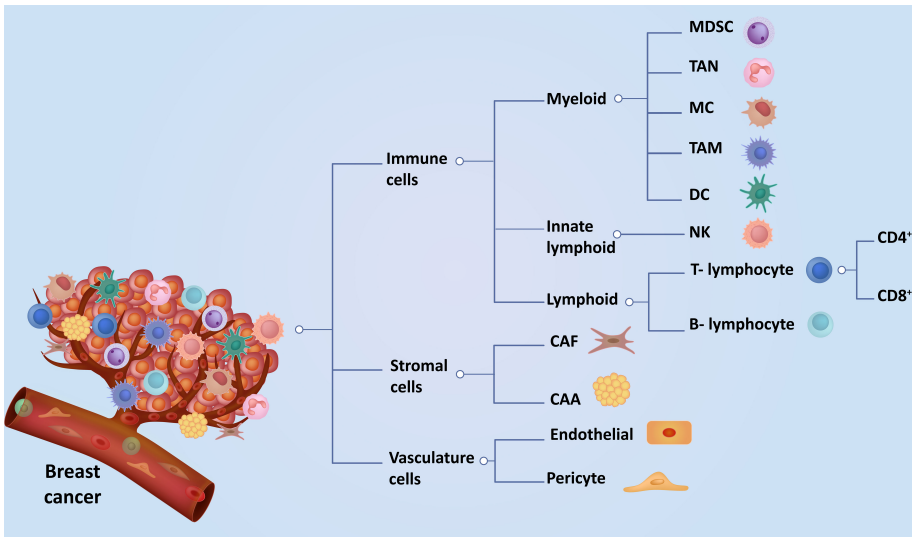
The breast cancer tumor microenvironment (TME) is dynamic, with various immune and non-immune cells interacting to regulate tumor progression and anti-tumor immunity. It is now evident that the cells within the TME significantly contribute to breast cancer progression and resistance to various conventional and newly developed anti-tumor therapies. Both immune and non-immune cells in the TME play critical roles in tumor onset, uncontrolled proliferation, metastasis, immune evasion, and resistance to anti-tumor therapies. Consequently, molecular and cellular components of breast TME have emerged as promising therapeutic targets for developing novel treatments. The breast TME primarily comprises cancer cells, stromal cells, vasculature, and infiltrating immune cells. Currently, numerous clinical trials targeting specific TME components of breast cancer are underway. However, the complexity of the TME and its impact on the evasion of anti-tumor immunity necessitate further research to develop novel and improved breast cancer therapies. The multifaceted nature of breast TME cells arises from their phenotypic and functional plasticity, which endows them with both pro and anti-tumor roles during tumor progression. In this review, we discuss current understanding and recent advances in the pro and anti-tumoral functions of TME cells and their implications for developing safe and effective therapies to control breast cancer progress.

KEYWORDS

tumor microenvironment, stroma, immune cells, breast cancer, metastasis



GRAPHICAL ABSTRACT

Introduction

Breast cancer is the most common and frequently diagnosed cancer in women worldwide, with more than 2 million new breast cancer cases reported annually (1). Globally, breast cancer is the leading cause of cancer deaths in women (2, 3). Based on gene profiling, breast cancer can be classified into five molecular subtypes - Luminal A, Luminal B, Human epidermal growth factor receptor 2 (HER2), Basal-like, and Triple-negative breast cancer (TNBC) (4). Although these classifications are not static, ongoing research may lead to refinements or the discovery of new subtypes. Molecular classification plays a crucial role in tailoring treatment strategies for breast cancer patients, helping oncologists choose the most effective therapies based on the specific molecular characteristics of the tumor (5). Additionally, the TNM staging system proficiently evaluates patients by effectively assessing the extent of the tumor (T), involvement of lymph nodes (N), and presence of metastasis (M). Due to differences in molecular characteristics of breast cancer sub-types, the use of biomarkers, histologic grade, HER2 expression, hormone receptor, and multigene panels have now been incorporated into the conventional TNM staging (6). The tumor microenvironment (TME) of breast cancer plays a central role in tumor progression, immune evasion, and resistance to conventional anti-cancer therapy (7). Breast TME mainly comprises cancer, immune, and stroma cells. Apart from cancer cells, the cellular components of breast TME can be broadly classified as immune cells (myeloid, innate lymphoid, and lymphocytes), stromal cells (fibroblasts and adipocytes), and vasculature cells (endothelial cells and pericytes) (Graphical Abstract). The various cellular components of breast TME exhibit intricate and dynamic interactions that significantly impact cancer progression, metastasis, immunosuppression, and resistance to both conventional and emerging immunotherapies (8, 9). The complex molecular and cellular interplay among the TME constituents provides essential nutrients, oxygen, and growth factors that facilitate efficient tumor cell proliferation and progression (10, 11). The surrounding stroma's cellular, genetic, structural, functional, and epigenetic alterations profoundly impact the plasticity and morphogenesis of epithelial cells, thereby contributing to tumorigenesis (12). Recent breakthroughs and extensive studies from preclinical studies (Table 1) and clinical trials (Table 2) have indicated that alterations in breast TME signatures can serve as valuable prognostic indicators and aid in the development of innovative anti-cancer therapies (27). Consequently, there has been a notable shift towards targeting the key components of the TME in the development of novel treatments (27, 28). In this review, we discuss the current understanding of cancer, stromal, vasculature, and immune cell interactions within the breast TME and their implications for developing novel, safe, and effective breast cancer treatments.

Breast TME

Breast TME is highly plastic and undergoes constant changes and stage-specific adaptations depending on numerous cancer cell-intrinsic and extrinsic factors. These alterations in the TME are characterized by networks of cytokines and growth factors, disrupted signaling pathways, and modified molecular signatures in the stroma (29). Extensive research on TME characterization has highlighted the crucial role of communication between tumor cells and stroma in driving breast cancer oncogenesis, progression, and metastasis (Figure 1) (30, 31). The breast tumor stroma comprises various components, including fibroblasts, immune cells,

TABLE 1 Selected pre-clinical studies showing the suppression of tumor progression by targeting TME-associated cells and effector molecules.

Model	Agent	Target	Antitumor Effect	Ref.
BALB/c mice	Radiotherapy	CXCL16, a chemokine that binds to CXCR6 on Th1 and activated CD8 effector T cells	Increased the migration of $CD8^+CXCR6^+$activated T cells to tumors	(13)
67NR mouse	Combination therapy (Radiotherapy + Immunotherapy)	Immune checkpoints CTLA-4 and PD-L1	Radiation in combination with anti-CTLA-4 and/or anti-PD-L1 blockade stimulates $CD8^+$ T cell-mediated anti-tumor immunity	(14)
4T1 mouse	Combination therapy (Immunotherapy + chemotherapy)	Immune checkpoint	Suppression of MDSCs leads to regression of tumor cells	(15)
BALB/c mice	Administration of the Toll-like receptor (TLR) 7/8 agonist 3M-052	TLR 7/8	Enhances interferon-driven tumor immunogenicity and suppresses metastatic spread in preclinical triple-negative breast cancer	(16)
MDA-MB-231 xenograft	Knockdown of lysyl oxidase (LOX) β-aminopropionitrile (BAPN), miRNA-142-3p	LOX inhibition	Overcome chemoresistance in TNBC	(17)
4T1 tumor-bearing mice	Doxorubicin	DC, $CD44^+$ Cancer stem cells	Immunotherapy	(18)
Mammary tumor-bearing mice	Macrophage recruitment blockade + Paclitaxel	TAM	Reprogram the TME to decrease primary tumor progression, reduce metastasis, and improves survival by $CD8^+$ T-cell–dependent mechanisms.	(19)
4T1-Neu mammary tumor-bearing mice	Docetaxel	MDSC	Docetaxel treatment polarized MDSCs toward an M1-like phenotype	(20)
MDA-MB-231 xenograft	Eribulin	TME vasculature	Vascular remodeling: Improved perfusion Increased microvessel density. Decreased mean vascular areas. Fewer branched vessels in tumor tissues,	(21)
MCF-7 xenograft	Paclitaxel	CAF	Improved local drug accumulation	(22)
BALB/c Neu-transgenic mouse	Local delivery of IL-21	TAM	Identified that abundant TAMs are a major extrinsic barrier for anti-Her2/neu Ab therapy and present a novel approach to combat this extrinsic resistance to skew TAM polarization from M2 to M1	(23)
MCF7 breast cancer cells	B7-H3 Knock-down	DC (B7-H3/CD 276)	Inhibit the proliferation of $CD4^+$ and $CD8^+$ T cells. Inhibit the release of IFN-γ by decreasing mTOR signaling	(24)
KBP-mice	PD-L1 inhibitors	PD-L1	PD-L1 blockade reverts the expression of PD-L1 in macrophages and synergizes with paclitaxel to reduce tumor growth in TNBC	(25)
C57BL/6 mice and nude mice	5-Fluorouracil (5FU)	MDSC	5FU selectively induced MDSC apoptotic cell death leading to IFN-γ production by tumor-specific $CD8^+$ T cells infiltrating the tumor and promoting T cell-dependent antitumor responses in vivo	(26)
MDA-MB-231 xenograft	Capecitabine + Eribulin	TME vasculature	Decreased hypoxia-associated protein expression of VEGF	(21)

endothelial cells, adipocytes, and pericytes (32). Throughout the progression of breast cancer, the stroma undergoes significant changes, including the formation of cancer-associated fibroblasts (CAFs), infiltration of immune cells, inflammation, angiogenesis, and remodeling of the extracellular matrix (ECM) (33, 34). These alterations disrupt the integrity of the basement membrane, facilitating the spread of tumor epithelial cells into the stroma (35). Since these various molecular and cellular components have a direct influence on breast cancer progression, they represent attractive targets for therapeutic development. The immune cells within the breast TME play a critical and dynamic role in cancer progression and anti-tumor immunity (36). Most immune cells are plastic in their functional phenotype and can adapt in response to local TME factors, allowing them to play dual pro or anti-tumor roles (37). Effector immune cells infiltrating the TME can directly eliminate neoplastic cells expressing neo-antigens on their surface and suppress tumor progression (Figure 2) (38). However, tumors employ numerous immune evasion strategies to impede immune cell infiltration and hinder their effector functions within the TME (37). The immune cell repertoire within the breast TME can be broadly classified as myeloid, innate lymphoid, and lymphoid cells. Myeloid cells include myeloid-derived suppressor cells (MDSCs), tumor-associated neutrophils (TANs), tumor-associated macrophages (TAMs), dendritic cells (DCs), mast cells (MCs),

TABLE 2 Selected clinical trials on breast TME-related targeting modalities (https://www.clinicaltrials.gov/).

Strategy/Rationale	Condition	Intervention	Identifier	Outcome measures	Status/ Stage
To quantify CD4 and CD8 in other to identify biomarker changes in the immune microenvironment induced by neoadjuvant chemotherapy	TNBC	Analysis of a list of biomarkers before and after sequential treatment with FEC100 or EC100 then taxane, and paclitaxel weekly	NCT04368468	Identifying biomarkers present in the residual disease would be a criterion to guide the choice of post-neoadjuvant adjuvant systemic treatment, so as to personalize it.	Completed
To evaluate the effects of orally administered reparixin on the TME, cancer stem cell (CSC) markers, and cytokine inflammation markers	HER2- metastatic breast cancer	Fixed dosage of Paclitaxel+ three increasing dosage of Reparixin were used	NCT02001974	Explores the safe dose limit in treating MUC1-positive advanced breast cancer	Phase 1
To evaluate the role of soluble immune checkpoints in predicting the response to neoadjuvant therapy	Breast cancer	Immune checkpoint measurement	NCT05519397	Measurement of sCD25 (IL-2Ra), 4-1BB, B7.2 (CD86), Free Active TGF-β1, CTLA-4, PD-L1, PD-1, Tim-3, LAG-3, Galectin-9	Completed
To differentially compare the breast TME between Luminal A and TNBC with and without Radiation Treatment	Luminal A and TNBC	The mean percent change in TILs in tumor tissue from initial core biopsy samples will be compared with pathology samples from definitive surgery after irradiation between the two different breast cancer sub-types	NCT03165487	Identifying these differences in proteins may allow them to be used in the future as markers to predict the likelihood of tumors recurring.	Recruiting
To determine the clinical response in patients with HER2/neu-positive stage I-III breast cancer and bone marrow micrometastases treated with the drugs of interest	HER2/ neu-positive	Bevacizumab, Trastuzumab, Carboplatin, Docetaxel	NCT00949247	To study how well giving docetaxel and carboplatin together with trastuzumab and bevacizumab works in treating patients with stage I, stage II, or stage III breast cancer and bone marrow micrometastases.	Early Phase 1
To evaluate the T Cell response to a peptide-based vaccine in patients with breast cancer	Breast cancer	Biological: 9 Peptides from Her-2/neu, CEA, & CTA	NCT00892567	To study the concentrations of Persistent Organics Pollutants in both adipose tissue and serum samples from breast tumor patients	Phase 1
To evaluate the impact of single dose versus three doses of Stereotactic Radiation Therapy (SBRT) prior to surgery	Early-stage breast carcinoma	Radiation: Stereotactic body radiation followed by lumpectomy	NCT02212860	Immune priming: (Quantify TILs, PDL-1, neutrophils, and macrophages) Measure angiogenesis (VEGF), proliferation (Ki67), hypoxia (HIF1/ HIF2), and invasion (SDF-1) markers	Completed
To evaluate the effects of MK-3475 (Pembrolizumab) on the breast tumor microenvironment	TNBC	Merck 3475 Pembrolizumab	NCT02977468	To determine if immune modulation therapy with MK-3475 will increase TILs in newly diagnosed TNBC tumors will alter the expression of immune tolerant markers [including PD-L1], within the primary tumor.	Phase 1
To evaluate the effect of Palbociclib plus Letrozole in Hormone receptor-positive residual disease after neoadjuvant chemotherapy	Hormone Receptor (HR) Positive/ HER2 Negative	Palbociclib, Letrozole	NCT04130152	Study the changes in TILs and PDL-1 following treatment with palbociclib plus letrozole, after neoadjuvant chemotherapy	Early Phase 1
To comparatively evaluate the efficacy, safety, and pharmacokinetics of atezolizumab (MPDL3280A) administered with nab-paclitaxel	TNBC	Atezolizumab (an anti-PDL1 antibody) + Nab-Paclitaxel	NCT02425891	Atezolizumab plus nab-paclitaxel prolonged progression-free survival among metastatic TNBC in the intention-to-treat population and the PD-L1–positive subgroup.	Phase 3

etc. Natural killer (NK) are innate lymphoid cells with cytotoxic effector functions and play a crucial role in anti-tumor immunity. Lymphoid cells include B lymphocytes and numerous subsets of T-lymphocytes that play a central role in tumor-antigens-specific anti-tumor immunity (39).

In subsequent sections, we will briefly discuss the interplay of immune and non-immune (stromal and vasculature) cells and how these complex interactions can be strategically targeted to develop novel, safe, and highly effective therapies for breast cancer patients.

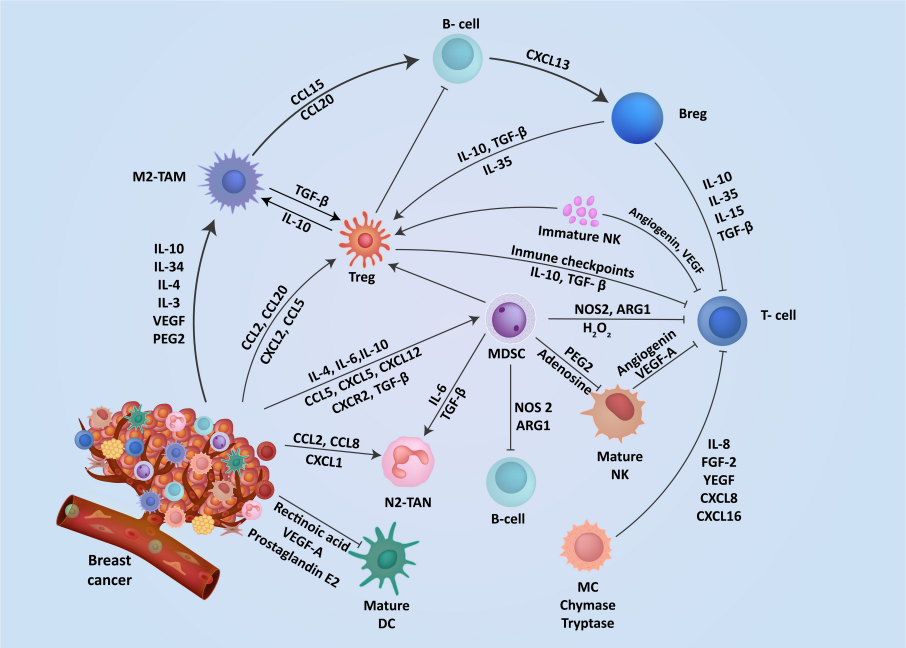


FIGURE 1
The interplay of mediators aid immunosuppression in breast TME. The TME contains a range of resident cells playing a key role in the progression and metastasis of breast cancer cells. These resident cells and their associated secretory elements and receptors including cytokines, chemokines, and stimulatory growth factors are shown. Cells in the TME exhibit a diverse network of mediators that actively engage in promoting an immunosuppressive TME.

Myeloid cells in breast TME

Myeloid-derived suppressor cells

Myeloid cells, derived from hematopoietic stem cells in the bone marrow, play a crucial role in initiating innate and adaptive immune responses (40). However, these cells undergo impaired differentiation during cancer progression, resulting in immature phenotypes with reduced phagocytic capacity and immunosuppressive function (41). MDSCs are prominent cell types in the breast TME that rapidly proliferate and promote tumor progression, angiogenesis, and metastases (42, 43). When activated, MDSCs contribute to immunosuppression and cancer invasiveness through increased

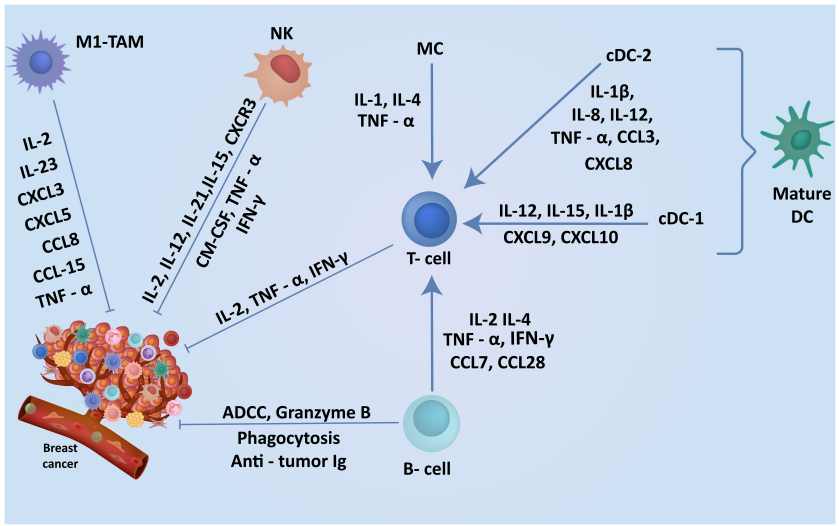


FIGURE 2
Cells in breast TME regulate the induction of robust anti-tumor immunity. The TME contains a range of anti-tumor cells including TILs, DCs and macrophages in the breast playing a key role in the breast cancer suppression. The expression of these cells within the breast cancer TME and understanding their anti-tumor function may enhance the discovery of new markers associated with specific subtypes leading to earlier diagnosis and better clinical outcomes.

production of reactive nitrogen species (RNS), reactive oxygen species (ROS), and arginase 1 (ARG1) expression (44, 45). Human MDSCs in the bloodstream can be classified into two types: granulocytic MDSCs (G-MDSCs) and monocytic MDSCs (M-MDSCs) (46). G-MDSCs are further categorized based on cell surface marker expression as $CD11b^+CD14^-CD66^+$ and $CD11b^+CD14^-CD15^+$. Similarly, M-MDSCs are characterized by the cell surface markers $CD11b^+CD14^+CD15^-$ (47). MDSCs exhibit low expression of Human Leukocyte Antigen–DR isotype (HLA-DR) and CD14, the cell surface receptors essential for proper immune responses to antigens, resulting in an immune response defect (43, 48). The activation and recruitment of MDSCs in the TME is mediated through increased production of specific chemokines, cytokines, and factors, including IL-4, IL-6, IL-10, IL-12, IL-13, CCL5, CCL2, CXCL2, CXCL5, CXCL12, vascular endothelial growth factor (VEGF)-A, transforming growth factor (TGF)-β, and granulocytic-colony stimulating factor (G-CSF) in TME (49–52). These molecules are critical in shaping the tumor microenvironment and promoting MDSC-mediated immune suppression. MDSCs have been found to play a crucial role in the immunosuppressive microenvironment by facilitating the development of $CD4^+Foxp3^+$ regulatory T (Treg) cells and promoting immunosuppressive phenotype in macrophages (53, 54). Additionally, MDSCs express CD40, increasing Treg-mediated tumor immune tolerance (55). CD40, a member of the tumor necrotic factor (TNF) receptor superfamily, is expressed on antigen-presenting cells (APCs), while its ligand (CD40L) is primarily expressed on activated T and B cells (56). The interaction between CD40 and CD40L promotes the development of adaptive immunity (57). When exposed to increased stimulation by IFN-γ, G-MDSCs upregulate the expression of CD40 and MHC II, leading to the induction of Tregs and the suppression of T cell proliferation (55). MDSCs also contribute to angiogenesis, maintain cancer stem cells (CSCs), and inhibit $CD8^+$ T cell activation through the expression of nitric oxide synthase 2 (NOS2) and ARG1 (58, 59). Using microarray analysis, Hix et al. compared the low-aggressive TM40D and highly aggressive TM40D-MD mouse mammary carcinoma cells and discovered a positive correlation between tumor-recruited $CD33^+$ myeloid cells and the progression of human breast cancer from DCIS to IDC (60). Additionally, they found a significant association between $CD33^+$ MDSCs and poor prognosis and worsened overall survival (OS) in the ER^- subtype (61). Furthermore, the transcriptional factor deltaNp63 enhanced the recruitment of MDSCs and correlated with poor prognosis and metastasis in TNBC (62). A pre-clinical study revealed the major role of $CXCR2^+$ MDSCs, a subtype of MDSCs, in breast cancer metastases (63). Moreover, MSDCs indirectly regulate immune response and hinder cancer immunotherapy by interacting with other components of the TME (64). The critical role of TME MDSCs in causing immunosuppression and resistance to cancer immunotherapies during breast cancer progression underscores the need for further comprehensive studies to successfully develop innovative immunotherapies.

Tumor-associated neutrophils

Neutrophils, comprising 50-70% of circulating leukocytes, represent the body's primary defense against infections (65). Additionally, they play a crucial role in tumor progression by infiltrating the TME. The TME regulates the recruitment and polarization of neutrophils, allowing them to develop either an anti-tumor (N1) or pro-tumor (N2) phenotype in response to cytokines present in the TME (66, 67). N1 polarized neutrophils exhibit a robust immune profile characterized by elevated levels of TNF-α, CCL3, ICAM-1, and reduced arginase expression. On the other hand, N2 TANs overexpress several chemokines, including CCL2, CCL8, CXCL1, CXCL2, etc (68). The increased NADPH oxidase activity of N1-like neutrophils leads to the generation of cytotoxic ROS, which can effectively target tumor cells (69). Despite the critical role of neutrophils in the TME (70, 71), further studies are warranted to investigate molecular and cellular networks that drive immunosuppressive phenotype in neutrophils in TME. Studies have demonstrated the preferential migration of neutrophils into specific breast tumor subtypes, such as hormonal negative ductal adenocarcinoma and TNBC (72). Additionally, TGF-β has been shown to promote the N2 phenotype in neutrophils infiltrating the TME (66). TANs in the TNBC TME are a source of proangiogenic factors and matrix metalloproteinase (MMP)-9, a protease crucial in ECM remodeling (73). MMPs and gelatinase B/MMP-9 actively degrade the extracellular matrix, promoting tumor invasiveness and metastasis (74, 75). MMP-9 also contributes to angiogenesis and tumor progression by releasing VEGF-A and inhibiting anti-angiogenic molecules (70). TANs' inability to express tissue inhibitors of metalloproteinase-1 (TIMP-1) enhances the angiogenic potential of neutrophil-derived MMP-9 in the TME, unlike cells expressing the MMP-9/TIMP-1 complexes (76). Recent findings have shown that in the presence of CD90, TIMP-1 expressed by TANs induces epithelial-mesenchymal transition (EMT) in breast cancer, facilitating metastasis (77). As a result, a significant reduction in the spread of cancer has been observed through CD90 blockade (77). Several strategies can be employed to target TANs, including preventing neutrophil migration to tumors, hindering their polarization into N2-type, and targeting neutrophil-associated mediators (71, 78). However, further studies are warranted to characterize TANs' pro-tumoral role during breast cancer progression properly.

Mast cells

MCs demonstrate a vital role in both innate and adaptive immunity. Positioned within epithelial and mucosal tissues throughout the body, MCs effectively regulate various immune and non-immune cell types, including T and B lymphocytes, endothelial cells, fibroblasts, macrophages, and DCs (79). Notably, MCs exhibit a dual function in breast cancer progression (80). Their ability to produce anti-tumoral cytokines, such as IL-1, IL-4, IL-6, and TNF-α facilitates $CD8^+$ priming and maturation (81). Conversely, MCs can assume pro-tumor roles by increasing the production of immunoregulatory molecules, including IL-8, fibroblast growth factor (FGF)-2, TGF-β, VEGF-A, CXCL8, and CXCL16 (82). Such effector molecules released by MCs hinder immunity, degrade the ECM, and enhance tumor vascularization, thus modifying the TME (82, 83).

In the context of breast TME, MCs actively promote cell proliferation, invasiveness, and metastases, ultimately correlating

with a poor prognosis (84). Additionally, MCs are crucial in promoting angiogenesis through secretion of angiogenic cytokines (85). MC stabilizer, disodium cromolyn, has demonstrated its ability to induce an anti-tumor effect by effectively inhibiting the production of VEGF and platelet derived growth factor (PDGF) (86). The infiltration of human MC subpopulations within the TME can be classified based on their expression of the proteases chymase, tryptase, or tryptase-chymase (87). The involvement of chymase and tryptase in ECM remodeling and the production of angiogenic factors highlights their significant role in promoting invasiveness (88). The functions of tryptase and chymase MCs in breast cancer are specific to subtypes. Research by Glajcar et al. revealed a significantly higher presence of the MC tryptase-chymase subset in luminal A and B tumors compared to $HER2^+$ and TNBC, indicating relevance in these subtypes (89). Various studies have also shown the contribution of $tryptase^+$ MCs to tumor progression in TNBC and luminal A breast cancer (89, 90). Although MC stabilizers and protease inhibitors have been successfully used in other cancers, their clinical effectiveness in breast cancer remains uncertain (91). Conversely, a recent report suggested the increased infiltration of MCs is associated with lower tumor grade, reduced tumor proliferation, and decreased HER2 overexpression (92). Further studies are needed to fully comprehend MC function and explore their potential as therapeutic targets in breast cancer.

Tumor-associated macrophages

TAMs are abundant immune cells within the TME (93). Human blood monocytes undergo differentiation into naïve macrophages (M0) and subsequent polarization into M1 and M2 phenotypes mediated by IFN-γ and IL-4, respectively (94, 95). M1 macrophages are highly phagocytic and are associated with $CD4^+$ polarization towards IFN-γ producing Th1 cells (95). M1-like macrophages possess the capability to induce acute inflammatory responses through the production of inflammatory cytokines and chemokines such as IL-2, IL-12, IL-23, TNF-α, CXCL3, CXCL 5, CCL8, CCL15, as well as reactive nitrogen and oxygen intermediates, which exert antitumor effects (96, 97). Resident macrophages play a critical role in host defense (98). However, macrophage populations in TME adapt to an anti-inflammatory, M2-like phenotype (99). The recruitment of TAMs to the TME is promoted by stromal and tumor cells' production of chemokines and growth factors (100). Peripheral blood monocytes derived from the bone marrow are recruited to the tumor site and undergo differentiation into TAMs (101). The CSF is an integral factor in regulating the recruitment of macrophage populations (102). The recruitment of peripheral blood monocytes to the tumor site is facilitated through chemokine receptors expressed on monocytes and chemokine gradient in TME. One such example is the binding of CCL2 to CCR2 and CCR5 receptors on monocytes, leading to monocyte recruitment to the TME (103). Another example is the binding of CCL20 to CCR6 receptors (104).

In breast cancer, the polarization of monocytes to TAMs is influenced by various factors, including tumor-derived factors produced by breast cancer cells and other cells in the TME (105). Monocyte differentiation into TAMs is mediated by VEGF-A and IL-4 (106). M2 TAM differentiation can occur through IL-4 secreted from Th2 cells and IL-10 derived from Tregs (107). IL-10 inhibits the production of pro-inflammatory chemokines by macrophages and promotes the self-polarization of TAMs (108). Furthermore, alternative M2 activation of TAMs is elicited by IL-34 and IL-13 derived from Th2 cells, eosinophils, or basophils (102). These macrophages serve as a significant source of proteolytic enzymes that facilitate the destruction of the ECM and promote neoplastic cell invasion (74).

TAMs contribute to immune evasion by producing IL-10, EGF, and TGFβ (99, 109). The EGF produced by TAMs actively stimulates the proliferation of breast carcinoma cells (110), whereas TAM-produced IL-10 promotes the accumulation of tumor cells at distant sites (111). Furthermore, TGFβ originating from TAMs enables monocyte efflux (112). Also, TAMs facilitate tumor cell growth, angiogenesis, metastasis, and immune evasion by recruiting Tregs (113). TAMs can also establish cancer stem-cell niches, leading to tumor chemotherapy resistance (114). In TNBC, TAMs consistently activate hepatic leukemia factor (HLF) through the IL-6-TGF-β1 axis. HLF transactivates gamma-glutamyltransferase 1 (GGT1), which promotes ferroptosis and cisplatin resistance, ultimately driving malignancy in tumor cells (115). High infiltration of TAMs is associated with a worsened prognosis in breast cancer patients (116). TAMs and DCs play a pivotal role in inducing and regulating effector T cell and Treg responses within the TME, thereby influencing resistance to recently developed immune-checkpoint blockade (ICB) therapies (93). The Wnt/β-catenin pathway is critical for several biological processes (117). However, its dysregulation has been associated with the development of cancer and other diseases. TAMs and DCs activate the Wnt/β-catenin pathway to induce immune tolerance, inhibiting effector T-cell responses and promoting regulatory T-cell responses (118). Consequently, targeting the Wnt/β-catenin pathway holds promise for effective therapeutic interventions in breast cancer (119). Research on TAMs has led to the development of macrophage-focused treatment approaches, which are currently undergoing clinical trials for breast cancer (120). These strategies involve suppressing macrophage recruitment, reprogramming TAMs towards an anti-tumor phenotype, and enhancing macrophage-mediated phagocytosis or tumor cell killing (100, 116).

Dendritic cells

DCs are critical in maintaining immune surveillance and achieving a delicate equilibrium between protective immunity and immune tolerance (121). However, tumors exploit these mechanisms to regulate anti-tumor immunity (122). DCs can be categorized into various subsets based on their location, phenotype, and antigen presentation abilities (123). As professional APCs, DCs are pivotal in initiating and activating anti-tumor T cell responses in tumor-draining lymph nodes and the TME (124). During breast cancer progression, DCs engage in phagocytosis of apoptotic tumor cells, process and present tumor antigens on MHC-I and MHC-II molecules, migrate to local lymph nodes, and present antigens to naive $CD4^+$ and $CD8^+$ T cells to elicit an anti-tumor immune response (125, 126). Additionally, DCs in the TME secrete

chemokines and cytokines that play a crucial role in recruiting and activating effector $CD4^+$ and cytotoxic $CD8^+$ T cells for effective anti-tumor immune responses (127).

Transcriptional profiling has identified specific subsets of DCs in both normal breast tissue and breast TME (128). While the nomenclature and classification of DCs in the TME can be complex, DCs in TME can be broadly classified into three subsets, including plasmacytoid DCs (pDCs), monocytic DCs (moDCs), and conventional DCs (cDCs), which are further classified as cDC-1 and cDC-2 (129, 130). pDCs play a significant role in cross-presenting tumor antigens on MHC-I molecules to initiate cytotoxic $CD8^+$ T Lymphocytes (CTL)-mediated anti-tumor responses (131). pDCs also secrete large amounts of type I interferons (IFN-α/β) (131). Recent studies have shown that pDCs in breast TME promote Treg responses, negatively impacting prognosis and survival rates (132, 133). Additionally, gene expression analysis has revealed that pDC-related genes are among the top genes associated with an increased risk of breast cancer metastasis (134). However, other studies have contradicted these findings, highlighting a better prognosis and increased survival linked to pDCs (135–137). Circulating monocytes can differentiate into moDCs within the TME and are primarily responsible for inducing $CD4^+$ T cell-mediated responses (138). However, the immunosuppressive TME often leads to a tolerogenic phenotype in moDCs, increasing pro-tumor Treg responses (139). cDC-1 and cDC-2 in the TME play a critical role in capturing tumor antigens to activate $CD8^+$ and $CD4^+$ effector T-cell responses (134). cDC-1 can be identified by the expression of markers such as IRF8, BDCA3, BATF3, CLEC9A, and CD103 (140). They produce cytokines (IL-12, IL-15, IFN-β) and chemokines (CXCL9 and CXCL10) to mount a robust immune response (128). In the luminal and TNBC subtypes, cDC-1 has been associated with improved disease-free survival (DFS) and positive patient outcomes through its activation and expansion of CD103, enhancing the tumor response to therapeutic programmed death-ligand 1 (PD-L1) and BRAF inhibition (141, 142). cDC-2 express various markers such as IRF4, CD11b, SIRPα, CLEC10A, and CD1C, and produce IL-1β, IL-6, IL-8, IL-12, TFN-α, CCL3, and CXCL8 to activate anti-tumor T cell responses (143, 144).

DCs have been found to exhibit a dual pro-tumoral and anti-tumoral role depending on the cytokine milieu in the TME and their maturation state (145, 146). For instance, immature DCs support angiogenesis in rapidly growing angiogenic tumors, while mature DCs suppress angiogenic characteristics (147). Additionally, infiltration of mature DCs in primary tumors is associated with reduced metastasis and improved clinical outcomes (148). The increased expression of CD83, a marker for mature DCs, is strongly linked to improved survival in node-positive tumor patients, particularly in TNBC patients with mature $CD11c^+$ (149, 150). Moreover, the presence of $CD83^+$ in the peri-tumoral region of IDC lesions suggests a potential role for mature DCs, while immature $CD1a^+$ DCs are found at tumor edges (151). Previous studies indicate that the immature DC phenotype promotes primary tumor progression to IDC (148, 152). Furthermore, elevated levels of pro-tumor molecules like VEGF-A and prostaglandin E2 in the TME hinder DC maturation, thereby inhibiting T-cell proliferation (153, 154). In response, therapeutic strategies targeting VEGF-A, such as anti-VEGF-A antibodies like bevacizumab, have shown promise in promoting T cell and DC infiltration in TNBC (155). Extensive research has unveiled the pivotal role played by Wnt/β-catenin signaling in the advancement of breast cancer, spanning both tumor cells and immune cells (156). The upregulation of Wnt ligands triggers the activation of canonical β-catenin signaling in DCs, thereby facilitating the generation of IL-10, TGF-β, and retinoic acid (RA) synthesizing enzymes (122, 157). This augmented production of immunoregulatory molecules by DCs within the TME fosters the development of Treg responses, overshadowing Th1 and CTLs (156, 158). These findings underscore the potential of targeting DCs in breast cancer progression as a viable therapeutic strategy, capable of stimulating robust anti-tumor immunity and suppressing regulatory T-cell responses.

Innate lymphoid cells in breast TME

Natural killers

NKs are innate lymphoid cells that can directly eliminate tumor cells by releasing anti-tumor cytokines and cytolytic granules (159). Their development primarily occurs from hematopoietic stem cells (HSCs) in the bone marrow, although other origin sites, such as the thymus and liver, have been proposed (160). Essential transcriptional factors for NK cell precursors include Nfil3, Id2, and Tcf1, while maturation relies heavily on Smad4, Tox, Eomes, Gata3, T-bet, and Runx3 (161–163). Cytokines also play a crucial role in NK cell development and maturation (164–166). For example, IL-7 is responsible for generating $CD122^+$ NK progenitors from HSCs, while IL-15 is critical for the development of NKs from $CD122^+$ NK progenitors into mature NKs (mNKs) (167, 168). Additionally, IL-17 modulates the activities of IL-15 (166). Functionally, mNKs can be differentiated into two major subtypes: $CD56^{bright}CD16^{dim}$ NK subtypes, which make up approximately 90% and are involved in cytotoxicity, and $CD56^{dim}CD16^{bright}$ NK subtypes, which make up the remaining 10% and are responsible for antibody-dependent cell-mediated cytotoxicity (ADCC) (169). Unlike T-lymphocytes, NK cells recognize target cells expressing aberrant cell surface proteins, such as virus-infected or tumor cells, through their Fc receptors (170). The binding of NK cell Fc receptors to antibody-coated target cells leads to targeted killing through ADCC. Moreover, NK cells can eradicate cells that lack or display diminished MHC class I molecules on their cell surface, a common strategy that cancer cells employ to avoid CTL responses (171).

Tumor-infiltrating NK cells engage in immunosurveillance using a combination of activating and inhibitory receptors, effectively identifying and eliminating target cells while sparing healthy ones (172, 173). This process is facilitated by the production of various cytokines and chemokines, including TNF-α, IFN-γ, IL-2, IL-12, IL-21, IL-15, IL-18, CXCR3, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which actively promote anti-tumor immunity (174, 175). Additionally, the receptors on NK cells can selectively target tumor cells by recognizing growth factors like

PDGF, thereby triggering the release of IFN-γ and TNF-α to inhibit tumor growth (176). Although NK cells exhibit anti-tumor capabilities, they can also produce immunosuppressive cytokines that hinder anti-tumor immunity. NK cells secrete angiogenic factors like VEGF-A and angiogenin, which contribute to the progression of breast cancer (177). A recent study uncovered a new mechanism of cancer immune evasion, which involves inhibiting NK cells' cytotoxic granule machinery by chitinase-3-like protein 1 (CHI3L1) (178). This protein, synthesized by tumor cells, plays a significant role in inflammation, tissue injury, and remodeling responses (179). Analysis conducted *in vitro* revealed elevated levels of CHI3L1 in the sera of trastuzumab-resistant patients compared to responders (178). CHI3L1 inhibits NK cell cytotoxicity and ADCC by disrupting the cytotoxic machinery, preventing lytic granule polarization to the immune synapse, and hindering downstream JNK signaling, a crucial process for cancer cell apoptosis (180). Furthermore, administering CHI3L1 *in vivo* weakens the control of NK cell-sensitive tumors while blocking CHI3L1 in conjunction with ADCC effectively treats $HER2^+$ xenografts in mice (178).

NK cell exhaustion has been observed in an immunosuppressive TME and characterized by reduced activating receptors, decreased production of effector cytokines (181), impaired signaling/transcriptional pathways, hypoxia (182), low pH (183), upregulation of inhibitory receptors like NKG2A, TIM-3, PD-1, TIGIT, LAG-3, KIR (184, 185), and the presence of Tregs (186), Bregs (187), and MDSCs (188). This NK cell exhaustion phenotype presents a significant obstacle to developing NK cell-targeting immunotherapies. However, new strategies are being developed to combat NK cell exhaustion and enhance their anti-tumor function. For example, IL-21 treatment increases IFN-γ and granzyme B levels through $Tim\text{-}3^+PD\text{-}1^+$NK cells, reversing NK cell exhaustion (189). This highlights the potential therapeutic approach of using IL-21 to restore NK cell immunity function (190). In addition, IL-15 plays a crucial role in NK cell proliferation and survival (191). However, repetitive exposure to IL-15 during cancer treatment can diminish viable cell cycle signaling, decreased tumor control, and reduced fatty acid oxidation, resulting in NK cell exhaustion (192–194). Alternatively, an immunotherapy with membrane-bound IL-15 (mbIL15) is proposed (193, 195). By linking the human IL-15 gene to the CD8α transmembrane domain gene, mbIL15 can be created. NK cells expressing mbIL15 have been shown to activate cell cycle signaling and exhibit higher cytotoxicity against leukemia, lymphoma, and sarcoma *in vitro* and *in vivo* mouse xenograft tumor models (193). Expression profiling of NK cells can help identify dysfunction and exhaustion markers relevant to each breast cancer subtype. However, further studies on NK cell exhaustion in breast cancer are necessary.

Moreover, TME has demonstrated the capacity to modify the functionality and phenotype of NK cells (196). In a recent study by Mamessier et al., the dysfunctional tendencies of tumor-infiltrating NK cells in invasive and non-invasive breast cancer were characterized (197). Their findings unveiled a gradual reduction in the expression of NK cell activating receptors, such as NKp30, NKG2D, DNAM-1, CD16, CD226, and 2B4, as breast cancer progressed. Conversely, there was an upregulation of the inhibitory receptor NKG2A, which diminishes NK cell cytotoxic function and evasion of NK cell-mediated anti-tumor immunity (197, 198). Another study revealed a decline in the levels of NKp46, a lysis receptor responsible for direct tumor cell elimination, within the TME compared to normal cells (199). Immunotherapies targeting NK cells encompass various strategies to improve their activity, including promoting ADCC with mABs (200), blocking inhibitory signals (201), utilizing cytokines to augment NK cell proliferation and cytotoxicity through CAR NKs (202), IL-15 (203), and adoptive transfer of NK cells (204). In recent years, adoptive cell therapy strategies have emerged as a promising approach for utilizing NK cells (205). These immunotherapies entail the isolation, activation, and expansion of immune cells, which are then reintroduced into patients to combat tumor cells. A noteworthy application of this technique involves equipping NK cells with cancer-targeting CARs (206). However, the potential of engineered NK cells is hindered by immunometabolism limitations caused by factors such as hypoxia and cytokine stimulation in the TME (194, 207). Further studies are needed to understand how NK cell immunometabolism in TME regulates their anti-tumor properties.

Lymphoid cells in breast TME

T- lymphocytes

Tumor-infiltrating lymphocytes (TILs) in TME regulate the induction of robust anti-tumor immunity, immunosuppression, efficacy of ICB therapy, cancer metastasis, and resistance to novel combinational ICB therapies (208). The TILs found in the TME primarily consist of CTLs, B cells, NK T cells, and $CD4^+$ T helper cell subsets, including IFN-γ-producing $CD4^+$ (Th1) cells, IL-4-producing $CD4^+$ (Th2) cells, $Foxp3^+CD4^+$ regulatory T cells (Tregs) (209). Recent advancements in sub-type classification of TILs, using techniques such as flow cytometry, genomic approaches (single-cell RNA-seq, 10X genomic sequencing), and ICB therapies targeting T cells, have resulted in an increased emphasis on identifying TILs and potential immunological prognostic biomarkers specific to different subtypes of breast cancer (210, 211). Despite the ability of Th1 and CTLs to stimulate strong anti-tumor immunity, the TME employs various immune evasion strategies to suppress the infiltration, activation, and effector functions of CTLs and Th1 cells, inhibiting host anti-tumor effector responses. One extensively studied mechanism involved in this process is the upregulation of inhibitory receptors on T cells and higher expression of inhibitory ligands by tumor cells and APCs within the TME (212). APCs define the T cell differentiation and activation through tumor antigen presentation on MHC molecules to T cell receptors (TCR), expression of CD80 and CD86 ligands, which bind to co-receptors (such as CD28, ICOS, PD-1, CTLA4), and secretion of specific cytokines that define the fate of T cell differentiation (213). These co-signaling receptors can stimulate or inhibit T cell activation and effector functions. Examples of inhibitory receptors include PD-1, CTLA-4, LAG3, and TIM-3 (214). These receptors are crucial in maintaining immune balance and preventing excessive T cell activation during infections. However, tumors

highly promote the expression of co-inhibitory receptors on T cells in TME to promote immune evasion. PD-1, for instance, binds to PD-L1 or PD-L2 ligands expressed by various immune cells or cancer cells to facilitate immune evasion (214). PD-1 possesses an inhibitory immunoreceptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based switch (ITSM) motif in its cytoplasmic tail (215). When T cells engage with tumor cells and APCs, PD-L1 phosphorylates ITIM/ITSM, resulting in the recruitment of TCR-phosphorylating kinase, cytosolic tyrosine phosphatases (SHP-1 and SHP-2), and the inhibitory tyrosine kinase (216). As a result, the PI3K/Akt and Ras/MEK/Erk pathways necessary for initiating T cell activation are weakened. Recent research has shown the potential of blocking the PD-1/SHP-2 interaction as a novel approach to PD-1 inhibition (217). Accordingly, several monoclonal antibodies (mAbs) targeting PD-1 (pembrolizumab and nivolumab) and PD-L1 (atezolimumab) interaction have received FDA approval for the treatment of various lethal cancers including metastatic melanoma, Hodgkin's lymphoma, head and neck squamous cell carcinoma, and breast cancer, among others (218).

In breast TME, infiltrating T cells demonstrate an upregulation of PD-1, while APCs (DCs and macrophages) and tumor cells exhibit higher expression of PD-L1 (219). The expression of PD-1 on $CD4^+$ TILs is correlated with the invasiveness of breast cancer (220). Moreover, recent studies have shown decreased $CD4^+$ and $CD8^+$ T lymphocyte infiltration in DCIS and IDC breast cancer subtypes (221). These findings suggest that the reduced number of T lymphocytes in TME contributes to the transition of TNBC and $HER2^+$ cancer subtypes from DCIS to IDC, resulting in a poor prognosis and worsened overall survival (OS) (222). Another recent study revealed the efficacy of CD3-HAC, a bifunctional fusion protein engineered to target EA1-mesenchymal stromal cells against metastatic breast cancer (223). CD3-HAC specifically binds to PD-L1-positive tumor cells to attenuate the impact of PD-1/PD-L1 on T cells exposed to MDA-MB-231, leading to enhanced T cell activation and stimulated lymphocyte-mediated lysis both *in vitro* and *in vivo* (223). In addition to immune evasion, the heightened expression of PD-1 on T cells indicates T cell exhaustion. $CD8^+$ T cell exhaustion was initially identified in mice infected with chronic lymphocytic choriomeningitis virus (LCMV) infection (224). In this condition, the chronic presence of viral antigens constantly activates and stimulates $CD8^+$ T cells, resulting in a decline in their effector functions (224, 225). In the TME, immune cells experience continuous stimulation from tumor antigens (226). Consequently, their metabolism and transcription profile change, ultimately leading to functional exhaustion (227). Immune cell exhaustion in TME is characterized by persistent tumor antigens stimulation, reduced proliferation capacity, enhanced inhibitory receptor expression, and decreased production of effector cytokines such as IL-2, TNFα, or IFN-γ (228).

In a comprehensive cohort study of breast cancer patients, it was discovered that despite the prevalence of T lymphocytes in IDCs, a significant portion of T cells exhibited reduced activity or were inactive due to exhaustion. These exhausted T cells displayed heightened expression of co-inhibitory receptors, PD-1 and CTLA-4, and diminished levels of active anti-tumor T cell subsets, CD62-L and CD127 (229). Phenotyping and functional analysis studies unveiled a distinctive T cell differentiation subset associated with exhaustion (230). It was observed that the underlying transcriptional mechanisms differed between effector T cells and exhausted T cells (231). This distinction was reflected in the expression of phenotypic markers, with effector $CD8^+$ T cells exhibiting high levels of CD44 and killer cell lectin-like receptor subfamily G member 1 (KLRG1), while exhausted T cells displayed low or intermediate levels of these markers (232). Conversely, inhibitory receptor markers were highly expressed on exhausted T cells compared to effector $CD8^+$ T cells (231). Additionally, exhausted T cells exhibited disparate expression of the transcription factors EOMES and T-bet, whereas effector $CD8^+$ T cells expressed both simultaneously (233). The TME plays a critical role in inducing functional exhaustion in $CD8^+$ T cells by promoting the cell surface expression of CD39, an immunosuppressive molecule (234). $CD39^+CD8^+$ T lymphocytes displayed an exhausted phenotype characterized by reduced production of IFNγ, TNF-α, and IL-2 and increased expression of co-inhibitory receptors such as PD-1 and CTLA-4. Targeting $CD39^+$ appears promising in restoring T cell function and as a potential therapeutic intervention (234, 235). Revitalization of exhausted $CD8^+$ T lymphocytes can be achieved through the inhibition of PD-1:PD-L1 interaction (236), CTLA-4 (237), and LAG-3 (238). Clinical studies that block the PD-1/PDL-1 inhibitory pathway to restore $CD8^+$ T cell ability to proliferate and carry out its cytotoxic functions have been reported in other cancers. For instance, while pembrolizumab and atezolizumab are effective PD-1/PDL-1 inhibitors in second-line advanced non-small cell lung cancer (NSCLC), avelumab and durvalumab were effective in late-phase clinical testing (239). Another clinical study proposed donor lymphocyte infusion (DLI) targeting T cell exhaustion in hematology malignancy (240). In a clinical study, their findings reveal that patients who received DLI had a significant increase in CD8 cell counts, while the levels of CD4 T cells and B cells remained unaffected, indicating the potential of DLI to reverse $CD8^+$ T cell exhaustion (241). However, the use of DLI alongside other T cell exhaustion revitalization methods has been suggested (242). While research on T cell exhaustion in breast cancer subtypes remains limited, future investigations aimed at revitalizing exhausted T cells and enhancing active T lymphocyte proliferation hold immense potential for the development of safe and effective immunotherapies against breast cancer.

The presence of regulatory T lymphocytes (Tregs), specifically the Foxp3 expressing subtype, is associated with a negative prognosis in breast cancer patients (243). Tregs express co-inhibitory receptors such as PDL-1, CTLA-4, and PD-1, which promote local immunosuppression and contribute to the spread of breast cancer (244, 245). Targeting Tregs can lead to a breakthrough in immunotherapy. Current strategies developed to inhibit Tregs' harmful impact in the TME include inhibiting their recruitment, favoring their transformation into effector $CD4^+$ T-cell subsets, blocking their expansion, depleting Tregs, and impeding their suppressive function (246). Further research and clinical trials are needed to fully understand the dynamics of T cell exhaustion and explore the use of combination therapies that can enhance T cells' effector and cytotoxic functions.

B- lymphocytes

B lymphocytes are primary mediators of humoral immunity. In the induction of adaptive immunity, B cells stimulated by antigens, along with the assistance of helper T cells, undergo differentiation into antibody-secreting plasma cells, initiating adaptive immune responses (247). In tumors, B lymphocytes are commonly found in the lymph nodes and invasive margins (248). Their impact on tumor onset and progression can be positive, negative, or passive (249). Upon activation by antigens, B cells undergo differentiation into antibody-secreting plasma cells. There are five subtypes of human immunoglobulins (Ig): IgG, IgA, IgM, IgD, and IgE (250). Among these five Ig types, IgG accounts for approximately 75% of the antibodies found in human serum (251). Despite being highly preserved, IgG is classified into four, namely IgG1 – IgG4, which exhibit varying effector functions based on their interaction with Fcγ receptors (FcγR) (252). Activation of FcγR-expressing cells triggers ADCC and phagocytosis of tumor cells (253). Conversely, when expressed by tumors, IgG-FcγR interaction can promote tumor progression (254, 255). A study conducted by Ma et al. revealed an abundance of IgG-expressing cancer cells in 68 breast cancer cases, encompassing 40 primary cancers and 28 metastatic cancers (256). Their findings demonstrated that IgG-expressing breast cancer cells exhibit more aggressive biological behavior, indicating the progression and metastasis of breast cancer. Moreover, the formation of circulating immune complexes (CICs) from the Ag-Ab complex can activate FcγR on myeloid cells, leading to the generation of MDSCs (257). These MDSCs effectively suppress the anti-tumor function of $CD4^+$ and $CD8^+$ T cells (42). B cells actively induce tumor cell apoptosis by producing granzyme B, a potent cytolytic molecule (258). These granzyme B-producing B cells can perform vital effector and regulatory functions during immune responses (258). Notably, granzyme B derived from carcinoma sources has been observed to effectively eliminate tumor cells *in vitro* (259). However, it is worth noting that the presence of granzyme B in breast tumor tissue can degrade the TCR-zeta subunit in the TCR, thereby impeding TCR assembly, expression, and anti-tumor signaling. This phenomenon occurs particularly in continuous antigen exposure and chronic inflammation (260). Moreover, the production of cytokines such as IL-2, IL-4, IL-6, IL-7, IFN-γ, IFN-α, TNF-α, CCL7, and CCL28 can stimulate an anti-tumor response (261, 262). These vital molecules are crucial in B cell maturation, differentiation, and survival (261). Notably, CCL28 and CCL27 direct the migration of plasma cells to mucosal sites during breast cancer anti-tumor response, correlating with improved prognosis (263). Conversely, other chemokines produced by B cells like CCL5, CCL20, and CCL1 are known to attract TAMs, Tregs, and MDSCs and induce EMT in breast cancer cell (264).

