Int. J. Pharm. Investigation, 2026; 16(2):523-535.
https://www.jpionline.org

Review Article

Cancer Stem Cells in Breast Cancer: Unlocking New Frontiers in Therapeutic Strategies

Qamre Alam[1,*], Reem Saeed Alazragi[2], Alaa Al-Kathiri[3], Alrayan Abass Albaz[4], Jawaher Mohammad Alshehri[5], Fatimah Ali Alsaffar[6], Dalia Alammari[7,8], Saleh Alqahtani[9]

[1]Department of Molecular Genomics and Precision Medicine, EXPRESSMED Diagnostics and Research, Zinj, BAHRAIN.
[2]Department of Biological Sciences, College of Science, University of Jeddah, Jeddah, SAUDI ARABIA.
[3]College of Medicine, King Saud bin Abdulaziz University for Health Sciences, King Abdullah International Medical Research Center, Jeddah, SAUDI ARABIA.
[4]Department of Oncology and Human Metabolism, Molecular Medicine Genetics, The Medical School, The University of Sheffield, Beech Hill Road, Sheffield, S10 2RX, UNITED KINGDOM.
[5]Department of Optometry, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, SAUDI ARABIA.
[6]Department of Medical Laboratory Science, AlGhad College for Applied Medical Sciences, 4177 Al Nabighah Al Dhubyani - Al Zahour District, Dammam, SAUDI ARABIA.
[7]Department of Immunology and Pathology, Faculty in Medicine Program, Ibn Sina National College for Medical Studies, Jeddah, SAUDI ARABIA.
[8]Health Professions Education Center, Ibn Sina National College for Medical Studies, Jeddah, SAUDI ARABIA.
[9]Department of Haematology, King Faisal Medical City for Southern Regions, Abha, SAUDI ARABIA.

ABSTRACT

This review thoroughly examines Breast Cancer Stem Cells (BCSCs) discussing their involvement, in tumor advancement and spread and their resistance to treatment while also discussing methods for targeting them for elimination purposes. We delve into indicators as well as the signaling pathways that control their ability to regenerate and survive; we also explore different treatment methods such as small molecule inhibitors and immune based therapies along with newer technologies, like RNA interference techniques, CRISPR/Cas9 applications and drug delivery systems driven by nanotechnology. Furthermore, it deals with the difficulties presented by adaptability, tumor diversity and interactions, within the microenvironment alongside the requirement for enhanced models and successful clinical application. The analysis brings into focus existing progressions, constraints and forthcoming paths in BCSC focused treatments underscoring their ability to improve results, in breast cancer therapy.

Keywords: TNBC, Signaling Pathways, Cancer Vaccine, OncomiRs, Drug Delivery.

Correspondence:
Dr. Qamre Alam
Molecular Genomics and Precision Medicine Department, EXPRESSMED Diagnostics and Research, Zinj, BAHRAIN.
Email: alamqa2022@gmail.com

Received: 03-09-2025;
Revised: 22-10-2025;
Accepted: 16-12-2025.

INTRODUCTION

Breast cancer is the most frequent cancer and the leading cause of cancer mortality among women globally. It is a very complex disease consisting of different molecular subtypes like luminal A, luminal B, HER2-enriched and Triple-Negative Breast Cancer (TNBC) which have different behavior and response to treatment (Testa *et al.*, 2020). Nevertheless, there has been a significant enhancement in the early diagnosis and treatment of the disease, relapse, metastasis and therapy resistance are still a problem.

Surgery, chemotherapy, radiotherapy, hormonal therapy and targeted therapy are the conventional breast cancer treatments. Endocrine therapies such as tamoxifen and aromatase inhibitors are effective in hormone receptor positive breast cancers while trastuzumab is used in the treatment of HER2 positive cases (Montagna *et al.*, 2019). However, TNBC does not have hormone receptors and HER2 expression is treated poorly and is aggressive and likely to recur. One of the biggest problems in breast cancer therapy is the development of resistance to the drugs which can be intrinsic (before the treatment) or acquired during the course of treatment (Nikolaou *et al.*, 2018). The processes that lead to the development of resistance include genetic mutations, epigenetic changes, the activation of signaling pathways and interactions with the tumor microenvironment. These mechanisms allow cancer cells to become resistant to the cytotoxic effects of the drugs resulting in treatment failure and disease progression. Metastasis is another important issue because breast cancer can spread to other parts of the body including the lungs, liver, bones and brain. Metastatic Breast Cancer (MBC) is generally incurable and current therapies are mainly palliative, aimed at increasing the length of life and enhancing the quality of life. Cancer

DOI: 10.5530/ijpi.20260153

Stem Cells (CSCs) have been found to play a crucial role in the propagation of tumors, metastasis and therapy resistance, and are a rare subset of cells in the tumor (Yadav *et al.*, 2019). These CSCs possess the ability to self-renew, to differentiate and to escape from the conventional treatments, and thus are an important candidate for new treatment strategies.