The immunosuppressive B cell subtype, Bregs, produce IL-10, IL-35, IL-15, and TGF-β cytokines that suppress $CD8^+$ T-cell cytotoxicity, Treg recruitment, and M2/Th2 polarization (265–268). A study with a mouse 4T1 model of breast cancer demonstrated that the secretion of IL-10 by B lymphocytes acts in a TGF-β-dependent manner to promote the conversion of naive $CD4^+$ T cells to $Foxp3^+$ Tregs (269). Also, a chemokine, CXCL13, functions to recruit B cells to TME, where they differentiate into Bregs and stimulate EMT in tumor cells (270). A study showed that nano-trapping CXCL13 reduces Bregs differentiation, leading to prolonged cancer-free survival (271). Bregs-specific phenotype PD-1-PD-$L1^+CD19^+$ has been reported to exert the greatest suppressive effects on T effector cells (272). Two separate groups, Campbell et al. and Miligy et al. in 2017, revealed that B cells with phenotypes $CD19^+$, $CD24^+$, and $CD38^+$ were correlated with increased tumor proliferation and risk of recurrence in breast cancer subtypes ER^-, PR^- and $HER2^+$ (273, 274). The findings from their studies also suggested that $CD20^+$ is a prognostic marker for better patient outcomes. Conversely, numerous studies have shown that $Foxp3^+$ Tregs also express $CD20^+$ and can be indicative of poor prognosis in breast cancer (273–277). Hence, conducting in-depth research to accurately define and differentiate the $CD20^+$ anti-tumor role in B cells and the pro-tumor role in T cells is necessary.

Stromal cells in breast TME

Cancer-associated fibroblasts

CAFs are heterogenous cells that demonstrate their significance in various aspects of breast cancer, including growth, metastasis, response to treatment, and resistance to anti-cancer therapies (278). These cells derive from a range of sources, including normal fibroblasts, myofibroblasts, mesenchymal cells, stellate cells, fibrocytes, pericytes, smooth muscle cells, preadipocytes, or bone marrow-derived cells (279). Additionally, recent research by Flores et al. has identified $CD34^+$ stromal cells/telocytes as another origin of CAFs, particularly in the invasive lobular carcinoma (ILC) subtype (280). Throughout tumor progression, CAFs contribute to the production of crucial structural proteins like elastin and collagen type I-V, which are involved in basement membrane formation (281), inflammation (282), epithelial differentiation (283), and angiogenesis (284). Moreover, CAFs produce MMPs, which are responsible for the degradation of the ECM and play a role in ECM homeostasis (285). Increased proliferation and secretion of growth factors, immunomodulatory factors, and ECM proteins have also been observed in CAFs and linked to their role in breast cancer (286). These CAF-specific markers can be used to identify breast cancer biomarkers and hold significant importance in diagnosis, prognosis, and the development of novel therapeutic approaches against breast cancer (287, 288). CAF biomarkers are not exclusive to CAFs, thereby requiring a comprehensive characterization to accurately define CAFs. Notably, biomarkers such as α-SMA, vimentin, desmin, cadherin-11, integrin α1β1, and MMPs are utilized for identifying CAFs originating from myofibroblast (286). However, there remains a lack of clear understanding regarding the detailed characterization of pro-tumor phenotypes of CAFs and their associated biomarkers. Initial studies employing SNP array analyses, multi-gene sequencing, and whole exome sequencing have reported the absence of somatic mutations in CAF phenotypes (289, 290). Subsequent findings have suggested somatic mutations and loss of heterozygosity as indicative of CAFs in the tumor stroma (291, 292).

Furthermore, additional reports have demonstrated that epigenetic modifications, such as DNA methylation, may be responsible for maintaining the CAF phenotype and contributing to cancer cell growth and progression (293, 294). Hence, further studies are required to precisely characterize the pro-tumor properties of CAFs.

CAFs secrete growth factors such as TGF-β, EGF, FGF-2, TNF-α, platelet-derived growth factor (PGDF), and VEGF-A, and express cell surface and extracellular matrix proteins (295, 296). Extensive research links CAFs to breast cancer progression, with studies showing that CAFs secrete SDF-1/CXCL12 and HGF, both of which promote breast cancer growth and metastasis (297). HGF activates c-Met on tumor cells, leading to enhanced metastasis, while SDF-1 facilitates tumor growth and angiogenesis through the CXCR4 receptor on breast carcinoma cells. These functions promote the transition of breast carcinoma from ductal carcinoma *in situ* (DCIS) to invasive ductal carcinoma (IDC) (297). Recent targeting of HGF/c-Met interaction has emerged as a significant breakthrough in breast cancer therapy (298). Additionally, SDF-1 secretion by breast CAFs contributes to the proliferation of breast cancer stem cells ($CD44^+CD24^-$) and the induction of drug resistance (299, 300). Therefore, targeting SDF-1 holds great promise for breast cancer therapeutics. Furthermore, CAFs play a crucial role in immune evasion by regulating the miR-92/PD-L1 pathway during breast cancer progression (301, 302). Molecular profiling of CAFs in breast tissue and carcinoma has identified differentially expressed genes (DEGs) that can serve as diagnostic and prognostic biomarkers and be targeted for developing new therapies (303, 304). Notably, high PDGF expression by CAFs indicates a shorter median survival for breast cancer patients (305).

Numerous oncogenic and immune cell signaling pathways within the TME cross-regulate CAFs and immune cells (Figure 3), promoting tumor progression, immunosuppression, and drug resistance (306). These pathways encompass TGF-β/Smad, Wnt/β-catenin, EGFR, TGF-β, PI3k/AKT/mTOR, JAK/STAT3, etc (307). Shangguan et al. previously demonstrated that inhibiting the TGF-β/Smad signaling pathway in human bone marrow mesenchymal cells hinders their differentiation into CAFs (308). Additionally, suppressing the EGFR signaling pathway, a crucial factor in EbbB/HER subtype metastasis has shown potential to inhibit CAF-associated cancer stemness (309). Therapies targeting CAFs have proved effective in overcoming treatment resistance in $HER2^+$ breast cancer, with increased expression of NK-IL2RS, NK, and NKT cell signatures before treatment correlating with improved response to anti-HER2 mAbs-based therapy (310). Therapeutic targeting of CAF signaling pathways within the TME presents a promising approach for achieving breast cancer remission. Considering the significant role of CAFs in breast cancer metastasis and the complexity of cancer cell molecular signatures (311), further research and clinical trials are imperative to establish their potential utility in breast cancer prognosis and therapeutic intervention.

Cancer-associated adipocytes

CAAs are adipocytes that actively reside near cancer cells, promoting crucial communication by releasing factors that can induce localized and systemic effects (312). Adipocytes in the TME can change in response to signals from cancer cells, leading to the formation of CAAs. These CAAs may release fatty acids into the surrounding tissue which can be taken up by breast tumor cells (313). The increased demand for energy and building blocks for rapidly dividing cancer cells makes fatty acids a valuable substrate for their metabolic needs. Within the TME, fatty acids undergo β-oxidation and serve as the principal source of ATP which promotes tumor survival and proliferation (314). Breast cancer cells can utilize fatty acid oxidation (FAO) as a metabolic pathway to oxidize fatty acids and generate energy. This process becomes particularly relevant in situations where other energy sources, such as glucose, are limited. Enhanced fatty acid metabolism, including FAO, has been associated with increased tumor aggressiveness in breast cancer (315). Fatty acids not only serve as an energy source but also play a role in various signaling pathways that can influence cell survival, proliferation, and

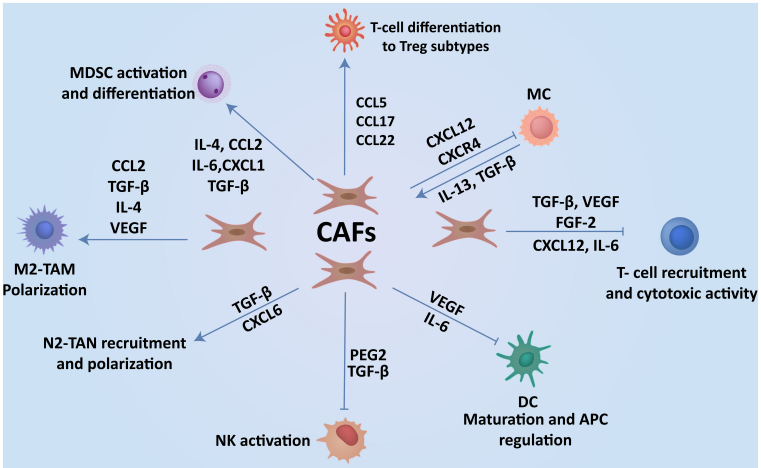


FIGURE 3
CAFs-immune cell interplay contributes to breast cancer progression. Interaction of CAFs with immune cells via the production of cytokines and soluble factors create an immunosuppressive TME, which enhances the progression of cancer to metastasis.

invasiveness. Fatty acids can also activate specific lipid signaling pathways within tumor cells leading to changes in gene expression and metabolic pathways (316, 317). For instance, fatty acids can activate peroxisome proliferator-activated receptors (PPARs) beside other nuclear receptors, which can regulate genes involved in lipid metabolism, inflammation, and cell growth (318). PPARs are a group of nuclear receptors that play a crucial role in the regulation of fatty acid metabolism and energy homeostasis (319). Activation of PPARs occurs when ligands, such as fatty acids or their derivatives, bind to the receptors. Once activated, PPARs form heterodimers with retinoid X receptors (RXRs) and bind to specific DNA sequences called PPAR response elements (PPREs) in the promoters of target genes (320). This binding regulates the transcription of genes involved in lipid metabolism, energy homeostasis, and inflammation (321). In hormone receptor-positive breast cancer, estrogen receptor-positive (ER+) tumors can be influenced by adipose tissue-derived factors. Fatty acids and other adipokines may affect the growth and behavior of ER+ breast cancer cells (322). Although all adipose depots can secrete inflammatory factors, such as TNF, IL-6, IL-1β, and TGF-β (323) obese visceral adipose primarily releases excessive fatty acids, cholesterol, triglycerides, hormones, and adipokines, closely associated with metabolic dysfunction and unfavorable cancer outcomes (324). Additionally, adipocytes can contribute to chemotherapeutic drug resistance, as their co-culture with fibroblasts can deactivate the effectiveness of anti-cancer drugs by metabolizing them into less potent secondary metabolites (325). Understanding the metabolic interactions between CAAs and breast cancer cells, specifically involving fatty acids and FAO, has implications for developing targeted therapies. Researchers are exploring ways to disrupt these metabolic pathways as potential strategies to inhibit tumor growth and improve treatment outcomes.

Furthermore, TME's plasticity allows for transdifferentiation, a process whereby cells undergo a significant shift in their identity, thereby acquiring new transcriptional or morphological characteristics typical of a different cell lineage. Microenvironmental cues including neighboring cells, extracellular matrix, blood vessels, and immune cells can induce shifts in cancer cell phenotypes (326). Emerging research has shown that cancer cells, can exhibit plasticity and undergo transdifferentiation, which can contribute to tumor heterogeneity and complicate treatment strategies (327). Despite these challenges, researchers are exploring ways to harness lineage plasticity for therapeutic purposes. In a research conducted by Ronen et al, to capitalize on the plasticity of cancer cells, breast cancer cell differentiation was redirected towards a non-malignant and non-proliferative adipocyte fate (328). In this study, the utilization of Rosiglitazone and an MEK inhibitor as part of the therapy appears to be particularly effective against aggressive characteristics of breast cancer cells, consequently inhibiting metastasis. The transdifferentiated adipogenesis-induced cancer cells, MTDECad and 3T3-L1 cells formed become functional post-mitotic adipocytes which have comparable characteristics with functional adipocytes. For instance, both differentiated cell types express the adipocyte-specific markers C/EPBa, PPARg2, and fatty acid binding protein 4 (FABP4), and they secrete the adipocyte-specific adipokine adiponectin (328). Therapeutic strategies need to consider the evolving nature of cancer cells and the potential for phenotypic changes under different microenvironmental conditions. Generally, CAA significantly influences various aspects of breast cancer, including risk, progression, migration, metastasis, and resistance to existing treatments (329). Therefore, targeting the interaction between adipose tissue and breast cancer may be a promising approach to overcoming immune tolerance and drug resistance.

Vasculature cells in breast TME

Endothelial cells

ECs are a constitutive part of the cardiovascular system and are critical to homeostasis, angiogenesis, and immune response (330). They regulate the passage of substances through tight cell junctions and line the basement membrane of capillaries (331). ECs, along with a basal lamina and strategically positioned pericytes, form the structure of blood vessel walls (332). ECs facilitate intravasation, allowing cancer cells to migrate into the blood vessel lumen, a critical step in cancer metastasis (333). Tumor growth relies on a blood supply, and during rapid growth, tumors stimulate neovascularization by weakening the basement membrane of existing blood vessels (334). Upon the secretion of angiogenic factors like VEGF-A, PDGF, hypoxia-inducing factors (HIF-1), and MMPs, the basement membrane degrades. This basement membrane degradation triggers the migration of endothelial cells and pericytes to the tumor region, contributing to TME angiogenesis (334). Additionally, tumor-associated hypoxia, mediated by HIF-1α and HIF-2α, plays a role in malignant conversion and metastasis, as well as influencing immune cell functions within the TME (335). Schneider & Miller's study revealed that angiogenesis precedes the progression of mammary hyperplasia to malignancy in breast cancer (336). They demonstrated that transfection of tumor cells with angiogenic stimulatory peptides promoted tumor growth, invasiveness, and metastasis (337). Clinical outcomes have substantiated the efficacy of anti-angiogenic therapy as a viable treatment approach. However, the use of antiangiogenic drugs in conjunction with conventional chemotherapy in metastatic breast cancer has shown limited clinical impact on overall survival (337). It is essential to conduct further studies by addressing potential obstacles, such as toxicity, drug resistance, and alternative angiogenesis mechanisms, in order to optimize the effectiveness of anti-angiogenic therapies in breast cancer progression.

Recently, correlation between neurogenesis and angiogenesis in breast TME has been linked to aggressive breast cancer breast cancer (338). Tumors release neurotrophic factors that can initiate innervation, a process that imitates angiogenesis (339). Hence, tumor neurogenesis is intricately linked to metastasis, as the presence of ingrown nerve endings can release neurotransmitters that significantly enhance the development of metastatic cells (339). In an immunohistochemistry analysis of carcinoma breast tissues, it was observed that protein gene product (PGP) 9.5 protein was present in 61% of IDC tissues compared to fibroadenoma and DCIS, particularly in ER-negative and node-negative subtypes (340). PGP 9.5, a ubiquitin-carboxyl hydrolase, is an enzyme expressed throughout the stages of differentiation in nerve tissue of mice brains and is a useful marker for detecting central nervous system damage (341). Likewise, in both ER-

negative and node-negative subgroups of breast IDC, a significant association was observed between PGP 9.5 expression and higher microvessel density (MVD), compared to less expression of PGP 9.5 and MVD identified in DCIS. The analysis reveals a clear correlation between neurogenesis and angiogenesis, particularly in ER-negative and node-negative subtypes of breast cancer (340). In a human breast cancer cohort study, a significant association between neurogenesis, consolidated neuro-angiogenic signature, and high-grade breast cancer features was observed (342). Single cell-based spatial mapping with imaging mass cytometry was used to identify the colocalization of neural and vascular structures, indicating the presence of neurovascular niches within tumor tissue. Cancer cells can release various signaling molecules, including growth factors and cytokines, that play a role in recruiting both sprouting axons (microaxons) and endothelial cells (microvessels) to the TME (343). This phenomenon is referred to as neurotropism, and it has been observed in several types of cancers, including breast cancer (343, 344). The exact mechanisms by which cancer cells influence axon recruitment are still an area of active research. The coexistence and potential coregulation of microaxons and microvessels suggest a complex interplay between neural and vascular elements within the tumor stroma.

Pericytes

Pericytes are mural cells that envelop blood vessels and reside adjacent to the endothelial cells lining the capillaries. Pericytes play a crucial role in the development and stabilization of the vasculature through TGF-β signaling activation (345). Also, pericytes actively enhance the physical stability and support of endothelial tubule function during the initial phase of angiogenesis by co-occupying endothelial tubules (346). Within the TME, the tumor vasculature serves multiple functions, such as supporting tumor growth and facilitating metastasis to distant organ sites (347). Notably, breast cancer is a highly vascularized tumor with extensive pericyte coverage (347). Targeting angiogenesis during breast cancer progression can be approached by inhibiting the vessel-stabilizing properties of vascular pericytes (348, 349). Depleting pericytes has been shown to increase intra-tumoral hypoxia and lung metastasis in advanced-stage hypoxic tumors with pre-established vasculature (348). The presence of perfusion defects in breast cancer blood vessels is associated with vessel dilation, tortuosity, and inadequate perivascular coverage (347, 350). This abnormal vascular system is partly attributed to morphological and molecular alterations in pericytes and significant population heterogeneity (347). The presence of pericytes in the primary TME impedes cancer progression and metastasis (350). Distinguishing pericytes can be achieved through morphological characteristics and molecular markers, including α-SMA, desmin, PDGFR-β, CD248, NG2, and angiopoietin-2 (349, 351). Many of the pericyte markers are used in several studies to calculate the mean microvascular pericyte coverage index (MPI). For instance, α-SMA expression in breast cancer yielded an estimated MPI range of 32%-80%. Other markers such as NG2, PDGFRβ, desmin, and CD248 have also been employed for MPI measurement (351). Other markers such as NG2, PDGFRβ, desmin, and CD248 have also been applied in MPI measurement (350). Many anti-angiogenic treatments involve targeting endothelial cells or proangiogenic factors to suppress neovascularization cause tumor cell death. In the context of anti-angiogenic treatments, simultaneously targeting both endothelial cells and pericytes has been suggested (352). According to some studies, non-selective elimination of pericytes may not provide benefits but may instead promote tumor aggressiveness and metastasis. Therefore, gaining a comprehensive understanding of pericyte heterogeneity in response to changes in the TME can inform effective pericyte targeting strategies (351, 353).

Conclusion and future directions

The current research findings on the interaction between the TME and cancer cells have significantly advanced our understanding of their crucial roles in cancer progression and treatment response. Traditionally, treatment strategies for breast cancer predominantly focused on promoting tumor cell death. However, the emergence of immunotherapy has revolutionized cancer treatment by incorporating anti-tumor immune responses and targeting TME cells. Successful research of some clinical trials targeting breast TME has provided a promising outlook for utilizing these cells in cancer therapies (Table 2). The cells within the breast TME can either act against or promote tumor cells; in certain conditions, they may exhibit dual roles. The ability of TME cells to switch from anti-tumor to pro-tumor functions poses a significant challenge for immunotherapy. The anti-tumor and pro-tumor functions of these cells primarily depend on specific mediators such as cytokines, chemokines, and growth factors in TME. The interplay between these mediators generated by the cellular components modulates the TME towards either an anti-tumor or an immunosuppressive environment. Despite the initial findings on breast TME, future studies should focus on understanding the evasion of anti-tumor immunity and exploiting TME cell mediators to target cancer cells. Immunotherapy has emerged as a critical component in the treatment of various types of cancer, including breast cancer.

ICB therapies have shown remarkable efficacy when used alone or in combination with other treatment modalities. Reprogramming CTLs through ICB immunotherapies has been successful, but the resistance caused by TAMs to recently developed ICB therapies remains challenging. Therefore, there is a need for targeted inhibition of TAMs to enhance tumor cell-killing capacity, as well as further investigation into repolarizing TAMs towards an anti-tumor phenotype. Moreover, the emerging field of immunometabolism and understanding how TME regulates metabolism in immune cells to suppress anti-tumor immunity is crucial to developing novel immunotherapies and overcoming resistance against conventional and ICB therapies. The potential of adoptive immunotherapy, specifically equipping NK cells with cancer-targeting CARs, is hindered by immunometabolism. Extensive research is required to understand how regulating the metabolism of NK and other immune cells in TME can promote their anti-tumor activities. Furthermore, there is a pressing need for further investigation into the recovery of exhausted T-cells and NK cells to promote their effector functions. The complexity and heterogeneity of TME cells, such as the CAFs and

pericytes, present challenges in their proper characterization. The recent advances in next-generation sequencing, metabolomics, and bioinformatics, which study cancer progression at both tissue and single-cell levels, can be employed to identify novel breast cancer stage-specific biomarkers, functional phenotype of immune and non-immune cells in TME, resistance to cancer therapies, and development of novel targeted immunotherapies. Such investigations will lead to improved stage-specific breast cancer diagnosis, the development of innovative TME cell-specific targeted immunotherapies with fewer side effects, and overall improved quality of life and survival for women with highly metastatic breast cancer.

Author contributions

TA: Conceptualization, Writing – original draft, Writing – review & editing. RM: Writing – original draft. AA: Writing – original draft. SP: Writing – review & editing. PM: Writing – review & editing. LA: Writing – review & editing. AS: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. AA and PM were supported by a grant from the Breast Cancer Research Foundation of Alabama (project number: 0142-22P).

Acknowledgments

We thank Ferrin Antony, Auburn University for giving feedback during manuscript preparation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 25 March 2024
DOI 10.3389/fonc.2024.1374674

OPEN ACCESS

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RECEIVED 22 January 2024
ACCEPTED 07 March 2024
PUBLISHED 25 March 2024

CITATION
Wang F, Wang J, Zhang H, Fu B, Zhang Y, Jia Q and Wang Y (2024) Diagnostic value of circulating miR-155 for breast cancer: a meta-analysis.
Front. Oncol. 14:1374674.
doi: 10.3389/fonc.2024.1374674

Diagnostic value of circulating miR-155 for breast cancer: a meta-analysis

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Backgrounds: The value of circulating microRNA (miR)-155 for breast cancer (BC) diagnosis may differ in different studies. Therefore, we conducted this systematic review and meta-analysis to evaluate the potential application of circulating miR-155 in the diagnosis of BC.

Methods: Articles published before December 2023 and in English were searched in these databases: PubMed, Web of Science, Medline, EMBASE and Google Scholar. A summary of sensitivity, specificity, positive likelihood ratios (PLR), negative likelihood ratios (NLR), and diagnostic odds ratio (DOR) were calculated from the true positive (TP), true negative (TN), false positive (FP) and false negative (FN) of each study. Additionally, the summary receive-operating characteristics (SROC) curve was constructed to summarize the TP and FP rates.

Results: The pooled parameters calculated were as follows: sensitivity, 0.93 (95% CI: 0.83-0.97); specificity, 0.85 (95% CI: 0.74-0.92); PLR, 6.4 (95% CI: 3.4-11.9); NLR, 0.09 (95% CI: 0.04-0.20); and DOR, 74 (95% CI: 22-247). The analysis showed a significant heterogeneity (sensitivity, I^2 = 95.19%, $p < 0.001$; specificity, I^2 = 95.29%, $p < 0.001$; DOR, I^2 = 92.9%, $p < 0.001$). The SROC curve was with an area under curve (AUC) of 0.95 (95% CI: 0.93-0.97).

Conclusion: Circulating miR-155 has a potential in the diagnosis of BC.

KEYWORDS

breast cancer, miR-155, meta-analysis, microRNA, systematic review

Introduction

Breast cancer (BC) is the most common cancer in women globally, accounting for 31% of all cancers (1). The risk of developing BC over a woman's lifetime is approximately 1 in 8 (1). It is estimated that there will be 297,790 new cases of invasive BC and 43,170 women will die from BC in the United States in 2023 (2). Almost 20% of global BC patients occurred in China, with an estimated age-standardized incidence rate of approximately 60 cases per 100,000 women in 2040 (3, 4). The incidence of BC in China has increased rapidly in recent decades. Early detection is important for a better prognosis because there are few signs and symptoms in the early stage.

Although mammography is widely considered the gold standard for breast cancer detection, it is not without its drawbacks. It is associated with pain, anxiety, and radiation exposure (5), which can deter some individuals from undergoing regular screenings. Additionally, the effectiveness of mammography is limited in women with dense breasts (6), further reducing its reliability as a screening tool. Furthermore, mammography has been found to be particularly inaccurate in patients below the age of 40, leading to underdiagnosis (7). This is concerning because the incidence of triple-negative tumors, which have worse prognostics, is higher in this age group. Early detection is crucial for improving overall cancer survival rates, as it allows for timely intervention before the cancer has a chance to metastasize (8). To overcome the limitations of imaging techniques, the analysis of biomarkers has emerged as a promising approach for early breast cancer diagnosis. Biomarkers such as the human epidermal growth factor receptor 2 (HER2), the KI-67 protein, and estrogen receptors (ERs) are commonly used for prognosis and to guide systemic treatment decisions. In recent years, miRNAs have also gained momentum as potential biomarkers for breast cancer.

MicroRNAs (miRNAs) are a class of small endogenous RNAs that are 19-25 nucleotides in length (9). MiRNAs contribute to the post-transcription regulation of target messenger RNA (mRNA) via mRNA degradation or translation repression (10). Studies have proven that miRNAs play an important role in a variety of biological processes, including inflammation, cell-cycle regulation, cell differentiation, apoptosis, and migration (11). Besides, various studies have demonstrated that miRNA dysregulation is relevant to cancer progression, such as miR-34a in myeloma and miR-145 in solid tumors (12). And miRNA has the merits such as stable quality, easy acquisition of samples and numerous sources of samples (13). Currently, miRNA biomarkers are not utilized in clinical practice due to the significant challenge of translating them from the laboratory into validated diagnostic tests. Among the miRNAs that have been found to be deregulated in BC, microRNA-21 (miR-21) and miR-155 have been identified as the most commonly associated with BC. However, it is important to note that these miRNAs are not highly specific for diagnostic purposes (14).

MiR-155 is an important oncogenic miRNAs in human cancers including in BC (15). Abnormal expression of miR-155 has been found in multiple cancers, such as lung and cervical cancer (16, 17). The expressions of miR-155 and SOCS3 were opposite in lymphoma and pancreatic cancer cells (18, 19). MiR-155 could affect the proliferation and apoptosis of bladder cancer cells via the GSK-3β/β-catenin pathway (20). These may indicate the important role of miR-155 in cancers. Recent studies have shown that the expression level of circulating miR-155 in BC tissues was significantly higher than in normal tissues, and the level of plasma miR-155 in BC patients was also significantly higher than in healthy controls (21, 22). However, the value of circulating miR-155 for BC diagnosis may differ in different studies. Therefore, we conducted this systematic review and meta-analysis to evaluate the potential application of circulating miR-155 in the diagnosis of BC.

Methods

Search strategy

Articles published before December 2023 and in English were searched in these databases: PubMed, Web of Science, Medline, EMBASE and Google Scholar by two researchers (Wang F and Wang J). Search terms used were: ("miR-155" OR "microRNA-155") AND ("breast cancer"). In addition, duplicates were removed. A total of 384 articles were screened in the study.

Selection criteria

The study included all articles based on these inclusion criteria as follows: (i) Studies that involved human patients with BC, (ii) studies in which expression of miR-155 was measured in plasma or serum. Additionally, when true positive (TP), true negative (TN), false positive (FP), and false negative (FN) regarding the diagnostic value of circulating miR-155 for BC could not be acquired or calculated from a study, the study would be excluded. Moreover, we dropped secondary processing of literature such as reviews and meta-analysis articles. Additionally, we excluded case studies without group-level statistics. Two researchers (Wang F and Wang J) independently read the abstracts and full texts.

Data collection

Two investigators (Wang F and Zhang H.J) read titles and abstracts of articles. We collected data as follows: Author, publication years, study location, sample type, sample size, sensitivity, specificity, cut-off value, detection method and endogenous control. In each selected article, we collected TP, TN, FP and FN directly or calculated them according to the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Meta-analysis for studies

All the statistical analysis was conducted using STATA 12.0 software and Meta-Disc Version 1.4. A summary of sensitivity, specificity, positive likelihood ratios (PLR), negative likelihood

ratios (NLR), and diagnostic odds ratio (DOR) were calculated from the TP, FP, FN, and TN of each study. Additionally, the summary receive-operating characteristics (SROC) curve was constructed to summarize the TP and FP rates (23). Q test was used to estimate heterogeneity between studies, and computed I^2 was used to assess the amount of variation derived from heterogeneity. With invariably high heterogeneity (Q test, $p \leq 0.05$), random effects models were used to generate a summary effect size of these studies; Inversely, fixed effects models were performed to summarize effect size in the absence of between-study heterogeneity (Q test, $p > 0.05$).

Results

Search results

Figure 1 showed the initial search results and selection process. Table 1 showed the characteristics of the finally included 16 studies. Data were collected from 16 studies (22, 24–38) for the diagnostic studies with miR-155 for BC (BC group: n = 1,377, control group: n = 716).

Meta-analysis results

Circulating miR-155 showed a diagnostic value for BC. As shown in forest plot (Figures 2, 3), the pooled parameters calculated were as follows: sensitivity, 0.93 (95% CI: 0.83-0.97); specificity, 0.85 (95% CI: 0.74-0.92); PLR, 6.4 (95% CI: 3.4-11.9); NLR, 0.09 (95% CI: 0.04-0.20); and DOR, 74 (95% CI: 22-247). The analysis showed a high heterogeneity (sensitivity, $I^2 = 95.19\%$, $p < 0.001$; specificity, $I^2 = 95.29\%$, $p < 0.001$; DOR, $I^2 = 92.9\%$, $p < 0.001$). Figure 4 shows the SROC curve, with an area under the curve (AUC) of 0.95 (95% CI: 0.93-0.97). The symmetrical Deek's funnel plot showed a publication bias ($p < 0.01$, Supplementary Figure 1).

Serum miR-155 showed a diagnostic value for BC. As shown in forest plot (Figures 5A, 6A), the pooled parameters calculated were as follows: sensitivity, 0.94 (95% CI: 0.85-0.97); specificity, 0.89 (95% CI: 0.76-0.96); PLR, 8.6 (95% CI: 3.6-20.4); NLR, 0.07 (95% CI: 0.03-0.17); and DOR, 123 (95% CI: 31-478). The analysis showed a high heterogeneity (sensitivity, $I^2 = 94.56\%$, $p < 0.001$; specificity, $I^2 = 96.62\%$, $p < 0.001$; DOR, $I^2 = 92.9\%$, $p < 0.001$). Supplementary Figure 2 showed the SROC curve, with an AUC of 0.97 (95% CI: 0.95-0.98). The symmetrical Deek's funnel plot showed a publication bias ($p < 0.01$, Supplementary Figure 3).

Plasma miR-155 showed a diagnostic value for BC. As shown in forest plot (Figures 5B, 6B), the pooled parameters calculated were as follows: sensitivity, 0.87 (95% CI: 0.43-0.98); specificity, 0.72 (95% CI: 0.67-0.76); PLR, 3.1 (95% CI: 2.1-4.5); NLR, 0.18 (95% CI: 0.03-1.25); and DOR, 17 (95% CI: 2-163). The analysis showed a high heterogeneity for sensitivity and DOR (sensitivity, $I^2 = 94.81\%$, $p < 0.001$; DOR, $I^2 = 92.9\%$, $p < 0.001$). The analysis showed a low heterogeneity for specificity ($I^2 = 0.0\%$, $p = 0.062$). Supplementary Figure 4 showed the SROC curve, with an AUC of 0.73 (95% CI: 0.95-0.98). The symmetrical Deek's funnel plot showed a publication bias ($p < 0.03$, Supplementary Figure 5).

Discussion

In our study, we found that the pooled sensitivity and specificity of circulating miR-155 were 0.93 (95% CI: 0.83-0.97) and 0.85 (95% CI: 0.74-0.92), respectively. The DOR and AUC of miR-155 were 74 (95% CI: 22-247) and 0.95 (95% CI: 0.93-0.97). A recent study showed that the sensitivity and specificity were 0.49 and 0.895 for carcinoembryonic antigen (CEA), 0.521 and 0.837 for carbohydrate

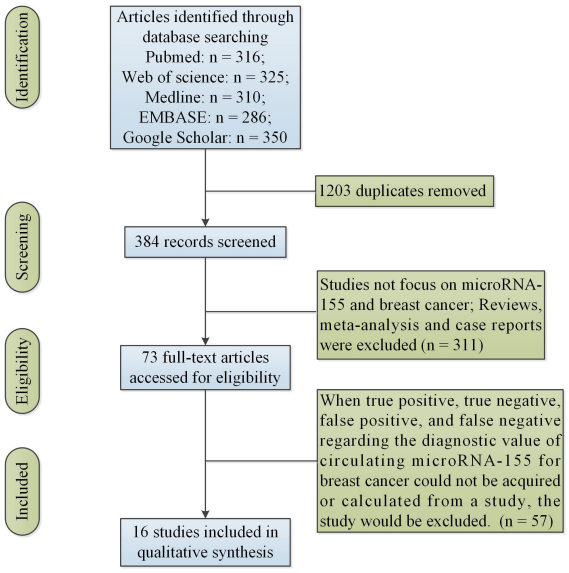

FIGURE 1
Flow of information through the different phases of a systematic review.

TABLE 1 Characteristics of all included studies.

Reference	Country	Sample type	Sample size		Age of BC patients	Stage of BC	Sensitivity (%)	Specificity (%)	Cut-off	Detection Method	Endogenous control
			Case	Control							
Zhao et al. (2012) (24)	China	Serum	20	10	54	I: 1; II: 19	100	90	0.95	Real-time qPCR	RNU6B
Sun et al. (2012) (25)	China	Serum	103	55	51	I: 29; II: 36; III: 30; IV: 8	65.0	81.8	1.911	Real-time qPCR	cel-miR-39
Mar-Aguilar et al. (2013) (26)	Mexico	Serum	61	10	52.9	I: 13; II: 14; III: 34	94.4	100	7.92	Real-time qPCR	18S RNA
Eichelser et al. (2013) (27)	Germany	Serum	152 M0:120 M1:32	40	65	I: 69 II-IV: 51	M0: 70.6 M1: 85.3	M0: 42.7 M1:70	NR	qPCR	miR-16
Shaker et al. (2015) (28)	Egypt	Serum	100	30	25-75	NR	94.1	100	3.1585	Real-time qPCR	SNORD
Zhang et al. (2016) (29)	China	Plasma	106	106	56.9 ± 6.7	I: 11; II: 43; III: 32; IV: 20	66.0	68.9	0.321	Real-time qPCR	NR
Han et al. (2017) (30)	China	Serum	99	21	45.38	I: 49; II: 36; III: 14	100	51.02	-1.171	Real-time qPCR	NR
Fan et al. (2018) (31)	China	Serum	49	19	43	NR	100	60	NR	Real-time qPCR	miR-10b, miR-222
Huang et al. (2018) (32)	China	Serum	158	107	51.19±10.39	II-IV	Training Set: 83.3 Validation Set: 40.6	Training Set: 80 Validation Set: 87	Training Set: 1.4980 Validation Set: 0.7615	Real-time qPCR	GAPDH
Swellam et al. (2018) (33)	Egypt	Serum	80	30	52	I-II: 33III: 47		97.1	NR	Real-time qPCR	RNU6-2
Shaheen et al. (2019) (34)	Pakistan	Plasma	37	34	NR	NR	100	73.53	NR	Real-time qPCR	miR-16
Swellam et al. (2019) (35)	Egypt	Serum	96	86	50	I-II: 21III: 71	95.8	96.5	NR	Real-time qPCR	RNU6-2
Hosseini Mojahed et al. (2020) (22)	Iran	Serum	36	36	47:64 ± 8:18	I: 8; II: 17; III: 11	77.78	88.89	1.40	Real-time qPCR	SNORD47
Itani et al. (2021) (36)	Lebanon	Plasma	41	32	53 ± 11.88	0	78	75	10.54	Real-time qPCR	miR-16
Papadaki et al. (2021) (37)	Greece	Plasma	140	20	55 (27–82)	0	56.5	69.1	0.405	Real-time qPCR	NR
Mohamed et al. (2022) (38)	Egypt	Serum	99	40	48	I: 14; II: 36; III: 40; IV: 9	86.9	90	7.5	Real-time PCR	NR

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; miR, microRNA; NR, not reported; qPCR, quantitative polymerase chain reaction.

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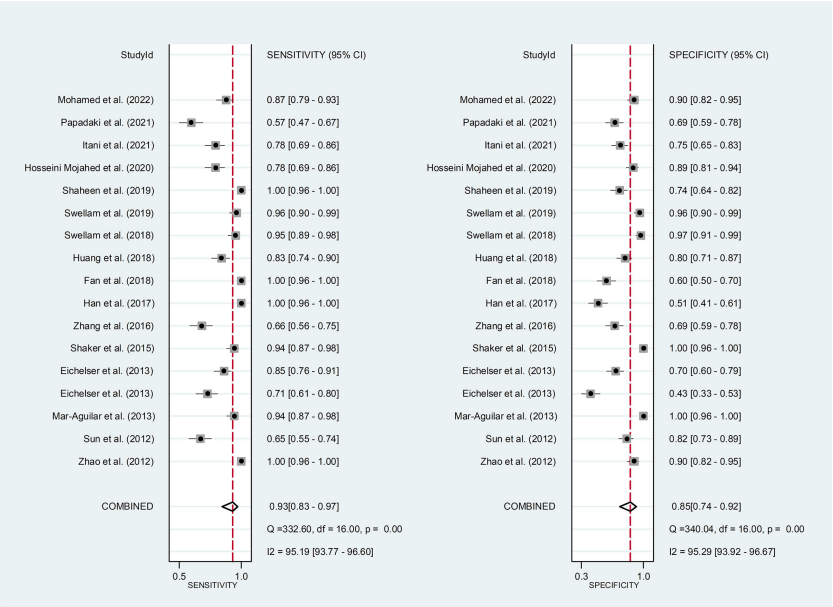

FIGURE 2
The sensitivity and specificity of circulating miR-155 in the diagnosis of BC. BC, breast cancer; miR-155, microRNA-155.

antigen (CA)15-3, and the AUC of CEA and CA15-3 was 0.669 (95%CI: 0.595-0737) and 0.839 (95%CI: 0.777-0.889), respectively (35). It is widely recognized that the AUC should be in the region of 0.97 or above to demonstrate excellent accuracy (39). Compared to CEA and CA15-3, miR-155 has better sensitivity and specificity and may probably be suitable for the screening of BC. Therefore, in this meta-analysis, our result showed that miR-155 may be an excellent potential biomarker in the diagnosis of BC. The present study reported that serum miR-155 showed a high diagnostic value for BC (sensitivity, 0.94 (95% CI: 0.85-0.97); specificity, 0.89 (95% CI: 0.76-0.96); AUC, 0.97 (95% CI: 0.95-0.98)), whereas that plasma miR-155 showed a medium diagnostic value for BC (sensitivity, 0.87 (95% CI: 0.43-0.98); specificity, 0.72 (95% CI: 0.67-0.76); AUC, 0.73 (95% CI: 0.95-0.98)). The subgroup analyses based on specimen types revealed that serum had a higher diagnostic value compared to plasma, suggesting that serum may be a more suitable source of clinical specimens for BC detection. Specifically, miR-155 in serum demonstrated a more precise diagnostic value than in plasma. This discrepancy may be attributed to the coagulation process, which can influence the extracellular miRNA spectrum in the blood and

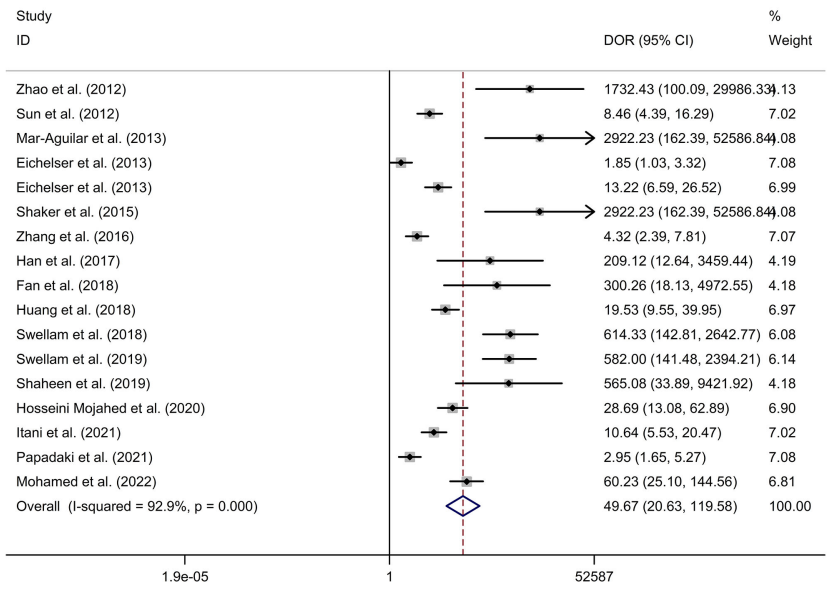

FIGURE 3
The DOR of circulating miR-155 in the diagnosis of BC. BC, breast cancer; DOR, diagnostic odds ratio; miR-155, microRNA-155.

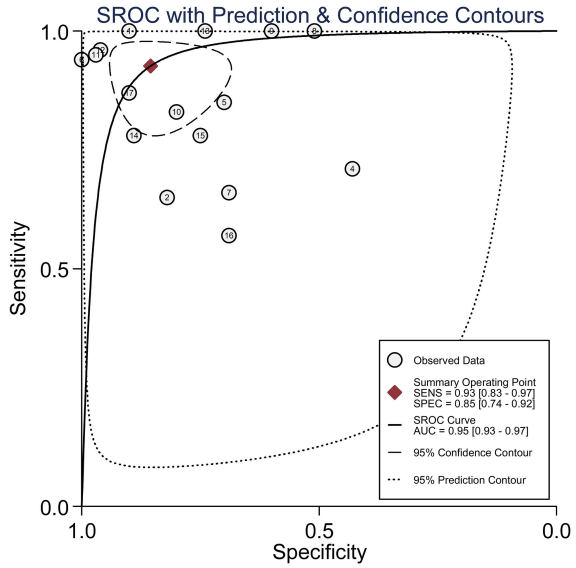

FIGURE 4
The SROC curve with AUC of circulating miR-155 in the diagnosis of BC. AUC, area under the curve; BC, breast cancer; DOR, diagnostic odds ratio; miR-155, microRNA-155; SROC, summary receiver operator characteristic.

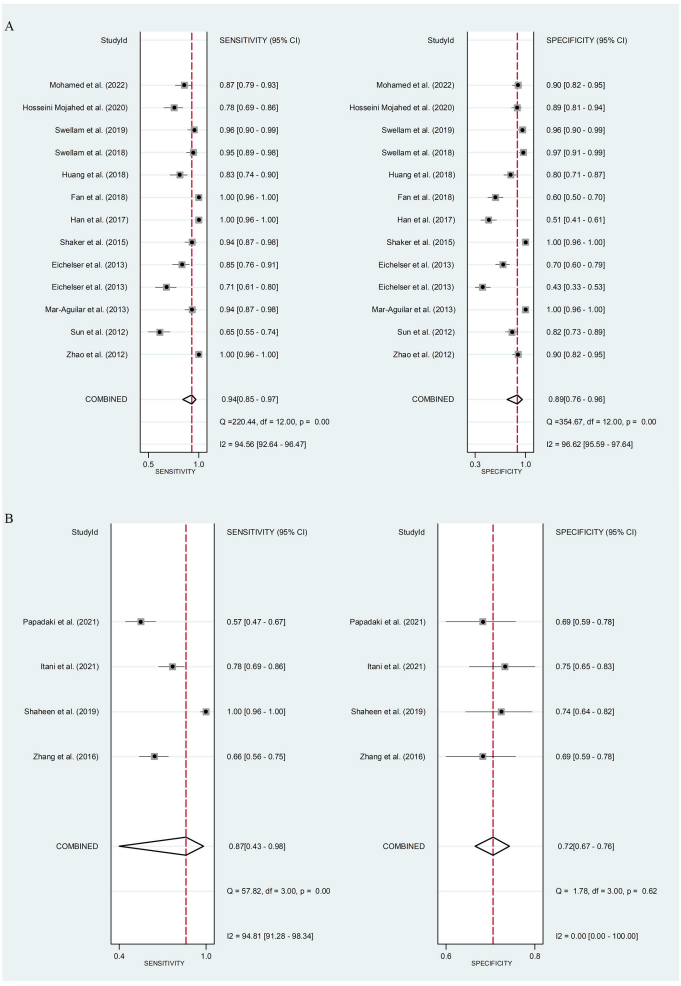

FIGURE 5
The sensitivity and specificity of serum miR-155 **(A)** or plasma miR-155 **(B)** in the diagnosis of BC. BC, breast cancer; miR-155, microRNA-155.

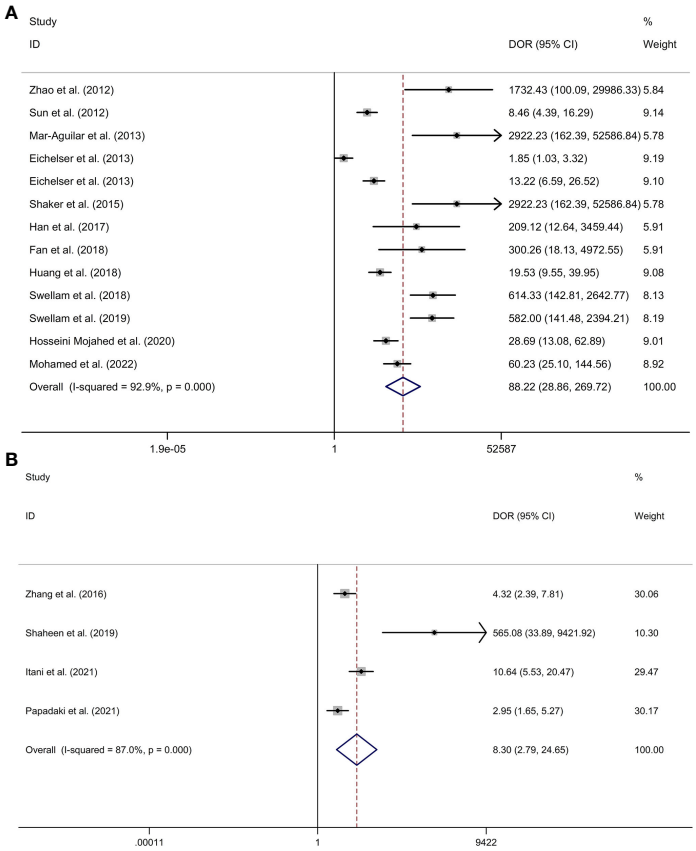

FIGURE 6
The DOR of serum miR-155 **(A)** or plasma miR-155 **(B)** in the diagnosis of BC. Abbreviations: BC, breast cancer; DOR, diagnostic odds ratio; miR-155, microRNA-155.

consequently lead to varying miRNA expression levels in different specimens (40). Furthermore, differences in detection or normalizing methods between the two specimen types could also influence the diagnostic value (40). However, it is important to note that only four studies were included in the assessment of plasma miR-155 for BC diagnosis, which may have an impact on the overall clinical conclusion. Thus, more studies were essential to explore the diagnostic value of circulating miR-155 for BC.

The association between microRNAs and cancer has been a research focus in recent years, especially some most frequently studied microRNAs, such as miR-155. Recently published basic research results attempted to explain the association between miR-155 and the development of BC. Kim et al. reported that miR-155 was a key regulator of glucose metabolism in breast cancer via phosphoinositide-3-kinase regulatory subunit alpha (PIK3R1)-FOXO3a-cMYC axis and down-regulation of miR-155 could inhibit the growth of tumor *in vivo* (41). Wang et al. showed that miR-155 played a vital role in regulating the function of dendritic cells in BC, and miR-155 deficiency promoted BC growth in mice (42). MiR-155 was proved to play an important role in the proliferation and migration of BC cells via the down-regulation of suppressors of cytokine signaling (SOCS)1 and up-regulation of matrix metalloproteinase (MMP)16 (43). MiR-155 has also been studied as the potential prediction biomarker of early BC recurrence and therapy resistance (44, 45).

Our meta-analysis result was consistent with previous meta-analysis results published in 2014 (46). However, the previous meta-analysis only included three studies and the sample size of included studies was small. In our meta-analysis, we updated published articles regarding the association between circulating miR-155 and BC to obtain more accurate results. Some limitations still should be noticed. First, the lack of some important information from original published articles limited our research such as TNM-stage, lymph node metastasis, the level of estrogen receptor alpha (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER)-2. However, Zeng et al. reported that the expression of miR-155 was related to lymph node metastasis, and the status of ER, PR and HER-2 (47). Second, previous studies have demonstrated that circulating miR-155 was also associated with not only cancer, but also some other diseases, such as pre-eclampsia pregnancies (48), coronary artery disease (49), and multiple sclerosis (50). MiR-155-related diseases may influence the expression of miR-155 among the included samples and affect the accuracy of relevant results. So it should be noted that if we apply miR-155 as the screening BC, we should avoid the impact of miR-155-related diseases.

Conclusions

Up until now, we can demonstrate that circulating miR-155 has a potential in the diagnosis of BC. Before circulating miR-155 can be applied to clinical diagnosis, more large-scale clinical studies should be conducted in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

FW: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. JW: Writing – original draft, Methodology, Investigation, Data curation. HZ: Writing – review & editing, Investigation, Formal analysis, Data curation. BF: Writing – original draft, Formal analysis, Data curation. YZ: Writing – review & editing, Methodology. QJ: Writing – original draft, Methodology, Investigation. YW: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1374674/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 18 April 2024
DOI 10.3389/fonc.2024.1381251

OPEN ACCESS

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RECEIVED 03 February 2024
ACCEPTED 08 April 2024
PUBLISHED 18 April 2024

CITATION
Long L, Fei X, Chen L, Yao L and Lei X (2024) Potential therapeutic targets of the JAK2/STAT3 signaling pathway in triple-negative breast cancer. *Front. Oncol.* 14:1381251. doi: 10.3389/fonc.2024.1381251

Potential therapeutic targets of the JAK2/STAT3 signaling pathway in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) poses a significant clinical challenge due to its propensity for metastasis and poor prognosis. TNBC evades the body's immune system recognition and attack through various mechanisms, including the Janus Kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway. This pathway, characterized by heightened activity in numerous solid tumors, exhibits pronounced activation in specific TNBC subtypes. Consequently, targeting the JAK2/STAT3 signaling pathway emerges as a promising and precise therapeutic strategy for TNBC. The signal transduction cascade of the JAK2/STAT3 pathway predominantly involves receptor tyrosine kinases, the tyrosine kinase JAK2, and the transcription factor STAT3. Ongoing preclinical studies and clinical research are actively investigating this pathway as a potential therapeutic target for TNBC treatment. This article comprehensively reviews preclinical and clinical investigations into TNBC treatment by targeting the JAK2/STAT3 signaling pathway using small molecule compounds. The review explores the role of the JAK2/STAT3 pathway in TNBC therapeutics, evaluating the benefits and limitations of active inhibitors and proteolysis-targeting chimeras in TNBC treatment. The aim is to facilitate the development of novel small-molecule compounds that target TNBC effectively. Ultimately, this work seeks to contribute to enhancing therapeutic efficacy for patients with TNBC.

KEYWORDS

triple-negative breast cancer, receptor tyrosine kinase, Janus Kinase 2, signal transducer and activator of transcription 3, small molecule compounds

1 Introduction

Globally, breast cancer stands as the most prevalent malignant tumor (1). Among its subtypes, triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) expression, constitutes approximately 10%-15% of all breast cancer cases (2). The treatment of TNBC presents significant challenges, notably its propensity for early metastasis (3) and a comparatively poorer prognosis than other breast cancer subtypes (4). Current clinical strategies for TNBC primarily employ taxanes and anthracycline-based cytotoxic drugs. However, these approaches frequently encounter obstacles in the form of chemotherapy resistance, and the absence of effective small-molecule targeted therapies, constituting primary impediments in TNBC clinical management (5).

Reassessing classic drug targets is crucial for advancing new precision medicine strategies. The JAK2/STAT3 pathway, clinically validated as a therapeutic target for inflammation-related conditions, has shown promise through its inhibitors in treating inflammatory and autoimmune diseases. This success paves the way for novel clinical therapy developments (6, 7). Extensive research has established a strong association between aberrations in the JAK2/STAT3 signaling pathway and key oncogenic processes such as proliferation, invasion, and metastasis in various malignancies, including TNBC. Notably, activation of this pathway has been observed in multiple solid tumors, TNBC included (8–12). Targeted inhibition of the JAK2/STAT3 signaling has demonstrated efficacy in curtailing TNBC cell proliferation, invasion, and migration (13), knockdown of JAK2 or STAT3 in triple-negative breast cancer cells significantly reduced cell proliferation, invasion and migration (14–21), tumor volume and distant metastasis were significantly inhibited in a mouse model of triple-negative breast cancer with conditional knockout of JAK2 or STAT3 (22–25). Moreover, the downregulation of this pathway has been shown to counteract paclitaxel (PTX) resistance (26). Thus, targeting JAK2/STAT3 emerges as a promising therapeutic strategy for treating TNBC and overcoming challenges associated with PTX resistance.

Abbreviations: TNBC, Triple-negative breast cancer; JAK2, Janus Kinase 2; STAT3, Signal transducer and activator of transcription 3; ER, Estrogen receptor; PR, Progesterone receptor; HER-2, Human epidermal growth factor receptor 2; PTX, paclitaxel; RTKs, Receptor tyrosine kinases; EGFRs, Epidermal growth factor receptors; VEGFRs, Vascular endothelial growth factor receptors; IGFRs, Insulin-like growth factor receptors; PDGFRs, Platelet-derived growth factor receptors; FGFRs, Fibroblast growth factor receptors; LIFR, Leukemia inhibitory factor receptor; IL-6R, Interleukin-6 cell factor receptor; IL-13R, Interleukin-13 cell factor receptor; GP130, Glycoprotein 130 receptor; EGF, Epidermal growth factor; MEK, Mitogen-activated protein kinase; ERK, Extracellular signal-regulated kinase; PI3K, Phosphoinositide 3-kinase; AKT, Protein kinase B; VEGF, Vascular endothelial growth factor; PDGF, Platelet-derived growth factor; FGF, Fibroblast growth factor; TBx3, T-box transcription factor 3; TYK2, Tyrosine kinase 2; PROTAC, Proteolysis targeting chimera; PD-L1, Programmed death-ligand 1; c-MET, Cellular mesenchymal-epithelial transition; PARP, Poly ADP-ribose polymerase; AR, Androgen receptor; EZH2, Enhancer of zeste *homolog 2*; CDK, Cyclin-dependent kinases.

The signaling cascade of the JAK2/STAT3 pathway is predominantly mediated through receptor tyrosine kinases (RTKs), JAK2, and the transcription factor STAT3. RTKs are single-pass transmembrane proteins ubiquitously expressed across various cell types, including those within the tumor microenvironment. Characteristically, all RTKs possess a conserved structural composition: an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain (27). Upon ligand binding, RTKs undergo dimerization on the cell membrane and phosphorylate tyrosine residues on the receptors, facilitating their recognition and binding by downstream proteins with SH2 domains, such as JAK2. RTK dimerization brings the associated JAK2 kinase into proximity, enabling their activation through reciprocal tyrosine phosphorylation. Activated JAK2 then stimulates the RTKs to generate binding sites for STAT3. STAT3 binds to RTKs through its SH2 domain and undergoes phosphorylation under the influence of JAK2. The phosphorylated STAT3 forms homodimers and enters the nucleus to induce downstream signal transduction, effectuating various physiological or pathological roles (28). Given the characteristics of this signaling pathway, targeting its components to treat TNBC represents an effective strategy for precision therapy (Figure 1).

Consequently, this review systematically summarizes the roles of the JAK/STAT3 pathway in the pathogenesis of TNBC and the current advances in research on small-molecule compounds targeting the JAK/STAT3 signaling pathway as a therapeutic approach for TNBC.

2 Receptor tyrosine kinases in TNBC

Receptor tyrosine kinases represent a diverse class of enzyme-linked cell surface receptors with a high affinity for growth factors, cytokines, and hormones. These receptors not only bind specific ligands but also function as protein kinases, phosphorylating tyrosine residues on target proteins. RTKs are categorized into 20 distinct families based on the types of ligands they bind (29). TNBC expresses various RTKs, including epidermal growth factor receptors (EGFRs) (30), vascular endothelial growth factor receptors (VEGFRs) (28), insulin-like growth factor receptors (IGFRs) (31), platelet-derived growth factor receptors (PDGFRs) (32), fibroblast growth factor receptors (FGFRs) (33), leukemia inhibitory factor receptor (LIFR) (34), interleukin-6 cell factor receptor (IL-6R) (35), interleukin-13 cell factor receptor (IL-13R) (36), and glycoprotein 130 receptor (GP130) (37). Before ligand binding, RTKs exist on the cell surface as inactive monomers. Homologous ligand binding induces receptor dimerization, activating their intrinsic kinase activity (38).

2.1 Epidermal growth factor receptors: regulator of progression, metastasis, and cancer stem cells in TNBC

Epidermal growth factor receptors, the receptor for epidermal growth factor (EGF), is a key member of the HER family, which also

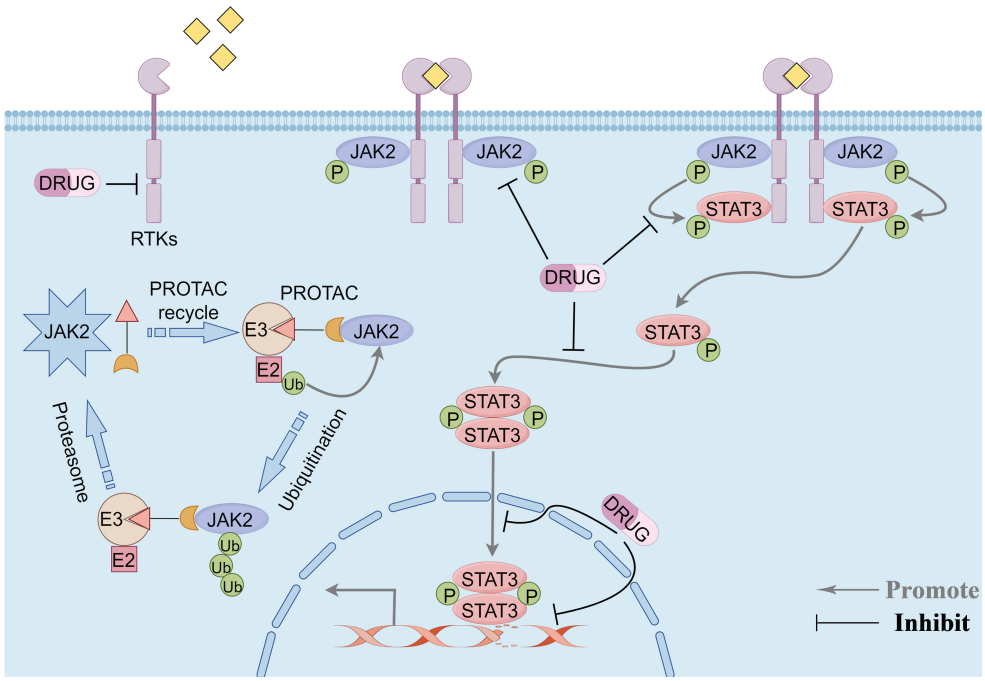

FIGURE 1
Overview of the JAK2/STAT3 pathway signaling modality and potential therapeutic targets of the pathway. Phosphorylation signaling blockade and protein-targeted degradation pathways. By Figdraw.

includes Her-2, Her-3, and Her-4 (39). EGF binding to EGFR induces receptor dimerization, a critical step leading to the autophosphorylation of tyrosine residues on the activated receptor. This activation allows the receptor to recruit various signal sequence proteins, transmitting biological signals from the extracellular milieu to the intracellular domain. These signaling cascades culminate in gene transcription, modulating key cellular processes such as proliferation, differentiation, and apoptosis. In cancer, EGFR contributes to tumor progression by promoting invasion and metastasis and stimulating tumor angiogenesis (40). EGFR activates complex signal transduction pathways with primary pathways including mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) (41), JAK2/STAT3 (42), and phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) (43). Dysregulation of these pathways is intricately linked to tumor development, invasion, and metastasis. EGFR is overexpressed in several malignant tumors, including lung, colon, liver, and breast cancers (44–47). In the context of cancer prognosis, EGFR overexpression is associated with shorter recurrence times, increased recurrence rates, and reduced survival durations (48). In TNBC, the positive expression rate of EGFR is notably higher than in non-TNBC, with over 40% of patients with TNBC exhibiting EGFR overexpression, a factor closely correlated with TNBC prognosis (49, 50). Targeted inhibition of EGFR expression has demonstrated anti-cancer effects in TNBC (51). EGFR expression is also implicated in CD44+ cell aggregation, with its inhibition disrupting cancer stem cell assembly in TNBC. These lines of evidence suggest a link between EGFR expression and the progression of CD44+-mediated cancer stem cells (52).

2.2 Vascular endothelial growth factor receptors: regulator of angiogenesis and cancer stem cells in TNBC

Vascular endothelial growth factor receptors, the receptor for vascular endothelial growth factor (VEGF), comprises three primary types: VEGFR1, VEGFR2, and VEGFR3. VEGF induces angiogenesis by binding to VEGFR-2, enhancing the survival, proliferation, migration, and adhesion of endothelial cells (53). However, in pathological contexts, particularly in cancer, VEGFR expression is linked to the promotion of tumor angiogenesis and metastasis (54, 55). Numerous studies have documented the overexpression of VEGFR in a range of malignant tumors, including lung, colon, breast, liver, and ovarian cancers (56–60). In TNBC, elevated VEGF levels correlate with increased metastasis, poor treatment response, and decreased survival rates (61). Upregulation of VEGFR in TNBC is linked to heightened cell proliferation, while its downregulation inhibits this proliferation (62). A notable randomized cohort study has indicated a strong association between high VEGFR expression and 5-year and 10-year breast cancer-specific survival rates in patients (63). These findings underscore the potential of targeting the VEGF/VEGFR axis as a promising approach in the targeted therapy of TNBC. Research employing primary breast cancer mouse models and models of spontaneous breast cancer metastasis has revealed elevated VEGFR expression levels in metastatic breast cancer compared to non-metastatic forms (64). Additionally, VEGFR expression correlates with cancer stem cell characteristics. By activating the VEGFR2/STAT3 pathway, VEGF induces the

upregulation of Myc and Sox2 expression, thereby promoting the self-renewal of breast cancer stem cells. The autocrine action of VEGF can establish a positive feedback loop, diminishing the efficacy of anti-angiogenic drugs and enhancing cancer stem cell renewal (28). Consequently, targeting VEGFR expression emerges as a potentially effective therapeutic strategy for the regulation of breast cancer stem cells.

2.3 Platelet-derived growth factor receptors: regulator of endothelial cell differentiation and cancer stem cells in TNBC

Tumor blood vessel development is crucial to tumor growth, making angiogenesis a potential target in cancer therapy (65). Platelet-derived growth factor receptors, which binds to platelet-derived growth factor (PDGF), exists in two forms: PDGFRα and PDGFRβ. PDGFR activation, contingent upon PDGF interaction, initiates various intracellular signaling pathways. While PDGFR contributes to vascular repair after tissue damage (66), it also promotes cell proliferation within tumor tissues (67). Studies involving mouse models with differential PDGF gene expression have yielded insightful observations. Specifically, tumors in mice with PDGF gene deficiency exhibit reduced pericyte recruitment, whereas tumors in mice with PDGF overexpression demonstrate increased pericyte recruitment. These findings suggest that tumors recruit pericytes through paracrine PDGF secretion, interacting with PDGFR, facilitating blood vessel maturation, and synergizing with VEGF-mediated angiogenesis, contributing to tumor vascularization (68). Extensive research indicates that PDGFR is overexpressed in various malignant tumors, including lung, colon, breast, and ovarian (69–72). In TNBC, PDGFRβ plays a notable role in mediating endothelial cell differentiation and vasculogenic mimicry in tumor cells (32). Further studies have identified a link between PDGFRβ expression in TNBC, and cancer stem cells, where FOXC2 induces cancer stem cell characteristics and metastasis by upregulating PDGFRβ expression (73). These findings position PDGFR as a promising therapeutic target for TNBC.

2.4 Fibroblast growth factor receptors: regulator of cell proliferation and cancer stem cells in TNBC

Fibroblast growth factor receptors, the receptor for fibroblast growth factor (FGF), comprises four subtypes: FGFR1, FGFR2, FGFR3, and FGFR4, collectively forming the FGFR family. Upon binding with FGF, FGFR is activated and modulates multiple intracellular signaling pathways crucial to various biological processes, including angiogenesis and lymphangiogenesis (74). Studies have highlighted the association of FGFR expression in various solid tumors with tumor cell proliferation (75–79). High-throughput sequencing has identified FGFR gene mutations in approximately 7.1% of malignant tumors, with breast cancer exhibiting the second-highest frequency after urothelial carcinoma (80). In TNBC, FGFR3 expression is observed, and inhibition of the FGFR3 signaling pathway reduces TNBC cell invasion and migration (81). Targeting and blocking the FGFR pathway can significantly enhance T cell infiltration and suppress tumor growth in TNBC (33). Some studies have revealed that estrogen can stimulate breast cancer stem cell proliferation via the paracrine FGF/FGFR/T-box transcription factor 3 (TBx3) signaling pathway, and inhibiting this pathway curtails cancer stem cell expansion in TNBC (82). These findings highlight the potential of targeting the FGFR pathway as a therapeutic approach in TNBC.

3 Activation of the JAK2/STAT3 signaling pathway in TNBC

Janus Kinase 2, a member of the JAK family of non-receptor tyrosine kinases, includes JAK1, JAK3, and tyrosine kinase 2 (TYK2). JAK3 is predominantly expressed in hematopoietic cells, while JAK1, JAK2, and TYK2 exhibit broader expression across various tissues (83). JAKs mediate a range of disease processes, including immune system disorders (84), hematologic conditions (85), and various malignancies (86, 87). Signal transducer and activator of transcription (STAT) protein family comprises members such as STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. Notably, STAT3 is implicated in the promotion of tumor growth and the induction of immunosuppression (88–90). The JAK2/STAT3 signaling pathway is ubiquitously expressed in cells and vital in physiological functions (91), such as cell proliferation, differentiation, apoptosis, and immune regulation (92). Beyond its physiological roles, this pathway is significantly implicated in various pathologies, notably in cancer and autoimmune disorders. In breast cancer, particularly TNBC, JAK2/STAT3 signaling is known for its excessive activation (93). Advanced research has facilitated a more precise molecular classification of TNBC, identifying the mesenchymal subtype characterized by heightened JAK2/STAT3 activity (8). This insight offers a new direction and foundation for the personalized clinical treatment of TNBC, focusing on targeting the JAK2/STAT3 signaling pathway. Research has elucidated that the JAK2/STAT3/Cyclin D2 signaling pathway is pivotal in promoting cancer stem cell proliferation (94). Specifically, in TNBC, studies have demonstrated that downregulating the JAK2/STAT3 pathway can significantly inhibit cancer stem cell proliferation (95). Furthermore, additional research has indicated that suppressing this pathway may reduce TNBC cell proliferation and migration (13).

4 The role of the JAK2/STAT3 signaling pathway in multidrug resistance in TNBC

The lack of effective targeted therapies, necessitating reliance on taxanes and anthracycline cytotoxic drugs significantly hinders the treatment of TNBC. However, the emergence of multidrug resistance during treatment poses a formidable challenge to this approach (5). For instance, the activation of the JAK2/STAT3

signaling pathway in nasopharyngeal carcinoma has been demonstrated to induce forkhead box M1 transcription, thereby enhancing resistance to PTX (96). Subsequent studies have revealed the contribution of the JAK2/STAT3 pathway to the development of PTX resistance by upregulating anti-apoptotic gene expression. Targeting this pathway has proven effective in reversing PTX resistance in ovarian cancer (97). In a model of PTX-resistant cells, researchers observed differential expression of the JAK2 gene, suggesting its potential role as a candidate gene linked to PTX resistance in ovarian cancer cell lines (98). Further studies indicate that the downregulation of JAK2/STAT3 signaling pathway can counteract PTX resistance in TNBC (26). Furthermore, a separate research effort found that JAK2 inhibitors can directly bind to the drug efflux protein P-gp in resistant cell lines, thus impeding P-gp-mediated drug efflux (99). Collectively, these studies underscore the significance of the JAK2/STAT3 signaling pathway in the development of multidrug resistance. Consequently, targeting this pathway, either as a standalone therapy or in combination with PTX, presents a promising strategy for the treatment of PTX-resistant TNBC.

5 Current therapeutic applications of the JAK2/STAT3 signaling pathway in TNBC

Recent research has elucidated the pivotal role of the JAK2/STAT3 signaling pathway in driving the proliferation, invasion, and migration of TNBC. These findings position the JAK2/STAT3 pathway as a promising therapeutic target for TNBC management. In response to these insights, numerous preclinical and clinical studies are actively exploring the development of inhibitors targeting RTKs, JAK2, and STAT3. These inhibitors are categorized based on their mode of action into traditional small molecule inhibitors in the occupation-driven mode and proteolysis targeting chimera (PROTAC) molecules based on ubiquitin-mediated protein degradation in an event-driven mode. Traditional small molecule inhibitors in the occupation-driven mode function by occupying the active site or binding site of the target protein with small molecule compounds. This action blocks its interaction with downstream signaling molecules, inhibiting its function. On the other hand, PROTAC molecules employ a ligand linker to bind the target protein with an E3 ubiquitin ligase, leveraging the ubiquitin-proteasome system to drive the degradation of the target protein (Figure 2).

5.1 Occupation driven mode: application of small molecule inhibitors in TNBC

5.1.1 RTKs inhibitors

Currently, the U.S. Food and Drug Administration (FDA) has not approved RTK inhibitors for the treatment of TNBC. Both monoclonal antibodies and small molecule inhibitors are progressing through preclinical and clinical research stages. Preclinical studies have shown that cetuximab can effectively reduce cancer stem cells in TNBC and inhibit tumor growth (100). However, clinical trials reveal a more complex picture. For instance, a study investigating the combination of cetuximab and cisplatin in metastatic TNBC reported benefits in fewer than 20% of patients. Genomic analyses revealed limited efficacy due to cetuximab-induced activation of alternative bypass pathways. Combining cetuximab with inhibitors targeting downstream elements of the EGFR pathway is proposed for enhanced benefits in patients with TNBC (101). Preclinical research has demonstrated that bevacizumab, a VEGFR inhibitor, effectively suppresses TNBC growth *in vivo* (102). However, the adjunctive use of bevacizumab with chemotherapy did not improve overall survival rates in early-stage patients with TNBC compared to chemotherapy alone. Similarly, tocilizumab, an interleukin-6 receptor (IL-6R) inhibitor, exhibits potential anti-TNBC properties in preclinical studies (103), but its clinical efficacy in TNBC treatment remains unreported and warrants further investigation.

Several RTK small molecule inhibitors have been reported in clinical studies for the treatment of TNBC (Table 1). Apatinib, a highly selective VEGFR inhibitor, has exhibited promising efficacy in a Phase II clinical trial for patients with TNBC combined with chemotherapy. The results highlighted not only its effectiveness but also a manageable safety profile (104). Furthermore, combining Apatinib with a programmed death-ligand 1 (PD-L1) inhibitor in another Phase II trial resulted in favorable outcomes with a controllable safety profile (105). Integration of Apatinib with a PD-L1 inhibitor and Eribulin in a multicenter Phase II trial demonstrated significant therapeutic benefits in treating advanced TNBC, notably extending its efficacy to PD-L1–negative patients (106). Anlotinib, identified as a small molecule inhibitor targeting the VEGFR, displayed promising results in advanced TNBC treatment. Specifically, a Phase Ib clinical trial revealed that Anlotinib, when employed in a chemotherapy-free regimen alongside a PD-L1 inhibitor, effectively treated previously advanced patients with TNBC. This combination not only demonstrated favorable efficacy but also maintained a manageable safety profile (107). Another Phase II clinical trial combining Anlotinib with standard chemotherapy for metastatic TNBC demonstrated therapeutic benefits with manageable safety (108).

Gefitinib, a small molecule EGFR inhibitor, along with neoadjuvant chemotherapy, showed a higher pathological complete response rate in a Phase II clinical trial for patients with TNBC, especially in the chemotherapy and Gefitinib combination group. However, it is critical to note that patients receiving Gefitinib exhibited a higher incidence of toxic reactions, consequently leading to the discontinuation of the trial for those patients (109). In another Phase II clinical trial, Erlotinib, a small molecule EGFR inhibitor, was evaluated for its efficacy in treating metastatic TNBC. Patients in this trial initially received treatment with albumin-bound PTX combined with bevacizumab, followed by a maintenance regimen comprising both bevacizumab and Erlotinib. Notably, a significant proportion of participants in this trial exhibited partial tumor responses (110).

Research on RTK inhibitors in TNBC is expanding to include natural products such as Salidroside extracted from Rhodiola (Table 2).

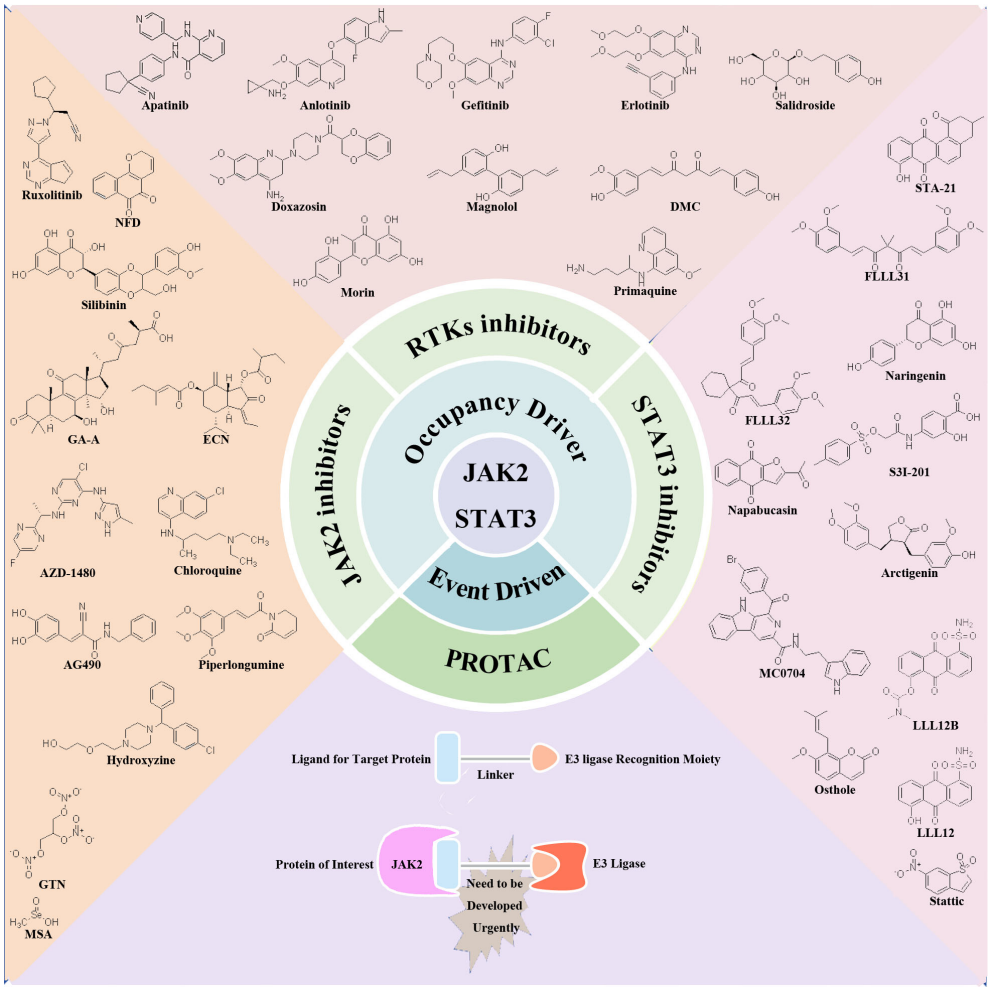

FIGURE 2
Potential therapeutic targets and inhibitors targeting JAK2/STAT3 signaling pathway for TNBC treatment. Occupation-driven mode: RTKs, JAK2 and STAT3-targeted inhibitors; event driven mode: small molecule compounds targeting JAK2 protein for ubiquitination degradation.

Preclinical studies have shown that Salidroside inhibits phosphorylation signaling pathways of EGFR/JAK2/STAT3, thereby impacting TNBC cell viability by binding to EGFR. Salidroside's therapeutic potential is highlighted by its selective efficacy, demonstrating minimal toxicity in normal breast epithelial cells (114). Doxazosin, primarily known as a vasodilator, has a dual-target mechanism, binding to cellular mesenchymal-epithelial transition factor (c-MET) and EGFR. This binding results in the inhibition of JAK2/STAT3 phosphorylation signaling. Research has demonstrated that doxazosin significantly affects TNBC cell proliferation, invasion, and migration, supported by *in vitro* and *in vivo* evidence. The efficacy of doxazosin in curbing TNBC lung metastasis was further substantiated through a mouse lung metastasis model (115).

Magnolol, a multifunctional lignan compound derived from the traditional Chinese herb Houpo, has demonstrated notable anti-cancer properties against TNBC. *In vitro* studies reveal that Magnolol effectively reduces the viability of TNBC cells. This inhibitory effect is primarily attributed to the suppression of phosphorylation signaling in the EGFR/JAK2/STAT3 pathway (116). The lead compound APP has also shown promising results in TNBC treatment. *In vitro* analyses indicate that APP induces apoptosis in TNBC cells. This apoptotic effect is mediated through the inhibition of EGFR/JAK2/STAT3 phosphorylation signaling, coupled with the regulation of apoptotic proteins (117). Demethoxycurcumin (DMC), a principal variant of curcumin predominantly found in the rhizomes of turmeric, has garnered attention in the context of TNBC research. *In vitro* studies have illuminated its potential in modulating TNBC cell viability. DMC achieves this by inhibiting EGFR protein expression levels. Furthermore, its mechanism involves the inhibition of specific phosphatases, thereby sustaining EGFR activation. This suggests that DMC's influence on TNBC cells might result from its regulation of multiple signaling pathways (118). Morin, a flavonoid compound derived from plants, has shown potential in the treatment of TNBC. Studies indicate that Morin, particularly when used in conjunction with doxorubicin, promotes apoptosis in TNBC cells. This synergistic effect is attributed to the inhibition of EGFR/STAT3 phosphorylation signaling (119, 120).

Primaquine, an antimalarial drug, has been observed to inhibit TNBC cell viability and migration *in vitro*. This inhibition is linked

10.3389/fonc.2024.1381251

TABLE 1 Targeting JAK2/STAT3 signaling pathway for TNBC in preclinical studies.

Compd.	Target	Effects/ Adverse Reactions	Citation
Apatinib	VEGFR	Combined with chemotherapy in a phase II clinical trial study, excellent results were achieved in patients with TNBC and were safe and manageable; Combination with a PD-L1 inhibitor in a phase II clinical trial study showed favorable results with a manageable safety profile in patients with advanced TNBC; Combination of PD-L1 inhibitors and eribulin shows promising results in the treatment of advanced TNBC in a multicenter phase II clinical trial study.	(104–106)
Anlotinib	VEGFR	Combination with a PD-L1 inhibitor in a phase Ib clinical trial showed favorable efficacy in previously treated patients with advanced TNBC with a manageable safety profile; Combination chemotherapy for treatment of metastatic TNBC achieves efficacy and is safe and controlled in phase II clinical trial studies.	(107, 108)
Gefitinib	EGFR	Efficacy achieved in combination with neoadjuvant chemotherapy in TNBC patients in a randomized phase II clinical trial study, but the trial was terminated due to toxic events.	(109)
Erlotinib	EGFR	Combined bevacizumab maintenance therapy reduces tumor load in most patients in a phase II clinical trial study.	(110)
Ruxolitinib	JAK2	In a phase II clinical trial study, treatment of TNBC as a single agent did not meet efficacy endpoints; Combination capecitabine has no benefit over capecitabine alone for TNBC in a phase II clinical trial study; Combined PTX is better than PTX alone for TNBC in a phase I clinical trial study.	(111–113)

TABLE 2 Targeting RTKs to modulate the JAK2/STAT3 signaling pathway in preclinical studies for the treatment of TNBC.

Compd.	Target	*In Vivo* Or *In Vitro*	Citation
Salidroside	EGFR	*In vitro*	(114)
Doxazosin	EGFR/ c-MET	*In vitro* and *in vivo*	(115)
Magnolol	EGFR	*In vitro*	(116)
4-(adamantan-1-yl)-2-(3-(2,4-dichlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl) thiazole (APP)	EGFR	*In vitro*	(117)
Demethoxycurcumin	EGFR	*In vitro*	(118)
Morin	EGFR	*In vitro*	(119, 120)
Primaquine	EGFR	*In vitro* and *in vivo*	(121)
Centipeda minima Extract (CME)	EGFR	*In vitro* and *in vivo*	(122)
CAPE-*p*NO2	EGFR	*In vitro* and *in vivo*	(123)
Phospho-aspirin-2 (PA-2)	EGFR	*In vitro* and *in vivo*	(124)
Deguelin	EGFR/ c-MET	*In vitro* and *in vivo*	(125, 126)
Picrasidine G	EGFR	*In vitro*	(127)
Regorafenib	VEGFR/ PDGFR	*In vitro* and *in vivo*	(128)
Bazedoxifene	GP130	*In vitro* and *in vivo*	(129, 130)
Raloxifene	GP130	*In vitro*	(131)
EC359	LIFR	*In vitro* and *in vivo*	(34, 132)
Chikusetsusaponin IVa Butyl Ester (CS-IVa-Be)	IL-6R	*In vitro*	(133)

to the suppression of EGFR/STAT3 phosphorylation signaling. However, the specific mechanisms by which Primaquine impedes TNBC growth *in vivo* remain to be explored further (121). Additionally, Centipeda minima Extract (CME), an extract from the Centipeda minima, has demonstrated efficacy in regulating TNBC cell behavior by modulating the phosphorylation signaling of multiple pathways, notably the STAT3 pathway. This modulation occurs through the inhibition of EGFR expression, thereby promoting apoptosis in TNBC cells (122). CAPE-pNO2 has also been identified as a potent inhibitor of proliferation and migration in TNBC by suppressing EGFR phosphorylation and the regulation of STAT3 and AKT phosphorylation signaling. This dual effect has been observed in both *in vitro* and *in vivo* studies (123).

Similarly, PA-2, another compound under investigation, has demonstrated its ability to promote apoptosis in TNBC cells. It achieves this through the inhibition of EGFR phosphorylation and by modulating the phosphorylation signaling of the PI3K/AKT and STAT3 pathways (124). Deguelin also contributes to this growing field of TNBC therapeutics. It influences the expression of both EGFR and c-Met, leading to the downregulation of phosphorylation signaling across several pathways, including STAT3, AKT, ERK, and NFκB. This comprehensive action results in a marked impact on the viability of TNBC cells (125, 126). Picrasidine G, a naturally derived dimeric alkaloid, has shown efficacy in inhibiting the vitality

of TNBC cells *in vitro* by suppressing the EGFR/STAT3 phosphorylation signaling pathway (127). Regorafenib, another compound under study, exerts its anti-cancer effects by inhibiting key receptors such as VEGFR and PDGFR. This inhibition impacts the STAT3 phosphorylation signaling (128).

Bazedoxifene presents a different angle in TNBC treatment. By targeting GP130, it influences the STAT3 phosphorylation signaling pathway. This strategic inhibition inhibits TNBC both *in vitro* and *in vivo* (129, 130). Raloxifene, a compound known for its influence on the GP130 receptor, has been shown to inhibit the vitality of TNBC cells *in vitro*. This effect is achieved through the modulation of the STAT3 phosphorylation signaling pathway (131). EC359, another promising agent, binds to the leukemia inhibitory factor receptor (LIFR), inhibiting the LIFR/STAT3 phosphorylation signaling, thereby curbing TNBC proliferation both *in vitro* and *in vivo* (34, 132). Similarly, CS-IVa-Be targets cancer cells through the inhibition of IL-6R, impacting the JAK2/STAT3 phosphorylation signaling pathway. This specific action has been observed to inhibit TNBC *in vitro* (133).

In summary, RTK inhibitors can inhibit the signaling of the JAK2/STAT3 pathway. However, due to the activation of bypass pathways, clinical trial results suggest that combining these inhibitors with chemotherapy may be more beneficial for patients with TNBC.

5.1.2 JAK2 inhibitors

Directly targeting JAK2 emerges as a strategic approach for modulating the JAK2/STAT3 signaling pathway in TNBC treatment. However, to date, JAK2 inhibitors have not received FDA approval for use in TNBC therapy. Ruxolitinib, a JAK1/JAK2 inhibitor, is being evaluated for its clinical efficacy in treating TNBC in several clinical trials. In a Phase II clinical trial, Ruxolitinib monotherapy did not meet its efficacy endpoint for TNBC treatment (111). Further research explored the potential of Ruxolitinib in combination with chemotherapy drugs. While combining Ruxolitinib with Capecitabine did not enhance overall survival, another trial pairing it with PTX showed improved clinical efficacy, outperforming PTX monotherapy (112, 113) (Table 1).

In preclinical TNBC studies, several small molecules exhibit promise as JAK2 inhibitors (Table 3). For instance, Glyceryl Trinitrate (GTN), a vasodilator, inhibits STAT3 activation by blocking JAK2 phosphorylation, suppressing TNBC cell viability (134). Additionally, a range of compounds, including Withaferin A (WA) (135), Naphtho[1,2-b]furan-4,5-dione (NFD) (136), Ganoderic acid A (GA-A) (137), Methylseleninic Acid (MSA) (138), and AZD1480 (139), have been identified to inhibit the phosphorylation and signal transduction of the JAK2/STAT3 pathway, reducing the viability of TNBC cells. Recent research findings highlight the efficacy of JAK2 small molecule inhibitors in TNBC. For instance, the JAK2 inhibitor AG490 has been shown to reduce TNBC cell viability by modulating the phosphorylation and signal transduction of STAT3 and AKT (140, 141). Additionally, 3-Deoxy-2β,16-dihydroxynagilactone E (B6) interacts with the FERM-SH2 domain of JAK2, inhibiting downstream STAT3 phosphorylation and reducing TNBC cell viability (142). Another study highlights the effectiveness of ECN in suppressing TNBC cell viability by targeting the JAK2/STAT3 signaling pathway. ECN has also demonstrated the ability to inhibit TNBC tumor growth *in vivo* (143).

TABLE 3 Targeting the JAK2 for TNBC in preclinical studies.

Compd.	Target	*In Vivo* Or *In Vitro*	Citation
Glyceryl Trinitrate (GTN)	JAK2	*In vitro* and *in vivo*	(134)
Withaferin A (WA)	JAK2	*In vitro*	(135)
Naphtho[1,2-b]furan-4,5-dione (NFD)	JAK2	*In vitro*	(136)
Ganoderic acid A (GA-A)	JAK2	*In vitro*	(137)
Methylseleninic Acid (MSA)	JAK2	*In vitro* and *in vivo*	(138)
AZD1480	JAK2	*In vitro*	(139)
AG490	JAK2	*In vitro*	(140, 141)
3-Deoxy-2β,16-dihydroxynagilactone E (B6)	JAK2	*In vitro*	(142)
7β-(3-Ethyl-*cis*-crotonoyloxy)-1α-(2-methylbutyryloxy)-3,14-dehydro-Z-notonipetranone (ECN)	JAK2	*In vitro* and *in vivo*	(143)
Chloroquine	JAK2	*In vitro* and *in vivo*	(95)
Silibinin	JAK2	*In vitro*	(144, 145)
Piperlongumine	JAK2	*In vitro* and *in vivo*	(146)
Hydroxyzine	JAK2	*In vitro*	(147)

Chloroquine enhances PTX therapeutic efficacy in TNBC by inhibiting JAK2/STAT3 pathway phosphorylation, impacting autophagy processes (95). Additionally, Silibinin suppresses TNBC cell invasive and migratory capabilities *in vitro* by downregulating JAK2/STAT3 pathway phosphorylation (144, 145). Piperlongumine, a bioactive alkaloid known for its antioxidant and anti-tumor properties, has been found to inhibit TNBC cell proliferation and migration by inhibiting JAK2/STAT3 pathway phosphorylation (146). Similarly, Hydroxyzine, primarily recognized as a histamine H1 receptor antagonist, has demonstrated the capability to induce apoptosis in TNBC cells through the inhibition of JAK2/STAT3 phosphorylation (147).

JAK2 inhibitors have shown significant efficacy in inhibiting TNBC *in vitro*. Clinical trial data *in vivo* also suggest that a combination of these inhibitors with the chemotherapy drug paclitaxel could be a promising therapeutic approach for TNBC. However, concerns about the safety of JAK2 inhibitors, as evidenced by FDA warnings, underscores the necessity for alternative therapeutic strategies targeting the JAK2/STAT3 pathway.

5.1.3 STAT3 inhibitor

Several STAT3 small molecule inhibitors have been reported in preclinical studies for the treatment of TNBC (Table 4). The therapeutic strategy to inhibit STAT3 involves targeting multiple stages of its functional cycle, including phosphorylation, dimerization, nuclear translocation, and DNA binding activities. This approach leverages the nuclear translocation signal of STAT3.

Stattic, a non-peptidic small molecule, has demonstrated notable anti-TNBC effects by selectively targeting STAT3, inhibiting its activation, dimerization, and nuclear translocation. This inhibition is facilitated through Stattic's binding to the SH2 functional domain of STAT3 (148, 149). Similarly, STA-21, another small molecule inhibitor, induces apoptosis in TNBC cells by inhibiting DNA binding activity and dimerization of STAT3 (150). FLLL31 and FLLL32, derivatives of curcumin, have been identified as selective inhibitors of STAT3. They achieve this by binding to the SH2 functional domain of STAT3, thereby inhibiting its phosphorylation and DNA binding activities. Notably, these compounds have shown potential in synergistically inhibiting TNBC cell proliferation when combined with doxorubicin. *In vivo* studies further indicate that FLLL32 can effectively suppress TNBC growth by downregulating STAT3 phosphorylation levels (151). Pyrrolidine sulfonamide derivative **6a** selectively inhibits STAT3 activation at phosphorylation and transcription levels, reducing TNBC cell viability in response to IL-6 stimulation (152). LLL12, a non-peptidic, cell-permeable small molecule, selectively targets STAT3 by inhibiting its DNA binding activity and phosphorylation through SH2 domain binding. It induces apoptosis in TNBC cells and suppresses TNBC growth *in vivo* by downregulating STAT3 phosphorylation levels (153). LLL12B, a prodrug of LLL12, is activated in the tumor microenvironment by tumor-associated plasmin, which cleaves its aminoformate bond to release active LLL12. LLL12B exhibits improved pharmacokinetic properties compared to its parent compound, LLL12. However, additional research is required to fully elucidate the comparative *in vivo* and *in vitro* pharmacology of these compounds, particularly their respective abilities to bind to STAT3 (15).

Naringenin, a naturally occurring compound, reduces TNBC cell viability by binding to the SH2 domain of STAT3, suppressing STAT3 phosphorylation. In combination with cyclophosphamide, naringenin has demonstrated enhanced efficacy in inducing apoptosis in TNBC cells (154). S3I-201, a selective STAT3 inhibitor probe, targets the SH2 functional domain of STAT3, inhibiting its DNA binding activity and dimerization. *In vitro* studies have revealed that S3I-201 significantly diminishes the TNBC cell viability and inhibits tumor growth by reducing STAT3 phosphorylation (155). Napabucasin, a targeted therapeutic agent, selectively inhibits the DNA binding activity and phosphorylation of STAT3 by binding to its SH2 functional domain. *In vitro* studies have demonstrated Napabucasin's capability to reduce TNBC cell viability (156). Additionally, a series of compounds, such as 7a (157), SLSI-1216 (158), H182 (159), SMY002 (160), MC0704 (161), ZSW (162), and Acetyl-cinobufagin (163), have been identified to selectively inhibit STAT3 phosphorylation by binding to its SH2 domain and

TABLE 4 Targeting STAT3 for TNBC in preclinical studies.

Compd.	Target	*In Vivo* Or *In Vitro*	Citation
Stattic	STAT3	*In vitro*	(148, 149)
STA-21	STAT3	*In vitro*	(150)
FLLL31	STAT3	*In vitro*	(151)
FLLL32	STAT3	*In vitro* and *in vivo*	(151)
Pyrrolidinesulphonylaryl molecules (6a)	STAT3	*In vitro*	(152)
LLL12	STAT3	*In vitro* and *in vivo*	(153)
LLL12B	STAT3	*In vitro* and *in vivo*	(15)
Naringenin	STAT3	*In vitro*	(154)
S3I-201	STAT3	*In vitro*	(155)
Napabucasin	STAT3	*In vitro*	(156)
Coumarin-benzothiophene1, 1-dioxide conjugates compound(7a)	STAT3	*In vitro* and *in vivo*	(157)
SLSI-1216	STAT3	*In vitro*	(158)
H182	STAT3	*In vitro* and *in vivo*	(159)
SMY002	STAT3	*In vitro* and *in vivo*	(160)
MC0704	STAT3	*In vitro* and *in vivo*	(161)
ZSW	STAT3	*In vitro* and *in vivo*	(162)
Acetyl-cinobufagin	STAT3	*In vitro* and *in vivo*	(163)
Arctigenin	STAT3	*In vitro* and *in vivo*	(164)
KYZ3	STAT3	*In vitro* and *in vivo*	(165)
Dihydrotanshinone	STAT3	*In vitro* and *in vivo*	(166)
DT-13	STAT3	*In vitro* and *in vivo*	(167)
Cucurbitacin E	STAT3	*In vitro*	(168–170)
Niclosamide	STAT3	*In vitro* and *in vivo*	(171–173)
SG-1709	STAT3	*In vitro*	(174)
SG-1721	STAT3	*In vitro* and *in vivo*	(174)
Nifuroxazide	STAT3	*In vitro* and *in vivo*	(175, 176)
LLY17	STAT3	*In vitro* and *in vivo*	(177)

(Continued)

TABLE 4 Continued

Compd.	Target	*In Vivo* Or *In Vitro*	Citation
6Br-6a	STAT3	*In vitro* and *in vivo*	(178)
Pyrimethamine	STAT3	*In vitro* and *in vivo*	(179, 180)
Pectolinarigenin	STAT3	*In vitro* and *in vivo*	(181)
Flubendazole	STAT3	*In vitro* and *in vivo*	(182, 183)
Eupalinolide J	STAT3	*In vitro*	(184, 185)
Betulinic acid	STAT3	*In vitro* and *in vivo*	(186)
Carfilzomib	STAT3	*In vitro* and *in vivo*	(187)
WP1066	STAT3	*In vitro*	(188)
Rhus coriaria extract	STAT3	*In vitro* and *in vivo*	(189)
FZU-03,010	STAT3	*In vitro*	(190)
Disulfiram	STAT3	*In vitro*	(191)
Schisandrin B	STAT3	*In vitro* and *in vivo*	(192)
Osthole	STAT3	*In vitro* and *in vivo*	(193)
Brevilin A	STAT3	*In vitro* and *in vivo*	(194)
Arnicolide D	STAT3	*In vitro* and *in vivo*	(195)
Eucannabinolide	STAT3	*In vitro* and *in vivo*	(196)
Pulvomycin	STAT3	*In vitro* and *in vivo*	(197)
R001	STAT3	*In vitro* and *in vivo*	(198)
Salinomycin	STAT3	*In vitro*	(199, 200)
Ethanolic extract of Origanum syriacum	STAT3	*In vitro*	(201)
Apigenin	STAT3	*In vitro* and *in vivo*	(202)
AG-014699	STAT3	*In vitro*	(203)

suppressing TNBC cell viability *in vitro*. Arctigenin, a bioactive lignan isolated from the seeds of Arctium lappa, inhibits STAT3 in TNBC cells by binding to its SH2 domain, thereby disrupting hydrogen bond connections between DNA and STAT3. This disruption prevents STAT3's binding to genomic DNA, effectively reducing TNBC cell viability (164). Similarly, KYZ3, a derivative of cryptotanshinone, binds to the SH2 domain of STAT3, inhibiting its DNA binding activity and phosphorylation, leading to decreased TNBC cell viability *in vitro* (165). Research has identified a wide array of small molecules that exhibit the potential to inhibit TNBC cell viability. This includes Dihydrotanshinone (166), DT-13 (167), Cucurbitacin E (168–170), Niclosamide (171–173), SG-1709 (174), SG-1721 (174), Nifuroxazide (175, 176), LLY17 (177), 6Br-6a (178), Pyrimethamine (179, 180), Pectolinarigenin (181), Flubendazole (182, 183), Eupalinolide J (184, 185), Betulinic acid (186), Carfilzomib (187), WP1066 (188), Rhus coriaria extract (189), FZU-03,010 (190), Disulfiram (191), Schisandrin B (192), Osthole (193), Brevilin A (194), Arnicolide D (195), Eucannabinolide (196), Pulvomycin (197), R001 (198), Salinomycin (199, 200), the ethanolic extract of origanum syriacum (201), Apigenin (202), and AG-014699 (203). This inhibition is attributed to their ability to suppress STAT3 phosphorylation. However, direct evidence demonstrating their binding to STAT3 is currently lacking.

While preclinical studies have identified various small molecule compounds as potential STAT3 inhibitors, TTI-101 stands out as the sole compound advancing into Phase I clinical trials. Various groups of researchers are investigating the efficacy and safety of TTI-101 in patients with advanced breast cancer and those with inoperable solid tumors.

5.1.4 Adverse effects of JAK2/STAT3 pathway inhibition

Because the biological processes of normal cells also depend on the JAK2/STAT3 pathway, the long-term use of JAK2/STAT3 pathway inhibitors has certain toxic side effects. JAK2 inhibitors inevitably inhibit the normal hematopoietic function of the body, which can cause anemia, thrombocytopenia, and other adverse effects (including dizziness, headache, abdominal pain, diarrhea, and the secondary tumor).

Studies have shown that anemia and thrombocytopenia are the most common hematologic adverse effects when JAK2 inhibitors are used to treat myelofibrosis (204–210); in another study of Ruxolitinib as a drug treatment for true erythrocytosis, headache and diarrhea were the most common non-hematologic adverse effects (211); the immunosuppressive effects of JAK2 inhibitors are important in inducing infections, and in the JUMP study, the most common infection was pneumonia, followed by urinary tract infections and nasopharyngitis (205), and another study showed that 30 of 31 patients treated with Ruxolitinib developed infections, including several opportunistic infections (212); although JAK inhibitors can be used to treat hematologic cancers and inflammatory diseases, during treatment with these drugs, studies have found that some patients suffer from lymphomas and other malignancies, with a statistically significant 16-fold increase in the risk of B-cell malignancies in patients with myeloproliferative neoplasms treated with JAK1/2 inhibitors, and skin cancers being the most common secondary tumor (213); in addition to these symptoms, Ruxolitinib can also cause other adverse reactions such as abdominal pain, drowsiness, acute renal failure (211), and even some studies have reported that patients have died from cardiac arrest (204).

Since the JAK family mediates signaling of multiple cytokines and different receptors are associated with different JAKs, and comprehensive inhibition of the JAK family can result in a variety of side effects, the design and development of new targeted JAK2 inhibitors could provide a solution to these adverse effects.

5.2 Event-driven mode: application of PROTAC molecules based on ubiquitin-mediated protein degradation in TNBC

The regulation of the JAK2/STAT3 signaling pathway can be strategically achieved through the targeted degradation of the JAK2 protein employing PROTACs. These molecular constructs consist of a linker connecting two ligands, with one ligand binding to the target JAK2 protein and the other engaging with the ubiquitin E3 ligase. This dual binding facilitates the formation of a ternary complex, bringing the JAK2 protein and E3 ligase into close proximity. Subsequently, the target JAK2 protein undergoes ubiquitination, marking it for recognition by the proteasome system. This leads to the proteasomal degradation of JAK2 into peptide fragments, effectively nullifying its protein activity (214). Recent studies underscore the promising role of PROTAC molecules in TNBC treatment. MZ1, a small molecule PROTAC, targets BRD4 protein for degradation. Compared to JQ1, a conventional inhibitor targeting the protein domain, MZ1 exhibits superior anti-TNBC activity both *in vitro* and *in vivo*, which is attributed to its specific action in targeting BRD4 protein degradation (215). Another notable PROTAC molecule, NN3, is designed to target PARP1 protein degradation. Experimental findings indicate that NN3 demonstrates effective anti-TNBC activity *in vitro* and *in vivo*. Remarkably, NN3 retains its efficiency in degrading PARP1 protein even in the presence of point mutations, further underscoring its potential as an anti-tumor agent (216). Emerging research sheds light on the efficacy of PROTAC molecule 6n, designed to target the degradation of the AXL protein. Demonstrating a significant advantage over traditional AXL kinase inhibitors, 6n has shown superior anti-TNBC activity *in vitro* and *in vivo* (217). Similarly, YX-02-030, a PROTAC molecule targeting the MDM2 protein degradation, exhibits enhanced anti-tumor activity compared to specific MDM2 inhibitors. Notably, YX-02-030 achieves this therapeutic efficacy without causing harm to normal cells (218). TEP, a PROTAC molecule engineered to target c-Myc protein degradation, effectively inhibits the proliferation of TNBC cells by facilitating the specific degradation of the endogenous c-Myc/Max complex. Additionally, TEP enhances the sensitivity of TNBC cells to palbociclib, a cyclin-dependent kinase inhibitor (219). Another PROTAC molecule, CT-4, designed to target HDAC8 protein degradation, promotes apoptosis in TNBC cells through the targeted degradation of HDAC8 protein (220). The small molecule compound A4, a PROTAC developed based on DCAF16, specifically targets CDK4/6 protein degradation. Research demonstrates that A4 exhibits potent inhibitory activity against CDK4/6, offering a favorable safety profile in normal cells, which is considered superior to the established CDK4/6 inhibitor, palbociclib (221). The small molecule compounds 7f and PP-C8, which also function as PROTAC molecules, also target CDK12/13 degradation. Studies have indicated that these compounds effectively reduce TNBC cell viability by inhibiting the expression of CDK12/13 (222, 223). PROTAC molecules MS8815 and U3i, designed to target EZH2 protein degradation, induce ubiquitination and subsequent proteasome-dependent degradation of EZH2, effectively inhibiting TNBC cell growth (224, 225). Similarly, androgen receptor (AR)-PROTAC has shown efficacy in targeting AR-positive TNBC cells by mediating the ubiquitination and degradation of the AR, thereby inhibiting cell growth (226). Furthermore, C8, a PROTAC molecule developed based on the PARP1/2 inhibitor Olaparib, exhibits promising therapeutic potential against TNBC. It promotes PARP2 protein degradation, demonstrating effectiveness *in vitro* and *in vivo* (227).

Currently, PROTAC small molecules targeting the degradation of JAK2 protein have been reported. However, research indicates that the E3 ligase CUL5 mediates JAK2 protein degradation (228). Developing PROTAC small molecules that facilitate the binding of JAK2 protein to the E3 ligase CUL5 could be a feasible strategy for treating TNBC.

6 Conclusion

The JAK2/STAT3 signaling pathway, activated by cytokines, is central in governing fundamental cellular processes, including growth, differentiation, apoptosis, and immune responses. In TNBC, excessive activation of this pathway contributes to immune evasion by TNBC cells. This aberrant activation promotes tumor growth, facilitates metastasis, and develops drug resistance in TNBC. Therefore, the strategic therapeutic targeting of the JAK2/STAT3 signaling pathway emerges as a promising strategy for the effective treatment of TNBC. The JAK2/STAT3 signaling pathway presents multiple targets for therapeutic intervention in TNBC. Inhibition strategies focusing on RTKs, JAK2, and STAT3 effectively suppresses TNBC cell growth. Despite these promising results, clinical trials of inhibitors that bind to the active sites of these proteins have encountered challenges. These limitations can be attributed to several factors, including the activation of compensatory bypass pathways, overexpression of target proteins, emergence of point mutations within these targets, and the heightened expression of competitive ligands. Unlike conventional inhibitors, PROTAC molecules do not rely on sustained binding to the target protein to exert their inhibitory effect. This unique characteristic enables them to remain effective even in the presence of mutations in the protein's active binding site. A key advantage of PROTACs lies in their catalytic mechanism; following the facilitation of ubiquitination and degradation of the target protein, PROTAC molecules can be recycled. This recycling ability potentially allows for lower drug dosages, enhancing both the safety profile and therapeutic potential of these molecules. Consequently, employing PROTACs to target the JAK2/STAT3 pathway emerges as an exceptionally promising strategy for TNBC treatment. By developing PROTACs that specifically target JAK2 protein degradation, not only is TNBC growth inhibited through the downregulation of the JAK2/STAT3 pathway, but the typical toxic side effects associated with traditional JAK2 inhibitors are also likely to be mitigated.

Author's note

During the preparation of this work authors did not used any AI tool/service, and takes full responsibility for the content of the publication.

Author contributions

LL: Conceptualization, Resources, Writing – original draft. XF: Conceptualization, Resources, Writing – original draft. LC: Formal Analysis, Project administration, Writing – original draft. LY: Conceptualization, Supervision, Writing – review & editing. XL: Conceptualization, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 18 April 2024
DOI 10.3389/fonc.2024.1356014

OPEN ACCESS

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RECEIVED 14 December 2023
ACCEPTED 25 March 2024
PUBLISHED 18 April 2024

CITATION
Nicolis O, De Los Angeles D and Taramasco C (2024) A contemporary review of breast cancer risk factors and the role of artificial intelligence. *Front. Oncol.* 14:1356014. doi: 10.3389/fonc.2024.1356014

A contemporary review of breast cancer risk factors and the role of artificial intelligence

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Background: Breast cancer continues to be a significant global health issue, necessitating advancements in prevention and early detection strategies. This review aims to assess and synthesize research conducted from 2020 to the present, focusing on breast cancer risk factors, including genetic, lifestyle, and environmental aspects, as well as the innovative role of artificial intelligence (AI) in prediction and diagnostics.

Methods: A comprehensive literature search, covering studies from 2020 to the present, was conducted to evaluate the diversity of breast cancer risk factors and the latest advances in Artificial Intelligence (AI) in this field. The review prioritized high-quality peer-reviewed research articles and meta-analyses.

Results: Our analysis reveals a complex interplay of genetic, lifestyle, and environmental risk factors for breast cancer, with significant variability across different populations. Furthermore, AI has emerged as a promising tool in enhancing the accuracy of breast cancer risk prediction and the personalization of prevention strategies.

Conclusion: The review highlights the necessity for personalized breast cancer prevention and detection approaches that account for individual risk factor profiles. It underscores the potential of AI to revolutionize these strategies, offering clear recommendations for future research directions and clinical practice improvements.

KEYWORDS

breast cancer, risk factors, artificial intelligence (AI), medical history, metabolic factors, reproductive and hormonal factors, lifestyle factors, environmental influence

1 Introduction

Over the past decade, breast cancer has remained a leading cause of mortality among women globally, driving an intensive search for effective prevention and early detection strategies. During 2020, more than 2.3 million women were diagnosed, of which 33.5% died (1). Despite significant advances in understanding biological mechanisms and risk factors of breast cancer, substantial challenges persist in the personalized clinical management and preventive intervention. This work aims to evaluate and synthesize the evidence available on breast cancer risk factors, ranging from genetic predispositions and lifestyle to environmental influences, with a particular interest in recent technological advancements, including AI, in predicting and detecting the disease. We pose two critical research questions: 1) What are the main risk factors associated with the development of breast cancer, and how do these vary among different populations and age groups? 2) How do recent technological advancements based on Artificial Intelligence (AI) help the detection and prevention of breast cancer? Guided by the hypothesis that the variability in breast cancer risk factors among different populations suggests that prevention and early detection strategies must be personalized, considering genetic, lifestyle, and environmental factors to be effective, this review seeks to identify areas of consensus and discrepancy in the scientific literature. Highlighting the need for personalized strategies that consider variability among populations and age groups, we aim to provide clear recommendations that guide future research and clinical practices towards more effective prevention and early detection of breast cancer.

The paper is organized as follows. In Section 2, the methodology for selecting and reviewing papers is described. Section 3 shows the results with particularly emphasis to the bibliometric study and risk factor categories. A discussion and some conclusions are in Sections 5 and 6, respectively.

2 Methodology

The methodology of the paper involved a comprehensive bibliographic development and analysis, which steps are described in Figure 1.

2.1 Literature search and eligibility criteria

Our review concentrated on studies published between 2020 and 2024, with a focus on breast cancer risk factors. We sourced these from databases like PubMed, Scopus, and Web of Science. We included research papers that provided insights into demographic, genetic, lifestyle, and environmental influences on breast cancer risk, alongside studies utilizing AI for enhancing risk prediction and classification. Exclusion criteria were set for articles published prior to 2020 and those not directly examining the outlined risk factors. English language has been mainly used for the selection.

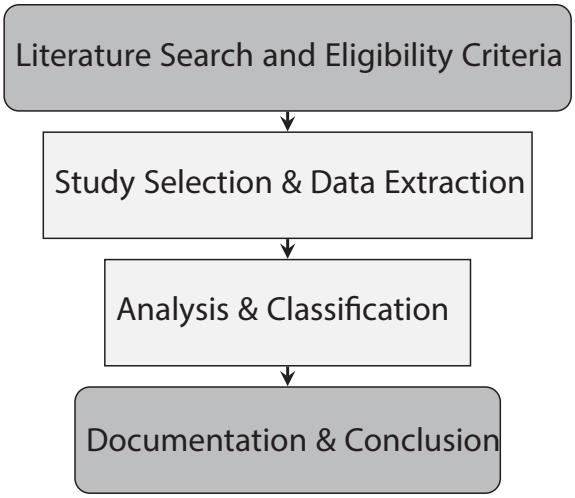

FIGURE 1
Flow chart of the methodology.

2.2 Study selection and data extraction

The study selection process meticulously filtered approximately 250 article by titles, abstracts and keywords, to determine their relevance to breast cancer risk factors and AI applications. A deeper process based on a complete reading of the papers narrowed the focus to 112 articles that met our inclusion criteria and offered important information on the topic. This approach ensured that only the most relevant studies were included, providing a detailed exploration of breast cancer risk factors and the role of AI in risk management. A bibliometric analysis was realized for setting frequencies and relationships among risk factors. Finally, these risk factors were systematically classified into categories, as detailed in Table 1.

2.3 Analysis and classification

This classification was based on the analysis of risk factors available in various articles, which were then grouped according to characteristics to derive the respective classifications. Regarding risk factors, they were classified into groups corresponding to "Demographic and Genetic Factors", "Reproductive and Hormonal Factors", "Metabolic Factors", "Medical History" and "Lifestyle and Environmental Factors." Additionally, a new independent category was created to group papers that include studies with artificial intelligence models, named "Use of AI in Risk Prediction". A simple Natural Language Processing (NLP) word count was used to identify the risk factors most frequently mentioned in each paper.

2.4 Documentation and conclusion

This methodology involved the following steps: conducting an exhaustive literature search across major scientific databases;

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TABLE 1 Keywords and descriptions for breast cancer risk factors and AI research.

Risk Factor	Keywords and search
Demographic and Genetic Factors	
Age	*Breast cancer age risk, age-related breast cancer*
Race or ethnicity and geographic location	*Breast cancer ethnicity, racial disparities in breast cancer, geographic variation breast cancer*
Family History	*Family history breast cancer risk, hereditary breast cancer*
Genetic mutations	*HER2 (Human Epidermal Growth Factor Receptor 2), Genetic and Molecular Factors*
Economic factors	*Socioeconomic status breast cancer risk, Socioeconomic impact breast cancer, economic disparities breast cancer*
Reproductive and Hormonal Factors	
Menarche (age at first menstruation)	*Menarche breast cancer risk, hormonal exposure breast cancer*
Menopause (age at menopause)	*Menopause breast cancer risk, hormonal exposure breast cancer*
Breastfeeding and Parity (number of fullterm pregnancies)	*Breastfeeding breast cancer risk reduction, parity breast cancer correlation*
Hormonal factors (use of hormone replacement therapy, contraceptives, etc	*HRT (hormone replacement therapy) breast cancer risk, contraceptives breast cancer*
Metabolic Factors	
Diabetes	*Insulin resistance breast cancer, glycemic control breast cancer, type 2 diabetes*
Metabolism	*Thyroid function breast cancer,*
Medical History	
Breast density	*Breast density cancer risk, mammographic density breast cancer*
Other cancers and diseases	*Comorbidity breast cancer risk, second primary cancer breast cancer*
Lifestyle Factors	
Alcohol consumption	*Alcohol consumption breast cancer risk*
Cigarette smoking	*Smoking and breast cancer link*
Obesity and Body Mass Index (BMI)	*obesity breast cancer correlation, dietary factors affecting breast cancer*
Poor nutrition	*Dietary fats and breast cancer, Nutritional deficiencies and breast cancer*
Physical inactivity	*Exercise breast cancer risk reduction, physical inactivity breast cancer*
Stress, anxiety, or depression	*Stress and breast cancer risk, depression impact on breast cancer, anxiety breast cancer correlation*
Environmental Factors	
Exposure to radiation	*Radiation exposure breast cancer risk, ionizing radiation breast cancer*
Exposure to chemicals	*Endocrine disruptors breast cancer risk, chemical exposure and breast cancer*
Environmental pollutants and heavy metals	*Explore research on how air quality and exposure to pollutants correlate with breast cancer incidence, Research the relationship between exposure to industrial byproducts and breast cancer development*
Use of AI in Risk Prediction	
AI breast cancer detection, predictive models Breast Cancer.	

applying inclusion and exclusion criteria, and to narrow down the selection from approximately 250 papers to 112 most relevant papers; employing techniques for a more deep analysis of the risk factors mentioned across the selected papers and categorizing the identified risk factors into specific groups for a structured analysis. This methodology not only ensures a comprehensive understanding of the existing research landscape but also supports the identification of key risk factors for breast cancer, facilitating a more precise and evidence-based analysis.

3 Results

By applying the above methodology, we show the results of the a systematic literature review of the selected 112 papers and we describe the main findings for each category of risk according to Table 2.

3.1 Bibliometric analysis

In this section we provide a bibliometric analysis using the Bibliometrix package of R software (114).

In order to facilitate a deeper understanding of how keywords interconnect across the collection of reviewed papers, a keyword network graph is shown in Figure 2. The graph highlights the thematic ties and focal points within the research landscape under examination. In the Figure 2 we can see the most interconnected and frequent keywords are: female, breast tumor, breast cancer and breast neoplasms.

Figure 3 displays the distribution of bibliographic authors by country. In this chart, 'MCP' represents Multiple Country Publications, indicating research papers co-authored by individuals from various nations, while 'SCP' signifies Single Country Publications, denoting research executed solely by authors within the same country. This visual representation clearly indicates that the United States is at the forefront in terms

TABLE 2 Summary of risk factors and characteristics in breast cancer research literature.

Paper	Age	Race and geography	Family History	Genetic mutations	Economic factors	Menarche	Menopause	Breastfeeding and Parity	Hormonal factors	Diabetes	Metabolism	Breast density	Other cancers and diseases	Alcohol consumption	Cigarette smoking	Obesity and BMI	Poor nutrition	Physical inactivity	Stress, anxiety, or depression	Exposure to radiation	Exposure to chemicals	Pollutants and heavy metals	AI Models
(2)		•	•		•																		
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TABLE 2 Continued

Paper	Age	Race and geography	Family History	Genetic mutations	Economic factors	Menarche	Menopause	Breastfeeding and Parity	Hormonal factors	Diabetes	Metabolism	Breast density	Other cancers and diseases	Alcohol consumption	Cigarette smoking	Obesity and BMI	Poor nutrition	Physical inactivity	Stress, anxiety, or depression	Exposure to radiation	Exposure to chemicals	Pollutants and heavy metals	AI Models
(21)				•			•		•								•						
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TABLE 2 Continued

Paper	Age	Race and geography	Family History	Genetic mutations	Economic factors	Menarche	Menopause	Breastfeeding and Parity	Hormonal factors	Diabetes	Metabolism	Breast density	Other cancers and diseases	Alcohol consumption	Cigarette smoking	Obesity and BMI	Poor nutrition	Physical inactivity	Stress, anxiety, or depression	Exposure to radiation	Exposure to chemicals	Pollutants and heavy metals	AI Models
(40)														•									
(41)															•								
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TABLE 2 Continued

Paper	Age	Race and geography	Family History	Genetic mutations	Economic factors	Menarche	Menopause	Breastfeeding and Parity	Hormonal factors	Diabetes	Metabolism	Breast density	Other cancers and diseases	Alcohol consumption	Cigarette smoking	Obesity and BMI	Poor nutrition	Physical inactivity	Stress, anxiety, or depression	Exposure to radiation	Exposure to chemicals	Pollutants and heavy metals	AI Models
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Paper	Age	Race and geography	Family History	Genetic mutations	Economic factors	Menarche	Menopause	Breastfeeding and Parity	Hormonal factors	Diabetes	Metabolism	Breast density	Other cancers and diseases	Alcohol consumption	Cigarette smoking	Obesity and BMI	Poor nutrition	Physical inactivity	Stress, anxiety, or depression	Exposure to radiation	Exposure to chemicals	Pollutants and heavy metals	AI Models
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TABLE 2 Continued

Paper	Age	Race and geography	Family History	Genetic mutations	Economic factors	Menarche	Menopause	Breastfeeding and Parity	Hormonal factors	Diabetes	Metabolism	Breast density	Other cancers and diseases	Alcohol consumption	Cigarette smoking	Obesity and BMI	Poor nutrition	Physical inactivity	Stress, anxiety, or depression	Exposure to radiation	Exposure to chemicals	Pollutants and heavy metals	AI Models
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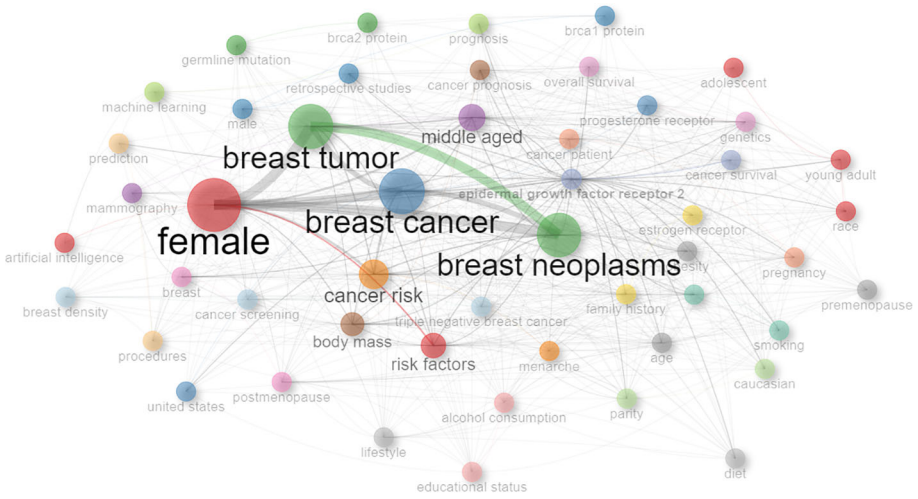

FIGURE 2
Keyword network visualization in breast cancer research.

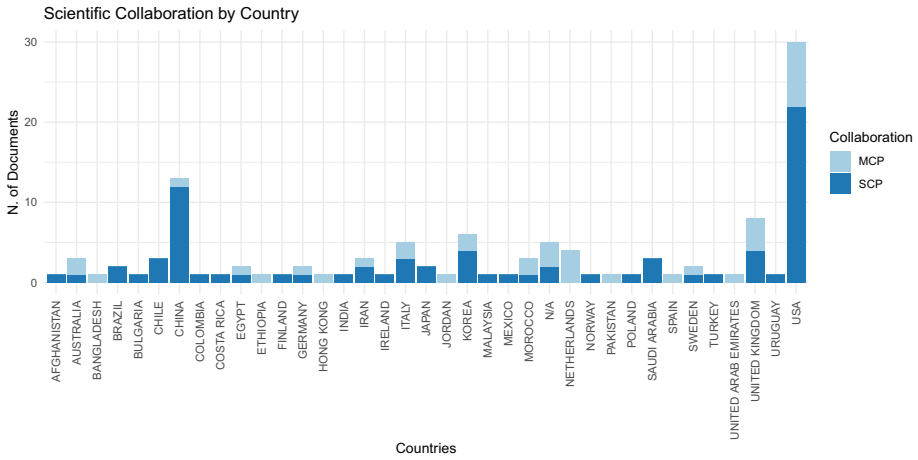

FIGURE 3
Distribution of countries of bibliographic authors.

of the volume of scientific publications, with significant contributions in both national (SCP) and international (MCP) collaborations, followed by China, evidencing a robust level of scientific output and cooperative engagement in these nations.

Conversely, the author network depicted in Figure 4 illustrates clustering among authors who have contributed to more than five publications. Those with a higher publication frequency are represented by larger circles, visually highlighting the most prolific contributors within the network.

3.2 Breast cancer risk factors

In this Section, we provide a detailed analysis of breast cancer risk factors identified by the reviewed works as represented in Table 2.

3.3 Demographic and genetic factors

- Age: Age plays a crucial role in breast cancer incidence and outcomes, particularly impacting middle-aged and older women. Studies like (53) and (33) investigate treatment efficacy and risk factors, especially in younger women. Demographic factors, including age, are highlighted by (67) and (110). Mortality rates, notably rising in women under 50 and over 70, are observed by (65), underscoring age's significance. Associations between reproductive history and breast cancer subtypes in women aged ≤50 are explored by (24) (42). focuses on mammographic density's relation to risk in women aged 40 to 74. Lastly (46), emphasizes age-specific preventive measures for women aged 30–39.

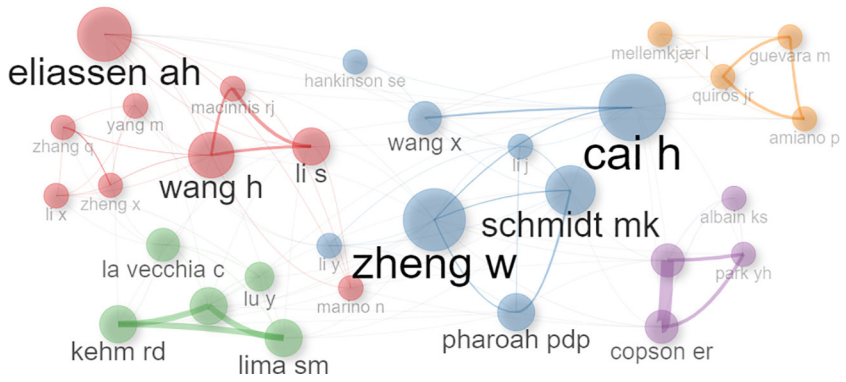

FIGURE 4
Co-authorship network analysis in scientific research.

- Race or ethnicity and geographic location: Research underscores significant variations in breast cancer predisposition across ethnicities and geographic locations, influenced by genetic, environmental, and socioeconomic factors. Studies like (112) emphasize diverse risk prediction models' necessity, especially for Asian women. Disparities persist despite similar treatments, as shown by (4) among Black and White women. Meanwhile (12), and (18) identify genetic susceptibility in Egyptian and Arab populations. Geographical variations, highlighted by (29), highlight the need to adopt personalized approaches. These findings emphasize the multifaceted nature of breast cancer risk and treatment strategies across diverse populations.
- Family History: The presence of a family history significantly impacts the assessment and management of breast cancer risk (110). reveals that 35.5% of women with a familial history face a high lifetime risk, yet only 23.9% receive enhanced screening (13). demonstrates the effectiveness of machine learning, achieving 77.78% precision in risk prediction. In addition (77), identifies specific germline variants linked to susceptibility. Furthermore, the integration of polygenic risk scores with family history, as demonstrated by (91), significantly alters surveillance recommendations. Overall, these findings underscore the crucial role of family history in personalized breast cancer care and risk management.
- Genetic mutations, such as BRCA1 (Breast Cancer Gene 1) and BRCA2 (Breast Cancer Gene 2): Genetic mutations, particularly in BRCA1 and BRCA2 genes, significantly increase hereditary breast cancer risk. Studies like (92) analyze the role of germline CHEK2 (Checkpoint Kinase 2) variants, while (97) advocate personalized prevention strategies (98). identifies genetic loci associated with contralateral breast cancer risk, and (3) explores molecular links between obesity and breast cancer. These findings emphasize the multifactorial nature of breast cancer, requiring tailored risk assessment and management.
- Economic factors: Economic factors significantly impact breast cancer risk and outcomes (86). reveals disparities in access to systemic anticancer therapies based on geographic and sociodemographic factors. Similarly (36), notes a social gradient in cancer incidence in Costa Rica (51). links higher education levels to increased breast cancer risk (2). emphasizes local demographic factors in TNBC (Triple-Negative Breast Cancer) treatment, while (32) highlights access disparities in Colombia. Finally (70), stresses the importance of socio-demographic indices and public health policies in addressing breast cancer burden in developing countries.

3.4 Reproductive and hormonal factors

- Menarche (age at first menstruation): Early menarche increases breast cancer risk due to prolonged hormonal exposure (26). links higher anti-Müllerian hormone levels to early menarche, indicating elevated risk. Conversely (72), suggests later menarche protects against certain breast cancer subtypes. Lifestyle changes, like plant-based diets, are crucial in mitigating risk, as emphasized by (49).
- Menopause (age at menopause): Late menopause increases breast cancer risk due to prolonged hormonal exposure (111). links menopausal hormonal changes to chemotherapy side effects severity. Conversely (20), emphasizes fat distribution's role in postmenopausal breast cancer risk (26). associates lower anti-Müllerian hormone levels with earlier menopause, indicating elevated risk. Conversely (72), suggests later menopause as a risk factor for certain breast cancer subtypes. Lifestyle factors like higher BMI and caloric intake heighten post-menopausal breast cancer risks, as noted by (49).
- Breastfeeding and Parity (number of full-term pregnancies): Parity and breastfeeding reduce breast cancer risk (80). analyzes parity's influence across birth cohorts, showing changing risk patterns (26). links anti-Müllerian hormone levels to age at menarche and parity, aiding risk assessment

(64). studies parity's impact on breast cancer incidence, highlighting rising rates in younger women (72). meta-analysis reveals subtype-specific risks, emphasizing tailored prevention strategies.

- Hormonal factors (use of hormone replacement therapy, contraceptives, etc.): Hormonal factors like hormone replacement therapy and contraceptives influence breast cancer risk (3). highlights obesity's role in breast cancer, especially in postmenopausal women (10). emphasizes hormonal imbalances' impact, urging further research (59). finds no significant difference in breast cancer risk with Hormone Replacement Therapy among BRCA mutation carriers. These findings emphasize the importance of hormonal markers like estrogen and progesterone receptors in breast cancer treatment (3, 10, 59). Additionally (21), and (72) explore lifestyle factors like diet and reproductive behaviors, highlighting hormonal influences on breast cancer risk.

3.5 Metabolic factors

- Diabetes: Elevated levels of insulin can promote cellular proliferation and reduce apoptosis, thus facilitating the development and progression of mammary neoplasms (3). elucidate obesity's pivotal role in breast cancer (BC) risk, particularly postmenopausal women, citing hormonal imbalances and insulin resistance among its mechanisms. They reveal how obesity-driven molecular changes, like increased estrogen and insulin levels, contribute to BC via specific signaling pathways. Conversely (34), find a significant correlation between genetic predisposition to Type 2 Diabetes Mellitus (T2DM) and poorer breast cancer-specific survival (HR = 1.10, 95% CI = 1.04–1.18, P = 0.003), emphasizing the potential causal impact of T2DM on BC outcomes.
- Metabolism: Metabolic processes play a crucial role in modulating breast cancer risk, significantly influencing hormonal levels and cellular dynamics. Alterations in metabolism, including imbalances in lipid and glucose metabolism, can lead to endocrine changes and alterations in the cellular microenvironment that favor mammary carcinogenesis. Metabolism plays a crucial role in breast cancer risk, with various factors influencing susceptibility (113). found that high-density lipoprotein cholesterol (HDL-C) significantly affects breast cancer risk, suggesting a metabolic component to cancer development (9). identified associations between insulin-like growth factor 1 (IGF-1) levels and fasting blood glucose with breast cancer risk, emphasizing the complexity of metabolic factors. Additionally (13), integrated genetic mutations and demographic factors to predict breast cancer risk, highlighting the importance of considering metabolic pathways in risk assessment. These findings underscore the multifaceted nature of metabolism-related risk factors in breast cancer susceptibility (113) (9) and (13).

3.6 Medical history

- Breast density: Breast density complicates cancer detection in the sense that it can make more difficult for mammograms to identify cancerous tumors due to the tissue's thickness or opaqueness. Additionally, high breast density is considered an independent risk factor for developing breast cancer. This is because denser breast tissue contains more connective and glandular tissues, which can potentially hide tumors and it is also associated with a higher likelihood of cancer development (11). found a sixfold risk difference between densest and least dense categories (42). investigated this relationship across a cohort of 21,150 women, confirming the effectiveness of automated density assessments in predicting breast cancer risk. Similarly (69) emphasizes higher risk in younger women with lower BMI (46). explores mammography-based risk assessment for early screening. These studies underscore the importance of considering mammographic density in breast cancer risk assessment and screening.
- Other cancers and diseases: The presence of other cancers may indicate heightened risk for breast cancer (107). developed prognostic nomograms for breast cancer patients with lung metastasis (66). addressed disparities in colorectal and breast cancer screenings (83). revealed screening rate disparities among females with schizophrenia (106). noted a slight increase in primary lung cancer risk post-radiotherapy for breast cancer.

3.7 Lifestyle factors

- Alcohol consumption: Alcohol consumption significantly increases breast cancer risk, even with moderate intake (85). revealed odds ratios between 1.82 to 5.67, indicating a notable association (40). highlighted a high prevalence (18.34%) of risky drinking among Australian women, exceeding weekly guidelines. These studies emphasize the importance of preventive measures. These findings underscore the link between alcohol intake and breast cancer risk, highlighting the need for preventive measures (35, 51).
- Cigarette smoking: Cigarette smoking contributes to breast cancer risk, with global estimates from (41) showing it accounted for 5.1% of deaths and 5.2% of DALYs in 2019. They emphasize anti-tobacco policies, particularly in low SDI regions (80). found smoking's heightened impact in

younger Asian cohorts, highlighting the need for tailored prevention strategies.

- Obesity and Body Mass Index (BMI): Obesity, particularly postmenopause, significantly increases breast cancer risk, impacting hormonal levels and inflammation. Studies like (3) highlight obesity's role in altering molecular pathways, while (102) emphasize its association with higher estrogen levels, especially in postmenopausal women (19). stresses lifestyle interventions for reducing breast cancer risk in obese postmenopausal women. Additionally (71), found BMI significantly influences breast cancer prognosis, particularly in premenopausal women with specific cancer subtypes.
- Poor nutrition: Poor nutrition, characterized by diets high in fats and sugars, increases breast cancer risk. Studies like (103) highlight the positive impact of tailored lifestyle interventions, while (16) suggest higher plasma vitamin D levels may offer protection (21). and (49) emphasize the association between Western diets and increased risk, contrasting with the protective effect of plant-based diets. Additionally (62), and (94) address dietary misconceptions and socio-demographic factors influencing nutritional risk, advocating for comprehensive approaches in breast cancer care.
- Physical inactivity: Physical inactivity increases breast cancer risk, while exercise helps regulate hormones and maintain a healthy weight. Studies like (19) emphasize its benefits in reducing recurrence risk. Tailored interventions, as shown by (103), positively impact survivors' quality of life (49). link low physical activity to higher risk, especially in post-menopausal women. Additionally (91), propose personalized surveillance integrating lifestyle factors for better outcomes.
- Stress, anxiety, or depression: Chronic stress may impact breast cancer risk (57). links stress, anxiety, and depression to reduced quality of life in survivors (103). shows positive outcomes in QoL (Quality of Life) indicators with home-based interventions despite pandemic challenges.

3.8 Environmental factors

- Exposure to radiation: Exposure to ionizing radiation, like from radiotherapy, elevates breast cancer risk, especially when received at a young age. Studies explore various factors (38): concluded that exposure to chest radiation therapy significantly elevates breast cancer risk, with individuals who have undergone such treatments facing a notably higher likelihood of developing the disease. Similarly (57), mention that receiving chest radiation therapy was significantly associated with a higher risk of breast cancer, with an Adjusted Odds Ratio (AOR) of 6.43, indicating a more than sixfold increase in risk compared to those who had not received such therapy (98). found that genetic variations can influence an individual's susceptibility to radiation toxicity (106). discusses lung cancer risk post-radiotherapy (111); links menopause to chemotherapy side effects; and (22) reported a high radiodermatitis incidence (98.2%) in breast cancer patients undergoing radiotherapy, with BMI and statin use affecting severity, and hydrogel showing protective effects.
- Exposure to chemicals: Chemicals like endocrine disruptors may disrupt hormonal balance, potentially contributing to breast cancer (105). evaluates CDK4/6 inhibitors' toxicity in metastatic breast cancer, stressing personalized treatment strategies due to varying drug profiles.
- Environmental pollutants, specific exposures and heavy metals: Environmental pollutants, including heavy metals and air pollution, contribute to breast cancer risk (6). found altered levels of metals like copper and cadmium in breast cancer patients (96). investigated air pollution's association with postmenopausal breast cancer risk, finding a significant 18% risk increase with a 10 μg/m3 rise in PM10 levels in 2007.

4 The role of artificial intelligence models for detecting breast cancer

The integration of artificial intelligence (AI) in breast cancer management spans various aspects, including diagnosis, recurrence prediction, survival rate estimation, and treatment response assessment. Studies like (5) demonstrate the effectiveness of machine learning models, achieving 80.23% accuracy in diagnosing early-stage breast cancer. Key risk factors identified for breast cancer included levels of glucose, age, and resistin. This approach demonstrates the potential of machine learning in enhancing breast cancer diagnostic processes by effectively selecting critical risk factors. Similarly (8), utilizes NLP and machine learning to predict breast cancer recurrence, emphasizing the efficacy of the OneR algorithm. The main clinical data used in the paper for predicting breast cancer recurrence involve a wide range of factors extracted from electronic health records (EHR). These include diagnostic symptoms, medications, lab results, medical recommendations, past medical history, procedures, family history, imaging, endoscopic assessments, anesthesia types, allergies, and other clinical documents. NLP algorithms were developed to extract these key features from the medical records. Notably (81), highlights Support Vector Machine (SVM) as the most accurate algorithm for breast cancer prediction, achieving an accuracy of 97.2%. The characteristics of the cell nuclei present in the images, are used as inputs for the SVM. They include, Radius, Texture, Area, Perimeter, Smoothness, Compactness, Concavity, Concave points, Symmetry, and Fractal dimension. These attributes are determined from the digitized images and serve as the basis for the SVM model to classify instances into benign or malignant categories.

For detection purposes, most of the papers use mammography images for training deep learning models, by assuming these algorithms are able to detect anomalies in the breast tissue. In this context, a comprehensive review is provided by (14) focusing on various ANN models such as Spiking Neural Network (SNN), Deep Belief Network (DBN), Convolutional Neural Network (CNN), Multilayer Neural Network (MLNN), Stacked Autoencoders (SAE), and Stacked De-noising Autoencoders (SDAE). The review highlights the effectiveness of these models in improving diagnosis accuracy, precision, recall, and other metrics, with particular success noted in models like ResNet-50 and ResNet-101 within the CNN algorithm framework. Instead, clinical data have been considered by (17) which developed a Machine Learning (ML) system for classifying breast cancer and diagnosing cancer metastases using clinical data extracted from Electronic Medical Records (EMRs). The best results have been obtained by a decision tree classifier which achieved 83% accuracy and an AUC (Area Under the Curve) of 0.87, demonstrating the potential of ML models based on blood profile data to aid professionals in identifying high-risk metastases breast cancer patients, thereby improving survival outcomes.

Regarding treatment response assessment (28), employs CNNs to predict treatment response in breast cancer patients undergoing chemotherapy, achieving high accuracies for various parameters. The study integrates both imaging and non-imaging data for the inputs of the models included longitudinal multiparametric MRI data (dynamic-contrast-enhanced MRI and T2-weighted MRI), demographics, and molecular subtypes. The use of advanced imaging techniques alongside clinical and molecular data indicates the need for a personalized treatment planning and assessment in breast cancer care (73). demonstrates deep learning's superior performance in risk identification compared to traditional Machine Learning (ML) methods. Important inputs for their models include age, resistin levels, global burden of disease (GBD) relative risk upper values, glucose, adiponectin, high BMI (binary), MCP-1, leptin, relative risks from meta-analyses, obesity (binary), and insulin levels. These inputs were selected based on their relevance and low redundancy for predicting breast cancer, highlighting the potential of deep learning to complement traditional screening methods by identifying individuals at risk non-invasively and affordably. In survival rate prediction (63), evaluates ML's role, highlighting challenges like data preprocessing and model validation. review 31 studies, mainly from Asia, to predict the 5-year survival rate of breast cancer. It is highlighted that among the papers reviewed, the most used algorithms are decision trees (61.3%), artificial neural networks (58.1%) and support vector machines (51.6%), where clinical and molecular information was used to build predictive models (73). used a database of 116 women, of which 52 were healthy and 64 had been diagnosed with breast cancer. The information included demographic and anthropometric data. The application of Deep Learning was considered the best evaluated method for breast cancer prediction, among algorithms such as SVM, Neural Networks, Logistic Regression, XGBoost, Random Forest, Naive Bayes and Stochastic Gradient. Lastly, studies like (88) predict patient satisfaction post-mastectomy, revealing that 45.2% of women experienced improved satisfaction with their breasts. These findings underscore the potential of AI in enhancing various aspects of breast cancer management, from diagnosis to patient satisfaction assessment. A novel approach that integrates Machine Learning (ML) algorithms with Explainable Artificial Intelligence (XAI) has been recently developed to enhance the understanding and interpretation of predictions made by ML models. In the context of breast cancer research (95), introduced a Hybrid Algorithm combining ML and XAI techniques aimed at preventing breast cancer. This innovative methodology enables the identification and extraction of key risk factors, such as high-fat diets and breastfeeding habits, to accurately differentiate between patients with and without breast cancer among Indonesian women. Risk indicators, such as auxiliary nodes and breast density, can also be extracted by the images by using deep learning (7, 56, 84).

5 Discussion

Upon reviewing multiple studies on breast cancer and its associated risk factors, several key findings emerge.

- Demographic and genetic factors play a crucial role in influencing breast cancer risk. This review highlights the crucial impact of age, with a notable increase in breast cancer incidence and outcomes, particularly affecting middle-aged and older women, as well as younger demographics in certain contexts. The significance of race, ethnicity, and geographic location is underscored, emphasizing the variability in breast cancer predisposition across different populations due to a mix of genetic, environmental, and socioeconomic factors. Family history and specific genetic mutations, such as BRCA1 and BRCA2, are identified as key risk determinants, necessitating personalized prevention and management strategies. Economic factors also emerge as crucial, with disparities in access to care and outcomes spotlighted. Collectively, these findings underscore the necessity for tailored breast cancer prevention and treatment approaches that consider the intricate interplay of demographic and genetic factors.
- Early menarche, late menopause, parity, breastfeeding, and hormonal therapies like hormone replacement therapy and contraceptives highly influence breast cancer risk. These factors are intricately linked with hormonal exposure over a woman's lifetime, affecting her breast cancer susceptibility. This review emphasizes the need for awareness and consideration of these factors in breast cancer risk assessment, suggesting lifestyle modifications and preventive strategies tailored to individual reproductive histories and hormonal exposure profiles.
- The relationship between metabolic factors, such as diabetes and overall metabolism, play an important role in the context of breast cancer risk. In particular, conditions like insulin resistance and alterations in lipid and glucose metabolism can influence breast cancer development by affecting hormonal levels and cellular processes. Our review suggests that understanding the impact of these metabolic factors is crucial for developing targeted prevention

strategies and emphasizes the need for further research to explore the intricate connections between metabolic health and breast cancer risk.

- Medical history, specifically breast density and the history of other cancers, can influence breast cancer risk. In particular, dense breast tissue can obscure mammograms, making detection more challenging, and emphasizes the independent risk factor that high breast density presents. Additionally, the history of other cancers may indicate an elevated risk for breast cancer. This work underscores the importance of considering an individual's medical history in breast cancer risk assessments and the need for personalized screening strategies.
- Lifestyle factors such as alcohol consumption, cigarette smoking, obesity, poor nutrition, and physical inactivity, highlight their significant roles in increasing breast cancer risk and the necessity of addressing these modifiable risk factors through public health interventions and individual lifestyle changes to reduce breast cancer incidence. This review underscores the potential of preventive measures and lifestyle modifications in mitigating breast cancer risk, emphasizing the importance of holistic approaches in breast cancer prevention strategies.
- Environmental factors like radiation exposure, chemicals, and pollutants, play a significant role in breast cancer risk. The cited works emphasize the need for awareness and protective measures against these exposures. Highlighting the complexity of breast cancer etiology, our work calls for comprehensive research to better understand the interactions between environmental factors and genetic predisposition, and for public health strategies to minimize exposure and mitigate breast cancer risk.
- The description of role of artificial intelligence (AI) models in detecting breast cancer illustrates the significant potential AI has in enhancing diagnostic accuracy, predicting recurrence, estimating survival rates, and assessing treatment response. Highlighting various studies, this review shows that machine learning algorithms, such as Support Vector Machines (SVM) and Convolutional Neural Networks (CNNs), have achieved notable success. This discussion emphasizes AI's transformative impact on breast cancer management, advocating for further research and integration of AI technologies to tailor detection and treatment approaches, ultimately improving patient outcomes.

A detailed description of the results of each work will be presented in Section 3.2. This analysis advocates for a multifaceted approach to prevention, screening, and treatment, reflecting the complex nature of breast cancer risk factors.

6 Conclusion

Our research reveals a breakthrough in early detection of breast cancer with machine learning models demonstrating an impressive diagnostic accuracy of 80.23%. The bibliographic review and analysis of the last 5 years in this field allowed us to identify the transformative impact of AI both in the identification of risk factors and in the improvement of diagnostic accuracy. Our analysis, unlike previous studies such as those by (69) (89), and (35), goes beyond updating risk factor inventories to show the fundamental role of sophisticated risk algorithms. AI. These tools, particularly SVM, have achieved an accuracy rate of up to 97.2% in locating breast cancer, which is a significant leap over traditional diagnostic methods by using a wider range of datasets, including images and clinical details including risk factors for your diagnosis.

Future explorations should delve into AI's ability to tailor breast cancer detection and treatments, thereby improving patient-specific outcomes.

Author contributions

ON: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. DA: Writing – original draft, Software, Investigation, Formal analysis, Data curation, Conceptualization. CT: Writing – review & editing, Project administration, Funding acquisition.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was funded by the ANID FONDAP 152220002 (CECAN).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1356014/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 26 April 2024
DOI 10.3389/fonc.2024.1361128

OPEN ACCESS

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RECEIVED 25 December 2023
ACCEPTED 10 April 2024
PUBLISHED 26 April 2024

CITATION
Deng C, Wu Z, Cai Z, Zheng X and Tang C (2024) Conservative medical intervention as a complement to CDT for BCRL therapy: a systematic review and meta-analysis of randomized controlled trials.
Front. Oncol. 14:1361128.
doi: 10.3389/fonc.2024.1361128

Conservative medical intervention as a complement to CDT for BCRL therapy: a systematic review and meta-analysis of randomized controlled trials

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Background: The effect of first-line complex decongestive therapy (CDT) for breast cancer-related lymphedema (BCRL) depending on various factors forces patients to seek additional treatment. Therefore, this meta-analysis was conducted to evaluate the effect of different conservative medical interventions as a complement to CDT. This is the first meta-analysis that includes various kinds of conservative treatments as adjunctive therapy to get broader knowledge and improve practical application value, which can provide recommendations to further improve BCRL patients' health status.

Methods: RCTs published before 18 December 2023 from PubMed, Embase, Cochrane Library, and Web of Science databases were searched. RCTs that compared the effects of conservative medical intervention were included. A random-effects or fixed-effects model was used based on the heterogeneity findings. Study quality was evaluated using the Cochrane risk of bias tool.

Results: Sixteen RCTs with 690 participants were included, comparing laser therapy, intermittent pneumatic compression (IPC), extracorporeal shock wave therapy (ESWT), electrotherapy, ultrasound, diet or diet in combination with synbiotic supplement, traditional Chinese medicine (TCM), continuous passive motion (CPM), and negative pressure massage treatment (NMPT). The results revealed that conservative medical intervention as complement to CDT had benefits in improving lymphedema in volume/circumference of the upper extremity [SMD = −0.30, 95% CI = (−0.45, −0.15), $P < 0.05$, $I^2 = 51\%$], visual analog score (VAS) for pain [SMD = −3.35, 95% CI (−5.37, −1.33), $P < 0.05$, $I^2 = 96\%$], quality of life [SMD = 0.44, 95% CI (0.19, 0.69), $P < 0.05$, $I^2 = 0$], and DASH/QuickDASH [SMD = −0.42, 95% CI (−0.70, −0.14), $P < 0.05$, $I^2 = 10\%$] compared with the control group. Subgroup analysis revealed that laser therapy and electrotherapy are especially effective ($P < 0.05$).

Conclusion: Combining conservative medical interventions with CDT appears to have a positive effect on certain BCRL symptoms, especially laser therapy and electrotherapy. It showed a better effect on patients under 60 years old, and laser therapy of low to moderate intensity (5–24 mW, 1.5–2 J/cm^2) and of moderate- to long-term duration (≥36–72 sessions) showed better effects.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=354824, identifier CRD42022354824.

KEYWORDS

breast cancer-related lymphedema, BCRL, meta-analysis, systematic review, adjunctive therapy

1 Background

Breast cancer (BC) is the most frequently diagnosed cancer among women around the world although the incidence rate continues to rise and the overall mortality rate has declined with the advancement of early screening and diagnosis in many high-income countries (1). As the 5-year survival rate is almost 90% (2), there is an increasing number of BC survivors suffering long-term complications brought by surgery and radiation-related therapeutic exposure. BC-related lymphedema (BCRL) is one of the most seen complications among the survivors, with up to 40% of BC survivors suffering from upper extremity complications especially if lymphedema axillary lymph node procedures are applied (3). BCRL is associated with swelling in the limbs, persistent inflammation, pain, numbness, and restricted mobility (4), causing great distress in physical and mental function, and patients consider it as a medical burden (5, 6).

Since BCRL remains both incurable and debilitating (7), it is still challenging to confirm effective and safe therapy for patients. Currently, the first-line therapy for lymphedema is complex physical therapy/complex decongestive therapy (CDT), comprised of two phases: the first phase includes manual lymphatic drainage (MLD), compression therapy (bandages/garments/pumps), remedial exercise, and skin and nail care (8); the second phase includes lifelong self-care maintenance. Even though CDT has been proven as the most widely used treatment for lymphedema, its effectiveness depends greatly upon the therapist's experience and overall patient compliance with the complex self-care requirement (9), which may cause low adherence in the long term because of repeated and tedious procedures. Furthermore, using compression therapy alone shows limited benefit to edema over a long-term period (10). The benefits of CDT will be greater if applied in the early lymphedema stage (stage I) (11) or less than 1 year of lymphedema duration (12). The severe condition of BCRL might hinder the effect of CDT, thus forcing patients to seek additional treatment (13), e.g., surgical treatment such as lymphaticovenular anastomosis (10, 14) and various conservative treatments to better improve the overall status of BCRL patients. Due to these limitations, a multidisciplinary approach and additional treatment strategies to treat BCRL systematically are necessary to be explored to optimize treatment efficiency and contribute to a more complete and efficient treatment, improving the quality of life as well as the adherence of the survivors (15).

To date, no studies have investigated the effectiveness of combinations of various conservative medical interventions and CTD. Our study was therefore designed to evaluate the effect of conservative interventions combining CDT in improving the symptoms of BCRL patients, and the results may help practitioners choose more efficient treatments. The hypothesis is that a combination of certain conservative interventions in these patients would improve the symptoms more significantly compared with CDT alone.

2 Methods

2.1 Eligibility criteria

All available randomized controlled trials (RCTs) met the following criteria: 1) types of studies: RCTs; 2) types of participants: women patients with BCRL and no restrictions on their BC and lymphedema stage, nationality, and age; 3) types of intervention: conservative treatment including physical therapy/oral supplements. Standard care (ST) was defined as any combination of skin care, exercises, compression method, and part of CDT. Giving the patients with only health education or guidance will be defined as none; 4) types of outcomes: primary outcomes were changes in edema, such as volume/circumference of the arm (lower scores mean better effect). Secondary outcomes included quality of life (QoL); VAS for pain; disabilities of the arm, shoulder, and hand (DASH/QuickDASH); and grip strength; and 5) language: English.

The exclusion criteria were as follows: 1) case reports, reviews, study protocols, conference abstracts, commentaries, and letters; 2) duplicate articles; 3) animal experiments; 4) studies that used surgical intervention and oral medicine (such as diosmin) and

compared instruments like bandages/kinesio taping/garments aiming to compare different brands; studies that compared exercises were also excluded because our focus was on medical practice rather than self-care practice and studies that used intervention in the control group other than CDT/standard care/none; 5) any study design except RCT; 6) unavailable original full text; 7) language except English; and 8) studies with outcome only shown in graphs without concrete data form and failure to contact the authors to obtain the data.

2.2 Information sources and search strategy

Four relevant English literature databases (Embase, Cochrane Library, Web of Science, PubMed) were searched for all relevant citations published until 18 December 2023. We established search strategies that combined Medical Subject Headings (MESH term) and random words related to BCRL, interventions of interest (treatment/therapy), and RCTs. Furthermore, the reference lists of the included studies were manually reviewed to look for additional relevant manuscripts. The specific search strategies are shown in Appendix 1.

2.3 Selection process

Two reviewers (CYD, ZGW) independently screened the titles and abstracts of identified citations and full texts of potentially eligible studies. Disagreements were resolved by discussion or third-party (XYZ) adjudication when necessary.

2.4 Data collection and data items

Two reviewers (CYD, ZJC) independently extracted the study data, including the name of the first author, publication year, participant characteristics (country and age), sample size, intervention and the comparison treatments, baseline, course of treatment, outcomes, adverse events (AEs), and dropout. Lymphedema volume was measured as volume calculated from the circumference, water displacement and bioimpedance spectroscopy. Two researchers (CYD, ZJC) checked the extracted data for consistency, and a third researcher (XYZ, CZT) arbitrated any dispute.

2.5 Study risk of bias assessment

The Cochrane Risk of Bias Assessment tool (Cochrane Collaboration) was used to assess the risk of bias. The following types of bias were assessed: 1) random sequence generation, 2) allocation concealment, 3) blinding of participants and personnel, 4) blinding of outcome assessment, 5) incomplete outcome data, 6) selective reporting, and 7) other bias. Each item was classified into three types: low risk, high risk, and unclear risk. The quality of the included trials was evaluated by two reviewers (CYD, ZJC) independently, and disagreements were resolved by a third researcher (ZGW).

2.6 Effect measures

Continuous data were expressed as the mean ± standard deviation (SD) and summarized as a mean difference (MD) or standardized mean difference (SMD). Considering the primary outcomes reflected by the volume (ml/cm^3) or circumference (cm) of the arm, the different units can cause great differences in data size; therefore, SMD with 95% confidence intervals (CIs) was used to analyze the primary outcome.

Heterogeneity among the studies was detected using P and I^2 statistics. A random-effects model was adopted when heterogeneity was observed ($P < 0.05$ and/or $I^2 > 50\%$); otherwise, a fixed-effects model was adopted. If the pooled result included clinical heterogeneity, subgroup analysis was performed to search for the source of heterogeneity. Clinical heterogeneity was defined as differences in participants, treatment, outcome characteristics, or research setting.

All data were analyzed using the Excel 2016 (Microsoft) and RevMan 5.3 (Cochrane Collaboration, Oxford, UK) software.

3 Results

3.1 Study selection

The PRISMA flow diagram (Figure 1) displays the selection process. We found 1,057 articles by searching the databases (PubMed, Cochrane Library, Web of Science, Embase). After excluding 374 duplicate articles, two researchers conducted independent review and exclusion. Based on the title and abstract, 625 articles did not meet the selection criteria and were excluded, and 8 reports could not be retrieved.

Of the remaining 50 papers, 34 were excluded for the following reasons: in 17 studies, the control intervention was not CDT/standard care/none; 1 study used surgical intervention as combination; 1 study focused on follow-up research; and 15 articles did not report relevant concrete outcomes; specifically, the outcome data were shown in graphs/proportion in percentage/patients' number without specific curative effect outcome data. Finally, 16 articles were included in our analysis.

3.2 Study characteristics

A total of 16 randomized controlled trials with 690 participants were ultimately included in this meta-analysis, of which 4 studies (16–19) adopted laser therapy; 3 studies (20–22) adopted IPC; 2 studies (23, 24) adopted ESWT; 1 study with three arms adopted electrotherapy (25) and ultrasound (25); 1 study with three arms adopted diet (26) or diet combined with a synbiotic supplement (26); 3 studies adopted the traditional Chinese medicine (TCM) method including sliding cupping (27), acupuncture (28), and

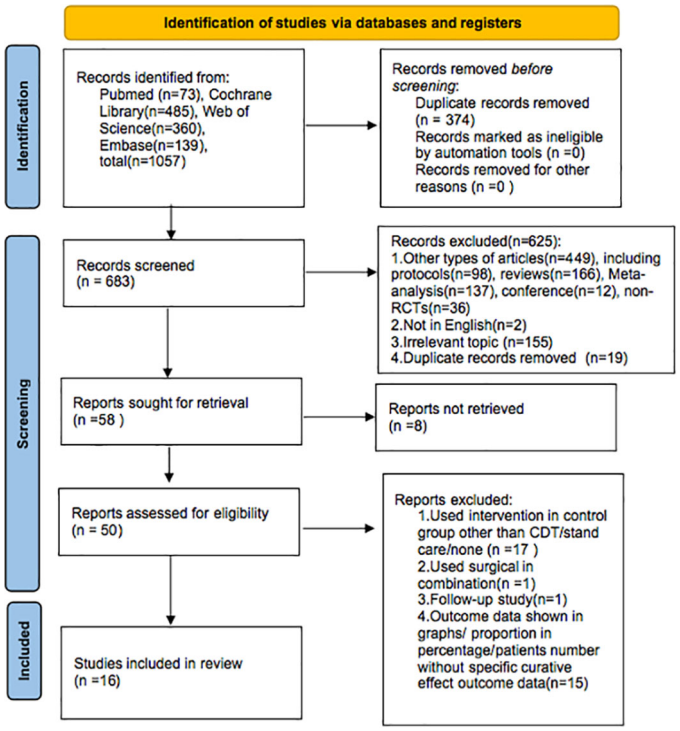

FIGURE 1
The PRISMA flow diagram.

Tuina in combination with moxibustion (29); 1 study adopted continuous passive motion (CPM) (30); and 1 study adopted negative pressure massage treatment (NMPT) (31).

Four RCTs (21, 22, 24, 30) were conducted in Turkey, three RCTs (20, 28, 31) were conducted in the USA, two RCTs (25, 26) were conducted in Iran, two RCTs (16, 19) were conducted in Egypt, three RCTs (17, 27, 29) were conducted in China, one RCT was conducted in Germany (18), and one RCT was conducted in Korea (23).

The results of the 16 RCTs involved changes in arm circumference (16, 23–25, 27–29) and/or volume (17–20, 22, 24–26, 29–31). The secondary outcomes in the included RCTs were VAS for pain (18, 21, 23, 25), quality of life (18, 26, 30), DASH (17, 30, 31) or QuickDASH (21, 23, 24), grip strength (16, 18, 21), bioimpedance (28, 31), BMI (26), range of motion (ROM) (16, 19, 20) of the shoulder, tissue resistance (17, 20), and skin thickness (23, 27). The summarized characteristics of the 16 RCTs are presented in Table 1.

3.3 Risk of bias in the studies

The risk of bias assessment is shown in Figure 2. All the included trials mentioned randomization: 12 studies described the randomization method in detail such as the random number table (27), block randomization (24, 26), block permutation method (25), random number table using block randomization (30), computer-generated program (16, 18, 21, 28, 29, 31), and the Bebbington method (17) and were defined as low risk of bias; 3 studies (19, 20, 23) had no concrete description of randomization and were defined as unclear risk of bias; and 1 study (22) randomized the patients according to admittance time and was defined as high risk of bias.

Furthermore, six studies (16, 18, 21, 28, 29, 31) used a computer-generated program in randomization to ensure the allocation concealment; two studies (25, 29) used sequentially numbered, sealed, and opaque envelopes; and one study (30) used an uninvolved researcher to assign patients. These studies were defined as low risk. The rest of the studies did not describe the allocation concealment and were defined as unclear risk.

As for performance blinding, blinding of the treating physiotherapist and patients in some interventions such as ESWT (24), IPC (20–22), CPM (30), NPMT (31), electrotherapy (25), ultrasound (25), acupuncture (28), sliding cupping (27), and Tuina in combination with moxibustion (29) was impossible considering the nature of the studies and according to the description of the authors; therefore, these were defined as high risk. Laser therapy (16–18, 31) (both active and placebo laser devices were similar in terms of weight, emitted sounds, and optical appearance to guarantee strictly controlled double-blinded conditions) and capsules (26) (the placebo capsule was comprised of lactose and was equal to the synbiotic capsule in terms of appearance, color, shape, size, smell, taste, and packaging) were performed in a blinded manner according to the description of the authors and were defined as low risk.

As for assessment blinding, six studies (16, 21, 22, 25, 29, 31) had a clear description of the blinding of outcome assessments and

TABLE 1 Characteristics of the included studies.

No.	References	Country	Age	Sample size (I/C)	Baseline	Outcomes	Course	AE	Dropout
1	Ahmed 2011 (16)	Egypt	I: 54.76 ± 3.33 C: 53.36 ± 3.56	LLLT (25)/ST (25)	2~8 cm interlimb circumference difference	1. Circumference 2. Grip strength 3. ROM	3 times/week for 12 weeks	UK	I: 4 withdrawn [cellulitis (n = 1), poor adherence (n = 3)] C: 4 withdrawn [cellulitis (n = 2), poor adherence (n = 2)]
2	Bao 2018 (28)	USA	I: 65 (54, 71) C: 58 (49, 70)	Acupuncture (40)/ none (42)	>2 cm interlimb circumference difference in at least one of two sites (10 cm above or 5 cm below the olecranon process). Lymphedema diagnosed as stage II	1. Circumference 2. Bioimpedance	Twice/week for 6 weeks	Bruises 45 (58%) Hematoma 2 (2.6%) Pain 2 (2.6%) Skin infection 1 (1.3%)	I: N = 4 (lost to follow-up (n = 1), withdrew due to AE (n = 2), withdrew consent (n = 2)] C: Withdrew consent (N = 5)
3	Cebicci 2021 (24)	Turkey	I: 51.61 ± 6.6 C: 57.90 ± 6.9	ESWT (11)/CDT (12)	No description	1. Volume 2. Circumference 3. QuickDASH	3 times/week for 4 weeks	UK	I: N = 1 (1 lost to follow-up and withdrawn from the study) C: N = 2 (1 discontinued the treatment, 1 lost to follow-up)
4	Hemmati 2022 (25)	Iran	I: 48.96 ± 10.12 C: 49.13 ± 10.5	Electrotherapy + CDT (13)/CDT (13)	>2 cm interlimb circumference difference and/or >10% difference in volume between upper extremities	1. Volume 2. Circumference 3. VAS for pain	5 times/week for 2 weeks	UK	0
5	Hemmati 2022 (25)	Iran	I: 49.32 ± 10.15 C: 49.13 ± 10.5	Ultrasound + CDT (13)/CDT (13)	>2 cm interlimb circumference difference and/or >10% interlimb volume difference	1. Volume 2. Circumference 3. VAS for pain	5 times/week for 2 weeks	UK	0
6	Khalaf2012 (19)	Egypt	I: 49.13 ± 2.58 C: 48.66 ± 2.31	He–Ne laser + CDT (15)/CDT (15)	No description	1. Volume 2. ROM	3 times/week for 6 months	UK	0
7	Kizil 2018 (30) 50	Turkey	I: 55.50 (40–73) C: 58.00 (35–75)	CPM + CDT (16)/ CDT (16)	>2 cm interlimb circumference difference	1. Volume 2. DASH 3. QoL (FACT-B4)	Once/day for 15 consecutive days	UK	I: N = 2 (2 lost to follow-up)
8	Kyeong 2020 (23)	Korea	I: 53.13 ± 10.85 C: 52.24 ± 8.60	ESWT + CDT (15)/ CDT (15)	>2 cm interlimb circumference	1. Volume 2. Circumference	Twice/week for 3 weeks	No AEs occurred	0

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TABLE 1 Continued

No.	References	Country	Age	Sample size (I/C)	Baseline	Outcomes	Course	AE	Dropout
					difference and a volume difference >200 ml	3. VAS for pain 4. QuickDASH 5. Skin thickness			
9	Lampinen 2021 (31)	USA	I: 64.24 ± 13.69 C: 60.34 ± 10.65	NPMT (15)/MLD (13)	>150 ml interlimb volume difference, ≥2 cm interlimb circumference difference at any of the measured locations, or a lymph edema index (L-Dex) score of ≥7.1	1. Volume 2. L-Dex 3. DASH	2–3 times/week for 4–6 weeks, 12 sessions in total	No AEs occurred	I: *N* = 2 (1 fracture, 1 skin problem) C: *N* = 2 (1 leukemia, 1 commute challenge)
10	Lau 2009 (17)	China	I: 50.9 ± 8.6 C: 51.3 ± 8.9	LLLT (11)/none (10)	>200 ml interlimb volume difference	1. Volume 2. Tissue resistance 3. DASH	12 sessions of LLLT in 4 weeks	UK	0
11	Storz 2017 (18)	Germany	I: 61.06 ± 9.66 C: 59.37 ± 10.16	LLLT (20)/none (20)	In both groups, median pain intensity was 4 at baseline. QoL measured using MMSQ and MQoL was slightly higher in the active laser group than in the sham group (82.75 vs. 79.88 and 6.43 vs. 6.28). Regarding grip strength, both groups were nearly identical. Median limb volume difference was higher in the placebo group (160.46 ml/cm^3) than in the active laser group (91.63 ml/cm^3); however, this difference was not statistically significant.	1. Volume 2. QoL 3. Grip strength 4. VAS for pain	2 times/week for 4 weeks	No AEs occurred	I: *N* = 3 (2 withdrawn: poor adherence; 1 withdrew: not interested) C: *N* = 1 (withdrawn for poor adherence)
12	Szuba 2002 (20)	USA	I: 68.8 ± 9.11 C: 65 ± 10.8	CDT + IPC (12)/ CDT (11)	>20% interlimb volume difference	1. Volume 2. Tissue resistance 3. ROM	Once/day for 10 days	No AEs occurred	0
13	Tastaban 2020 (21)	Turkey	I: 53.0 (43.0–58.0) C: 55.0 (48.0–58.0)	CDT + IPC (38)/ CDT (38)	>2 cm interlimb circumference	1. Volume 2. QuickDASH	5 days/week for 4 weeks	UK	0

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TABLE 1 Continued

No.	References	Country	Age	Sample size (I/C)	Baseline	Outcomes	Course	AE	Dropout
					difference or >10% interlimb volume difference	3. VAS for pain 4. Grip strength 5. Depression			
14	Uzkeser 2015 (22)	Turkey	I: 55 (42–75) C: 56 (37–75)	CDT + IPC (16)/ CDT (15)	>2 cm interlimb circumference difference or >10% interlimb volume difference	1. Volume	5 times/week for 3 weeks	UK	I: $N = 1$ (move to another city)
15	Vafa 2020 (26)	Iran	I: 53.80 ± 1.42 C: 53.24 ± 1.5	Diet + synbio + CDT (45)/CDT (45)	Stage I or II lymphedema	1. Volume 2. BMI 3. QoL	Once for 10 weeks	No AEs occurred	I: $N = 4$ [long distance from residence to the clinic ($n = 1$); follow-up with other physicians ($n = 2$); and unwillingness to continue the study ($n = 1$)] C: $N = 4$ (long distance from residence to the clinic ($n = 2$), unwillingness to continue the study ($n = 2$)]
16	Vafa 2020 (32)	Iran	I: 52.41 ± 1.19 C: 53.24 ± 1.5	Diet + CDT(45)/ CDT (45)	Stage I or II lymphedema	1. Volume 2. BMI 3. QoL	Once/day for 10 weeks	No AEs occurred	I: $N = 6$ [recurrent disease ($n = 1$); long distance from residence to the clinic ($n = 3$); follow-up with other physicians ($n = 1$); and unwillingness to continue the study ($n = 1$)] C: $N = 4$ [long distance from residence to the clinic ($n = 2$), unwillingness to continue the study ($n = 2$)]
17	Xiong 2019 (27)	China	I: 53.43 ± 11.87 C: 52.47 ± 11.27	Sliding cupping (30)/ CDT (30)	Sliding cupping: 15 mild degree of edema (difference <3 cm), 10 moderate edema (3~5 cm), and 5 cases of severe edema (≥5 cm)	1. Circumference 2. Skin thickness	Once/day for 14 days	UK	0

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TABLE 1 Continued

No.	References	Country	Age	Sample size (I/C)	Baseline	Outcomes	Course	AE	Dropout
18	Wang 2023 (29)	China	I: 58.45 ± 5.92 C: 59.30 ± 7.06	Tuina + Moxi/CDT	≥2 cm interlimb circumference difference or ≥120 ml interlimb volume difference	1. Volume 2. Circumference 3. VAS for swelling	Tuina for 20 min + moxibustion for 20 min, twice/week for 4 weeks	No AEs occurred	I: N = 3 [intolerance to moxibustion odor (n = 2), pneumonia (n = 1)] C: N = 2 (loss to follow-up)

No., number; I, intervention; C, control; BCRL, breast cancer-related lymphedema; LLLT, low-level laser therapy; Moxi, moxibustion; CDT, complex decongestive therapy; ESWT, extracorporeal shock wave therapy; IPC, intermittent pneumatic compression; CPM, continuous passive motion; NMPT, negative pressure massage treatment; AE, adverse event; UK, unknown.

were defined as low risk. Two studies (17, 28) mentioned research staff not being blinded to the treatment group and were defined as high risk. Eight studies (18–20, 23, 24, 26, 27, 30) did not mention blinding in assessment and were defined as unclear risk.

Nine studies (16, 18, 22, 24, 26, 28–31) reported dropouts with clear reasons, and seven studies (17, 19–21, 23, 25, 27) reported no dropouts. We assessed the risk of attrition bias in all these studies as low risk.

As for selective reporting, although all the included studies reported all outcomes in the methods section, the protocol was not available and was defined as unclear risk.

Furthermore, we assessed the other risks as low considering that the whole design of most of the included studies was relatively formal.

3.4 Primary outcome: arm volume/circumference change

3.4.1 Volume/circumference change and subgroup analysis

Of the 15 included studies, 4 studies (23–25, 29) reported both volume and circumference change of the limbs, 9 studies (17–22, 26, 30, 31) reported only volume change, and 3 studies (16, 27, 28) reported only circumference change. Considering the different units of volume (cm^3) or circumference (cm) may result in great differences in data size; standardized mean difference (SMD) with 95% confidence intervals (CIs) was used.

The results revealed that conservative medical intervention as a complement to CDT had benefits in improving lymphedema in volume/circumference of the upper extremity compared with that of the control group [SMD = −0.40, 95% CI = (−0.62, −0.18), P < 0.05, I^2 = 53%] (Figure 3).

3.4.2 Subgroup analysis

3.4.2.1 Age

According to age-grouped data, the age group <50 years old [SMD = −1.14, 95% CI = (−1.91, −0.37), P < 0.05, I^2 = 61%] and between 50 and 60 years old [SMD = −0.34, 95% CI = (−0.56, −0.13), P < 0.05, I^2 = 32%] showed a better effect; however, the age group >60 years old [SMD = −0.07, 95% CI = (−0.44, 0.29), P > 0.05, I^2 = 20%] had a relatively poor effect. It can be found that most heterogeneities come from the age group <50 years old (Figure 4).

3.4.2.2 Different treatments

Subgroup analysis based on different treatments showed that laser therapy [SMD = −0.78, 95% CI = (−1.56, −0.00), P = 0.05, I^2 = 77%] and electrotherapy [SMD = −1.85, P < 0.05, 95% CI = (−2.79, −0.90)] had better effect, and diet/diet + synbiotic, IPC, ESWT, ultrasound, TCM (sliding cupping, acupuncture, Tuina combined with moxibustion), NPMT, and CPM did not show a significantly better effect compared with the control group (P > 0.05) (Figure 5).

3.4.2.3 Dose-grouped and session-grouped laser therapy analysis

In a dose–subgroup analysis of laser therapy, we found that only low intensity (5 mW, 1.5/cm^2) [SMD = −1.13, 95% CI = (−1.65, −0.62), P < 0.05, I^2 = 0%] to moderate intensity (24 mW,

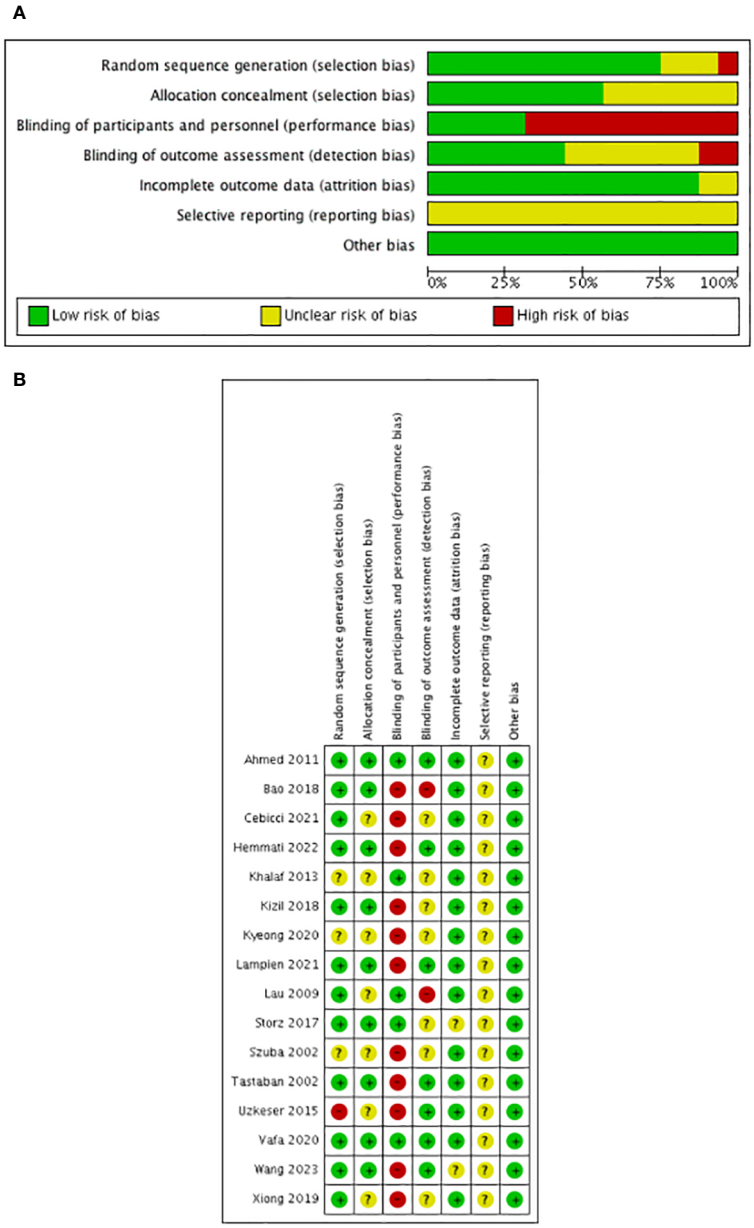

FIGURE 2
Risk of bias. **(A)** Overall quality assessment. **(B)** Individual quality assessment.

2 J/cm^2) [SMD = −0.97, 95% CI = (−1.88, −0.05), $P < 0.05$] (Figure 6A) and moderate term (36 sessions) [SMD = −1.08, 95% CI = (−1.75, −0.40), $P < 0.05$] to long term (72 sessions) [SMD = −1.21, 95% CI = (−2.00, −0.42), $P < 0.05$] significantly improved lymphedema compared with those of the control group (Figure 6B).

3.5 Secondary outcome 1: quality of life

Three studies (18, 26, 30) took four comparisons using QoL as the outcome measure, using the scales of Functional Assessment of Cancer Therapy for Breast Cancer (FACT-B4) (30), Lymphedema Life Impact Scale (LLIS) (26), McGill Quality of Life Questionnaire (MQoL), and the German version of the Multidimensional Mood State Questionnaire (MMSQ) (18). The meta-analysis displayed that QoL was significantly higher in the experimental group than in the control group [SMD = 0.44, 95% CI (0.19, 0.69), $I^2 = 0$], and a statistically significant difference was found ($P < 0.05$) (Figure 7).

3.6 Secondary outcome 2: DASH/QuickDASH

Six studies used the quick disabilities of the arm, shoulder, and hand (QuickDASH) (21, 23, 24) or DASH (17, 30, 31) questionnaire as the outcome (these are self-reported assessment tools that

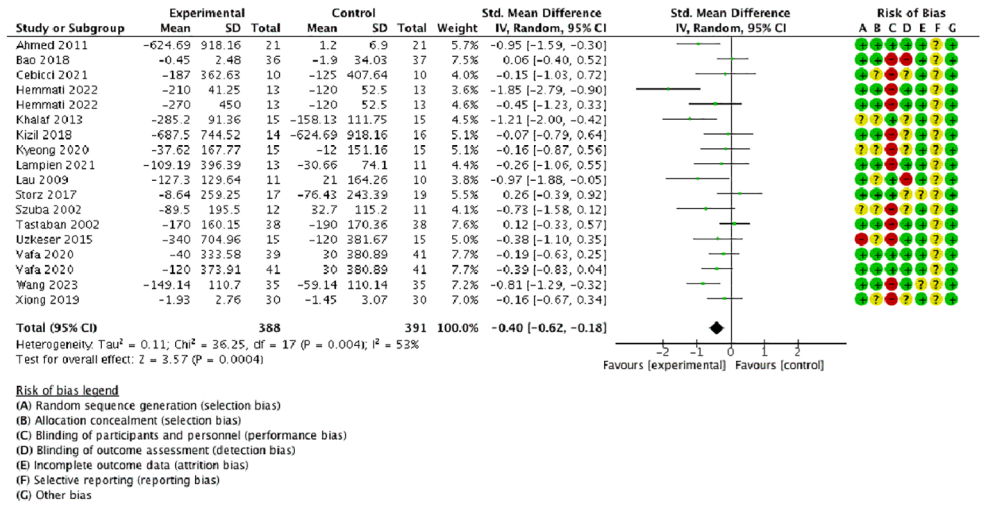

FIGURE 3
Forest plot of volume/circumference change.

measure physical function and symptoms in individuals with a musculoskeletal disorder of the upper limb). The scores indicated the level of disability and severity, ranging from 0 (no disability) to 100 (most severe disability).

The meta-analysis manifested that QuickDASH/DASH was significantly lower in the experimental group than in the control group [SMD = −0.42, 95% CI (−0.70, −0.14), I^2 = 10%], and a statistically significant difference was found ($P < 0.05$) (Figure 8).

3.7 Secondary outcome 3: VAS for pain

Four studies (18, 21, 23, 25) performed five comparisons using the visual analog scale (VAS) for pain as the outcome with a 0–10 numerical rating scale. The meta-analysis proved that VAS for pain was significantly decreased in the experimental group than in the control group [SMD = −3.35, 95% CI (−5.37, −1.33), I^2 = 96%], and a statistically significant difference was found ($P < 0.05$) (Figure 9).

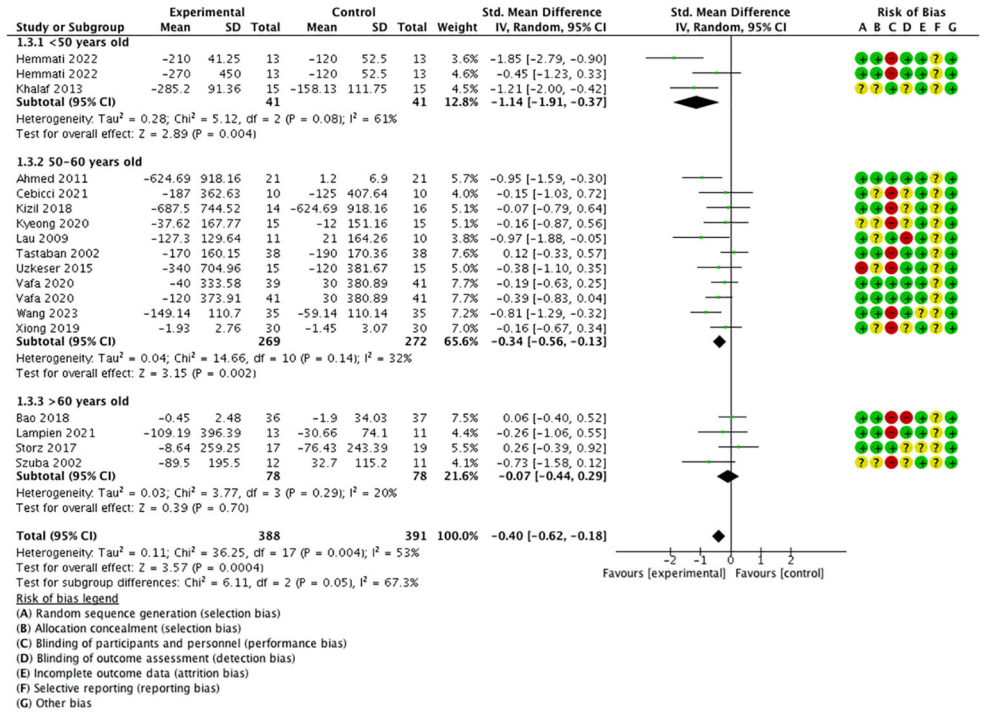

FIGURE 4
Forest plot of age-grouped analysis.

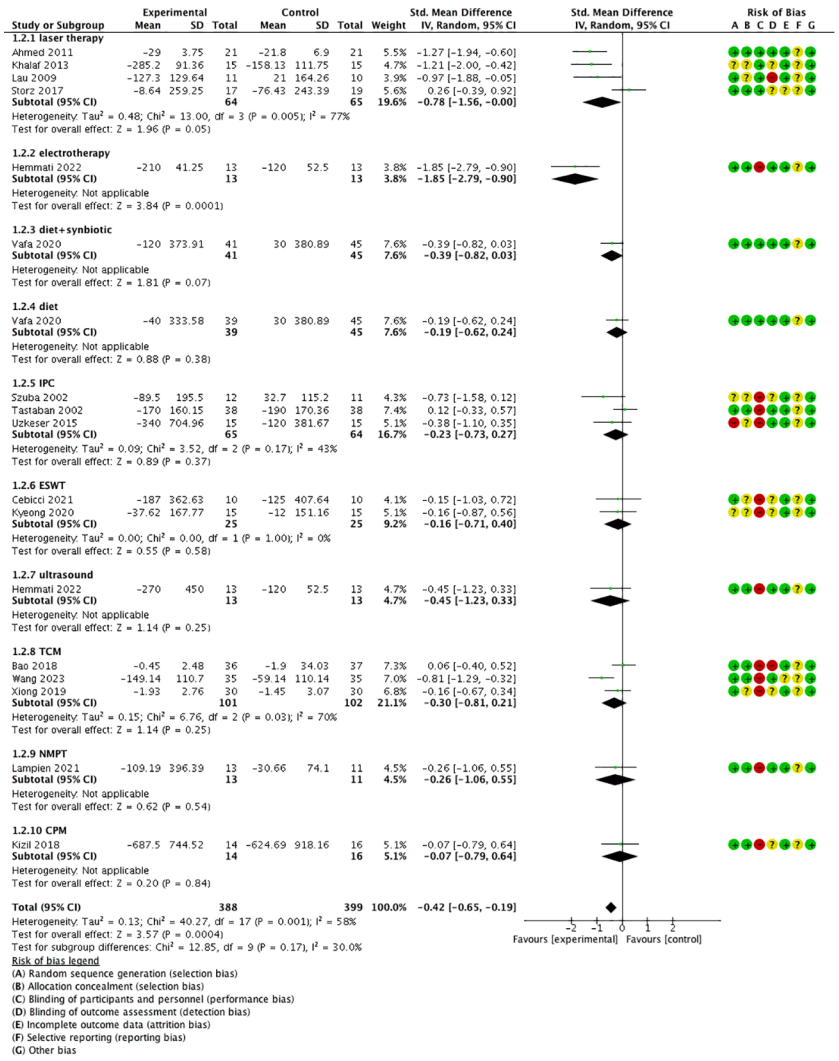

FIGURE 5
Forest plot of treatment-grouped analysis.

3.8 Adverse events

One study reported the AEs in detail (during acupuncture, bruises 45 cases, hematoma 2 cases, pain 2 cases, skin infection 1 case) and 7 studies reported no AEs (ESWT, IPC, LLLT, NPMT, diet, synbiotic). However, 10 studies did not mention AEs.

4 Discussion

4.1 Main findings

Our results showed that the addition of certain conservative medical treatments to CDT can exert a better effect in reducing the volume/circumference of the swollen limbs of BCRL patients, as well as relieve pain and disability and also improve the quality of life of the patients. Furthermore, we found among the various treatments that laser therapy and electrotherapy had the best effect in relieving swollen limbs, and we recommend laser therapy of 5–24 mW, 1.5–2 J/cm^2 intensity, and 36–72 sessions for BCRL patients to achieve the best effect based on the subgroup analysis findings. Moreover, patients <60 years old may show a better effect than elderly patients, indicating the importance for younger patients to receive relative treatments as it will be more efficient and economical.

4.2 Strengths and limitations

Our review has several strengths. First, most of the included articles were of moderate to high quality with relatively standardized design, which improved the confidence of the results. Second, the entire search strategy and data analysis process were relatively formal. Third, we included 16 eligible RCTs with 655 participants investigating the additional beneficial effect of conservative medical treatments on swelling, pain, disability, and quality of life. Fourth, this study was conducted using in-depth subgroup meta-analyses to evaluate potential sources of heterogeneity.

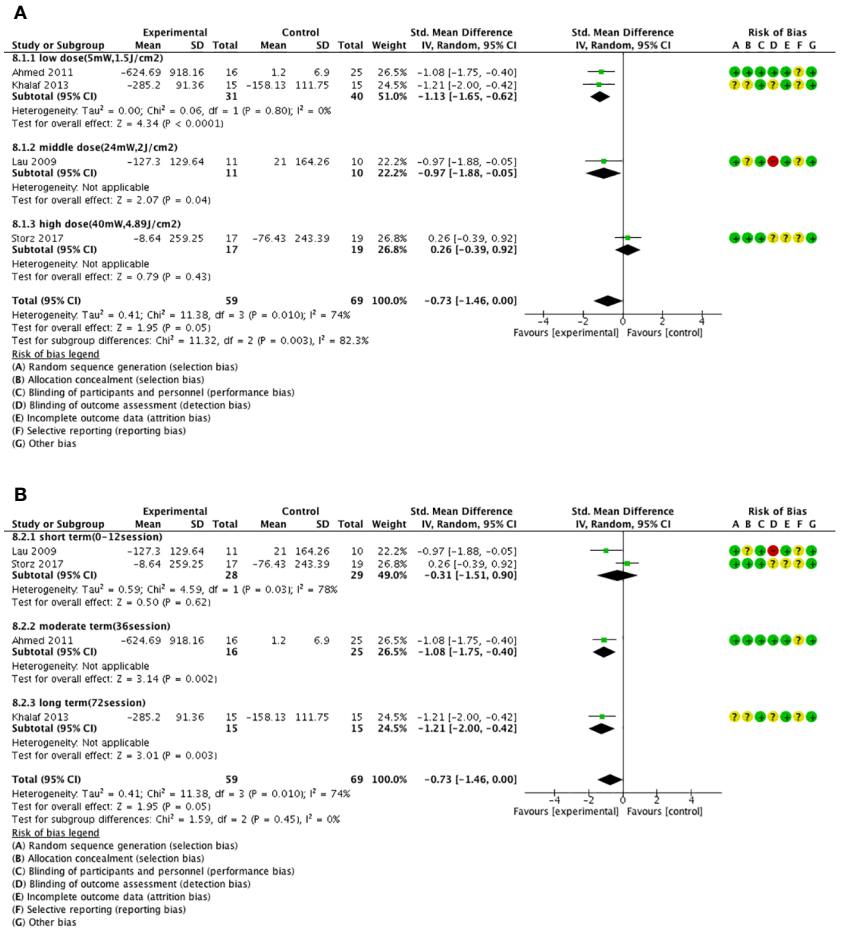

FIGURE 6
Forest plot of laser therapy subgroup analysis. **(A)** Forest plot of the laser dose–subgroup analysis. **(B)** Forest plot of the laser session–subgroup analysis.

However, this study has some limitations. First, some TCM treatments (including acupuncture/sliding cupping) did not show a significant overall effect in our study although some beneficial effects were observed (33), and this may have resulted from the lack of search of Chinese databases such as CNKI, VIP, and SinoMed, which may contain more research about TCM, and the selection and combination of acupoints also play an important role in the effectiveness of treatments. Second, this study was based on study-level data but not on patient-level data, and the analysis was based on small sample sizes, so the results should be interpreted carefully. Third, our study did not include gray literature such as some studies from the National Institutes of Health (NIH) and the World Health Organization (WHO). Fourth, this review just included RCT studies and excluded non-RCTs such as prospective pilot studies, and this might cause an underestimated effect of some treatments. Fifth, the exclusion criteria in the included studies did not mention the history of surgery to treat lymphedema, which may cause some efficacy differences.

4.3 Relation to previous studies

Several meta-analyses about BCRL treatment came to the following conclusions: one study concluded that conservative treatment interventions may not meaningfully improve lymphedema volume compared with standard care (34), and this study did not take CDT as the control group, which is the first-line treatment. Another study concluded that acupuncture and moxibustion (33) are effective in treating BCRL; however, different control methods were used; therefore, it is difficult to interpret the conclusions of the study. A third study concludes that ESWT combined with CDT could have significant effects (35); however, some non-RCT studies were included, which might affect the results. The present study, on the other hand, excluded non-RCT studies as the focus was on medical practice; furthermore, various kinds of conservative treatments were included to obtain broader knowledge, and CDT was used as a first-line treatment to improve its practical application value.

4.4 Future perspectives

Our findings indicate that it is meaningful to discover and promote conservative medical treatments in clinical practice to better help BCRL patients relieve their symptoms such as swollen limbs, pain, and disability and improve their life quality. However, there is still a lack of consensus on which patients will benefit from

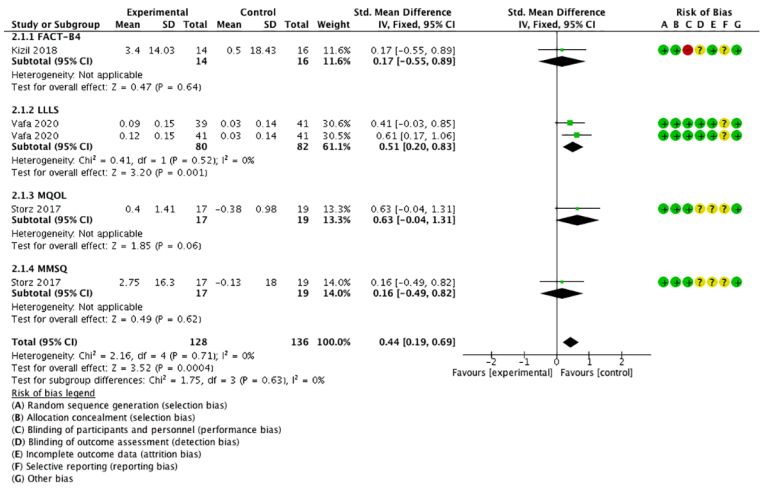

FIGURE 7
Forest plot of QoL.

each treatment option, and there are no guidelines on the appropriate time to start treatment, which can lead to treatment issues (36).

Low-level laser therapy (LLLT), also called photobiomodulation (PBM), may reduce inflammation, prevent fibrosis, and stimulate lymphangiogenesis (32). Electrical stimulation reduces edema by increasing muscle contraction and can increase lymph and blood flow up to 30-fold; moreover, muscle contraction favors the removal of intercellular proteins (37). However, there are relatively fewer studies about electrotherapy, and future studies can explore more about the efficacy and safety as well as the intervention time of applying electrotherapy.

The selection and combination of conservative medical treatments may play an important role in the effectiveness of treatments. At present, CDT is widely known as the most important treatment for BCRL. Previous meta-analyses (35) concluded that ESWT combined with CDT could significantly improve the volume of lymphedema in BCRL patients but not enough to replace CDT. Our review also supported the idea that a combination of conservative treatments with CDT could provide significant clinical benefits to BCRL patients but cannot replace CDT in treating BCRL.

Moreover, different measurements of the affected limbs by circumference or volume calculated by circumference or water displacement made it difficult to aggregate analyses. In future trials, it may be a better option to report the volume changes by water displacement to uniform the measurement, and water displacement is more direct than calculation by circumference. Conservative rehabilitation interventions need to be continued to develop studies to help guide therapeutic decisions that can promote health-related quality of life in BCRL women (38).

As for safety assessment, no adverse events occur when applying ESWT, IPC, LLLT, NPMT, diet, or symbiotic treatment, and most AEs come from acupuncture, indicating that practitioners should pay more attention in performing acupuncture and poor performance may also reduce the effect of acupuncture.

5 Conclusion

In conclusion, our research supports the efficacy of combining conservative medical intervention with CDT over utilizing CDT alone

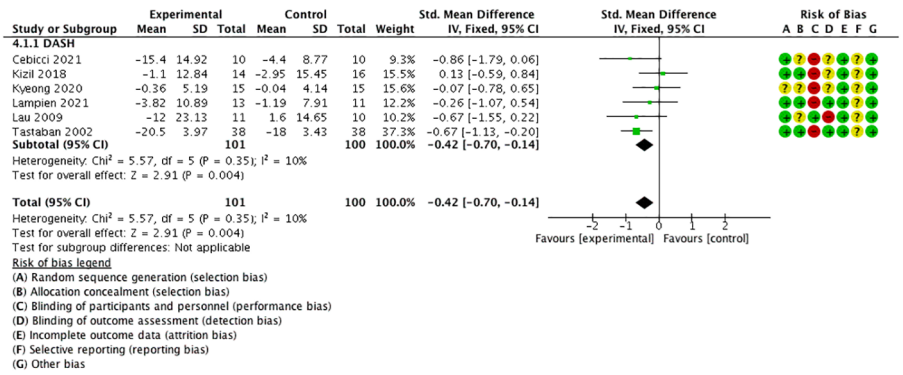

FIGURE 8
Forest plot of DASH and QuickDASH.

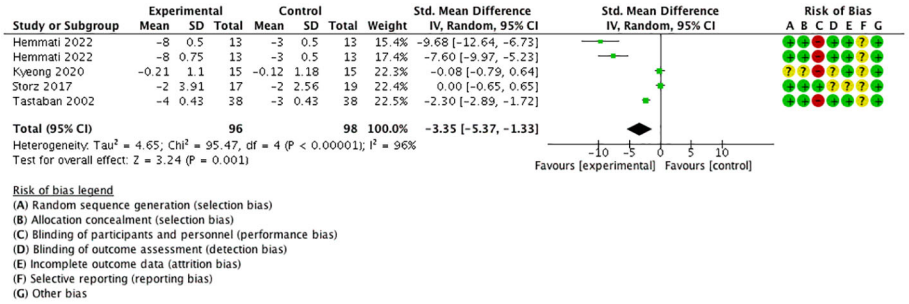

FIGURE 9
Forest plot of VAS for pain.

in improving the health status of BCRL patients (reducing lymphedema volume, pain, and functional disability and improving quality of life), particularly with the inclusion of laser therapy and electrotherapy. This combination showed better effects on patients under 60 years old, and laser therapy of low to moderate intensity (5–24 mW, 1.5–2 J/cm^2) and of moderate- to long-term duration (≥36–72 sessions) showed better effects.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

CD: Conceptualization, Data curation, Formal analysis, Software, Supervision, Writing – original draft, Writing – review & editing. ZW: Writing – original draft, Data curation, Formal analysis, Software. ZC: Writing – review & editing, Software. XZ: Writing – original draft, Funding acquisition, Supervision, Writing – review & editing. CT: Writing – review & editing, Conceptualization, Funding acquisition, Supervision.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by the National Natural Science Foundation of China (82174479,82205245) and the Natural Science Foundation for Guangdong, China (2023A1515011143).

Acknowledgments

The authors would like to thank XZ and Wen Hao for their excellent technical support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1361128/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 01 May 2024
DOI 10.3389/fonc.2024.1355353

OPEN ACCESS

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RECEIVED 18 December 2023
ACCEPTED 10 April 2024
PUBLISHED 01 May 2024

CITATION
Ali-Thompson S, Daly GR, Dowling GP, Kilkenny C, Cox L, McGrath J, AlRawashdeh MM, Naidoo S, Power C and Hill ADK (2024) A bibliometric analysis of HER2-positive breast cancer: 1987–2024. *Front. Oncol.* 14:1355353. doi: 10.3389/fonc.2024.1355353

A bibliometric analysis of HER2-positive breast cancer: 1987–2024

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Aim: The overamplification of human epidermal growth factor (HER2) in breast cancer (BC) has been the subject of numerous research publications since its discovery in 1987. This is the first bibliometric analysis (BA) conducted on HER2-positive (HER2+) BC. The purpose of this BA is to analyze the published research on HER2+ BC from 1987 to 2024, highlighting the most significant scientific literature, as well as the main contributing authors and journals, and evaluating the impact of clinical and lab-based publications on HER2+ BC research.

Methods: The Web of Science Core Collection (WoSCC) was searched using the terms "Breast cancer" OR "Breast carcinoma" OR "Breast tumor" AND "HER2 positive" OR "HER2+". The search was limited by publication year (1987–2024) and only full English articles were included. WoS returned 7,469 relevant results, and from this dataset, a bibliometric analysis was conducted using the "analyze results" and "journal citation report" functions in WoS and the VOSviewer 1.6.16 software to generate bibliographic coupling and co-citation analysis of authors.

Results: The analysis encompassed a total of 7,469 publications, revealing a notable increase in the annual number of publications, particularly in recent years. The United States, China, Italy, Germany, and Spain were the top five most prolific countries. The top five significant institutions that published HER2+ research were the University of Texas System, Unicancer, UTMD Anderson Cancer Center, Harvard University, and University of California System. *Breast Cancer Research and Treatment*, *Clinical Cancer Research*, and *Clinical Breast Cancer* were the top three notable journals with the highest number of HER2+ BC publications. Dennis Slamon (Nc = 45,411, H-index = 51) and Jose Baselga (Nc = 32,592, H-index = 55) were the most prolific authors. Evolving research topics include anti-HER2 therapy in the neoadjuvant setting, treatment of metastatic HER2+ BC, and overcoming therapy resistance.

Conclusion: This study provides an overview of HER2+ BC research published over the past three decades. It provides insight into the most cited papers and authors, and the core journals, and identifies new trends. These manuscripts have had the highest impact in the field and reflect the continued evolution of HER2 as a therapeutic target in BC.

KEYWORDS

HER2+, breast cancer, anti-HER2, trastuzumab, bibliometric, analysis

Introduction

Breast cancer (BC) has the highest incidence of all cancers worldwide and is the leading cause of cancer death in women (1). It accounts for 12.5% of new annual cancer cases and has an estimated mortality rate of 6.9% (2, 3). BC can be classified by molecular subtype based on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) on immunohistochemistry (4). These subtypes include luminal type A (ER+, PR+, HER2−), luminal type B (ER+, PR−/high Ki67, HER2+/−), HER2 subtype (ER−, PR−/low Ki-67, HER2+), and triple-negative breast cancer (TNBC) subtype (ER−, PR−/low Ki67, HER2−) (4). In the era of personalized medicine, BC molecular subtype hugely influences overall treatment, targeted therapeutics, and prognosis.

HER2+ BC constitutes approximately 10%–15% of BCs (2). HER2 is a tyrosine kinase receptor present on breast cells for the normal proliferation of breast tissue. The overamplification of HER2 leads to increased proliferation and activation of proto-oncogenic pathways (4). In comparison to the luminal subtypes, HER2+ BC has a higher proliferation rate, higher recurrence rate, and higher tendency to metastasize, with up to 30%–50% of HER2+ BC patients developing brain metastases (4). The result is that only TNBC has a worse prognosis than HER2+ BC (4).

The advent of targeted therapies for HER2+ BC has improved the outcomes and prognosis of the disease. The initial therapy, trastuzumab (Herceptin®), is a monoclonal antibody that directly targets HER2 (5). In its seminal trial, the addition of trastuzumab to chemotherapy saw an increase in median survival from 20.3 months to 25.1 months (p = 0.046) (5), revolutionizing the treatment of HER2+ BC. Anti-HER2 antibodies continue to have a pivotal role in the treatment of HER2+ BC. A 2022 population-based cohort study evaluating women with T1a/bN0M0 HER2+ BC reported that there was a 5-year disease-free survival of 94.8% in women who received adjuvant trastuzumab in comparison to 82.7% in women who did not receive trastuzumab (6). There was also a 5-year overall survival of 100% in the women who received trastuzumab compared to 90.4% of women who did not receive trastuzumab. Since the development of trastuzumab, new monoclonal antibodies (i.e., pertuzumab) have been developed with advancements in the cell signaling cascades leading to improved progression-free and overall survival (7). The current general first-line regimen for HER2+ BC is a single-agent chemotherapeutic in combination with trastuzumab and pertuzumab (8). Therapies such as tyrosine kinase inhibitors (e.g., lapatinib and neratinib) are also being used in the treatment of HER2+ BC. The treatment of HER2+ BC brain metastases is varied. Treatment decisions are often individualized based on expert opinion. However, treatment usually consists of a combination of systemic anti-cancer treatments, radiotherapy, and surgery (7).

In this study, we performed a bibliometric analysis of the most significant scientific literature published on HER2+ BC from 1987 to 2024 in order to evaluate the impact and analyze the trends of both clinical and lab-based research publications on this topic. While bibliometric analyses have been performed on several topics in BC (9–11), this is the first study undertaken to determine the most influential literature in HER2+ BC. This study provides a succinct analysis and summary of the most-cited papers, authors, and core journals on HER2+ BC, aiming to provide insight into the evolution of the HER2+ BC literature, and how this progression has impacted the treatment of HER2+ BC.

Materials and methods

The Web of Science Core Collection (WoSCC) was searched using the terms “Breast cancer” OR “Breast carcinoma” OR “Breast tumor” AND “HER2 positive” OR “HER2+”, yielding 20,049 results. The search was refined to include only English articles from publication years 1987–2024. This search gave 7,469 results. The WoSCC software was then used to categorize the results and retrieve the number of publications and H-index for authors, years, countries, and journals. The results were used to identify the current globally approved HER2+ BC treatment. The NIH clinicaltrials.gov database was used to identify the clinical trials and national clinical trial (NCT) number. Studies were exported to Microsoft Excel to chart a bar graph based on the number of publications per year. WoSCC Journal Citation Reports was used to record the 5-year Journal Impact factor and Eigenfactor Score for each journal. The VOSviewer 1.6.16 software was used to generate bibliographic coupling analyses, co-citation analysis of authors, and co-occurrence analysis of keywords.

Results

Annual number of publications

Figure 1 represents the annual number of articles published on HER2+ BC since its discovery in 1987. In total, 7,469 articles have been published on HER2+ BC. The annual publication rate has increased exponentially ($y = 73.173e^{0.1353x}$, $R^2 = 0.9238$), representing HER2’s continually evolving role in precision oncology.

Countries

A total of 101 countries have contributed to publication on HER2+ BC. The top three contributors have been the USA with 2,570 publications (34%), China with 1,291 publications (17%), and Italy with 761 publications (10%) (summary in Table 1). However, after the USA (H-index 180, Nc 192,016), Germany had the highest H-index (107) and Nc (65,528). VOSviewer was used to visualize co-authorship between countries. Countries with at least five publications were included (75 countries). Increasing node size represents the number of articles, and the thickness of the line represents the degree of cooperation. The USA occupied the central position and shared co-authorship with England, Germany, Spain, and China, among others. The three countries with the strongest link strength were the USA (2,570 articles, 19,2016 citations, total link strength 3,229), Germany

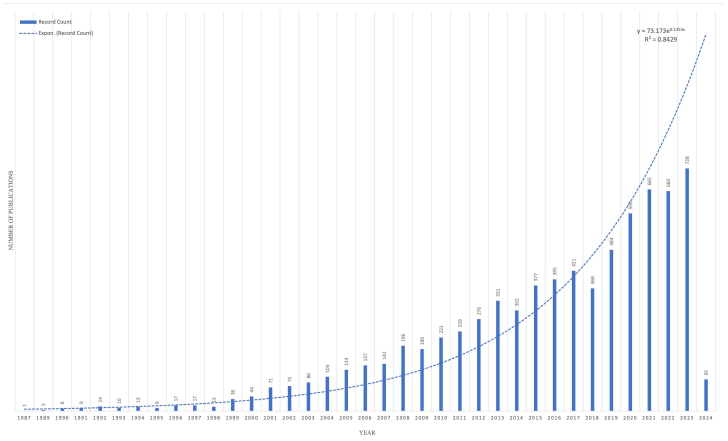

FIGURE 1
The trend in publication on HER2+ breast cancer since the discovery of HER2.

(630 articles, 65,528 citations, total link strength 1,937), and Spain (562 articles, 56,396 citations, total link strength 1,820). Despite China having the second most published articles, it ranked 14th in terms of cooperation with other nations (link strength 642) (summary in Figure 2A). The USA had its highest APY in 2016. Similar APY trends are observed in other Western countries. Conversely, China, along with several other Asian and Arab countries, had its highest APY in 2023 (summary in Figure 2B).

Journals and authors

Articles in the field of HER2+ BC were published in 1,038 journals; 120 of these journals published 10 or more articles. Table 2 shows the journals with the highest quantity of publications and number of citations (Nc). The top three journals with the highest quantity were *Breast Cancer Research and Treatment* (481), *Clinical Cancer Research* (221), and *Clinical Breast Cancer* (168). In respect to Nc and H-index, *Clinical Cancer Research* (Nc = 15,806, H-Index = 72) held the number one position, followed by *Breast Cancer Research* (Nc = 13,248, H-Index = 59) and *Annals of Oncology* (Nc = 12,352, H-Index = 68).

A total of 34,790 authors contributed to the field of HER2+ research of varying impacts. Table 3 shows the top 10 authors with the most publications and citations. The top three authors with the highest number of articles were Jose Baselga (91 articles), Seock-Ah Im (77 articles), and Nadia Harbeck 73 articles). However, the authors with the highest Nc and H-index, which gives insight into the citation impact and quality of research, were Dennis Slamon (Nc = 45,411, H-index = 51), Jose Baselga (Nc = 32,592, H-index = 55), and Axel Ullrich (Nc = 17,068 H-index= 7).

Co-cited references and top 10 cited papers

Figures 3A, B illustrate the density visualization and network visualization respectively of co-citation references in HER2 papers.

TABLE 1 The top 10 countries with the highest number of publications.

Rank	Countries/Regions	Count	% of 7,469	Nc	H-index
1	USA	2570	34	192,016	180
2	CHINA	1291	17	22,108	60
3	ITALY	761	10	47,795	93
4	GERMANY	630	8	65,528	107
5	SPAIN	562	7	56,396	96
6	JAPAN	528	7	21,915	61
7	FRANCE	467	6	41,782	85
8	ENGLAND	455	6	45,470	95
9	SOUTH KOREA	423	5	28,406	68
10	CANADA	397	5	49,656	82

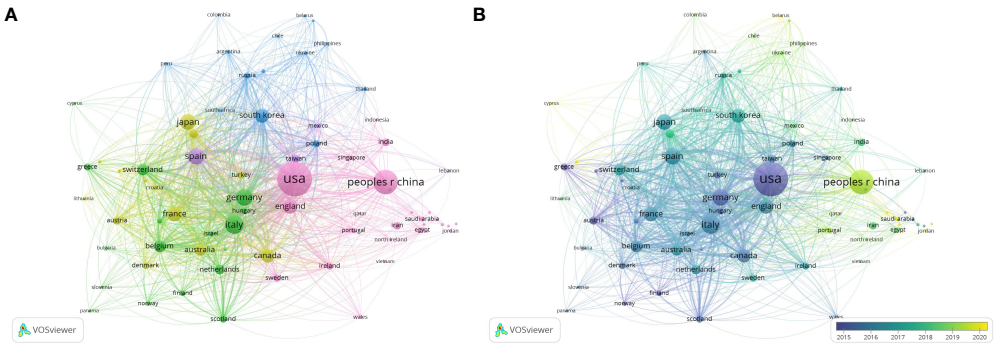

FIGURE 2
Visualization of countries involved in HER2+ BC research. **(A)** Visualization of cooperation between countries. **(B)** Visualization of temporal cooperation overlay between regions.

TABLE 2 The top 10 journals with the highest number of publications.

Rank	Journal	Count	% of 7,469	Nc	H-index	5-year impact factor	Eigenfactor
1	*Breast Cancer Research and Treatment*	481	6.44	13,248	59	4.4	0.02445
2	*Clinical Cancer Research*	221	2.96	15,806	72	12.5	0.11111
3	*Clinical Breast Cancer*	168	2.25	2,575	27	3.3	0.00582
4	*Breast*	160	2.14	2,901	31	4.1	0.00867
5	*Annals of Oncology*	159	2.13	12,352	68	32.4	0.10839
6	*BMC Cancer*	148	1.98	3,615	32	4.3	0.0517
7	*Frontiers in Oncology*	132	1.77	681	12	5.2	0.097
8	*PLoS One*	131	1.75	2,852	28	3.8	0.71257
9	*Anticancer research*	126	1.69	1,998	24	2.2	0.0148
10	*Cancers*	123	1.65	902	16	5.6	0.12236

TABLE 3 The top 10 authors with the most publications and Nc.

Rank	Authors	Record count	% of 7,469	H-index	Authors	Nc	H-index
1	J Baselga	91	1.2	55	DJ Slamon	45,411	51
2	SA Im	77	1	30	J Baselga	32,592	55
3	N Harbeck	73	0.98	32	A Ullrich	17,068	7
4	N Masuda	73	0.98	21	SG Wong	16,284	3
5	H Iwata	70	0.94	22	WJ Levin	16,203	2
6	BH Xu	68	0.91	20	S Shak	13,690	8
7	M Untch	67	0.9	41	GM Clark	12,967	9
8	SB Kim	66	0.88	25	WL Mcguire	12,216	8
9	HS Rugo	65	0.87	31	L Norton	11,591	17
10	A Prat	57	0.83	25	V Paton	10,850	3

The figure shows that the Slamon, DJ 1987 article has the largest cluster, indicating the highest citation co-citation count. Table 4 shows the top 10 co-cited references in HER2+ BC. The top 3 publications with the highest citation frequencies were all by the author Slamon, DJ (1987) (n = 1,749), (2001) (n = 1,417), and (1989) (n = 942).

The top 10 most cited papers on HER2+ BC are summarized in Table 5. The top three most cited papers were all written by Denis

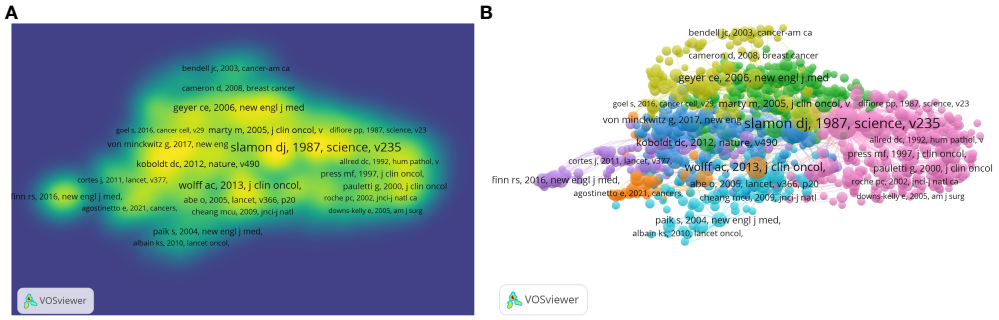

FIGURE 3
Visualization of article citations in HER2+ BC research. **(A)** Visualization of paper citation density. **(B)** Visualization of network co-citation between articles.

Slamon, representing the discovery of HER2 and its initial therapeutic implications. "Human-breast cancer—Correlation of relapse and survival with amplification of the HER-2 neu oncogene" was published in *Science* in 1987 (IF 56.9) and has been cited 9,708 times, "Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic BC that overexpresses HER2" was published in the *New England Journal of Medicine* (IF 158.9) in 2001 and has been cited 8,923 times, and "Studies of the HER-2/Neu proto-oncogene in human-breast and ovarian-cancer" was published in *Science* (IF-56.9) in 1989 and has been cited 6,186 times. Summarized in Figure 3, Slamon's 1987 paper holds the central position, with other seminal articles such as Von Minckwitz's paper "Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer", Geyer's paper "Lapatinib plus capecitabine for HER2-positive advanced breast cancer", and Goel's paper, "Overcoming therapeutic resistance in HER2-positive breast cancers with CD4/6 inhibitors".

TABLE 4 The top 10 co-cited references based on citation counts.

Rank	Citation count	References	DOI
1	1,749	Slamon DJ, 1987, *Science*, v235, p177	doi 10.1126/science.3798106
2	1,417	Slamon DJ, 2001, *New Engl J Med*, v344, p783	doi 10.1056/nejm2001031534411
3	942	Slamon DJ, 1989, *Science*, v244, p707	doi 10.1126/science. 2470152
4	772	Romond EH, 2005, *New Engl J Med*, v353, p1673	doi 10.1056/nejmoa052122
5	730	Piccart-Gebhart MJ, 2005, *New Engl J Med*, v353, p1659	doi 10.1056 /nejmoa052
6	627	Wolff AC, 2013, *J Clin Oncol*, v31, p3997	doi 10.1200/jco.2013.50.9984 10.5858
7	509	Vogel CL, 2002, *J Clin Oncol*, v20, p719	doi 10.1200/jco.2002.20.3.719
8	455	Geyer CE, 2006, *New Engl J Med*, v355, p2733	doi 10.1056 /nejmoa064320
9	453	Verma S, 2012, *New Engl J Med*, v367, p1783	doi 10.1056/nejmoa1209124
10	451	Gianni I, 2012, *Lancet Oncol*, v13, p25	doi 10.1016/ 1470-2045(11)70336-9

Bibliographic coupling analysis of institutions

A total of 9,577 institutions contributed to publication on HER2+ BC. The three institutions with the most publications were the University of Texas System (414), Unicancer (347), and University of Texas MD Anderson Cancer Centre (339). However, the University of California System had the highest Nc (61,133) (summary in Table 6). VOSviewer was used to visualize co-authorship and collaboration between institutions (Figure 4), including institutions with a minimum of five published articles (1,266). Memorial Sloan Kettering Cancer Center (213 articles, 29,574 citations, total link strength 994), Dana Farber Cancer Institute (151 articles, 18,227 citations, total link strength 890), and the University of Texas MD Anderson Cancer Center (204 articles, 13,888 citations, total link strength 890) had the most cooperation of any institutions in the field of HER2+ BC research. Similar to the distribution of publications by country, the institutions from the USA and Europe had their highest APYs ~2016, while Chinese institutions were most published ~5 years later.

Co-occurrence analysis of keywords

The top 20 used keywords are listed in Table 7. Unsurprisingly, the top keywords were "breast cancer" (2,824 articles, total link strength 2815), "trastuzumab" (2,077 articles, total link strength 2072), and "HER2" (1,673 articles, total link strength 1673). Network analysis of keywords, visualized using VOSviewer in Figure 5A, revealed that these terms, along with chemotherapy and expression, were central hubs. Figure 5B reveals that newer terms of importance include antibody–drug conjugates (ADCs), lapatinib plus paclitaxel, the HERA trial, and

TABLE 5 The top 10 most cited HER2+ research articles.

Rank	Title	First author	Senior author	Institution	Country	Journal	IF (2022)	Year	No. of citations
1	Human-breast cancer—correlation of relapse and survival with amplification of the HER-2 neu oncogene	Slamon, DJ	Slamon, DJ	University of California System	USA	*Science*	56.9	1987	9708
2	Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2	Slamon, DJ	Slamon, DJ	University of California System	USA	*New England Journal of Medicine*	158.5	2001	8293
3	Studies of the HER-2/Neu proto-oncogene in human-breast and ovarian-cancer	Slamon, DJ	Slamon, DJ	University of California System	USA	*Science*	56.9	1989	6186
4	Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer	Romond, EH	Geyer, CE	University of Pittsburgh	USA	*New England Journal of Medicine*	158.5	2005	4088
5	Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer	Piccart-Gebhart	Piccart-Gebhart	Institut Jules Bordet	Belgium	*New England Journal of Medicine*	158.5	2005	3822
6	Lapatinib plus capecitabine for HER2-positive advanced breast cancer	Geyer, Charles E.	Geyer, CE	Allegheny General Hospital	USA	*New England Journal of Medicine*	158.5	2006	2502
7	Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer	Vogel, CL	Vogel, CL	University of Miami	USA	*Journal of Clinical Oncology*	45.4	2002	2453
8	Trastuzumab emtansine for HER2-positive advanced breast cancer	Verma, Sunil	Verma, Sunil	University of Toronto	Canada	*New England Journal of Medicine*	158.5	2012	2327
9	Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease	Cobleigh, MA	Cobleigh, MA	Rush University	USA	*Journal of Clinical Oncology*	45.4	1999	2188
10	Adjuvant trastuzumab in HER2-positive breast cancer	Slamon, DJ	Slamon, DJ	University of California System	USA	*New England Journal of Medicine*	158.5	2011	1801

cardiac safety. The top 15 topics focused on in the last 10 years are shown in Table 8 and Figure 6. The top three discussion topics were adjuvant pertuzumab and trastuzumab, trastuzumab emtansine, and neratinib after trastuzumab-based adjuvant therapy.

Globally approved therapies for HER2+ BC

Table 9 shows a summary of targeted therapies and chemotherapy options used as interventions in HER2+ BC. Trastuzumab, the pioneering monoclonal antibody, was the first FDA-approved targeted therapy in 1998 for HER2+ BC, followed by lapatinib, pertuzumab, and trastuzumab emtansine. The table shows their associated clinical trials and FDA approval numbers. The drug pyrotinib is not approved by the FDA but is approved and used in China for the treatment of HER2+ metastatic BC. Margetuximab is a recently approved (2020) monoclonal antibody indicated for patients with metastatic HER2+ BC. Eribulin is a chemotherapeutic agent that, when combined with trastuzumab, can be used to treat HER2+ advanced BC.

Discussion

The HER2 gene was first discovered in mice in 1984; however, it was not until 1987 that Slamon et al. discovered the link between HER2

TABLE 6 The top 10 organizations with the highest number of publications.

Rank	Affiliations	Record count	% of 7,469	Nc	H-index
1	University of Texas System	414	5.54	48,023	90
2	Unicancer	347	4.65	34,654	81
3	UTMD Anderson Cancer Center	341	4.57	32,665	80
4	Harvard University	339	4.54	38,750	88
5	University of California System	310	4.15	61,133	87
6	Roche Holding	251	2.88	27,329	27
7	Memorial Sloan Kettering Cancer Center	233	3.12	32,562	74
8	Dana Farber Cancer Institute	220	2.94	30,963	73
9	Fudan University	167	2.23	5,121	29
10	Harvard Medical School	163	2.18	13,123	55

and BC (12, 13). Initially, HER2+ BC was associated with aggressive disease and poor outcomes; however, the discovery of trastuzumab and improvements in precision oncology have led to dramatic prognostic improvement (14). To our knowledge, this study is the first bibliometric analysis of HER2+ BC. Analyzing the most cited and influential articles in HER2+ BC outlines the evolution of HER2+ BC and helps visualize emerging research trends, serving as a guide for clinicians on the current state and future direction of HER2 research.

TABLE 7 Top 20 keywords in HER2+ research with the strongest strength links.

Rank	Keywords	Frequency	Total link strength
1	Breast cancer	2,824	2,815
2	Trastuzumab	2,077	2,072
3	HER2	1,673	1,673
4	Therapy	1,370	1,366
5	Expression	1,364	1,364
6	Chemotherapy	1,333	1,332
7	Survival	1,196	1,195
8	Amplification	642	642
9	Adjuvant chemotherapy	611	611
10	Efficacy	577	577
11	Receptor	571	570
12	Women	571	565
13	Resistance	565	542
14	Monoclonal antibody	543	541
15	Open-label	541	524
16	Immunohistochemistry	525	521
17	Pertuzumab	522	519
18	Lapatinib	520	483
19	Docetaxel	483	474
20	Metastatic breast cancer	474	446

The most cited paper on HER2+ BC is Slamon et al.'s 1987 paper (13), which established the correlation between HER2 and human BC. This paper found that 30% of breast tumors demonstrate amplification of the HER2 oncogene to greater than 20-fold even when other prognostic factors were controlled (13). Ultimately, the data from this study helped determine the impact of HER2 in the pathogenesis of BC. The next most cited papers focus on biological mechanisms aiming to ascertain the association between HER2 overexpression and prognosis. A 1989 study by J. Baselga et al. shed light on the role of Trastuzumab in enhancing the anti-tumor activity of paclitaxel and doxorubicin against HER2 amplified human BC xenografts (14). This publication precipitated a shift in focus from the efficacy of screening HER2+ BC towards exploiting HER2 as a therapeutic target. The importance of anti-HER2 therapies, both as single agents and in combination with other therapies, is demonstrated in this analysis with consistently high publication numbers. The current standard of care for adjuvant treatment of HER2+ BC, is dual anti-HER2 monoclonal antibodies (trastuzumab and pertuzumab) with docetaxel. This regimen was established largely on the basis of the promising results of the CLEOPTATRA trial, and a recent increase in citations highlights the magnitude of this trial's impact on HER2+ BC (15). The similarly cited HERA trial reported no benefit in 2 years of trastuzumab treatment over 1 year, leading to guideline changes (16). This level of citation again reflects the impact this research has had on the landscape of HER2+ BC.

A total of 101 countries contributed to papers on the topic of HER2+ BC. The USA was the highest contributor, a somewhat expected finding given the HER2 gene and the association of HER2 positivity with BC and trastuzumab were all discovered there. Other factors such as the availability of resources and funding likely facilitated this high ranking. The top affiliations were the University of Texas system, the University of California, Los Angeles (UCL), Genentech, Memorial Sloan Kettering Cancer Center and Harvard University. These institutions have a long-

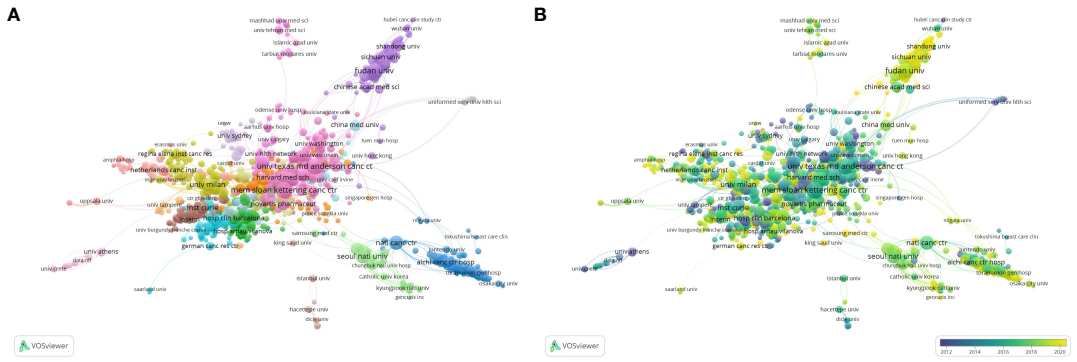

FIGURE 4
Visualization of institutions involved in HER2+ BC research. **(A)** Visualization of cooperation between institutions. **(B)** Visualization of temporal cooperation overlay between institutions.

standing interest in HER2+ BC research. Dennis Slamon, the founder of trastuzumab, is the chief of the division of Hematology-Oncology at UCLA, and Genentech is the company that developed trastuzumab.

"Metastatic breast cancer" is among the most commonly used keywords in the last 5 years, as despite overall treatment advances, the prognosis of metastatic BC remains poor. This is particularly relevant to HER2+ BC given the reduced efficacy of targeted HER2 in the metastatic setting. Development of resistance to anti-HER2 therapies has thus far posed an insurmountable therapeutic challenge, particularly in the context of advanced disease. However, the development of novel treatments including immunotherapy, cell-cycle inhibitors, and ADCs has improved outcomes of metastatic disease. ADCs have had particularly promising results in metastatic disease, and this is reflected by their high article publication and citation numbers of late. ADCs, such as trastuzumab deruxtecan (T-DXd) and trastuzumab emtansine (TDM-1), consist of a cell surface protein antigen, a cytotoxic agent, and a linker that combines them (17). The DESTINY-Breast03 trial, a multicenter randomized control trial (RCT), found that median progression-free survival with T-DXd was 28.8 months (95% CI 22.4–37.9) compared to 6.8 months (95% CI 5.6–8.2) in those treated with trastuzumab emtansine [hazard ratio 0.33 (95% CI 0.26–0.43), $p < 0.0001$] (18). TDXt is also currently being explored in the neoadjuvant setting for primary disease (19). The interest in research focused on overcoming resistance is clear in this study, with "resistance" identified as a top key term for the last 5 years. The centrality of Goel et al.'s article, "Overcoming therapeutic resistance in HER2-positive breast cancers with CD4/6 inhibitors", in citation analysis affirms this focus (20). Several mechanisms of treatment resistance are recognized including activation of the PI3K/Akt/mTOR signaling pathway in response to prolonged treatment course, which may cause resistance through expression of mutated PTEN or PIK3CA genes (21). Detailed understanding of this pathway, gained through scientific exploration, has led to the FDA approval of capisertib, an Akt inhibitor (22). This exemplifies the importance of cohesive international efforts to elucidate the interaction between signaling pathways, tumor proliferation, and treatment response at a molecular level to identify druggable targets that improve patient

FIGURE 5
Visualization of keywords in HER2+ BC research. **(A)** Visualization of network co-citation of keywords. **(B)** Visualization of temporal co-citation overlay between keywords.

TABLE 8 Top 20 keywords in HER2+ research 2020–2024.

Rank	Keywords	Occurrences
1	Breast cancer	1,032
2	Trastuzumab	685
3	Chemotherapy	483
4	Survival	484
5	Her2	466
6	Therapy	428
7	Pertuzumab	315
8	Open-label	296
9	Expression	375
10	Multicenter	250
11	Women	238
12	Efficacy	209
13	Resistance	211
14	Lapatinib	183
15	Docetaxel	173
16	Combination	166
17	Metastatic breast cancer	187
18	Safety	160
19	Receptor	167
20	Pathological complete response	142

outcomes. Other important mechanisms of resistance backed by numerous publications include Src mutations, MET mutations, and HER2 activating mutations (21).

Anti-HER2 therapy in the neoadjuvant setting is another focus of continually evolving HER2+ BC research. Dual trastuzumab and pertuzumab in combination with chemotherapy is prescribed both to downstage larger HER2+ primary tumors and to assess tumor response, guiding subsequent adjuvant therapies (23). The results of several ongoing trials assessing the efficacy of other therapeutic agents in achieving a pathological complete response and improving survival outcomes such as tyrosine kinase inhibitors, immunotherapy, and ADCs are eagerly awaited (19, 24, 25).

There are several limitations of this study. WoSCC was the only database that was used to search for manuscripts. While WoSCC has the broadest collection of literature and is one of the most widely used databases, it is possible that some articles have been omitted. Secondly, the level of evidence for each study was not evaluated as the present study aims to delineate the landscape of HER2+ BC research since its discovery in 1987 rather than provide a review of the literature itself. Lastly, the confounding factor of time since publication was not comprehensively analyzed, and thus, the citation numbers may be elevated in older publications as a result of having more time for citation rather than a true predominance of interest. An example is the omission of publications on HER2 vaccines in the treatment of BC. In 2022, NCT00436254 established the efficacy and safety of a plasmid DNA vaccine encoding the ERBB2 intracellular domain in late-stage HER2+ BC (26). NCT00791037 observed that T-cell infusion post HER2 DNA vaccine improved survival outcomes in patients with advanced disease (27). While failing to be recognized by the lists compiled in this study, this cutting-edge area of research may be among the most impactful in future management of HER2+ BC.

Conclusion

The present study illustrates the evolution of HER2 since the discovery of its link with BC. The discovery of anti-HER2 therapies and subsequent improvements in patient outcomes highlights the importance of both clinical and lab-based

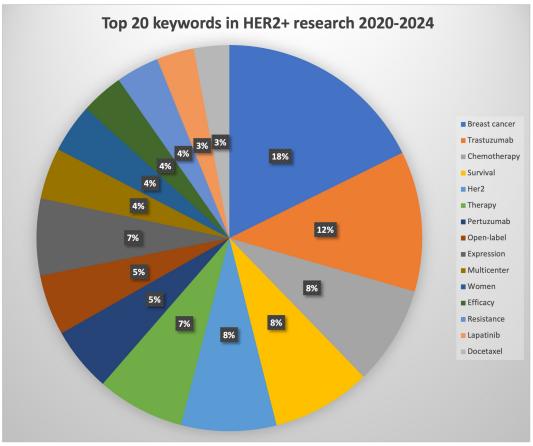

FIGURE 6
Pie chart visualization of the main topics of HER2+ articles focused on between 2020 and 2024.

TABLE 9 Table showing global approved anti-HER2+ therapies.

	Generic drug name	Brand drug name	Drug Class	Clinical Study	Approval year	Publication DOI
	Trastuzumab	Herceptin	Monoclonal Antibody	HERA trial (NCT00045032)	1998	doi:10.1056/NEJMoa052306
	Lapatinib	Tykerb	Tyrosine Kinase Inhibitor	EGF100151 trial (NCT00078572)	2007	doi:10.1056/NEJMoa064320
	Pertuzumab	Perjeta	Monoclonal Antibody	CLEOPATRA trial (NCT00567190)	2012	doi:10.1056/NEJMoa1113216
Targeted therapies	Ado-Trastuzumab emtansine(T-DM1)	Kadcyla	Antibody-Drug Conjugate	EMILIA trial (TDM4370g) (NCT00829166)	2013	doi:10.1056/NEJMoa1209124
	Neratinib	Nerlynx	Tyrosine Kinase Inhibitor	ExteNET trial (NCT00878709)	2017	doi:10.1016/S1470-2045(15)00551-3
	Pyrotinib	Pyrotinib Mesylate	Tyrosine Kinase Inhibitor	PHOEBE trial (NCT03080805)	2018	doi:10.1016/S1470-2045(20)30702-6
	Trastuzumab deruxtecan(T-DXdb)	Enhertu	Antibody-Drug Conjugate	DESTINY-Breast01 trial (NCT03248492)	2019	doi:10.1056/NEJMoa1914510
	Tucatinib	Tukysa	Tyrosine Kinase Inhibitor	HER2CLIMB trial (NCT02614794)	2020	doi: 10.1056/NEJMoa1914609
	Margetuximab	Margenza	Monoclonal Antibody	SOPHIA trial (NCT02492711)	2020	doi:10.1200/JCO.21.02937
	Doxorubicin	Adriamycin	Anthracycline chemotherapy agent	NCT00005970	1974	doi:10.1200/JCO.2011.36.7045
Chemotherapeutic agents	Paclitaxel	Taxol	Taxane chemotherapy agent	NCT00542451	1992	doi:10.1056/NEJMoa1406281
	Docetaxel	Taxotere	Taxane chemotherapy agent	NCT00003773	1996	doi:10.1200/JCO.2002.07.058
	Capecitabine	Xeloda	Antimetabolite chemotherapy agent	NCT00174893	1998	doi:10.1200/JCO.2006.09.6826
	Eribulin mesylate	Halaven	Microtubule inhibitor chemotherapy agent	EMBRACE trial (NCT00388726)	2010	doi: 10.1016/S0140-6736(11)60070-6

research in BC. The US and US-based institutions have continued to publish the most impactful articles on HER2+ BC. Emerging trends in HER2+ BC research are treatment of metastatic HER2+ BC, overcoming therapy resistance, and targeting HER2 in the neoadjuvant setting.

Author contributions

SA-T: Conceptualization, Formal analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing. GD: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. GD: Resources, Software, Writing – review & editing. CK: Conceptualization, Writing – review & editing. LC: Formal analysis, Writing – review & editing. JM: Formal analysis, Writing – review & editing. MA: Writing – review & editing. SN: Writing – review & editing. CP: Conceptualization, Supervision, Writing – review & editing. AH: Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

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frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 10 May 2024
DOI 10.3389/fonc.2024.1385577

OPEN ACCESS

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RECEIVED 13 February 2024
ACCEPTED 26 April 2024
PUBLISHED 10 May 2024

CITATION
Sharaf B, Hajahjeh A, Bani Hani H and Abdel-Razeq H (2024) Next generation selective estrogen receptor degraders in postmenopausal women with advanced-stage hormone receptors-positive, HER2-negative breast cancer.
Front. Oncol. 14:1385577.
doi: 10.3389/fonc.2024.1385577

Next generation selective estrogen receptor degraders in postmenopausal women with advanced-stage hormone receptors-positive, HER2-negative breast cancer

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Breast cancer is the most prevalent malignancy in women, and is characterized by its heterogeneity; exhibiting various subgroups identifiable through molecular biomarkers that also serve as predictive indicators. More than two thirds of breast tumors are classified as luminal with positive hormone receptors (HR), indicating that cancer cells proliferation is promoted by hormones. Endocrine therapies play a vital role in the effective treatment of breast cancer by manipulating the signaling of estrogen receptors (ER), leading to a reduction in cell proliferation and growth rate. Selective estrogen receptor modulators (SERMs), such as tamoxifen and toremifene, function by blocking estrogen's effects. Aromatase inhibitors (AI), including anastrozole, letrozole and exemestane, suppress estrogen production. On the other hand, selective estrogen receptor degraders (SERDs), like fulvestrant, act by blocking and damaging estrogen receptors. Tamoxifen and AI are widely used both in early- and advanced-stage disease, while fulvestrant is used as a single agent or in combination with other agents like the cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors (palbociclib, abemaciclib, ribociclib) or alpelisib for advanced-stage disease. Currently, SERDs are recognized as an effective therapeutic approach for the treatment of ER-positive breast cancer, showing proficiency in reducing and blocking ER signaling. This review aims to outline the ongoing development of novel oral SERDs from a practical therapeutic perspective, enhancing our understanding of the mechanisms of action underlying these compounds.

KEYWORDS

metastatic breast cancer, endocrine therapy, SERDS, aromatase inhibitors, fulvestrant, elacestrant, ESR1

1 Introduction

Breast cancer surfaces as a prevalent health issue, influencing a substantial number of women worldwide. According to GLOBOCAN 2020, it is the most commonly diagnosed cancer among women in 185 countries, and is a leading cause of mortality, too (1). The molecular heterogeneity of breast cancer makes it a challenging disease to treat, highlighting the need for active research to develop new drugs that can tackle the different tumor subtypes and at different phases throughout the disease course (2). Despite advances in treatment, metastatic breast cancer (MBC) remains an incurable disease, with a 5-year survival rate of 25% and a median overall survival (OS) of 3 years (3).

The current management of breast cancer is primarily determined by the human epidermal growth factor receptor-2 (HER2) and hormone-receptor (HR) status (4, 5). More than two-third of breast cancers are HR-positive/HER2-negative, and endocrine therapy (ET) represents a major treatment option for these patients (6, 7). The clinical profile of these drugs, with their high efficacy and tolerability (8) helped their wide adoption (6). Endocrine therapy comprises different classes of drugs including the selective estrogen receptor modulators (SERMs) like tamoxifen, the luteinizing hormone-releasing hormone (LHRH) agonists like leuprolide, goserelin and triptorelin, the AI like letrozole, anastrozole and exemestane, the CDK4/6 inhibitors (CDK4/6i) like palbociclib, ribociclib and abemaciclib, and the selective estrogen-receptor degraders (SERDs) like fulvestrant and the more recently introduced oral agents (9). Though SERMS are widely used therapy for patient with breast cancer, its efficacy is limited and almost 25% of patients with primary and advanced-stage disease develop resistance during the course of their treatment (10, 11).

The administration of fulvestrant as bilateral intramuscular injections in a suspension of castor oil on a monthly basis is required due to its limited oral bioavailability; pain at the injection sites, can occasionally be an issue and pose challenges when considering its use in adjuvant settings where prolonged hormonal therapy lasting 5-10 years may be necessary. Additionally, the monthly injections lead to a notable peak and prolonged trough in the drug concentration within the body, which may result in suboptimal degradation of estrogen receptors (ER) (12).

In this review, we shed light on this class of molecules to explore previous, current, and future clinical uses.

2 The mechanism of action

The primary goal of endocrine therapy for metastatic HR-positive breast cancer is to aggressively counteract the impact of estrogen on cancer cells. This is accomplished through a variety of potent strategies, including the profound reduction of estrogen levels throughout the body, which serves as the formidable mechanism behind the remarkable effectiveness of AI (13). An alternative tactic involves diminishing the binding between estrogen and its receptor, exemplifying the dynamic mechanism by which SERMs exert their influence (14). Lastly, ET can reduce the number of estrogen receptors in cancer cells by terminally blocking the receptor leading to its degradation and decrease the number of the receptors, which is how our drugs of interest, SERDs exert their action (15, 16). A visual representation of the mechanism(s) by which these three drug groups work is shown in Figure 1.

3 History

The evolution and exploration of SERDs can be traced back to the 1990s. However, due to limited effectiveness and significant toxicity, the initial program was discontinued (17). Undeterred, researchers persevered, leading to the development of fulvestrant in

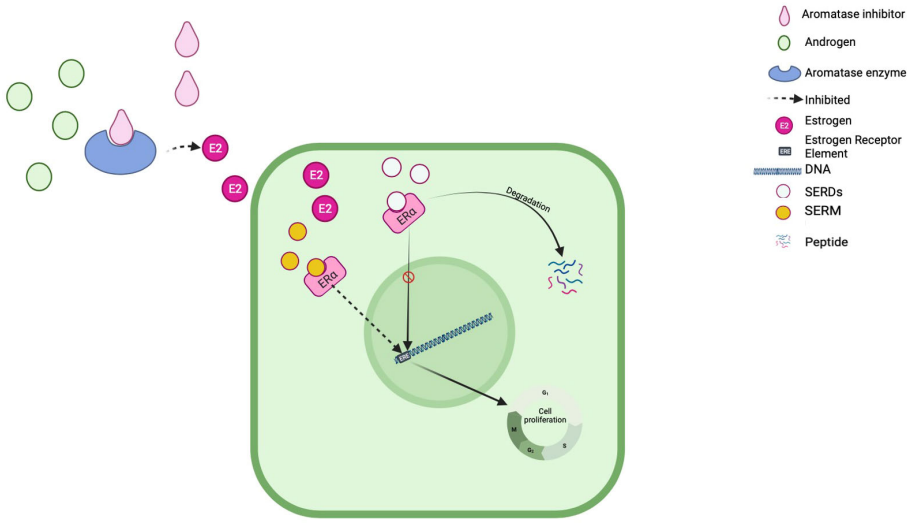

FIGURE 1
Mechanism of action.

the early 2000s. In 2002, fulvestrant received the Food and Drug Administration (FDA) approval for the treatment of advanced breast cancer (18). Subsequently, numerous clinical trials were conducted to assess the efficacy and safety of fulvestrant across various settings and phases of breast cancer therapy (19–21). As a result, in 2017, fulvestrant was granted approval in the frontline therapy for postmenopausal patients with advanced HR-positive breast cancer (22). More recently, the SOLAR-1 trial revealed that the combination of fulvestrant and alpelisib exhibited enhanced efficacy when compared to fulvestrant alone (23). This superiority was observed specifically in tumors with PI3K mutations that had experienced progression after prior endocrine therapy. Newer SERDs have shown promising results in preclinical studies and clinical trials, and their greater selectivity and potency, compared to their predecessors, indicating their potential to overcome resistance to ET (24, 25).

4 Candidates for SERDs

To be qualified for treatment with SERDs, patients must meet specific clinical features and their tumor should hold some molecular characteristics. The primary requirement is having a HR-positive breast cancer, along with developing resistance to other ET such AIs and SERMs (26). Additionally, patients should be in a postmenopausal state, since premenopausal women typically do not experience estrogen level changes affected by SERDs (23). It is generally advised to avoid prior exposure to SERDs, although in some cases, patients previously treated with fulvestrant may still be considered for other SERDs, although this is not commonly recommended (23).

5 SERDs in clinical trials

5.1 Fulvestrant

Fulvestrant is a synthetic steroid and a derivative of estradiol with an alkyl-sulfinyl moiety added to the endogenous estrogen receptor ligand (27). Unlike tamoxifen, it has pure anti-estrogenic effects and no apparent agonistic effects (14). It binds competitively to the estrogen receptor with a 100 times greater affinity than tamoxifen and causes downregulation of the receptor protein, which ultimately leads to complete interruption of estrogen-sensitive gene transcription (28, 29). This unique mechanism of action has demonstrated a clinical benefit rate (CBR) of 69% in postmenopausal women with tamoxifen-resistant breast cancer (30). In 2002, fulvestrant 250 mg was approved by the U.S. FDA for the treatment of ER-positive metastatic breast cancer in postmenopausal women with disease progression after antiestrogen therapy (either AI or tamoxifen) (31). The recommended dose was later revised to 500 mg after the demonstration of improved both progression-free survival (PFS) and OS, without increased toxicity versus fulvestrant 250 mg in the (CONFIRM) randomized, double-blind, phase III trial (32). Additionally, the randomized phase III FALCON trial, found that fulvestrant when given at 500 mg dose was more effective than anastrozole (33).

Fulvestrant was given a boost when it was approved to be used in combination with palbociclib, a CDK 4/6 inhibitor, in pre- and postmenopausal women to treat breast cancer progressing after ET (24). Consequently, fulvestrant in combination with ribociclib and abemaciclib have each been approved for HR-positive/HER2-negative MBC following the results of randomized Phase III studies (MONALEESA-3 and MONARCH-2), respectively; both showed improved PFS and OS (34, 35).

Although fulvestrant was generally tolerated as intramuscular injections once a month, its most common side effects were mild injection-site reactions, vasodilation, and hot flushes (36). Other well-known adverse events were asthenia, headache, gastrointestinal disturbances, urinary tract infections, and rashes (37). Moreover, recent studies have shown that fulvestrant 500 mg does not cause maximal ER downregulation *in vivo*, thus, a further increase in dose would increase the efficacy. However, that would require multiple injections each time which is less tolerable (14). In conclusion, the poor pharmacokinetic properties of fulvestrant and its injection-only administration route have directed the research community to find new oral SERDs with better pharmacokinetic properties and higher efficacy that could improve the clinical outcome (38, 39).

5.2 The new generation oral SERDs

The new generation oral SERDs are non-steroidal molecules that have an ER binding motif and a side chain with antiestrogenic and ER degrading activities. This side chain is either an acrylic acid or an amino acid (7). These drugs bind to the estrogen receptor and increase its hydrophobicity and instability, leading to its downregulation (14). The first developed SERD with an acrylic acid side chain was GW5638 in 1994 (40). However, this molecule and other oral SERDs with an acrylic acid side chain, including GDC-0810, AZD9496, and LSZ102 have all been discontinued as they did not show comparable or higher efficacy compared to fulvestrant (7, 14). On the other hand, new oral SERDs with basic side chains have achieved maximal ER degradation in multiple cell lines (7), in contrast to SERDs with acrylic acid side chains that do not degrade ER equally. These new SERDs have demonstrated potent activity against wild-type and mutant ER breast cancer and have reached phase III clinical trials (14).

5.2.1 Giredestrant

Giredestrant (GDC9545) was designed to overcome the poor clinical performance of the previous drugs like GDC-0810and GDC0927 (7, 8). Giredestrant efficacy has led to its evaluation in both early- and advanced-stage breast cancer in several clinical trials (8, 41). It has shown antitumor activity as a single-agent with tolerable side effect profile (42, 43). In early-stage disease, the phase II coopERA study had demonstrated superior efficacy of giredestrant over aromatase inhibitors in terms of Ki67 (a proliferation biomarker) suppression in ER+ breast cancer (8, 44). In the phase II acelERA trial, giredestrant was tested in metastatic breast cancer and showed a non-statistically significant improvement in PFS; 5.6 vs

5.4 months (HR, 0.81; 95% CI, 0.60-1.10; P = 0.1757) As a result, the trial did not meet its primary endpoint. However, the benefit was more evident in patients with *ESR1* mutation with 1.8 months difference in the median PFS (HR, 0.60; 95% CI, 0.35-1.03; P = .0610) (8, 45). The drug is still being evaluated in combination with CDK 4/6 inhibitors in the double-blind randomized persevERA trial in patients with ER+/HER2- locally advanced or metastatic breast cancer (6, 7), The LidERA Phase III multicentric trial, involving 4200 patients with early ER+/HER2- breast cancer, aims to assess the efficacy and safety of adjuvant giredestrant compared to physician's choice of adjuvant endocrine monotherapy as definitive treatment, providing crucial insights into the optimal therapeutic approach for this patient population (46). Table 1 shows summary of clinical trial of giredestrant.

5.2.2 Amcenestrant

Amcenestrant, also known as SAR439859, is a nonsteroidal SERD (8) that exhibits both acidic and basic properties and had demonstrated superior antagonism and degradation of estrogen receptors compared to other SERDs (47, 48). The AMEERA-1 and AMEERA-2 trials investigated the safety and efficacy of amcenestrant in postmenopausal women with advanced-stage breast cancer, particularly in heavily pre-treated patients (7). Amcenestrant has displayed excellent tolerability, with no dose-limiting toxicities. The trial has reported an objective response rate (ORR) of 10.9% and a clinical benefit rate (CBR) of 28.3%. The drug demonstrated comparable CBR in tumors expressing *ESR1* mutations (32.1%) and those without *ESR1* mutations (36.7%). This finding, based on a study involving 58 patients with known *ESR1* status, indicates that amcenestrant exhibits efficacy across both *ESR1*-mutated and *ESR1* wild-type tumors (49, 50).

In the AMEERA-3 trial, amcenestrant 400mg was compared to the standard treatment (EOC) in patients with metastatic ER-positive HER2-negative breast cancer. These patients had previously received two lines of hormonal therapy, one line of chemotherapy in the metastatic setting and were allowed CDK4/6i. The primary outcome, PFS, was nearly the same in both treatment arms, with durations of approximately 3.6 vs. 3.7 months. However, for patients with *ESR1* mutations, amcenestrant demonstrated a numerically favorable PFS of 3.7 months compared to 2.0 months with the standard treatment (HR, 0.9 [95% CI, 0.565 to 1.435]). Notably, the oral SERDs showed activity specifically in this patient subgroup (51).

Moreover, in the phase II AMEERA-4 trial, both amcenestrant 200 mg and 400 mg exhibited Ki67 suppression and demonstrated a good safety profile. This trial compared amcenestrant with letrozole at both doses (200 mg and 400 mg) in the neoadjuvant setting for postmenopausal women with operable ER+/HER2- breast cancer, specifically targeting patients with baseline Ki67 levels of 15% or higher. However, enrollment was voluntarily stopped early as informative data supporting adjuvant development became available, leading to the absence of formal statistical comparisons (52).

In the AMEERA-5 trial, amcenestrant was compared to letrozole in combination with palbociclib as a first-line treatment for metastatic HR+/HER2- breast cancer, with PFS as the primary endpoint. Unfortunately, according to the independent data monitoring committee, the combination of amcenestrant and palbociclib did not meet the pre-specified criteria for continuation compared to the control arm, resulting in the trial being stopped (6, 51, 53).

TABLE 1 Summary of clinical trial of Giredestrant.

Trial [Reference]	Masking	Other agents	Population (Sample Size)	Primary endpoint	Results
coopERA (36)	No masking	neoadjuvant window-of-opportunity phase, giredestrant 30 mg oral daily or anastrazole 1 mg QD then randomized to giredestrant or anastrazole for four 28-day cycles with 125 mg PO A Palbociclib on Days 1–21 for 16 weeks	Untreated early breast cancer postmenopausal women with ER+/HER2- breast cancer (n=221)	Change in Ki67 Scores from Baseline to Week 2	Giredestrant Ki-67 reduction 81% vs anastrozole 74%
acelERA (37)	No masking	giredestrant vs physician's choice of ET (fulvestrant or aromatase inhibitor	Previously treated ER +/HER2- locally advanced or metastatic breast cancer. (n=303)	PFS	Median PFS 5.6 months Giredestrant arm, 5.4 months Fulvestrant/AI arm (HR, 0.81; 95% CI, 0.60-1.10; P = .1757) In ESR1 mutant cohort: Median PFS:5.3 months vs 3.5 months (HR, 0.60; 95% CI, 0.35-1.03; P = .0610)
perevERA (49)	Double masking	Giredestrant plus palbocilib vs Letrozole plus Palbociclib	ER+/HER2- locally advanced or metastatic breast cancer. (n=978)	PFS	–
lidERA (46)	none	Giredestrant vs ET	Patients with early ER +/HER2- breast cancer underwent definitive treatment (n=4200)	Evaluating the Efficacy and Safety of Adjuvant Giredestrant Compared With Physician's Choice of Adjuvant Endocrine Monotherapy	

PFS, Progressive Free Survival; ER, Estrogen Receptor; HER2, human epidermal growth factor receptor-2; LHRH, Luteinizing Hormone-Releasing Hormone; HR, Hazzard Ratio.
"-" means not available.

As a consequence, the sponsor decided to terminate the phase III AMEERA-6 trial, which was designed to compare amcenestrant 200 mg with tamoxifen in the adjuvant setting. Furthermore, the sponsor made the decision to discontinue the global development of amcenestrant in August, 2022 (6, 54). Table 2 shows summary of clinical trials of amcenestrant.

5.2.3 Camizestrant

Camizestrant (AZD9833) is another new non-steroidal oral SERD. Its novel structure contributed to its increased potency and facilitated a unique pattern of gene regulation (8). In preclinical patient-derived xenograft models, it promotes ER degradation and inhibits tumor cell growth, including *ESR1*-mutant cells (55). Unlike fulvestrant, it has not shown any relative dose-dependent resistance in the *ESR1*-mutant cells (8).

5.2.3.1 Background of ongoing studies

In the phase I SERENA-1 trial, camizestrant monotherapy was assessed at different doses ranging from 25 mg to 450 mg in women with ER+/HER2- breast cancer. The patients were pre-treated with more than one line of endocrine therapy and less than two lines of chemotherapy (40). 46% of patients had detectable *ESR1* mutation in the baseline ctDNA samples. The results demonstrated good efficacy and dose-dependent safety profile. Evidence of clinical benefit was observed at all dose levels (56). The overall response rate (ORR) was 16.3%, and the clinical benefit rate (CBR) was 42.3%. In patients with *ESR1* mutations, 50% had a partial response or stable disease at 24 weeks of therapy (8, 47). Camizestrant-related side effects were mostly grade 1 and 2 visual and gastrointestinal disturbances, asymptomatic bradycardia, and fatigue. Dose-limiting toxicities were only observed at 300 mg and 450 mg doses (48). In the other two parts of the trial (C/D), camizestrant 75 mg was evaluated in combination with palbociclib. The results that were published in 2021 demonstrated efficacy and tolerability. None of the patients experienced camizestrant-related grade ≥3 toxicities. Furthermore, patients with prior heavy endocrine treatment had a clinical benefit rate of 28% (57). Due to the previous encouraging findings, several ongoing trials are evaluating camizestrant in multiple settings. The SERENA-3 trial is an ongoing randomized open-label phase II study that examines the biological effects of 75-150 mg camizestrant given once daily in early-stage treatment-naïve ER+/HER2- breast cancer (58). Moreover, the SERENA-4 and SERENA-6 trials are phase III

TABLE 2 Summary of clinical trials of Amcenestrant.

Trial [Reference]	Masking	Other agents	Population	Primary outcome	Results
AMEERA-1 (50)	No masking	Experimental: monotherapy partA/B: Part A Dose Escalation, Part B Dose Expansion Experimental: Amcenestrant/ Palbociclib: Arm #2 Part C Dose Escalation, Part D Dose Expansion Experimental: Amcenestrant/Alpelisib: Arm #3 Part F Safety Run-In, Part G Dose Expansion Experimental: Amcenestrant/ Everolimus: Arm #4 Part H Dose Escalation, Part I Dose Expansion Experimental: Amcenestrant/ Abemaciclib: Arm #5 Part J Dose Escalation, Part K Dose Expansion	Postmenopausal ER +/HER2- breast cancer (n=136)	DLTs, ORR, and Adverse events	Favourable safety profile, with no safety signals of bradycardia or eye disorders. Preliminary antitumor activity was observed (ORR: 10.9% and CBR: 28.3%)
AMEERA-3 (51)	No masking	Amcenestrant 400mg was compared to standard treatment (Fulvestrant, Letrozole, Exemestane, and Tamoxifen)#	Postmenopausal women with HR+/HER2- breast cancer with prior ET (n=290)	PFS	PFS was numerically similar between Amcenestrant and other drugs (median PFS 3.6 vs 3.7 months)
AMEERA-4 (52)*	Single	Amcenestrant at 200 mg or 400 mg was compared to Letrozole in neoadjuvant setting	Postmenopausal women with resectable stage I-III ER+/HER2- breast cancer (n=105)	Percent change from baseline in Ki67 level at Day-15	The geometric least squares (LSM) estimate of Ki67 reduction was 75.9% for Amcenestrant 400 mg, 68.2% for Amcenestrant 200 mg, and 77.7% for letrozole.
AMEERA-5 (53)*	Quadrable masking	Amcenestrant was compared to letrozole in combination with palbociclib	ER+/HER2- advanced breast cancer with no prior systemic treatment (n=1,068)	PFS	Trial stopped
AMEERA-6 (54)*	Quadrable masking	Compare Amcenestrant with Tamoxifen	ER+/HER2- early breast cancer who discontinued AI due to toxicity (n=3,738)	IBCFS	Trial stopped

ER, Estrogen Receptor; HER, Human Epidermal Growth Factor Receptor; ORR, Overall Response Rate; DLT, Dose-Limiting Toxicity; CBR, Clinical Benefit Rate; PFS, Progression-Free Survival; ET, Endocrine Therapy; IBCFS, Invasive Breast Cancer-Free Survival. LSM, The geometric least squares.
Prior use of CDK4/6 inhibitors was allowed.
*Enrolment was voluntarily stopped.

randomized double-blind ongoing studies. The primary endpoint of the SERENA-4 trial is the PFS in camizestrant plus palbociclib versus anastrozole plus palbociclib in *de novo* or recurrent ER+/HER2- breast cancer (59). In the SERENA-6 trial, camizestrant is being compared to aromatase inhibitors when combined with palbociclib or abemaciclib in ER+/HER2- metastatic breast cancer (60). Table 3 shows summary of clinical trials of camizestant.

5.2.3.2 SERENA-2 trial (camizestrant vs fulvestrant)

The SERENA-2 trial (61), a randomized, parallel-group, multicenter phase II study, comparing the safety and efficacy of 3 different doses of camizestrant with fulvestrant 500 mg in the treatment of postmenopausal women with ER+/HER2- advanced-stage breast cancer with disease recurrence or progression after at least one line of endocrine therapy (55). Furthermore, the study included patients with no more than one line of chemotherapy and no prior fulvestrant treatment while prior treatment with CDK4/6i was permitted (55). Eligible women who have met the inclusion criteria were then randomized into 4 intervention arms 1:1:1:1. The interventions were camizestrant 75 mg, 150 mg, 300 mg, or fulvestrant 500 mg (55). The primary endpoint was PFS as assessed by response evaluation criteria in solid tumors (RECIST) from the date of randomization to the date of disease progression or death (53). Other secondary outcomes measured were the objective response rate (ORR), duration of response (DoR), OS, clinical benefit rate at 24 weeks, and effect on health-related quality of life (HRQoL). In addition to other pharmacokinetic and pharmacodynamic effects of Camizestrant (47, 55, 62).

Results from the trial were presented at the 2022 San Antonio Breast Cancer Symposium (SABCS). Camizestrant at 75 mg and 150 mg doses have shown statistically significant improvement in PFS compared to fulvestrant. In the overall population, camizestrant at 75 mg and 150mg reduced the risk of disease progression by 42%, and 33%, respectively (63). The median PFS on camizestrant 75 mg was 7.2 months and 7.7 months for camizestrant 150 mg, but 3.7 months for fulvestrant. Outcomes were better in patients with *ESR1*-mutated tumors; camizestrant reduced the risk of death or disease progression by 67% at 75 mg and by 45% at 150 mg dose, compared to fulvestrant. Improvement in PFS was also demonstrated in patients previously treated with CDK4/6i and patients with lung and/or liver metastases (57). The most common treatment-related adverse events were photopsia (12.2%, 24.7%, 35.0%, and 0%) and bradycardia (5.4%, 26.0%, 40.0%, and 0%), for 75 mg, 150 mg, 300 mg camizestrant or fulvestrant (57).

5.2.4 Elacestrant

Elacestrant (RAD1901) is a novel oral SERD with a basic side chain first reported in 2015 (7). It was developed by Radius health for use in ER+ breast cancer (8). Elacestrant exhibits significant dose-dependent antitumor activity in preclinical models (64). In a phase I study, elacestrant at a dose of 400 mg once daily, demonstrated single-agent activity with confirmed partial responses in ER+ heavily pre-treated metastatic breast cancer patients with an acceptable safety profile (65). These data provided the rationale for the phase III EMERALD study comparing the efficacy and safety of elacestrant versus standard-of-care endocrine treatment (fulvestrant or AI) in patients with ER+, HER2– advanced breast cancer (66). In this multicenter study, 478 patients with ER-positive, HER2-negative advanced or metastatic breast cancer were enrolled; 228 (48%) of them had *ESR1*-mutated tumors (60). The study population had disease progression following one or two prior lines of endocrine therapy, including at least one line containing a CDK4/6i. As such, this is the only trial in the second line and beyond were all participants had previous exposure to CDK4/6i in metastatic setting Additionally, one prior line of chemotherapy in the advanced or metastatic setting were allowed (60). Patients were randomized to receive the investigator's choice of endocrine therapy (including fulvestrant or an AI) or elacestrant 345 mg orally once daily (60). Stratification factors were *ESR1* mutation status, prior treatment with fulvestrant and presence of visceral metastasis. *ESR1* mutational status was determined by analyzing blood circulating tumor deoxyribonucleic acid (ctDNA) using the Guardant360 CDx assay (60). PFS was the primary endpoint for efficacy assessment and showed statistically significant improvement with elacestrant; 30% reduction in risk of disease progression and death, and even greater benefit in *ESR1*-mutant with 45% risk reduction compared to standard of care (SOC) (60).

The duration of prior CDK4/6i had a positive impact on PFS when treated with elacestrant, whereas no such association was observed with the SOC; the median PFS for elacestrant group treated with 12 months or longer of CDK4/6i was 3.8 months, but higher (5.5 months) for those treated for 18 months or longer, compared to 1.9 months and 3.3 months, respectively in SOC group. In the *ESR1*-mutant group, the median PFS with at least 12 months of prior exposure to CDK4/6i approached 8.6 months with elacestrant compared to only 2.1 months with the SOC; a 53% reduction in the risk for disease progression or death, making the *ESR1* mutation and duration on previous CDK4/6i as predictive markers for response. The most frequently reported adverse events (occurring in at least 10% of patients) during the study included musculoskeletal pain, fatigue, nausea, vomiting, decreased appetite, diarrhea, headache, constipation, abdominal pain, hot flushes, and dyspepsia. Laboratory abnormalities include increased cholesterol and triglyceride levels, elevated liver enzymes (AST and ALT), anemia, decreased sodium levels and mild renal impairment (60).

5.2.5 Imlunestrant

Imlunestrant represents an innovative orally bioavailable SERD characterized by its pure antagonistic attributes, leading to continuous inhibition of estrogen receptor (ER)-dependent gene transcription and cell growth.

The EMBER trial, a multicenter, open-label phase Ia/b dose-escalation/expansion trial, included patients with ER+ advanced breast cancer (prior endocrine therapy sensitivity; ≤3 prior therapies for advanced breast cancer). The phase Ia/b of the EMBER trial is assessing the efficacy of imlunestrant alone and in combination with other agents for ER+/HER2- advanced breast cancer. At the recommended phase 2 dose (RP2D) of 400 mg once

TABLE 3 Summary of the clinical trials of Camizestrant.

Trial	Masking	Other agents	Population	Primary outcome	Results
SERENA-1 (57)	No masking	Parts A/B: use different doses of camizestrant Parts C/D examine camizestrant 75 mg in combination with Palbociclib 75 mg	Women with ER+/HER2- Advanced Breast Cancer (n=403)	Dose-limiting toxicity (DLT) and adverse events	DLT at 300 mg and 450 mg: G1: Visual disturbances, bradycardia, nausea, fatigue, dizziness, vomiting, asthenia
SERENA-2 (62)	No masking	Arm A: Camizestrant at 75 mg or 150 mg daily Arm B: Fulvestrant at 500 mg by intramuscular injection every 4 weeks	Postmenopausal women with advanced ER +/HER2- breast cancer (n=240)	PFS	Median PFS: Camizestrant 75 mg: 7.2 months Camizestrant 150mg: 7.7 months Fulvestrant: 3.7 months
SERENA-3 (58)	No masking	In Stage-1, randomized 1:1 to receive either 75 mg or 150 mg oral Camizestrant daily for 5-7 days, followed by a minimum 5-day pre-surgery washout Stage-2 will include participants across up to 3 treatment groups. Stage 3 will include two treatment groups.	ER+/HER2- primary breast cancer (n=132)	Change from baseline in ER expression	–
SERENA-4 (59)	Randomized, double-blind	(a) Camizestrant 75 mg, once daily, Palbociclib 125 mg, once daily for 21 days followed by 7 days off treatment (b) Anastrozole (1 mg, once daily), palbociclib (same as active arm),	ER+/HER2- advanced/ metastatic breast cancer with no prior treatment (n=1342)	PFS	–
SERENA-6 (60)	Randomized, double-blind	Step1:CDK4/6i (palbociclib or abemaciclib) with AI (letrozole or anastrozole) Step 2 (upon detection of ESR1 mut without clinical or radiological disease progression Randomized into 2 arms: Arm A: continue with same AI Arm B: Switch to camizestrant	ER+/HER2- breast cancer on current 1 line SOC with detectable ESR1 mutation. (n=302)	PFS	–
CAMBRIA-1 (67)	Randomized, Open label	Arm A: Continue standard ET of investigator's choice (aromatase inhibitors [AI; exemestane, letrozole, anastrozole] or tamoxifen) Arm B: Camizestrant	patients with ER+/HER2 - early breast cancer with intermediate or high risk for disease recurrence who completed definitive locoregional therapy (with or without chemotherapy) and standard adjuvant endocrine therapy (ET) for at least 2 years and up to 5 years. The planned duration of treatment in either arm of the study is 60 months (n=4300)	Invasive breast cancer-free survival (IBCFS)	
CAMBRIA-2 (68)	Randomized phase III	Arm A: Camizestrant Arm B: Standard Endocrine Therapy (Aromatase Inhibitor or Tamoxifen)	Patients With ER+/HER2- Early Breast Cancer and an Intermediate-High or High Risk of Recurrence Who Have Completed Definitive Locoregional Treatment and Have No Evidence of Disease (n=5500)	Invasive breast cancer-free survival (IBCFS) and main secondary endpoints include Invasive disease-free survival (IDFS), Distant relapse-free survival (DRFS), Overall survival (OS), Safety and Clinical Outcome Assessments (COAs).	

ER, Estrogen Receptor; HER, Human Epidermal Growth Factor Receptor; DLT, Dose-Limiting Toxicity; G1, Grade 1; PFS, Progression-Free Survival; SOC, standard-of-care; ESR1, Estrogen Receptor Gene 1.
"-" means not available.

daily (n= 69), the most common all-grade treatment-emergent adverse events (TEAEs) were nausea (33.3%), fatigue (27.5%), and diarrhea (23.2%). Across all doses, the incidence of treatment-related grade 3 adverse events was low (3.6%). No patient discontinued treatment due to a TEAE. In evaluable advanced breast cancer patients, the objective response rate (ORR) was 8.0% (6/75), and the clinical benefit rate (CBR) was 40.4% (42/104). Clinical benefit was observed regardless of baseline *ESR1* mutation status as determined by circulating tumor DNA sequencing (69).

Imlunestrant in combination with abemaciclib ± AI demonstrated acceptable safety and tolerability, ORR was 36% (10/28) for imlunestrant and abemaciclib vs 44% (15/34) for imlunestrant and abemaciclib plus AI. These findings suggest no additional toxicity of imlunestrant when administered with abemaciclib, along with comparable clinical benefit to that observed in MONARCH 2 (70).

Updated data on imlunestrant ± everolimus or alpelisib arms at ESMO 2023 reveals the following tumor response rates: 8% (6/76) for imlunestrant monotherapy (114 patients), 21% (6/28) for imlunestrant + everolimus (42 patients), and 58% (7/12) for imlunestrant + alpelisib (21 patients). Imlunestrant, either alone or in combination with everolimus or alpelisib, demonstrated strong efficacy in pre-treated ER+, HER2- advanced breast cancer. Toxicities were in line with the known safety profiles of alpelisib and everolimus (71).

EMBER-3 is a randomized phase 3 clinical trial investigating the efficacy of imlunestrant compared to the investigator's selected endocrine therapy, which includes either fulvestrant or exemestane. The study focuses on patients diagnosed with ER-positive, HER 2-negative, locally advanced, or metastatic breast cancer who have undergone prior treatment with endocrine-based therapy. The primary endpoint of the trial is PFS in both the intention-to-treat (ITT) population and in patients with *ESR1* mutation (72).

EMBER4 is a randomized, open-label, global phase 3 study comparing imlunestrant versus physicians' choice of ET, in patients who are at an increased risk of recurrence based on clinico-pathological features and who have received 2 to 5 years of standard adjuvant ET. Approximately 6,000 patients will be randomized 1:1 to receive imlunestrant (400 mg daily) for 5 years or physicians' choice of adjuvant ET (tamoxifen or an aromatase inhibitor). Study treatment duration is 5 years with Invasive Disease-Free Survival (IDFS) as primary outcome (73).

5.2.6 Palazestrant

OP-1250 is an innovative, orally available medication that acts as a complete estrogen receptor (ER) antagonist and selective ER degrader. OP-1250 entirely prevents estrogen from activating transcriptional activity and lacks any agonist effects on the ER. This drug exhibits strong binding affinity, ER degradation, and antiproliferative properties in ER-positive breast cancer models, often matching or exceeding the performance of other comparable treatments.

OP-1250 offers superior pharmacokinetic benefits with the ability to cross the blood-brain barrier. It has demonstrated strong efficacy in wild-type and ESR1-mutant breast cancer xenograft models. OP-1250 works well in combination with cyclin-dependent kinase 4 and 6 inhibitors in preclinical studies, leading to significant tumor shrinkage in intracranial breast cancer models and extending the lifespan of the test subjects.

international, multicenter phase III clinical trial OPERA-01 is designed to evaluate the safety and efficacy of palazestrant (OP-1250) as a monotherapy compared to standard endocrine treatments: either fulvestrant or an aromatase inhibitor (anastrozole, letrozole, or exemestane). It is an open-label, randomized, active-controlled study.

The trial is recruiting adult patients with advanced or metastatic breast cancer that is hormone receptor-positive (ER+) and HER2-negative. These patients must have experienced disease progression or relapse after one or two previous lines of standard-of-care endocrine therapy for metastatic breast cancer. One of these lines must have involved a combination of endocrine therapy and a CDK 4/6 inhibitor.

The initial dose-selection phase of the trial involves approximately 120 participants, who will be randomly assigned to one of two doses of palazestrant or to the standard endocrine therapy. Subsequently, around 390 participants will be randomly assigned to either the chosen dose of palazestrant or to the standard endocrine therapy (74).

Research is still ongoing for other agents such as borestrant, rintodestrant, and taragarestrant.

6 Discussion

The introduction of CDK4/6i, initially in the metastatic setting and more recently in the adjuvant setting, have made significant progress in the treatment of patients with HER2-negative, HR-positive breast cancer. However, researchers were hoping to find a more effective hormonal therapy that can beat both AI and fulvestrant when used alone or in combination with CDK4/6i. The intramuscular formulation of fulvestrant have highlighted the need for alternative solutions, too.

Furthermore, the growing understanding of the role of *ESR1* mutations in endocrine therapy resistance for SERMs and AIs has intensified the pursuit of oral SERDs as a potential solution. In addition to addressing these challenges, oral SERDs offer the added advantage of enhancing the efficacy of agents that target other molecules involved in cross-talks with ER pathways. This multifaceted approach positions oral SERDs as promising candidates to overcome these limitations and optimize treatment outcomes in endocrine therapy-resistant breast cancer.

Results from the phase III EMERALD trial indicate that elacestrant offers an improvement in 12-month PFS compared to standard of care therapy for patients with ER+ metastatic breast cancer who have experienced disease progression on prior endocrine therapy. Furthermore, the clinical benefit of elacestrant was particularly significant in patients with *ESR1* mutations (66). These findings are highly promising and suggest a potential paradigm shift towards the use of oral SERDs as an effective treatment option for ER+ breast cancer.

Camizestrant also has shown promising efficacy in treating ESR1 mutations in breast cancer, as evidenced by its significant improvement in progression-free survival (PFS) compared to fulvestrant. At doses of 75 mg and 150 mg, camizestrant reduced the risk of disease progression by 42% and 33%, respectively, in the overall population. For patients with ESR1-mutated tumors, camizestrant at these doses led to even greater reductions in the risk of death or disease progression—67% at 75 mg and 45% at 150 mg—compared to fulvestrant. These results highlight camizestrant's potential as a valuable therapeutic option for patients with ESR1 mutations, offering a meaningful advancement in breast cancer treatment.

Despite the progress made in understanding the role of *ESR1* mutations and the potential efficacy of SERDs, there are still important questions need to be to addressed in optimizing endocrine therapy. While *ESR1* mutations are associated with increased likelihood of response to SERDs due to tumor dependence on ER-mediated signaling, this association is not always reliable or perfect in clinical settings. In the EMERALD trial, approximately half of the patients demonstrated intrinsic resistance to ET, regardless of the treatment arm, underscoring the fact that some patients may not benefit from this approach. Moreover, the presence of polyclonal resistance poses an additional challenge, as tumors with *ESR1* mutations often have subclones harboring concurrent genomic alterations that could confer resistance through ER-pathway-independent mechanisms (66).

Characterizing metastatic tumors solely based on ER positivity or *ESR1* status is insufficient, and there is a need for improved methodologies to select patients who remain endocrine sensitive after CDK4/6i treatment. This may involve the use of genomic profiling panels or the identification of novel biomarkers that can more accurately characterize tumors susceptible to endocrine therapy. Advancements in this area of research can significantly impact patient care by identifying the most appropriate treatment strategies.

Two oral SERDs, namely amcenestrant and giredestrant, failed to meet their primary endpoints in separate clinical trials. The AMEERA-3 trial included patients who had previously failed CDK4/6i and two lines of hormonal therapy, while the *ESR1* mutation status was not assessed (51). Speculation arises that amcenestrant might still demonstrate superiority over the control arm if only patients with *ESR1*-mutated tumors were enrolled. Similarly, the acelERA trial that compared giredestrant with fulvestrant or aromatase inhibitor, did not achieve its primary endpoint of superior PFS for the study drug. However, subgroup analysis of patients with baseline *ESR1* mutations indicated 1.8 months difference in the median PFS (HR, 0.60; 95% CI, 0.35-1.03; p=0.0610) (8, 45). Notably, giredestrant exhibited positive outcomes in reducing Ki67 expression and inducing complete cell cycle arrest when used as neoadjuvant therapy in previously untreated patients with hormone receptor-positive, HER2-negative early breast cancer in the coopERA study (8, 44).

Concerns regarding the potential effects of oral SERDs on the cardiac conducting system and cornea were raised prior to the presentation of the EMERALD trial results. Earlier trials of camizestrant and giredestrant reported cases of bradycardia, and QT prolongation was observed with camizestrant and amcenestrant. Ocular toxicity was primarily associated with camizestrant and giredestrant. It is worth noting that the cardiac conducting system and cornea do not express ER. In the past, such toxicities were rarely observed in patients treated with other antiestrogen agents, including tamoxifen, AI, and fulvestrant. This suggests that the ocular or cardiac toxicities observed with specific oral SERDs are unlikely to be solely caused by on-target effects against ER. Reassuringly, the EMERALD trial did not report any ocular or cardiac toxicities (66). Nonetheless, the underlying mechanisms behind the occurrence of these side effects with certain oral SERDs while others do not cause them remain the subject of investigation and scrutiny.

Accordingly, in January, 2023, elacestrant was approved by the Food and Drug Administration (FDA) for postmenopausal women or adult men with ER-positive, HER2-negative, *ESR1*-mutated advanced or metastatic breast cancer with disease progression following at least one line of endocrine therapy. Additionally, Guardant360 CDx assay was approved as a companion diagnostic device to identify patients for treatment with elacestrant.

7 Conclusions

Endocrine therapy, including SRDs and aromatase inhibitors, play a vital role in the effective treatment of breast cancer; both in early- and advanced-stage disease. The recently introduced oral SERDs are promising players, alone or in combination with other agents like the CDK4/6i. The increase utilization of genomic profiling and novel biomarkers, may accurately characterize tumor subtypes that are more susceptible to specific endocrine therapy.

Author contributions

BS: Data curation, Validation, Writing – original draft, Writing – review & editing. AA: Data curation, Validation, Writing – original draft, Writing – review & editing. HH: Data curation, Validation, Writing – original draft, Writing – review & editing. HA-R: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 10 July 2024
DOI 10.3389/fimmu.2024.1410300

OPEN ACCESS

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RECEIVED 31 March 2024
ACCEPTED 28 June 2024
PUBLISHED 10 July 2024

CITATION
Alqathama A (2024) Natural products as promising modulators of breast cancer immunotherapy.
Front. Immunol. 15:1410300.
doi: 10.3389/fimmu.2024.1410300

Natural products as promising modulators of breast cancer immunotherapy

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Breast cancer (BC) is the most common malignancy among women and is considered a major global health challenge worldwide due to its high incidence and mortality rates. Treatment strategies for BC is wide-ranging and include surgery, radiotherapy, chemotherapy, targeted hormonal therapy and immunotherapy. Immunotherapy has gained popularity recently and is often integrated as a component of personalized cancer care because it aims to strengthen the immune system and enable it to recognize and eradicate transformed cells. It has fewer side-effects and lower toxicity than other treatment strategies, such as chemotherapy. Many natural products are being investigated for a wide range of therapeutic pharmacological properties, such as immune system modulation and activity against infection, auto-immune disease, and cancer. This review presents an overview of the major immune response-related pathways in BC, followed by detailed explanation of how natural compounds can act as immunomodulatory agents against biomolecular targets. Research has been carried out on many forms of natural products, including extracts, isolated entities, synthetic derivatives, nanoparticles, and combinations of natural compounds. Findings have shown significant regulatory effects on immune cells and immune cytokines that lead to immunogenic cancer cell death, as well as upregulation of macrophages and CD+8 T cells, and increased natural killer cell and dendritic cell activity. Natural products have also been found to inhibit some immuno-suppressive cells such as Treg and myeloid-derived suppressor cells, and to decrease immunosuppressive factors such as TGF-β and IL-10. Also, some natural compounds have been found to target and hinder immune checkpoints such as PD-L1.

KEYWORDS

breast cancer, immunotherapy, checkpoints, immune cells, natural products

1 Introduction

Breast cancer is thought to be the second most frequent cause of cancer-related deaths in women worldwide. It has been estimated that one in three women may be at risk of developing BC in their lifetime and it has also been predicted that by 2050 there will be 3.2 million new cases worldwide (1). Cancer research data estimated that 287,850 women in the USA would be diagnosed with BC in 2022, and that 43,250 women would die from the disease. These statistics place BC second only to lung cancer as the leading cause of women's cancer deaths (2). BC is a heterogeneous disease and classified into four categories based on the immunohistochemical expression of hormone receptors, with different behavior and responsiveness to treatment and clinical results (1). The categories are as follows. Group 1 (luminal A), which is characterized by estrogen receptor (ER) positive and progesterone receptor (PR) positive; Group 2 (luminal B) shows ER positive, PR negative, and human epidermal growth factor receptor positive (HER2+). The third group is HER2+ positive only, and Group 4 (basal-like), known as triple-negative (TNBC), lacks the expression of any of the above receptors (1, 3).

2 BC treatment

For breast cancer treatment, choice of strategy is based on the grade, stage, and BC molecular subtype in order to achieve optimal therapy outcomes. Surgery is the most standard approach for BC treatment in the form of mastectomy, either by total excision of the breast, or by lumpectomy in which breast-conserving surgery removes the cancerous tissue in part of the breast. In addition, radiotherapy, chemotherapy in both neoadjuvant and adjuvant chemotherapy forms, hormone therapy, and targeted therapy are the mainstream options for BC treatment (4).

Most conventional anticancer drugs kill tumor cells directly by interfering with basic cell functions to the degree that cells are no longer able to survive (4). Recent research has focused on the crucial role of the immune system in fighting cancer cells, leading to the emergence of immunotherapy to provide a transformative new method of comprehensive cancer treatment. Moreover, immunotherapy builds on the natural ability of the immune system to recognize and eradicate non-normal cells through the process of tumor immune surveillance. This involves a dynamic and orchestrated interplay of innate and acquired immune responses. Tumor cells avoid immune surveillance by suppressing the body's immune system in various ways, thus enabling tumor immune escape. To counteract this, combinations of immuno- and chemotherapies are being developed to strengthen immune system response to tumors and to minimize the negative effects of chemotherapy (4, 5).

Abbreviations: BC, Breast cancer; TNBC, Triple-negative breast cancer; HER2+, Human epidermal growth factor receptor positive; TME, Tumor microenvironment; DCs, Dendritic cells; NKs, Natural killer T cells; TAMs, Tumor-associated macrophages; TILs, Tumor infiltrating lymphocytes; MDSCs, Myeloid-derived suppressor cells; Tregs, Regulatory T cells; CTLs, Cytotoxic CD8 + T lymphocytes; IL, Interleukin; PGE2, Prostaglandin E2; TGF-β, Transforming growth factor-β; IFN-γ, Interferon-γ; APCs, Antigen-presenting cells; Th1, Type 1 helper; Th2, Type 2 helper; MHC-I, Major histocompatibility complex I; PD-L1, Programmed cell death-ligand 1; CTLA-4, Cytotoxic T lymphocyte-associated protein 4; PD-1, Programmed cell death 1; TAN, Tumor associated neutrophils; TIM-3, T-cell immunoglobulin and mucin domain containing protein-3; LAG-3, Lymphocyte-activation gene 3; ICI, immune checkpoint inhibitor; PBMC, Peripheral blood mononuclear cells; JAK, Janus kinase; STAT, Signal transducers and activators of transcription; NF-κB, Nuclear factor kappa B; ERK, Extracellular signal-regulated kinase; MAPK, Mitogen-activated protein kinases; Protein kinase B Akt; mTOR, Mammalian target of rapamycin; AMPK, AMP-activated protein kinase; RCQ, Curcumin, resveratrol, and quercetin.

3 Role of the immune system in cancer

The immune system is highly complex and consists of collections of cells, chemicals, and organs, such as the skin, lungs, and gastrointestinal tract, which protect the major organs and other areas from foreign antigens. The immune system is activated as a defense mechanism when it detects abnormalities, for example, underactivity, that could allow microbial infection (i.e. by bacteria, fungi, or parasites), or overactivity, that could lead to allergy or autoimmune disease. Major components of the immune system are immune cells of different types, such as lymphocytes, dendritic cells (DCs), monocytes/macrophages, and natural killer T cells (NKs) (6). The tumor microenvironment (TME) is composed of both cancerous and non-cancerous cells (such as immune cells, endothelial cells, and fat cells), which play a major role in transforming normal cells into tumor cells. As a large proportion of the TME consists of immune cells, they play an essential role in controlling pro- and anti-tumor immune responses; thus the characteristics of the TME are intrinsically related to the efficacy of immunotherapy (7).

In this review we gather together findings from recent *in vitro*, *in vivo* and clinical studies that evaluate the effects of various natural products used as immunotherapeutic agents on BC models. We first provide detailed insight into the role of the TME, immune cells, cytokines, and immune checkpoints in BC immune tolerance. This is followed by a detailed discussion of research evidence for the efficacy of natural products in triggering or improving immunogenic activity in different multi-cell and multi-biomolecular pathways, and in inhibiting or eradicating tumor cells. To conclude, we present a summary of the advantages and disadvantages of natural products as immunotherapeutic agents in BC.

4 Tumor immune microenvironment and breast cancer

Detailed knowledge of the TME is essential to the development of potential immunotherapeutic strategies, since its composition and characteristics strongly influence the genesis and progression of tumors (8). In BC, the TME is composed of either cellular, soluble, or physical components. Cellular types is classified into local

(intratumoral), regional (breast) and metastatic categories. Local cells of the TME include cancer cells as well as unfiltered inflammatory cells such as macrophages, lymphocytes, DCs, and neutrophils. The interaction between cancer cells and adjacent stromal cells, including stromal fibroblasts, vascular/lymphatic endothelial cells, adipocytes and endothelial, fall into the regional category, while metastatic cells include host cells at metastatic areas, e.g. lymph nodes and distant organs, that form new TMEs (9).

4.1 Immunosuppressive/immunostimulant cells and factors

The TME contains two major classes of immune cells: immuno-suppressive and immuno-stimulating. Their activity is dependent on innate and adaptive immune system responses (10). Several types of immune cells are active in cancer cell escape immune editing, such as tumor infiltrating lymphocytes (TILs). TILs have different functions and transcription factors, and occur as several subtypes, including Th cells (helper cells), CTLs (cytotoxic CD8+ T lymphocytes), and Tregs (regulatory T-cells). Other cells with a role in immune regulation are CD4+ effector T cells, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), DCs, NKs, and mast cells (11). Several immunosuppressive factors, such as prostaglandin E2 (PGE2), interleukin-10 (IL-10), and transforming growth factor-β (TGF-β), are secreted by these cells to regulate the immunosuppressive network (5).

Studies have shown a high number of TILs in BC, which are composed mainly of T cells of different classes, and fewer B cells (9). The adaptive immune response is supported by CD4+ T cells and CD3 + T cells, as well as CTLs. When APCs (antigen-presenting cells) such as macrophages and DCs detect tumor antigens, CTLs release granzyme and perforin, mediated by interferon-γ (IFN-γ) secretion, to eliminate cancer cells. In addition, IFN-γ and IL-12 signaling activate CD4+ T-cells, which are type 1 helper (Th1) cells, allowing APCs to license the differentiation of CD8 T-cells and clonal expansion. Th1 cell presence is associated with better clinical outcomes in BC patients as these cells activate CD8+ T lymphocytes, triggering their cytotoxic activity by freeing pro-inflammatory cytokines (9). Other T helper cells, Th2 and Th17, play a role in BC progression as well as follicular helper T-cells, which largely regulate the maturation of antigen specific B cells, enhancing local memory and elevating the development of tertiary lymphoid organs, leading to an immune response that targets local tumors (12). Major regulators of immune system homeostasis are Tregs, as their existence in the TME elevates immune system capability to act as an immunosuppressive through direct cell-cell contact suppression and immunosuppressive cytokines (IL-10, TGF-β) (13). Tregs, which express Foxp3, belong to a class of suppressive cells and act to suppress effector T cells, preventing immune-mediated rejection of tumors (14).

NKs are a class of APC which play a major role in immune tolerance in BC. They are cytotoxic innate lymphocytes which act to lysate and eradicate malignant cells, and this eradicating mechanism is independent of major histocompatibility complex I (MHC-I) molecules and antibodies. In order to avoid tumor-invading cytotoxic T lymphocytes detecting the presence of tumor cells, MHC-I expression on the surface of the tumor cell is often inhibited or eliminated. NK cell inhibitory receptors are able to detect this lack of MHC-I, and the immunogenic effect of NK cells is evident in their contribution to regulating the function of multiple immune cells, including DCs, macrophages, and T and B cells (15). Principal components of the TME are DCs, which are thought to be one of the most potent types of APC, and present antigens, including tumor-derived antigens, to T-cells. DCs have two major phenotypes with different surface protein expression, known as myeloid and plasmacytoid populations. DCs become mature and stimulate the immune system by interacting with T cells. Cancer cells, however, have the ability to inhibit the maturation of DCs, which results in tumor-infiltrating DCs having an underdeveloped phenotype. Tumor-derived antigen cross-presentation is therefore inhibited, co-stimulatory molecules show downregulation, so DC antitumor functions can be impaired (9).

Findings show that tumor-infiltrating B cells could have both pro-tumor and anti-tumor effects, which greatly depend on the components of the TME and BC phenotypes. Moreover, tumor-specific antigen recognition, antibody production, and APC functioning have all been shown to affect the anti-tumor properties of B cells (9). On the other hand, B cells have been shown to mediate tumor growth, as regulatory B cells express inhibitory molecules such as programmed cell death-ligand 1 (PD-L1) and FAS ligands, as well as anti-inflammatory mediators such as TGF-β, IL-10, and IL-35, resulting in the suppression of immune responses leading to cancer cell immune escape (16).

One of the main innate immune cells in BC are macrophages, which occur as two polarized phenotypes, the M1 alternative macrophages and the M2-like TAMs (9). M1 macrophages are activated by IFN-γ and tumor necrosis factor-alpha (TNF-α), which are released by Th1 cells, thus causing production of reactive oxygen species and release of pro-inflammatory cytokines (IL-12 and IFN-γ), processes which, in turn, stimulate anti-tumor activity (17). The other activated macrophage phenotype, M2-like TAMs, are switched on by Th2 cells cytokines such as IL-13, IL-10 and IL-4, leading to tumor progression, inducing angiogenesis and metastasis, and inhibiting the anti-tumor response (18). The development of the M2-like TAM macrophage is encouraged by the existence of IL-4 and IL-13 in the TME. Tumor cells can produce macrophage-derived chemokines such as C–C motif chemokine 22, which then bring monocytes into the tumors; if immuno-suppressive conditions prevail, these monocytes will then differentiate into TAMs (5). Immune cells called neutrophils also have the potential to promote or hinder tumor development. As tumor-associated neutrophils (TANs) they function as tumor-infiltrating immune cells. They exhibit different phenotypes (N1 and N2) in which N1 cells have the pro-inflammatory and anti-tumor properties of TANs, triggered by IFN-γ and IFN-β exposure (9). N2 neutrophils are triggered by TGF-β exposure, producing anti-inflammatory and pro-tumor TANs (18).

MDSCs are known as immunosuppressive populations and are characterized into two phenotypes; polymorphonuclear or granulocytic MDSCs and monocytic MDSCs. These populations are precursors of bone marrow and have the ability to suppress the immune response via the repression of CTLs and NKs and the

secretion of immunosuppressive cytokines including IL-10 and TGF-β, as well as the induction of expression of PD-L1 (19). Mast cells promote the growth of tumor cells via the secretion of H+, NO, chondroitin sulfate, and oxidized polyamines, leading to the induction of the non-degranulated mode of mast cells, resulting in an immunosuppressive effect. Moreover, the cytokines secreted by mast cells, such as histamine, IL-10, and TGF-β result in the suppression of effector T cells, and the secretion of PGE2 affects the migration and function of DCs, all of which strengthens immunosuppression within tumors (5).

Recent research suggests that certain types of human immune cells exhibit this kind of two-way oppositional mechanism in BC occurrence, that is, alterations in cell composition mean they are able either to encourage tumor growth or suppress it (20). In breast cancer, TILs can affect cancer cells and immune cells in different ways, depending on the stage of the cancer and the specific cell phenotype. This means either a pro- or anti-tumor response, leading either to the promotion of tumor cell proliferation and spread, or its suppression and destruction (21).

Studies in the BC microenvironment show that TILs can affect the response of cancer cells by either promoting tumor generation, or triggering suppression and apoptosis. Both CD4+ and CD8+ T cells can affect the adaptive immune response; however, when activated, CD8+ differentiates into CTLs, while CD4+ cells divide into sub-populations of T helper cells (Th1, Th2, Th17) (20). The CD8+ cell types cause tumor cell destruction and are dominant in the BC microenvironment, whereas CD4+ subpopulations can produce either pro- or anti-tumor activity. For example, Th1 CD4+ cells secrete pro-inflammatory mediators INF-γ and TNF-α, and trigger the anti-tumor activity of NKs, thus activating a powerful anti-cancer immune response (11, 20). In a contradictory fashion, the cell subtype Th2 CD4+ instead promotes tumorgenicity and encourages metastasis by releasing cytokines IL-4, IL-5, and IL-13, but it also releases IL-10, which can influence either the growth or destruction of cancer cells. Another subtype, Th17 CD4+, releases TGF-β, which is known to encourage cancer cell progression (20). Similarly, different phenotypes of TAMs may polarize to either M1-like or M2-like macrophages, which are linked with tumor progression and suppression respectively (22). The M1 phenotype promotes the apoptosis of cancer cells via CTL recruitment and activation of the adaptive immune responses, while M2 attracts Th2 and Treg cells, encouraging cancer cell growth, tissue remodeling and tumor angiogenesis (23). Other clinical research supports findings that M2-like macrophages promote BC cancer cell proliferation and overall negative outcomes (22).

4.2 Immunological checkpoints

Literature has shown that certain regulatory molecules play a physiological role in the suppression of self-immune responses. When these regulatory molecules, known as immune checkpoints, become dysregulated, they cause evasion of immune-mediated destruction. In pathological conditions such as BC, they include programmed cell death 1 (PD-1), cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and its ligands (PD-L1/2) and T-cell immunoglobulin and mucin domain containing protein-3 (TIM-3) (24). Targeting immune checkpoints with immune checkpoint inhibitors (ICIs) has brought advances in in cancer immunotherapy because ICIs accelerate the anti-cancer immune response to eradicate malignant tumors. ICIs can restore the suppressed immune cells as a recognizers of cancer cells by blocking immune checkpoint interference in the cross-talk between immune cells and cancer cells, thus reactivating natural immune responses in BC patients. Current clinical practice using ICI treatment shows dramatic improvement in survival rates in advanced-stage and metastatic cancers (25).

5 Natural products as immunotherapy agents in BC

Humankind has used natural products to treat disease for at least three thousand years (26). The pharmacologically active constituents of many different plant, animal, marine, and microbial organisms continue to provide a wide range of molecules that produce healing responses in the body (27). Many recent studies have demonstrated the potential anti-cancer activities of naturally-sourced compounds, leading to the development of new anti-cancer agents (28). Moreover, around 47% of current anti-cancer drugs are derived from natural compounds (5). The chemical diversity of natural products is reflected in their different effects on cancer cells, including cell proliferation inhibition, apoptosis induction, suppression of metastasis and angiogenesis, autophagy modulation and reversal of multidrug resistance. They are also able to manipulate the tumor microenvironment or fine-tune it to bring about immune response regulation to eradicate tumor cells (7). Methods of treating cancer with immunotherapy, such as ICI treatment, adoptive T cell transfer therapy, and cancer vaccination, have all been successfully combined with anti-cancer agents derived from natural products to improve treatment efficacy (26). Research into the beneficial effects of natural products in anti-cancer and immunomodulatory treatment suggests that many are valuable candidates for adjuvant use in tumor immunotherapy (5).

This extensive review presents and discusses research findings on 50 preparations including isolated entities, extracts, several combination and nanoparticle formulations, all of which have significant immunotherapeutic potential in targeting the TME, immune cells, cytokines, and immune checkpoints. Different chemical classes which have been found to modulate immune response include alkaloids, flavonoids, terpenes, phenolics, and peptides as illustrated in Figure 1 below. Figure 2 illustrate the biomolecular mechanisms that are dysregulated in BC and how natural products restore theses pathways to induce immunogenic tumor-cell death.

5.1 Phenolic compounds

Curcumin (1) is known as the chief constituent of the culinary spice turmeric. It demonstrates important biological properties, including anti-inflammatory, antimicrobial and anticancer activity.

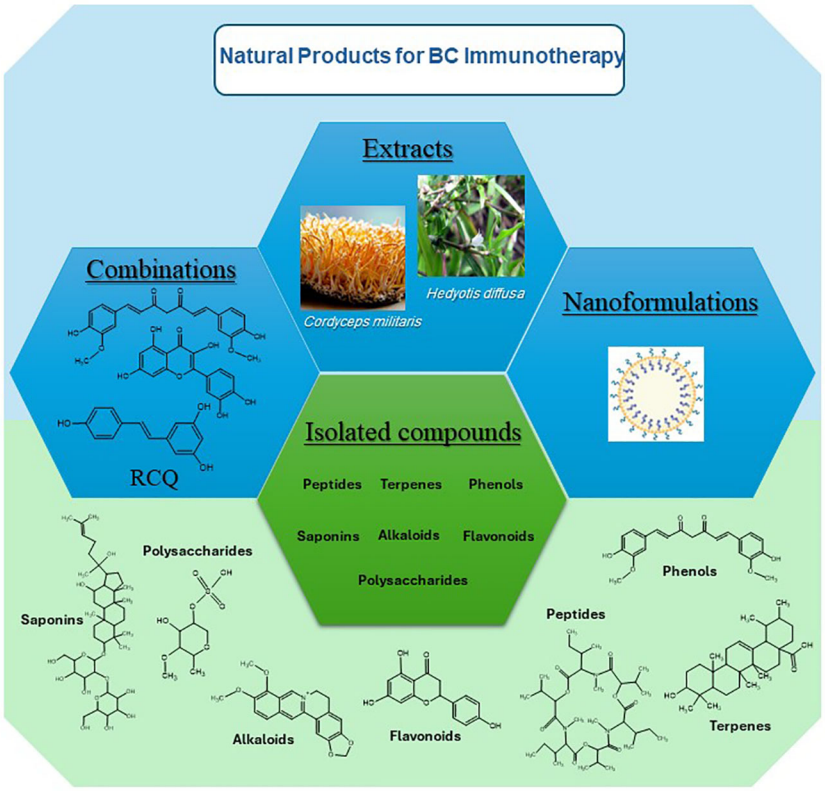

FIGURE 1
Different sources and chemical classes of immunotherapeutic natural products on BC.

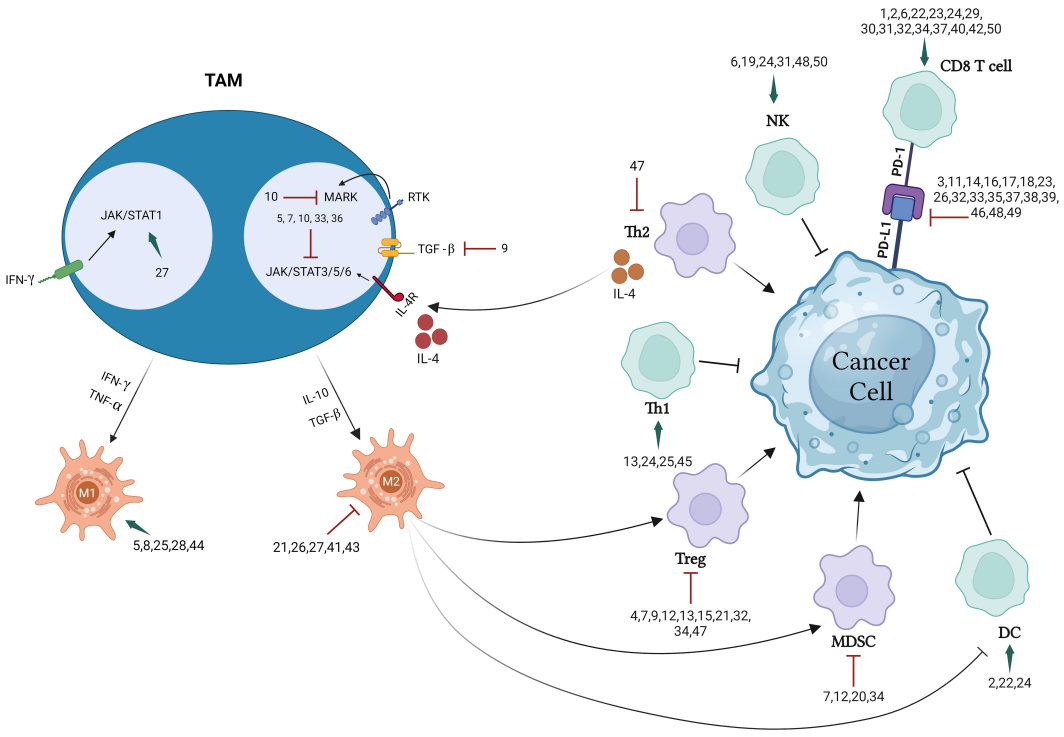

FIGURE 2
Natural products regulate biomolecular pathways to restore immunogenic tumor-cell death.

It can regulate immune response in BC as reported by Krishnamurthy et al., where curcumin and mannan, a component of the Aloe vera plant, inhibited the proliferative activity of immune cells, including peripheral blood mononuclear cells (PBMCs) such as lymphocytes, monocytes and macrophages (29). In some conditions the effects of curcumin are limited due to solubility issues, as it has poor solubility in neutral or acidic media and is unstable in alkaline conditions (30). However, delivery of curcumin to the target site in a sustainable and controlled manner can be achieved through nanotechnology, such as curcumin-loaded polymeric nanoparticles (2) and a nano-vaccine containing cytosine-phosphate-guanine and antigenic peptides. Injection of this formula in a BC model triggered immunogenic cell death of cancer cells and activation of DCs. DCs stimulation significantly improved tumor-specific CD8+ T-cell responses resulting in tumor inhibition (Table 1) (31).

Resveratrol (3) belongs to the class of stilbenes, and is recommended for a number of different pathological conditions. Its immunological effect on BC has been tested, revealing that resveratrol inhibits glyco-PD-L1-processing enzymes (α-glucosidase/α-mannosidase) and PD-L1 dimerization and blocks the PD-1/PD-L1 interaction. Thus, it in the process increases cytotoxic T-lymphocyte activity and restores T-cell immune function in tumor tissue (32). HS-1793 (4) is a derivative of resveratrol that has an effect on immune cells through changing lymphocyte proliferation and the Treg cell population in FM3A breast tumor-bearing mice. It was found to promote the activity of concanavalin A-induced lymphocytes in these mice. HS-1793 has also been found to cause changes in the subset of tumor-infiltrating T cells including the CD25+ cells, in a dose-dependent manner (33).

Vanillic acid (5) is an aromatic phenolic compound produced by several different plants, such as vanilla beans. It has been found to be of benefit in different pathological conditions due to its antioxidant and antibiotic effects. This phenolic compound exhibited anti-tumor properties in mouse models with 4 T1 breast tumors, with phagocytosis and apoptosis-induction occurring via the promotion of macrophage polarization to the M1 phenotype through IL-6R/Janus kinase (JAK) signaling (34). XK-81 (6) is a novel bromophenol obtained from *Leathesia nana* that was found to have an immunotherapeutic effect on BC cell lines. It elevated the number of CD8+ T cells and NKs and modulated the ratio of M1/M2 macrophage in tumor tissues. This was combined with an elevation of immune-related cytokines, including IL-12, TNF-α, and IL-1β in a macrophage cell line (35).

5.2 Terpenes

Ursolic acid (7) is triterpenoid compound that exists in different fruit and vegetables and is known for its poor solubility. Research has been conducted to develop liposome-loaded ursolic acid for BC immunotherapy. It has been found to modulate CD25+ forkhead box P3 (Foxp3+) T cells via the inhibition of signal transducers and

TABLE 1 List of natural products with immuno-tumor therapeutic effects on breast cancer model.

Natural Compound	Category	Immunomodulatory effect in BC	Reference
Curcumin (1)	Phenolic compounds	Inhibition of proliferative activity of some immune cells like PBMC and trigger Th2 activity	(29)
Curcumin nanoparticles (2)		Stimulation of DCs and tumor-specific CD8+ T-cells	(31)
Resveratrol (3)		Inhibition of PD-L1, restore T-cell function	(32)
HS-1793 (4)		Change subpopulations of tumor-derived T cells including the CD25+ and Treg population	(33)
Vanillic acid (5)		Promotion of the polarization of macrophages to a M1 phenotype	(34)
XK-81 (6)		Elevation of CD8+ T and NKs, modulation the ratio of M1/M2 macrophage and elevation of immune-related cytokines, including TNF-α, IL-1β, and IL-12	(35)
Ursolic acid (7)	Terpenes	Modulation of CD25+ Foxp3+ T cells via the inhibition of STAT5 phosphorylation, IL-10 secretion, reduced MDSC and Tregs	(36)
Anemoside A3 (8)		Shifting M2-TAM to M1-TAM phenotype and inhibiting TAM-TNBC crosstalk	(37)
Oridonin (9)		Attenuation of Tregs cells and expression of TGF-β receptor	(38)
Crassolide (10)		Reduction of the level of CD24 on the surface of cancer cells and blocked mitogen-activated protein kinase 14 activation and STAT	(39)
Triptolide (11)		Down-regulation of PD-1/PD-L1 pathway	(40)
Naringenin (12)	Flavonoids	Reduction of infiltrating MDSCs and Treg cells, the upregulation of INF-γ and IL-2-releasing T cells and reducing the production of TGF-β1	(41, 42)
Naringenin with cryptotanshinone (13)		Enhancing Th1 cells, modulating Treg cells activity through JAK2/STAT3 pathway	(43)

(Continued)

TABLE 1 Continued

Natural Compound	Category	Immunomodulatory effect in BC	Reference
Apigenin (14)		Inhibition of interferon-γ-induced PD-L1 expression	(44)
Salvigenin (15)		Reduction of IL-4 level, elevation of IFN-c with suppression CD25+Foxp3+ Treg cells	(45)
Myricetin (16)		Inhibition of PD-L1	(46)
Sativan (17)		Suppression of PD-L1, N-cadherin, snail and vimentin	(47)
Hesperidin (18)		Suppression on PD-L1 via inhibition of Akt and NF-κB signaling pathway	(48)
Berberine (19)		Elevation of infiltration of NKs	(49)
3,3′-Diindolylmethane (20)		Inhibition of MDSCs via downregulation STAT3 pathways and improving the therapeutic effect of PD-1 antibody	(50)
Prodigiosin (21)		Reduction of the number and differentiation of Tregs, inhibition of M2 polarisation and induction of TAM infiltration	(51)
Polyactin A (22)	Peptides	Stimulation of DCs, CD4+ and CD8+ T lymphocytes	(52)
Enniatin A (23)		Reduction of PD-L1 levels, promotion CD8+ T cell-dependent antitumor	(53)
Fucoidan (24)	Polysaccharides	Potentiation of CD8+ T cells, macrophages, DCs and NKs, elevation of pro-inflammatory mediators of Th1 and Tc1 cells (IFN-γ and TNF-α).	(54)
Nanoformulation of fucoidan with doxorubicin (25)		Shifting from M2 to M1 TAM phenotype polarization and increasing Th1 immune response	(55)
Combination of oligo-fucoidan and Olaparib (26)		Suppression of PD-L1 levels, subpopulations of CD44/CD24 and M2 macrophage, induction of antitumoral M1 macrophages	(56)
Lentinan (27)		Suppression of IL-4-induced M2 macrophage polarization and activation of JAK/STAT signaling pathway	(57)
Polysaccharide (SYQ) (28)		Induction of M2 to shift to anti-tumor M1 phenotypes	(58)
Polysaccharides GP (29)		Promotion of APC, cytotoxic T-lymphocytes and activated macrophages	(59)
Taccaoside A (30)	Saponins	Enhancement T lymphocyte (mTOR1)-Blimp-1 signal	(60)
Ginsenosides (31)		Increasing of NKs and memory T cell	(61)
Eribulin mesylate (32)	Miscellaneous	Reduction of PD-L1 and FOXP3	(62)
Sesamin (33)		Downregulation of PD-L1 expression through the suppression ERK JAK1/STAT signaling activity	(63)
Artemisinin (34)		Reduction of Tregs and MDSCs and increasing CD4+ IFN-γ+ T cells and cytotoxic T cells	(64)
Oleuropein (35)		Inhibition of PD-L1 expression	(65)
Salinomycin (36)		Restoring the proliferation of T cells and suppression of JAK/STAT pathway and IFN-γ-induced activation of the NF-κB pathway	(66)
Metformin (37)		Reduction of PD-L1 levels, activation of AMP-activated protein kinase activation and cytotoxic T cell activity	(67)
α-TOS nanoparticles (38)		Suppression of the expression of PD-L1 and modulation of cytotoxic lymphocytes	(68)
Neo-tanshinlactone (39)		Inhibition of PD-1/PD-L1 interaction	(69)
MZ58 (40)		Activation of CD8+ T cell	(69)
RCQ (41)		Shifting TAM cells to M2 TAMs and TANs to N2 TANs	(70)
Ethanolic extracts of *Cordyceps militaris* (42)	Extracts	Stimulation of proliferation of tumor-specific T cells, level of cytokines TNF-α, IL-1β and IL-6	(71)
XIAOPI extract formula (43)		Decreasing polarization and proliferation of M2-type macrophages	(37)
		Shifting of M2-TAM to M1-TAM phenotype	(37)

(Continued)

TABLE 1 Continued

Natural Compound	Category	Immunomodulatory effect in BC	Reference
Cordyceps sinensis, *Taraxacum mongolicum*, *Coriolus versicolor* (44)			
Coriolus versicolor (45)		Activation of DCs	(72)
Astragalus membranaceus (46)		Reduction of the expression of PD-L1 via the Akt/mTOR/ribosomal protein S6 kinase beta-1 pathway	(73)
Diospyros peregrina (47)		Suppression of CD25+ Foxp3+Treg cells, reduction of release of Th2 cytokine (IL-10 and IL-4) and elevation of the release of Th1cytokines, including (IL-12 and IFN-γ).	(74)
Sarcodon imbricatus (48)		Decreasing the expression of PD-L1 and elevation of IL-2, IL-6 and TNF-α, and NKs activity	(75)
Hedyotis diffusa and *Scutellaria barbata* (49)		Reduction of the expression of PD-L1, β-catenin, and cyclin D1 causing inactivation of MAPK and Akt signaling pathways	(76)
Camel milk (50)		Elevation CD+4, CD+8, NKs	(77)

activators of the transcription (STAT)5 phosphorylation and IL-10 secretion. It also reduced MDSC population and Tregs within tumor tissue, resulting in the correction of immunosuppressive conditions generated by the TME and the inhibition of tumor growth (36). Anemoside A3 (8), from the root of *Pulsatilla chinensis*, is a triterpenoid glycoside that suppresses progression of TNBC tumors via shifting of the M2-TAM to the M1-TAM phenotype and inhibiting TAM-TNBC crosstalk (37).

Oridonin (9) is a diterpenoid with anti-inflammatory and antitumor activities. It is obtained from the plant *Rabdosia rubescens* and is used in Chinese herbal medicine. This molecule modulates Treg differentiation both *in vitro* and *in vivo*, leading to the attenuation of Treg immunosuppressive ability. This mechanism depends on the reduction of TGF-β receptor expression (38). Crassolide (10) is a natural marine product belonging to the class of cembranoid diterpenes, and is produced by Formosan soft coral, *Lobophytum michaelae*. Tsai and colleagues investigated its potential immunogenic effects and found that crassolide induced immunogenic cancer cell death. It also reduced the expression of CD24 on the surface of cancer cells and blocked mitogen-activated protein kinase (MAPK) 14 activation and STAT activity (39). Another diterpene, triptolide (11), is derived from the vine *Tripterygium wilfordii* and is used in traditional Chinese medicine as an immunosuppressant in autoimmune diseases and inflammatory conditions (78). Research has shown the ability of triptolide to act as a controller to promote cancer cell-reactive immune responses via the suppression of interferon-γ-induced PD-L1 surface expression leading to down-regulation of the PD-1/PD-L1 pathway (40).

5.3 Flavonoids

Naringenin (12) is a flavanone that exists in citrus fruits and has immunomodulatory, antioxidant, antidiabetic, and hypolipidemic properties. Naringenin has been shown to decrease the infiltration of MDSCs and Treg cells in breast cancer cell lines, and to upregulate IL-2 and INF-γ -releasing T cells in spleen and lung tissue, demonstrating its use as an immunomodulatory agent. Qin's group revealed anticancer activity in tested animal model with an elevation of IL-2 and IFN-γ expressing T cells (41). Other research found that naringenin inhibited the transformation of lymphatic T cells into Tregs, which prevented *in vivo* pulmonary metastasis triggered by BC through the lowering of TGF-β1 production via the protein kinase C signaling pathway (42). Furthermore, a combination of cryptotanshinone and naringenin (13) caused a switch in immune response towards Th1 cells, thereby enhancing their activities and modulating Treg cell activity through JAK2/STAT 3 pathway in BC (43). Another flavonoid, apigenin (14), which is found in fruit and vegetables such as onions and oranges, has been found to boost immune system functioning. Apigenin has shown inhibitory effects on interferon-γ-induced PD-L1 expression as well as interferon-γ mediated STAT1 activation. One study investigated the immunogenic properties of apigenin and found that it intensified the anti-tumor immune response by increasing the proliferation of T cells (44). Salvigenin (15) is a flavonoid obtained from *Salvia miltiorrhiza* known to have cytotoxic and immunomodulatory properties. Its activity, in conjunction with the modulation of cytokine production of primed immune cells, was demonstrated by Noori's group, who found a significant rise in anti-cancer immunity and a reduction of tumor tissues in a BC mouse model. *In vivo* results showed the reduction of IL-4 and elevation of IFN-c in the models, accompanied by suppression of Foxp3+ Treg cells (45). Another flavonoid, myricetin (16), found in tea and in berry plants, showed significant inhibitory effects on PD-L1 in IFN-γ-treated MDA-MB-231 BC cells (46).

Sativan (17) is an isoflavane produced by *Spatholobus suberectus*, another plant which is used as a remedy in traditional Chinese medicine. Peng and colleagues revealed that treatment with sativan resulted in the downregulation of the expression of PD-L1 and epithelial-to-mesenchymal transition by up-regulating miR-200c. In addition to the suppression of PD-L1, N-cadherin, snail and vimentin levels decreased, indicating the inhibition of tumor migration and invasion (47). Hesperidin (18) is classified as a

flavanone glycoside and has various pharmacological effects on cardiovascular, neurological, and psychiatric conditions as well as on cancer. It is found naturally in citrus fruits. Kongtawelert and colleagues showed the suppressive effects of hesperidin on the mRNA and protein of PD-L1 in the MDA-MB-231 cell line, via inhibition of protein kinase B (Akt) and nuclear factor kappa (NF-κB) signaling (63). Sulaiman et al. investigated the properties of hesperidin via a nanoformulation of hesperidin loaded on gold nanoparticles. The results showed that the nanoformulation stimulated macrophage activity in Ehrlich ascites tumor cell-bearing mice (79).

5.4 Alkaloids

Berberine (19) is an alkaloid that is naturally present in a variety of plants, including barberry and oregon grape. It has various pharmacological properties, including anti-inflammatory, analgesic, hypolipidemic and antimicrobial activity. Upon exposure of BC 4T1 tumor-bearing mice to berberine, all immune-cell marker levels was significantly reduced except for CD8, and inflammatory markers were down-regulated. Furthermore, the infiltration of NKs was elevated in the treated group, revealing the immunogenic effect of berberine in BC (Table 1) (49). 3,3′-Diindolylmethane (20) is another natural alkaloid that has been investigated for its anti-cancer effects. It is formed during the autolytic breakdown of indole-3-carbinol, a reaction occurring in plants such as cruciferous plants. It possesses anti-tumor properties through its ability to inhibit tumor cell proliferation, suppress metastasis, and induce apoptosis of tumor cells. Moreover, Sun's group revealed the immunogenic effect of 3,3′-Diindolylmethane as an inhibitor of MDSCs via downregulation of miR-21 levels and subsequent activation of the phosphatase and tensin homolog/PIAS3-STAT3 pathways. In addition, by raising T-cell response, it promoted the production of beneficial PD-1 antibodies and slowed tumor growth, thus indicating its potential for cancer patients undergoing anti-PD-1 treatment (50). Prodigiosin (21) is a microbial alkaloid with a red pigment that is found in the gram-negative bacterium *Serratia marcescens*. It has anti-microbial and anti-tumor properties, and is able to regulate the TME by controlling immune cells and immune checkpoints. Furthermore, it has been found to reduce the number and differentiation of Tregs, thus preventing immune tolerance and enhancing antitumor functions via inhibition of heat shock protein 90 and survivin, and activation of p53. It also suppresses tumor growth via the inhibition of M2 polarization and induction of TAM infiltration (51).

5.5 Peptides

Polyactin A (22) is an antibiotic belonging to the class of polymannopeptides. It can be isolated after fermentation of the buccal α-hemolytic streptococci strain. This antibiotic affects immune cells via the induction of DCs maturation from PBMCs, and the mature cells *in vitro* could initiate a potent E75 peptide-specific CD8+ T-cell response. It may have the ability to trigger the E75-specific immunologic response *in vivo* as well as *in vitro*, and has been shown to significantly increase positive rates of CD4+ and CD8+ T lymphocytes (52). Enniatin A (23) is a cyclohexadepsipeptide obtained from the Fusarium species of fungi, with the ability to reprogram the TME. It has been shown to trigger immunogenic cell death in TNBC syngeneic mice. Moreover, it can reduce PD-L1 levels and promote CD8+ T cell-dependent antitumor activity by activating the chemokine-related receptor CX3C motif chemokine receptor 1 pathway (53).

5.6 Polysaccharides

Fucoidans (24), are sulfated polysaccharides that can be extracted from brown algae seaweeds such as *Cladosiphon okamuranus*. They have a range of properties that include antioxidant, immunomodulatory, and anti-cancer activity. In addition to their ability to induce cell cycle arrest and apoptotic death of BC cells, fucoidans have immuno-potentiating effects in immune cells. Moreover, they can modulate the activity of adaptive and innate immune responses via the potentiation of T cells, macrophages, DCs and NKs. A study involving co-culturing co-CD8+ T cells and human breast cancer cells (MCF-7) revealed that CD8+ T cells number and IFN-γ increased more in the fucoidan-treated group. In another study, by Jin et al., similarly immunogenic effects were found to occur through the promotion of both CD4+ and CD8+ T cell responses via the elevation of immunomodulatory mediators of Th1 and CD8+ T cells (IFN-γ and TNF-α). Fucoidans also activate the maturation of DCs, either by raising levels of CD40, CD86, and MHC-I and -II surface molecules or increasing cytokines such as IL-12 and TNF-α (70). Nanoformulations of fucoidan with doxorubicin (25) combine the cytotoxic effects of doxorubicin with the ability of fucoidans to moderate the tumor microenvironment by raising the immune response of Th1 and switching M2 TAM to the M1 TAM phenotype (55). Different formulations composed of oligo-fucoidan and Olaparib (26) were found to synergistically suppress PD-L1, resulting in repression of the oncogenic IL-6/p-epidermal growth factor receptor/PD-L1 pathway. Moreover, it decreased subpopulations of CD44/CD24, suppressed M2 macrophage intrusiveness and repolarized M2 to the M1-like (CD80high and CD86high) phenotypes and induced immunoactivity and antitumoral M1 macrophages (56).

Lentinan (27) is a polysaccharide obtained from the edible mushroom *Lentinus edodes*, which has antitumor and immuno-stimulating properties. It is able to enhance immune function in the treatment of BC, as demonstrated by Guan's group. The immunohistochemical findings showed that it reduced the mRNA expression of marker genes related to M2-type macrophage. It also suppressed M2 macrophage polarization induced by IL-4 cytokine. It activated the JAK/STAT signaling pathway, as shown by molecular docking, western blotting and siRNA transfection experiments (57). Polysaccharides from *Tetrastigma hemsleyanum* (SYQ) (28) have been found to induce the polarization of M2 to anti-tumor M1 phenotypes. This causes promotion of the macrophage polarization leading to the inhibition of BC cell proliferation (58). Polysaccharides (29) from *Ganoderma lucidum*

are known to boost the immune system. Polysaccharide fractions from the plant are reported by Zhao and colleagues to activate macrophages significantly, leading to inhibition of BC cells (80). Moreover, other studies have shown the ability of polysaccharides to promote the function of several immune cells such as APCs and mononuclear phagocytes, as well as increasing humoral and cellular immunity. The latter process includes the production of CTLs and activated macrophages (59).

5.7 Saponins

The steroidal saponin taccaoside A (30) is one of the principal phytochemicals in many herbs used in traditional Chinese medicine, and is known for its anti-cancer activity. It was found to exhibit significant activity against BC cells by increasing granzyme B through improving the signaling of the T lymphocyte mammalian target of rapamycin 1 (mTOR1)-Blimp-1, providing *in vivo* evidence of anti-tumor efficacy (60). Ginsenosides (31) belong to the class of saponins and are known for neuroprotective and anti-inflammatory properties. The main ginsenosides are obtained from the root of *Panax ginseng*, one of these being ginsenoside Rg3, extracted from Korean ginseng, which has the ability to induce apoptosis, enhance the activity of NKs and inhibit the NF-κB signaling pathway in BC model (81). Due to its insolubility in water and poor solubility in the intestine, nanoformulations of Rg3 with doxorubicin have been designed, in which Rg3 raises infiltration levels of memory T cells in the tumor microenvironment while the doxorubicin promotes immunogenic cancer cell death (61).

5.8 Miscellaneous

Eribulin mesylate (32) is a natural marine product extracted from the Japanese marine sponge *Halichondria okadai* which has been approved for use in metastatic cancer. Its immunomodulatory effect was investigated by Goto et al. in conjunction with locally advanced or metastatic breast cancer. Tumor biopsies from patients receiving eribulin treatment were collected and analyzed for the expression of immune markers including PD-L1, PD-L2, CD8 and the Treg marker FOXP3. Results showed a significant reduction of immunosuppressive drivers PD-L1 and FOXP3, leading to reduced immunosuppression in the TME (62). Sesamin (33) is a lignin extracted from the oil of *Sesamum indicum*, which is recognized for antioxidant and anti-inflammatory activities. Kongtawelert's group revealed that sesamin downregulated PD-L1 expression in both mRNA and protein in MDA-MB231 breast cancer cells. This was mediated by NF-κB and Akt, and also suppressed extracellular signal-regulated kinase (ERK) and JAK/STAT signaling activity (48).

Artemisinin (34) is sesquiterpene lactone obtained from the plant *Artemisia annua* that is used as an anti-malarial drug. Cao and colleagues explored its ability to suppress BC growth via its immunomodulatory activity. They found that artemisinin boosted T cell functioning, blocked the immunosuppressive effects of Tregs and MDSCs, and allowed CD4+ IFN-γ+ T cells and cytotoxic T cells to thrive, all of which hindered tumor growth *in vivo* (64). Oleuropein (35) is a glycosylated seco-iridoid, a type of phenolic bitter compound, extracted from *Olea europaea* L. It is known for its anti-inflammatory, anti-cancer antioxidant, neuroprotective, and anti-atherogenic properties. Hamed and colleagues revealed that oleuropein controls the miR-194/XIST/PD-L1 loop in TNBC, thus making it a promising nutritional epigenetic agent in cancer immunotherapy (65). Salinomycin (36) is an antibiotic obtained from the bacterial species *Streptomyces albus* that has been investigated for its anti-tumor activity in BC. It was found to suppress activation of the JAK/STAT pathway by IFN-γ and inhibit IFN-γ-induced activation of the NF-κB pathway by inhibiting IκB degradation and NF-κB phosphorylation. It also inhibited indoleamine 2,3 dioxygenase enzymatic activity, as shown by molecular docking, where salinomycin demonstrated nucleophilic attack in the catalytic domain of indoleamine 2,3 dioxygenase. In tumor tissue, *in vivo* research found that salinomycin activated cisplatin's anti-tumor properties and appeared to boost T cell production when co-cultured with BC cells treated with IFN-γ (66).

Metformin (37) is a known hypoglycemic drug that can be extracted from *Galega officinalis*. Results reported by Cha's group show that metformin has the potential to lower PD-L1 in breast cancer by activating protein kinases via AMP-activated protein kinase (AMPK). Blocking PD-L1 signaling enhances CTLs activity against tumor cells (67). Alpha-tocopheryl succinate (α-TOS) is one of the forms of vitamin E that is an effective anti-tumor agent. A nanoparticle delivery system was designed for α-TOS (38) which aimed to boost anticancer immunity through the suppression of IFN-γ-induced PD-L1 expression. It also enhanced tumor elimination via the modulation of cytotoxic lymphocyte infiltration into the TME (68). Neo-tanshinlactone (39) is a planar natural molecule having four rings. It is obtained from *Salvia miltiorrhiza* Bunge and is known as an ICI and as PD-1/PD-L1 interaction inhibitor. Zhang's group investigated the effect of different analogues of neo-tanshinlactone against TNBC. MZ58 (40) proved to be the best candidate in a subcutaneous transplantation tumor model as it showed less cytotoxicity toward T cells, activated CD8+ T cells, and reduced T cell exhaustion (69).

5.9 Combinations of natural compounds

In some cases, the effect of single compound can be strengthened by combining it with other phytochemicals. This phenomenon has been investigated in studies aimed at remodeling BC cells using the synergistic effects created in combining active chemical constituents (Table 1). For example, a combination of curcumin, resveratrol, and quercetin (RCQ) (41) was designed to manipulate the multi-layered interactions of cells and signaling pathways in a novel approach to phyto-immunotherapy in BC. The RCQ combination has been shown to remodel antitumor immunity in 4T1 breast cancer-bearing mice by shifting the immune balance toward an immune activation state by reversing the superiority of immunosuppressive infiltrating cells in the TME. This is achieved by the inhibition of the development of

TILs into immunosuppressive cells, including TAMs cells to M2 TAMs and TANs to the N2 TANs. It also enhanced the T cells accumulation and decreased the recruitment of macrophages and neutrophils in the TME (54).

5.10 Extracts

Ethanolic extracts of *Cordyceps militaris* (42) have shown immunogenic effects in stimulating the proliferation of tumor-specific T cells without inhibiting DCs functioning and T cell proliferation (82). Yang et al. isolated *C. militaris* immunoregulatory protein that suppresses the proliferation of 4T1 breast cancer cells. The immunoregulatory protein elevated the mRNA levels of cytokines IL-6, IL-1β and TNF-α in peritoneal macrophages (71). In addition, a traditional Chinese formula (XIAOPI) (43), composed of 10 plants, has shown the ability to reprogram the TME by decreasing the polarization and proliferation of M2-type macrophages. Similarly, other herbal extracts from *Cordyceps sinensis*, *Taraxacum mongolicum*, and protein-bound polysaccharides (from the *Coriolus versicolor* fungus) (44) suppressed progression of TNBC via shifting the M2-TAM to the M1-TAM phenotype, and inhibiting TAM-TNBC-talk (37). Regarding protein-bound polysaccharides from *C. versicolor* (45), several clinical studies have been conducted showing their significant immunological and oncological activity, with overall improvement of prognosis for BC patients. The mechanism of action involves a Th1 adaptive immune response and modulation of immunosuppressive TME via activation of DCs (72). A polysaccharide fraction obtained from *Astragalus membranaceus* (46) has been shown to increase immune response. This effect is linked to the inflammatory immune response at the tumor site. Detailed investigation of the mechanism showed reduction of the expression of PD-L1 via the Akt/mTOR/ribosomal protein S6 kinase beta-1 pathway (73).

The fruit extract of *Diospyros peregrina* (47) has been evaluated for its immunogenic effect on BC models. Findings show that the extract controlled and combatted BC, and suppressed the expression of Foxp3+Treg cells within tumor tissue. This was reflected in immune cell and cytokine activity, as the release of Th2 cytokines was reduced, including IL-10 and IL-4, and the release of Th1 cytokines, including IL-12 and IFN-γ, was elevated. This was accompanied by elevation of the activity of T-box transcription factor TBX21and the suppression of the expression of transcription factor FOXP3 and GATA binding protein 3 (74). *Sarcodon imbricatus* (48) as an aqueous extract, showed inhibitory effects on the growth, migration, and invasion capacity of BC cells. It decreased the expression of PD-L1 in BC models and elevated IL-2, IL-6, TNF-α, and NKs activity (75). Yang and colleagues prepared an ethyl acetate fraction from a mixture of *Hedyotis diffusa* and *Scutellaria barbata* (49) revealing their ability to reduce the expression of PD-L1, β-catenin, and cyclin D1, causing inactivation of MAPK and Akt signaling pathways (76).

Badawy et al. investigated the properties of camel milk (50) and its exosomes nanoparticles. *In vitro* as well as *in vivo* tests using oral and local injection, found that camel milk reduced breast tumor progression via several different mechanisms, including apoptosis induction, oxidative stress inhibition, suppression of several types of gene-related inflammation (IL1b, NF-κB), angiogenesis (vascular endothelial growth factor) and metastasis (matrix metalloproteinase-9, intercellular adhesion molecule 1). These anti-tumor effects were accompanied by higher immune response, evidenced by higher numbers of CD+4, CD+8, and NKs (77).

6 Perspectives and conclusion

This review demonstrates the potential role of natural products as immunotherapeutic treatments in BC. The risks and side-effects of modern cancer chemotherapy are well-known, and research into less debilitating treatments such as immunotherapy is increasing steadily. Plant, animal, microbial, and marine organisms continue to provide sources of structurally diverse and biologically active compounds that are able to regulate the human body's immune response. The findings of this review reveal the wide range of different immune cells, cytokines, and signaling pathways that can be modified and regulated to fight cancer tumors. Natural compounds can suppress immunosuppressive cells such as Tregs and MDSCs can significantly lower treatment response, as can immunosuppressive mediators such as IL-10 and TGF-β. Active compounds derived from natural products have been shown to effectively stimulate immune cells such as CD+8 cells, NKs, DCs and TAMs, which then block immune suppression in the TME. Active compounds also promote the secretion of anti-tumor immune factors (IFN-γ, TNF-α, IL-1). Research has shown that some can inhibit signaling pathways such as NF-κB, JAK-STAT, MAPK and Akt/mTOR, and stimulate immunogenic cancer cell death. Others have been shown to inhibit immune checkpoints such as PD-L1 in both *in vitro* and *in vivo* studies.

Immunomodulatory natural compounds can be delivered in a variety of different forms, such as extracts, isolated entities, synthetic derivatives, nanoformulations, and compound combinations. For example, extracts of *Coriolus versicolor, Cordyceps militaris* and *Astragalus membranaceus* successfully stimulated significant immunological and oncological activity with overall improvements in the prognosis for BC. Different classes showing chemical diversity, such as flavonoids, alkaloids, terpenes, peptides and polysaccharides exert modulatory effects on immune cells, immune cytokines and immune checkpoints. RCQ is an example of the successful combination of compounds, with its synergistic activity leading to inhibition of immunosuppressive cells in the TME and restoration of immune balance and immune activation to fight BC growth. Nanoformulations have also been successively designed and developed to combat immunosuppressive TMEs, an example being curcumin-loaded polymeric nanoparticles, which, once injected, triggers immunogenic cell death of cancer cells in BC models. The combination of immunotherapeutic compounds with chemotherapy shows outstanding effects on immune tolerance in BC. Furthermore, a nanoformulation of fucoidan combined with the chemotherapeutic drug doxorubicin significantly enhanced doxorubicin's effects and caused

manipulation of the immune landscape of the tumor to increase immune cell response against cancer cells.

Bioactive compounds have been shown, in *in vivo* and *in vitro* experiments, to have a positive effect on the immune system and its ability to fight cancer cells. This has been especially valuable when using TNBC and 4T1 BC mouse models, where natural products can be evaluated in terms of their immunotherapeutic activity on the TME in BC progression. Recent clinical studies have shown that natural compounds can provide adjunctive immune support in TNBC patients. For example, protein-bound polysaccharides from *C. versicolor* have demonstrated significant immunological and oncological activity, resulting in overall improvements in BC prognosis. Unfortunately, most natural active compounds are not readily translatable into clinical trials where pharmacokinetics, stereochemistry, and bioavailability are all considerations for efficient drug delivery. In addition, current restrictions applied to clinical trials may affect the extent to which some natural compounds can be used in drug combinations. As an example, the bioactive alkaloid matrine, found in plants of the Sophora species (e.g. *Sophora flavescens*), has been shown to cause autophagic and apoptotic death in BC cell lines. Despite demonstrating significant ability to regress tumors and suppress metastasis in TNBC mouse models, matrine has only been studied in a few clinical trials in China, and there has been no definitive positive consensus in the findings. In other clinical trials, BC patients were treated with aqueous extracts of *Sophora flavescens* and *Smila glabra* as part of a combination therapy alongside conventional chemotherapy. However, some of these studies revealed a better clinical response compared to control group than others while others showed no response. Nevertheless, the studies reported improvements in quality of life resulting from a decrease in chemotherapy toxicity.

Compared to conventional drugs, natural products exhibit several advantages; wide availability, fewer and less severe side-effects, diverse pharmacological and chemical properties including immunomodulation, apoptotic induction, proliferation suppression, and metastasis inhibition, which all together contribute to cancer cell death. These factors indicate the potential for naturally-occurring compounds to play a significant role in tumor immunotherapy. However, there are a few issues that need to be addressed. For example, in order to identify the most effective natural compounds, we need more comprehensive and in-depth research into immune-system signaling pathways with respect to tumor immunotherapy. Also, determining the range of safe dose is challenging, since potential toxicity to vital organs such as liver and kidneys needs to be taken into account. In addition, there are research challenges relating to variations in the TME and in tumor heterogeneity, as well as difficulties in elucidating the molecular mechanisms and identification of targets relevant to tumor immunity. Also, the bioavailability of some natural products could limit their applications *in vivo*, as repeated doses are required, leading to increased risk of toxicity. Using advanced techniques to overcome these challenges could improve the likelihood of successful cancer immunotherapy natural drug development. Natural products can be incorporated in unique drug delivery systems, computerized design techniques, and metabolomics. These could lead to fruitful future strategies for clinical breast cancer treatments.

Author contributions

AA: Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 06 August 2024
DOI 10.3389/fonc.2024.1419123

OPEN ACCESS

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RECEIVED 17 April 2024
ACCEPTED 23 July 2024
PUBLISHED 06 August 2024

CITATION
Li L, Liang Y, Li C, Huang M, Liang W and Qin T (2024) Comparison of endoscopic breast-conserving surgery versus conventional breast-conserving surgery for the treatment of early-stage breast cancer: a meta-analysis.
Front. Oncol. 14:1419123.
doi: 10.3389/fonc.2024.1419123

Comparison of endoscopic breast-conserving surgery versus conventional breast-conserving surgery for the treatment of early-stage breast cancer: a meta-analysis

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Introduction: This meta-analysis seeks to evaluate the efficacy and safety of endoscopic breast-conserving surgery (E-BCS) compared to conventional breast cancer surgery (C-BCS) in patients diagnosed with early-stage breast cancer.

Materials and methods: Four databases (Medline, Embase, Web of Science and CENTRAL) were searched published from establishment of database to January 30,2024, for articles studying E-BCS compared to C-BCS in patients diagnosed with early-stage breast cancer. Meta-analyses of procedure time, blood loss, length of incision, drainage duration, total postoperative drainage volume, average duration of hospital stay, positive rate of margin, complication rate, recurrence rate, metastasis rate and cosmetic scoring were performed.

Results: Totally 11 studies were included for meta-analysis. Compared with C-BCS, E-BCS exhibited significantly reduced incision length (WMD = -6.44, 95%CI: -10.78 to -2.11, P=0.004, I^2 = 99.0%) and superior cosmetic scoring (WMD = 2.69, 95%CI: 1.46 to 3.93, P=0.001, I^2 = 93.2%), but had significantly longer operation time (WMD = 34.22, 95%CI: 20.89~47.55, P=0.000, I^2 = 90.7%) and blood loss (WMD = 3.65, 95%CI: -3.12 to 10.43, P=0.291, I^2 = 86.8%). There was no significant difference in terms of recurrence rate, metastasis rate, positive rate of tumor resection margins, drainage duration, drainage volume, complication rate and hospital days.

Conclusions: Our research findings indicate that E-BCS is a viable and secure method for treating breast cancer in its early stages. E-BCS provides distinct advantages in terms of the length of the incision and the aesthetic result, without demonstrating an elevated recurrence rate or metastasis rate.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024535164, identifier CRD42024535164.

KEYWORDS

breast cancer, breast-conserving surgery, endoscopic, recurrence, meta-analysis

1 Introduction

Breast cancer is characterized by the excessive and unregulated proliferation of breast cells, resulting in the formation of tumors, and women bear a significant burden of this disease (1, 2). As of 2020, female breast cancer has overtaken lung cancer to become the most prevalent form of cancer globally, accounting for 11.7% of all cases. Additionally, it is now the fifth greatest cause of cancer-related deaths worldwide, responsible for 6.9% of all fatalities (3, 4). Patients diagnosed with early-stage breast cancer often have breast-conserving surgery (BCS) followed by adjuvant radiation therapy. This approach has been proven to be a viable and successful alternative to total mastectomy, and extensive research has demonstrated its safety (5–7). Nevertheless, conventional breast-conserving surgery (C-BCS) continues to have certain drawbacks in terms of its impact on breast aesthetics (8–10). From a clinical standpoint, there are still some patients who are not fully satisfied with the aesthetic outcome of breast-conserving surgery (11). To address the physiological and psychological needs of breast cancer patients and enhance patient satisfaction, clinical surgical treatment has been focused on achieving minimally invasive and aesthetically pleasing operations while ensuring safety. This has led to the advancement of new techniques for breast-conserving surgery, such as endoscopic breast-conserving surgery (E-BCS) (12).

During the 19th century, Dr. Desormeaux, a French physician, invented the first endoscopic technique for the examination of the urinary system and bladder, and also introduced this technique in the field of gynaecology (13, 14). As science and technology continue to advance, there is a growing number of therapeutic therapies that integrate endoscopic technology and microsurgery (15–17). Currently, endoscopic technology has emerged as a prominent technique in the realm of minimally invasive surgery and is extensively employed in breast surgery. This precise and minimally invasive approach preserves the functionality of the surgery area while significantly enhancing the aesthetic appeal of the breasts (18–20). Studies has shown that endoscopic assisted breast-conserving surgery offers a cosmetic benefit, leading to increased patient satisfaction and enhanced postoperative quality of life (21–26). Nevertheless, endoscopic technology poses challenges, and endoscopic breast surgery involves a sequence of specialized surgical techniques. There is a potential risk of tumor diffusion, and the safety of this operation in relation to tumor management lacks adequate supporting evidence (10). Hence, the application of E-BCS in the management of early breast cancer remains a subject of debate, necessitating additional validation of its safety and dependability.

Therefore, we conducted a meta-analysis to compare the effectiveness and safety of E-BCS versus C-BCS in patients with early-stage breast cancer.

2 Materials and methods

2.1 Search strategy

This meta-analysis adhered to the 2020 principles set forth by the Preferred Reporting Project for Systematic Review and Meta-Analysis (PRISMA). The study has been officially registered at PROSPERO under the registration number CRD42024529976. A thorough search was conducted in four databases, namely PubMed, Embase, Web of Science, and the Cochrane Library, to gather literature published until January 30, 2024. The search methodology followed the PICOS principle and employed a combination of MeSH terms and unrestricted text phrases. The search approach utilized involved combining the terms “Breast Cancer”, “endoscopic”, and “breast-conserving surgery”. Supplementary Material 1 provided a comprehensive overview of the search record.

2.2 Inclusion and exclusion criteria

Inclusion criteria were as follows (1) patients diagnosed as early-stage breast cancer; (2) patients in the intervention group received E-BCS; (3) patients in the control group received C-BCS; (4) at least one of the following outcomes were reported: operation time, intraoperative bleeding volume, incision length, postoperative drainage time, total postoperative drainage rate, complications, recurrence rate, positive tumor resection margin rate, hospital days, and cosmetic effect; (5) study design: randomized controlled trial, prospective study, and retrospective study.

The exclusion criteria are as follows: (1) other types of articles, such as case reports, protocols, letters, editorials, comments, reviews, meta-analyses; (2) Non breast cancer; (3) not E-BCS versus C-BCS; (4) duplicate patient cohort; (5) data cannot be extracted.

2.3 Selection of studies

The process of literature selection, which involved removing duplicate entries, was conducted using EndNote (Version 20; Clarivate Analytics). Two autonomous reviewers carried out the

initial search. The duplicate entries were eliminated, and the titles and abstracts were assessed to establish their relevancy. Each study was then categorized as either included or excluded. We resolved the issue by reaching a consensus. If the parties involved cannot reach an agreement, a third reviewer takes on the role of a mediator.

2.4 Data extraction

Two independent reviewers extracted data. The extracted data included: (1) Basic characteristics of studies included: author, nationality, year of publication; (2) Baseline characteristics of study subjects: age, sample size, tumor stage; (3) outcome indicators: operation time, intraoperative blood loss, incision length, postoperative drainage time, postoperative total drainage flow, postoperative complications, postoperative recurrence, tumor margin positive rate, hospital days, and cosmetic effect.

2.5 Quality assessment

Two autonomous reviewers evaluated the quality assessment in the trials that were included. We employed the Newcastle-Ottawa Scale (NOS) to evaluate the quality of retrospective literature in this study. In the event of any inconsistencies, the contested findings were resolved by engaging in collaborative deliberation.

2.6 Statistical analysis

The analyses were conducted using Stata 12.0. The continuous variables were compared using the weighted mean difference (WMD) and a 95% confidence interval (CI). The relative ratio (RR) was employed to compare binary variables, in conjunction with a 95% CI. The medians and interquartile ranges of continuous data were transformed into the mean and standard deviation. The statistical heterogeneity among the included studies was assessed using the Cochrane's Q test and the I^2 index. Given that the papers included in the study are obtained from public literature, it is generally more logical to opt for the random effect model as the initial choice. A p-value less than 0.05 was deemed statistically significant.

3 Results

3.1 Search results

The process of selecting and incorporating articles was depicted in Figure 1. A total of 235 publications were obtained from four databases, and an additional two articles were discovered by reviewing the bibliographies of the mentioned papers. A total of 11 articles (27–37) were included in the final meta-analysis, following the established criteria for inclusion and exclusion. The procedure of selecting and including the research was depicted in Figure 1.

3.2 Study characteristics

The meta-analysis comprised a total of 11 studies, consisting of 3 prospective studies and 8 retrospective investigations. The meta-analysis comprised a total of 2562 individuals, including 852

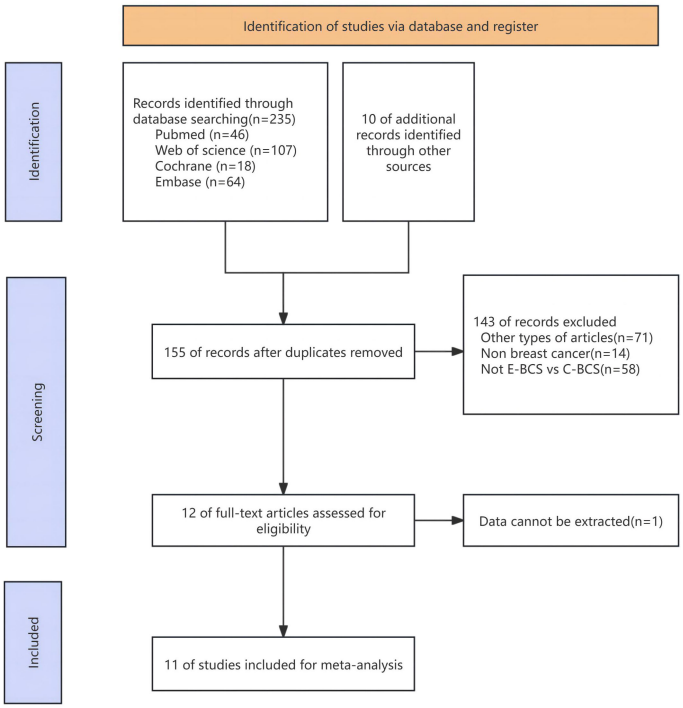

FIGURE 1
Flow chart of literature search strategies.

TABLE 1 Characteristics of the included studies.

Study, year	country	design	study period	group	cases	Age (Mean ± SD)	TNM stage (0/I/II)
Fang Xie 2022 (27)	China	R	2017~2019	E	63	52.8 ± 8.7	0/32/32
				C	117	54.0 ± 10.4	0/49/68
Zi-Han Wang 2018 (28)	China	R	2014~2015	E	35	50.8 ± 7.3	0/26/9
				C	35	51.0 ± 7.9	0/21/14
Hyung Seok Park 2011 (29)	Korea	R	2008~2010	E	40	51.1 ± 8.4	NA
				C	681	49.6 ± 9.5	NA
Hiroki Takahashi 2014 (30)	Japan	P	2009~2011	E	100	54.2 ± 10.7	4/61/35
				C	150	61.9 ± 14.3	12/74/64
Hung-wen Lai 2021 (31)	Taiwan	R	2011~2020	E	178	NA	NA
				C	24	NA	NA
Koji Yamashita 2006 (32)	Japan	P	2001~2005	E	80	53.7 ± 13.1	NA
				C	34	50.7 ± 13.0	NA
Nobuyuki Takemoto 2012 (33)	Japan	R	1997~2007	E	60	54.4 ± 12.5	5/42/13
				C	51	55.9 ± 11.4	5/36/10
Shinji Ozaki 2013 (34)	Japan	R	2005~2011	E	73	55.4 ± 10.0	14/36/23
				C	90	59.1 ± 12.1	11/43/36
Shou-Tung Chen 2021 (35)	Taiwan	R	2010~2020	E	149	NA	NA
				C	155	NA	NA
Hung-Wen Lai 2016 (36)	Taiwan	R	2009~2014	E	46	NA	NA
				C	322	NA	NA
Yinghui Liang 2020 (37)	China	P	2016~2018	E	28	43.39 ± 6.92	0/15/13
				C	51	48.46 ± 9.21	0/31/19

R, Retrospective study; P, Prospective study; E, Endoscopic Breast-conserving Surgery; C, Conventional Breast-conserving Surgery; NA, not available.

patients in the E-BCS group and 1710 patients in the C-BCS group. The studies included were conducted in multiple countries, including of China (27, 28, 31, 35–37), Japan (30, 32–34), and Korea (29). The comprehensive data and fundamental attributes of the patients involved in the study are provided in Table 1.

3.3 Quality assessment

The Newcastle-Ottawa Scale was utilized to evaluate the quality of studies included. Out of the 11 studies, 4 studies had a rating of 8 points while 7 studies received a rating of 7, suggesting that all of the included studies were of high quality. Table 2 provides the detail of the quality assessment.

3.4 Clinical outcomes

3.4.1 Operation time (min)

Operation time was reported in ten studies (27–36). The pooled results showed that C-BCS had a significantly shorter operation time than E-BCS (WMD = 34.22, 95%CI: 20.89~47.55, P=0.000, I^2 = 90.7%) (Figure 2).

3.4.2 Blood loss (ml)

Eight studies (27, 28, 30–35) reported blood loss. There was no statistically significant distinction observed between the two groups in terms of blood loss (WMD = 3.65, 95%CI: -3.12 to 10.43, P=0.291, I^2 = 86.8%) (Figure 3).

3.4.3 Length of incision (cm)

Length of incision was reported in three studies (27, 28, 31). The aggregated findings indicated a notable disparity between two groups, with E-BCS exhibiting a reduced incision length compared to C-BCS (WMD = -6.44, 95%CI: -10.78 to -2.11, P=0.004, I^2 = 99.0%) (Figure 4).

3.4.4 Drainage duration(day)

Drainage duration was recorded in four studies (27, 28, 32, 37). There was no statistically significant disparity in the duration of

TABLE 2 Quality assessment of included studies.

Study, year	Selection	Comparability	Outcome	Total score
Fang Xie 2022 (27)	***	**	***	8
Zi-Han Wang 2018 (28)	***	**	**	7
Hyung Seok Park 2011 (29)	****	*	**	7
Hiroki Takahashi 2014 (30)	****	**	*	7
Hung-wen Lai 2021 (31)	****	*	***	8
Koji Yamashita 2006 (32)	****	*	**	7
Nobuyuki Takemoto 2012 (33)	***	**	**	7
Shinji Ozaki 2013 (34)	****	**	**	8
Shou-Tung Chen 2021 (35)	***	**	***	8
Hung-Wen Lai 2016 (36)	****	*	**	7
Yinghui Liang 2020 (37)	****	*	**	7

drainage time between the two groups (WMD = 0.65, 95%CI: -0.10 to 1.41, P=0.089, I^2 = 79.0%) (Figure 5).

3.4.5 Total postoperative drainage volume (ml)

Three studies (27, 28, 32) reported total postoperative drainage volume. The pooled results showed that C-BCS had significantly lower total postoperative drainage volume than E-BCS (WMD = 62.9, 95%CI: 2.55~ 123.27, P=041, I^2 = 78.8%) (Figure 6).

3.4.6 Positive rate of tumor resection margins

Five studies (27, 29–31, 35) recorded the positive rate of tumor margins. There was no statistically significant difference between E-BCS and C-BCS (OR = 0.91, 95%CI: 0.30 to 2.80, P=0.872, I^2 = 53.1) (Figure 7).

3.4.7 Recurrence rate

Seven studies (27, 29–32, 34, 37) reported postoperative recurrence rate. There was no statistically significant disparity in recurrence rate between E-BCS and C-BCS (OR = 1.25, 95%CI: 0.37 to 4.17, P=0.721, I^2 = 0) (Figure 8).

3.4.8 Complication rate

Five studies (28–31, 37)reported the rate of postoperative complications. The pooled results indicated that there was no

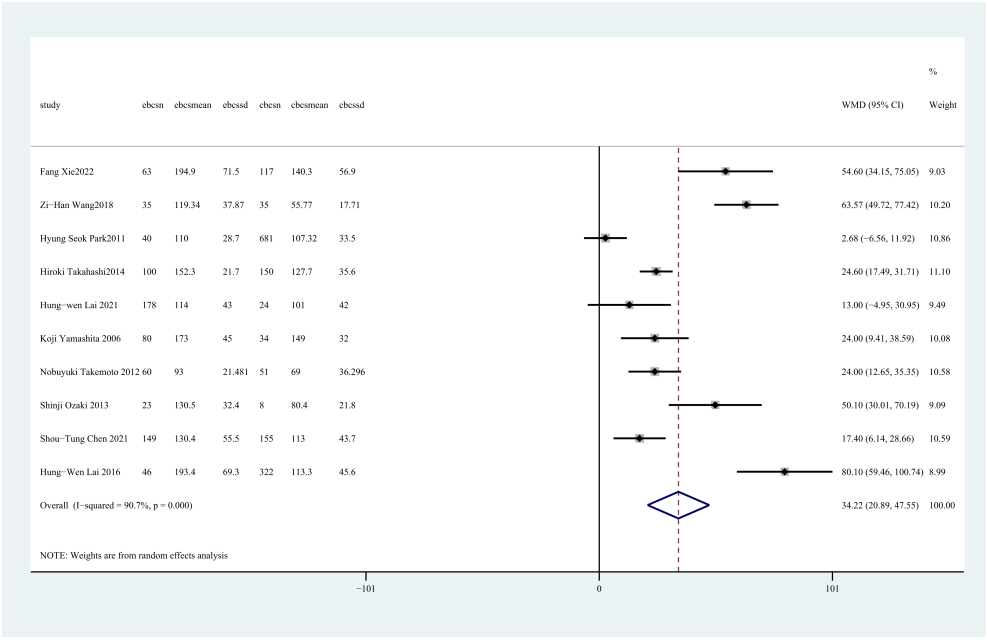

FIGURE 2
Forest plot of the meta-analysis for operation time.

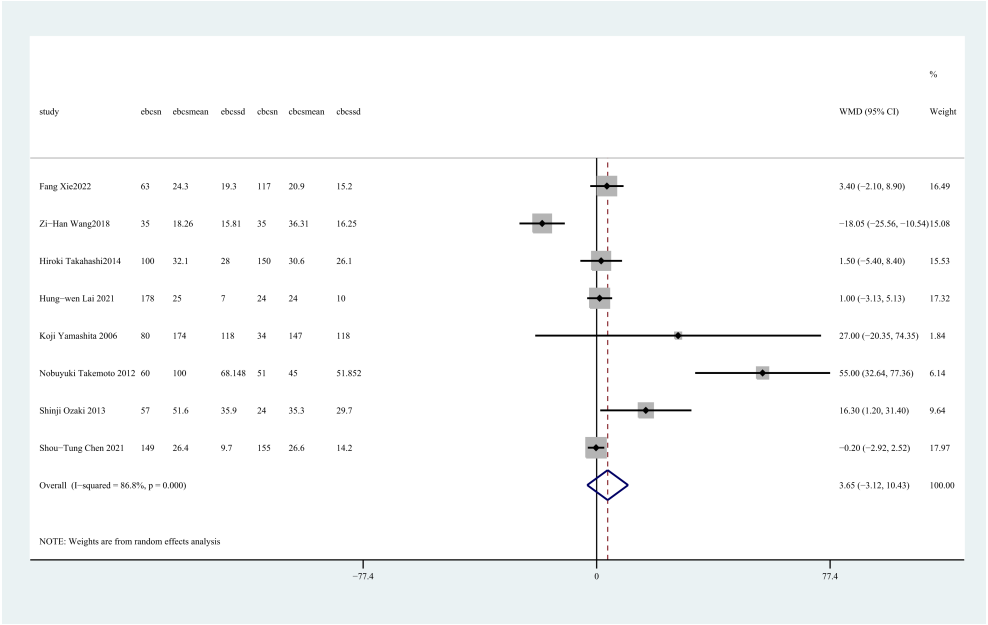

FIGURE 3
Forest plot of the meta-analysis for blood loss.

statistically significant difference between two groups (OR = 0.9, 95%CI: 0.45 to 1.79, P=0.756, I^2 = 27%) (Figure 9).

3.4.9 Cosmetic score

Three studies (28, 30, 34) reported cosmetic score. There was a notable disparity between two groups, with E-BCS exhibiting a superior cosmetic outcome compared to C-BCS (WMD = 2.69, 95% CI: 1.46 to 3.93, P=0.001, I^2 = 93.2%) (Figure 10).

3.4.10 Hospital days (day)

Two studies (30, 31) reported hospital days. There was no statistically significant difference between two groups regarding

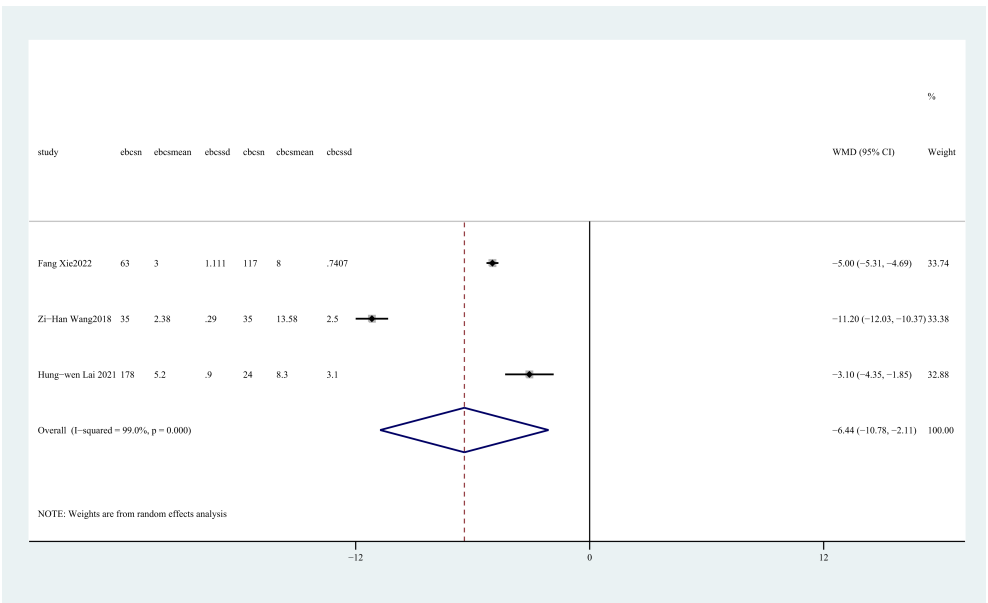

FIGURE 4
Forest plot of the meta-analysis for length of incision.

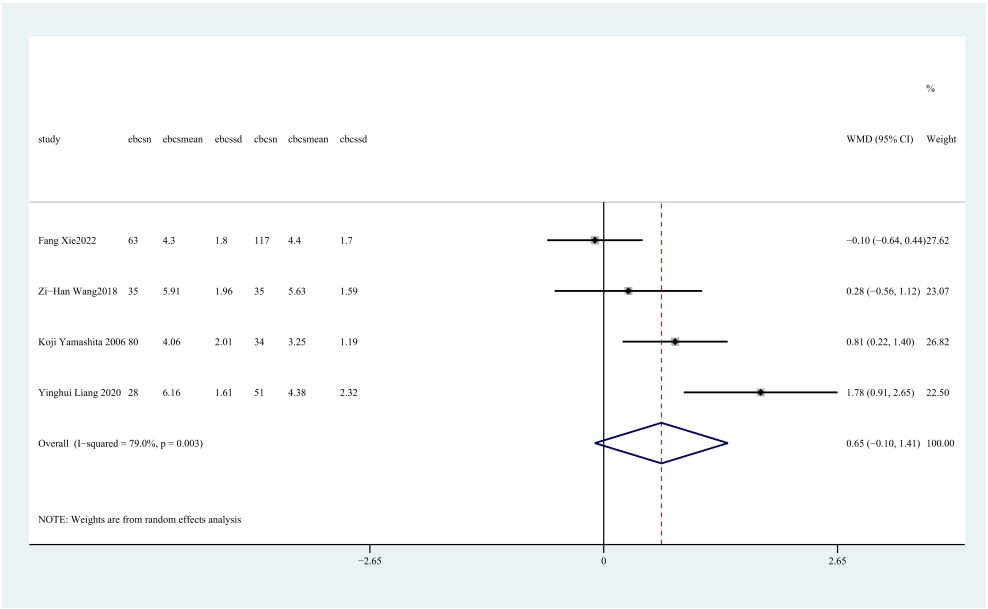

FIGURE 5
Forest plot of the meta-analysis for drainage duration.

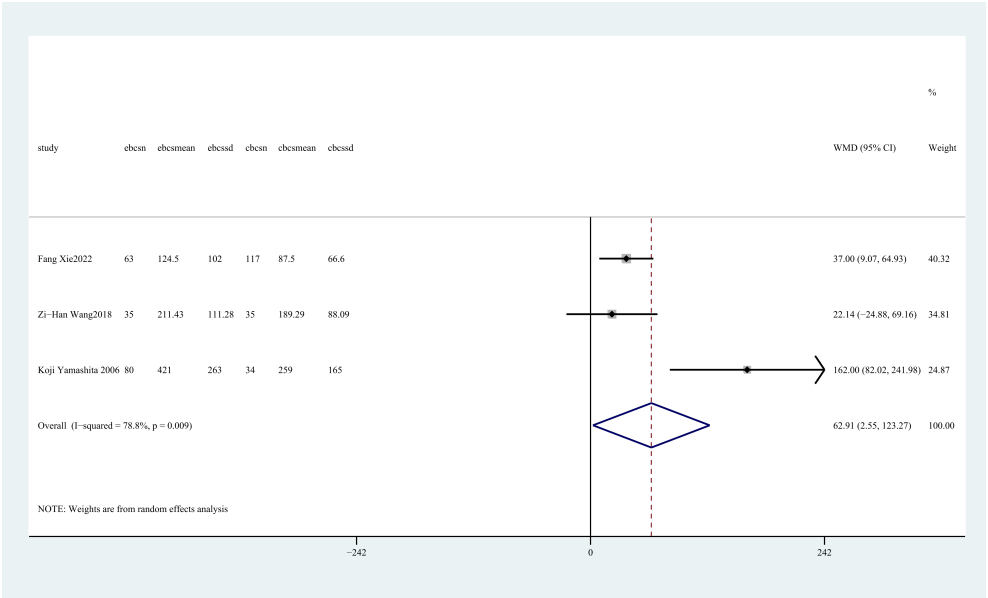

FIGURE 6
Forest plot of the meta-analysis for total drainage volume.

hospital days (WMD = -0.33, 95%CI: -1.02~0.35, P=0.343, I^2 = 82.3%) (Figure 11).

3.4.11 Metastasis rate

Two trials (27, 37) reported transfer rate. There was no statistically significant difference between two groups (OR = 0.44, 95% CI: 0.12 to 1.61, P=0.217, I^2 = 0) (Figure 12).

3.5 Publication bias

A funnel plot was performed to evaluate publication bias in relation to the recurrence rate (Figure 13). The bilateral symmetric funnel plot of the recurrence rate did not provide any substantial indication of publication bias.

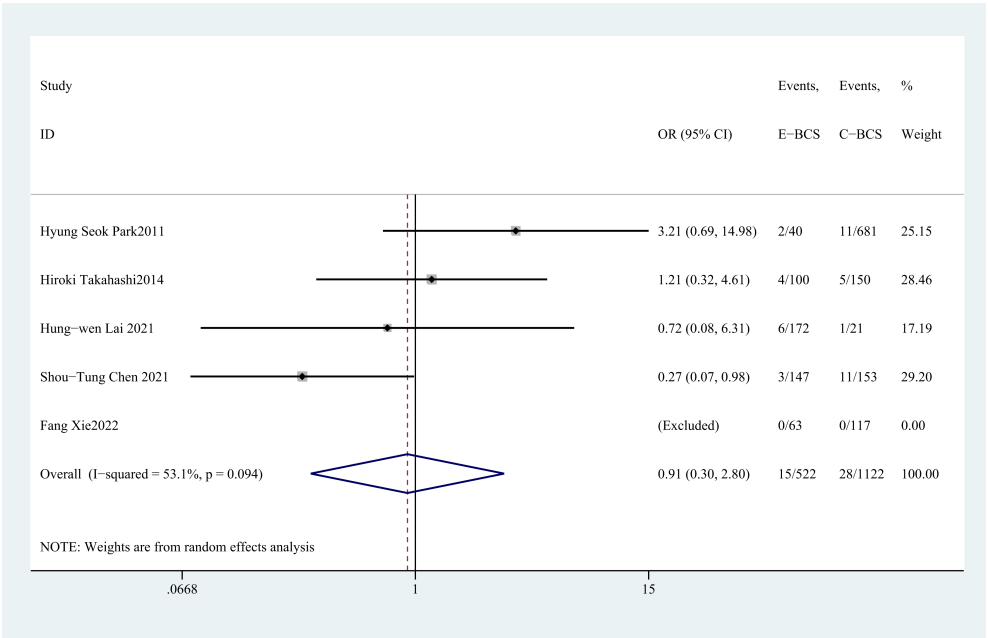

FIGURE 7
Forest plot of the meta-analysis for positive rate of tumor resection margins.

4 Discussion

BCS is the recommended treatment for women who have early-stage breast cancer (38–40). Nevertheless, C-BCS continues to have constraints in achieving satisfactory breast cosmetic results (8–10), so progressively falling short of meeting patient expectations (41). Endoscopy, a minimally invasive procedure, has been employed for over two decades (21, 22, 37, 42) in the management of breast cancer. The core principle involves utilizing discreet incisions in inconspicuous regions to enhance cosmetic outcomes (43). Endoscopic surgery has become increasingly prevalent in many surgical cases over the past decade and is now seen as a viable substitute for traditional open surgery. However, there are obstacles to endoscopic technology, and endoscopic breast surgery requires a

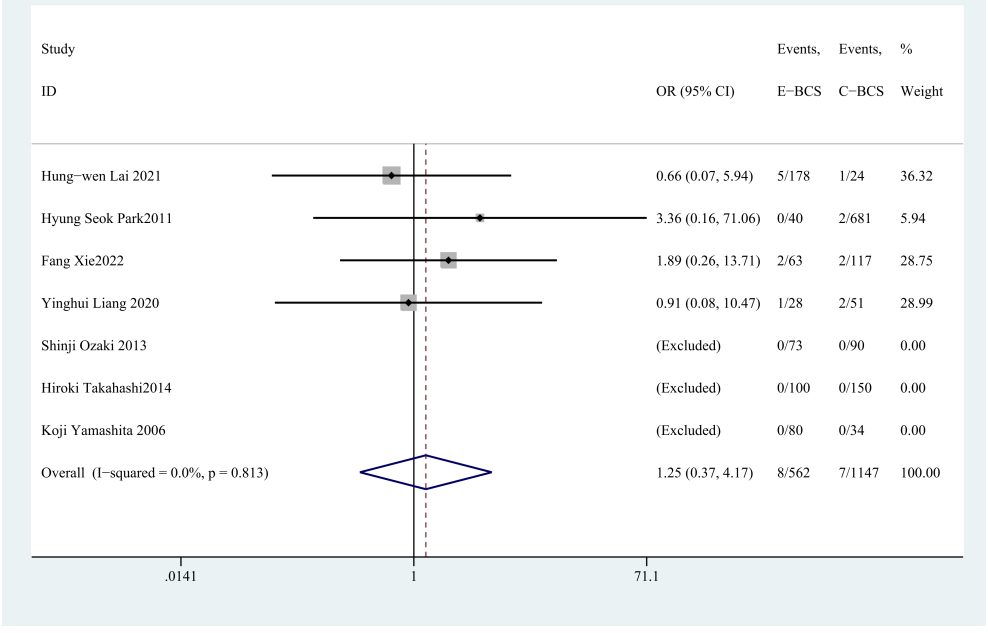

FIGURE 8
Forest plot of the meta-analysis for recurrence rate.

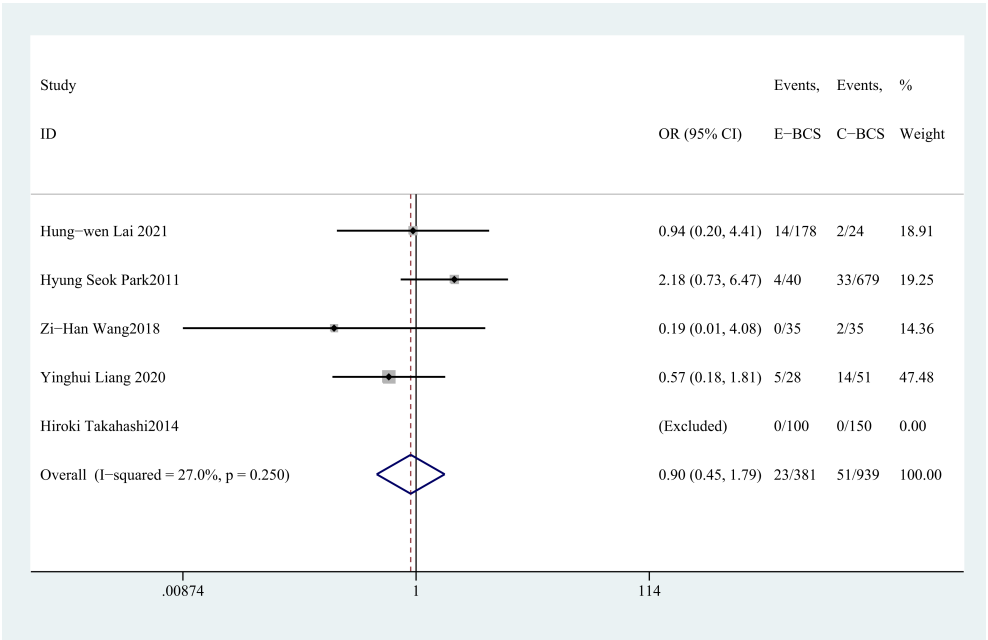

FIGURE 9
Forest plot of the meta-analysis for complication rate.

series of specialist surgical procedures. The operation's safety in regard to tumor treatment is not well-supported, and there is a chance of tumor diffusion (10). Therefore, the present meta-analysis was performed to compare the effectiveness and safety of E-BCS versus C-BCS in patients with early-stage breast cancer.

The oncological safety of E-BCS is frequently subject to scrutiny and skepticism due to doubts and worries regarding the effectiveness of resection performed through a small and inconspicuous incision (44). However, the findings of our study indicate that there was no statistically significant disparity between E-BCS and C-BCS in terms of the positive rate of tumor resection margins. Additionally, E-BCS did not exhibit a greater recurrence rate or metastasis rate when compared to C-BCS. In the E-BCS procedure, the surgeon benefited from carbon dioxide insufflation since it significantly expanded the available workspace for the surgical team, allowing them to execute the procedure with a

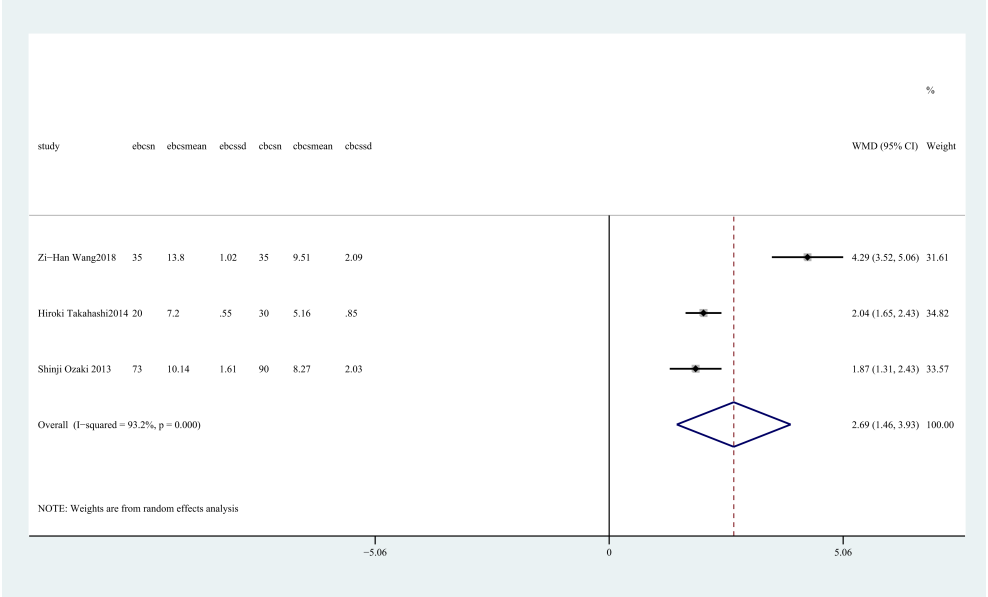

FIGURE 10
Forest plot of the meta-analysis for cosmetic score.

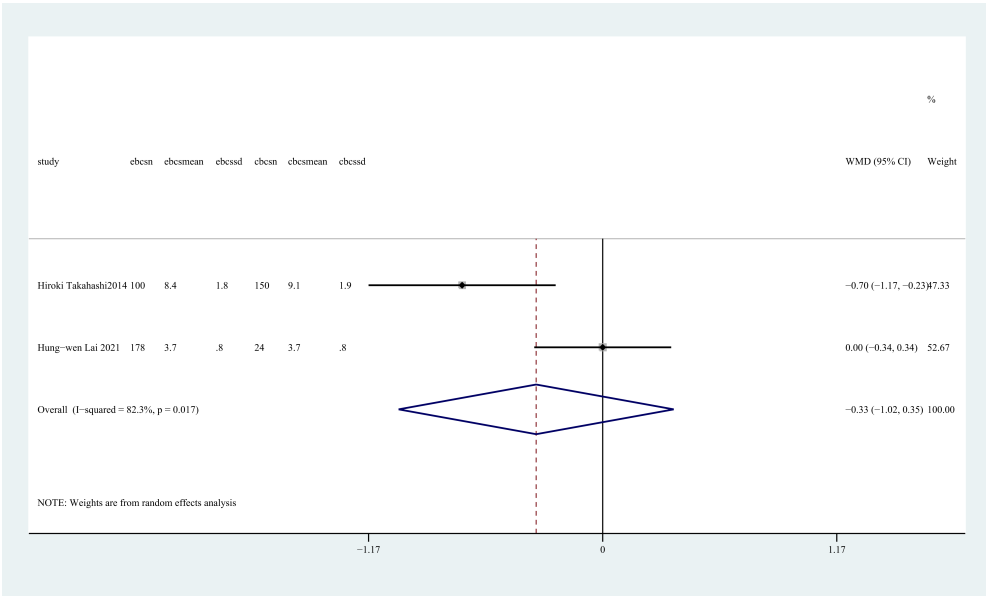

FIGURE 11
Forest plot of the meta-analysis for hospital days.

single small incision (27). Short- and medium-term oncological outcomes are not compromised since it enables tumor excision with appropriate margins. Improved visualization with light handle retractors and enhanced precision for wide excision are further benefits (43).

In comparison to C-BCS, E-BCS reduces surgical incision length and yields better cosmetic outcomes, as shown in the present meta-analysis. Consistent findings have been reported in earlier studies (43, 45–47). Compared to C-BCS, E-BCS offers a number of benefits. Firstly, the SIE-BCS offers an aesthetic benefit due to the shorter incisions required compared to C-BCS. The technique of creating the single-port incision by following the natural axillary wrinkles further enhances this benefit, as it is then concealed by the upper limb and the axillary fossa (27). The second advantage of endoscopic surgery over direct vision surgery is the increased area that can be used to separate the pectoralis major

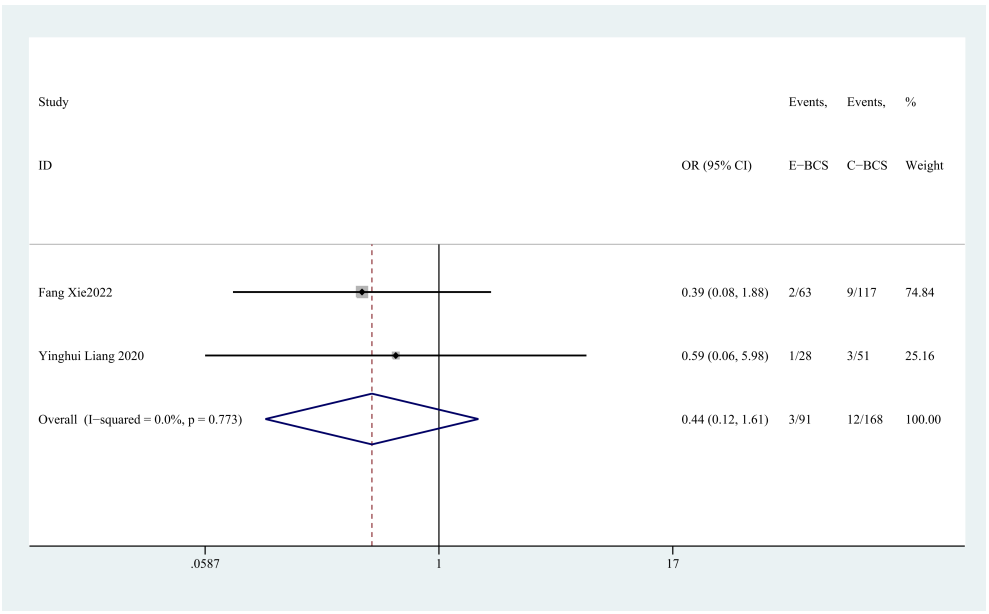

FIGURE 12
Forest plot of the meta-analysis for metastasis rate.

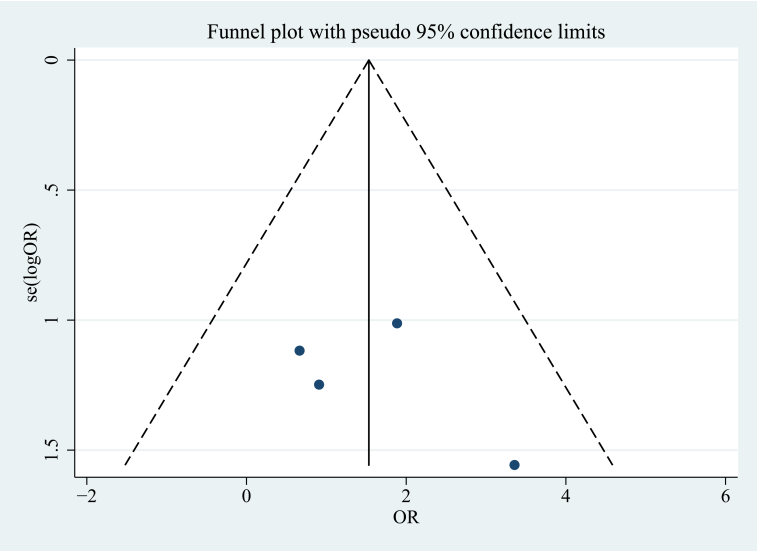

FIGURE 13
Funnel plot for recurrence rate.

muscle from its fascia. This, together with the broader skin flap that is created, enables suturing the surrounding dissected breast tissue more easier. This makes it easier for the space left behind after tissue removal to be adequately filled. Therefore, endoscopic surgery improves upon traditional breast-conserving surgery performed under direct vision in terms of cosmetic results by employing the aforementioned approaches (30).

Our findings indicated that E-BCS had a longer operation time than C-BCS. Several factors may contribute to it. To start, it's a novel surgical method, so doctors who have never done endoscopic surgery before still have a lot to learn (37). Secondly, the surgical field of E-BCS is restricted and necessitates additional time for both preparation and execution of the procedure (43, 46, 47). Eun-Kyu Lee et al (25) found that, with the exception of the 3 instances that had axillary node dissection, the early 9 cases had a substantially longer operational time (178 minutes) compared to the latter 8 cases (130 minutes) ($P < 0.001$).The duration of endoscopic surgery would be reduced if the surgeon completes a period of learning. In another study (44), it was found that using the CUSUM approach for learning curve analysis, a total of 15 cases were required to achieve a considerable reduction in operation time. Specifically, the mean operation time decreased from 208 ± 53 minutes to 121 ± 37 minutes. Once the first learning curve was overcome, the operation time continued to decrease as more case experience was gained.

As far as we know, this meta-analysis has included the largest number of articles that compare the outcomes of E-BCS and C-BCS in treating early-stage breast cancer. This could lead to a more reliable conclusion. The results of our study offer useful insights into the clinical outcomes of surgical techniques that contribute to clinical practice and research in the field of breast cancer. Nevertheless, we recognize the potential limitations of our study. Initially, we included only 11 articles that met our inclusion criteria. The results obtained from these studies were unstable due to the limited sample size. Furthermore, the meta-analysis was compromised to some extent by the fact that all the studies included were non-randomized controlled trials, which diminished its trustworthiness. Furthermore, the limited duration of the included studies was inadequate to gather a satisfactory number of target events, such as the rate of recurrence and metastasis. This may have led to the results being unreliable. Failure to account for confounding factors, such as variations in countries, case inclusion criteria, medical equipment, and surgical techniques, can lead to research heterogeneity and bias. Hence, in order to provide additional validation on the safety and effectiveness of E-BCS, it is imperative to conduct more multicenter, randomized controlled trials with extended follow-up periods.

In conclusion, our research demonstrated that E-BCS was both feasible and safe for treating early-stage breast cancer. E-BCS offers clear benefits in terms of incision length and cosmetic outcome, without showing an increased risk of recurrence or metastasis.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://doi.org/10.6084/m9.figshare.25596849.v1.

Author contributions

LL: Conceptualization, Formal analysis, Investigation, Writing – original draft. YL: Conceptualization, Formal analysis, Investigation, Writing – original draft. CL: Data curation, Supervision, Validation,

Visualization, Writing – original draft. MH: Data curation, Supervision, Validation, Visualization, Writing – original draft. WL: Funding acquisition, Resources, Writing – review & editing. TQ: Funding acquisition, Resources, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Scientific Research Foundation of Guangxi University of Science and Technology (20Z13), the Scientific Research Foundation of Guangxi Health Commission (Z-B20220927) and the Scientific Research Foundation of Guangxi Health Commission (Z-B20220930).

Acknowledgments

Everyone who contributed significantly to this study has been listed.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1419123/full#supplementary-material

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