CSCs are a subpopulation of tumor cells that have the capacity to self-renew and to differentiate into multiple cell types, and are thought to be involved in the initiation of a tumor, its progression, and the development of resistance to treatment. These cells are similar to normal stem cells but their signaling pathways are dysregulated to lead to tumorigenesis. CSCs were first found in haematological malignancies and have since been found in solid tumors including breast cancer (Kai *et al.*, 2010). The existence of CSCs explains tumor heterogeneity, treatment failure and recurrence. CSCs are thought to be derived from normal stem or progenitor cells that have undergone mutations that enable them to sustain self-renewal and to initiate tumorigenesis. In contrast to conventional cancer cells that have limited proliferative capacity CSCs can give rise to the entire tumor mass. In breast cancer, CSCs are defined by surface markers such as CD44+ / CD24-, Aldehyde Dehydrogenase 1 (ALDH1) (Zhang *et al.*, 2020). These markers are linked with aggressive tumor behaviour, poor prognosis and therapeutic resistance. Metastasis is the major cause of cancer death and is driven in part by CSCs. These cells have increased migratory and invasive capabilities that allow them to leave the primary tumor, survive in the circulation, and colonize distant organs. The EMT (epithelial-to-mesenchymal transition) is involved in this process, whereby epithelial-like cancer cells assume mesenchymal characteristics to enhance their motility and resistance to apoptosis. Breast cancer stem cells are highly EMT active and can thus avoid immune surveillance and form metastases.

CSCs are a significant cause of therapy resistance and are therefore a major problem in breast cancer management (Bai *et al.*, 2018). They have numerous mechanisms of resistance. The bulk tumor cells are usually proliferating rapidly, but CSCs are usually in a quiescent or relatively slow-cycling state and thus are relatively protected from the cytotoxic effects of chemotherapeutic agents that are designed to target mitochondria of actively proliferating cells. In addition, CSCs have high expression of ATP-Binding Cassette (ABC) transporters such as ABCG2 and P-glycoprotein that efflux the chemotherapeutic drugs from the cytoplasm decreasing the effectiveness of the drugs. Furthermore, CSCs have increased capacity to repair DNA damage which enables them to survive the genotoxic stress associated with radiation and chemotherapy. In addition, CSCs also manifest defective apoptotic pathways whereby they over express anti-apoptotic proteins like BCL-2, Survivin to name but a few that enable them to survive while under therapeutic pressure (Babikian *et al.*, 2020). Finally, CSCs are located in specific niches within the TME where stromal cells, immune cells, and the extracellular matrix provide cytokines that sustain them during therapy induced stress.

The main reason for targeting BCSCs is their role in tumor heterogeneity and plasticity. BCSCs can shift their phenotype between epithelial and mesenchymal states and, therefore, can easily change their phenotype in response to the alterations in the microenvironment. This plasticity makes BCSCs to evade the therapeutic pressure and remain a source of tumor recurrence even after aggressive treatments. Thus, the eradication of the pathways Controlling BCSC plasticity such as Wnt, Notch and Hedgehog signaling may be beneficial in decreasing tumor plasticity and enhancing the response to therapy. Moreover, BCSCs play an important role in metastasis which is the major cause of death from breast cancer. These cells have increased levels of invasion and migration, which enable them to leave the primary tumor, circulate in the bloodstream and form metastases in other organs. Conventional treatments are usually inadequate to kill these circulating tumor stem cells thereby leading to disease progression and recurrence. Inhibition of BCSCs with particular inhibitors or immunotherapeutic strategies may help to prevent metastatic spread and enhance patient survival time.

An additional crucial aspect of BCSC biology that makes them an attractive therapeutic target is their resistance to conventional therapies. Chemotherapy and radiotherapy affect primarily rapidly dividing cells; however, BCSCs are slow-cycling or quiescent and thus escape treatment. Furthermore, these cells have strong DNA repair mechanisms, drug efflux transporters at high expression levels, and resistance to apoptosis, which also contribute to therapeutic failure. Metabolic inhibitors, epigenetic modulators, and RNA-based therapies that attack this resistance mechanisms might make BCSCs more amenable to treatment and less likely to return. Last, the notion of targeting BCSCs offers an opportunity to enhance the standard therapies and patient results. Combining CSC-targeted therapies with conventional treatment may allow killing of both CSCs, as well as the differentiated cancer cells and prevent tumor regrowth and lead to better long term remission rates. If future breast cancer therapies are to move away from tumor control and towards cure, then targeting BCSCs may be required to eliminate tumor cell self-renewal.

Characteristics of BCSCs

Markers

Identifying and characterizing BCSCs is crucial to developing strategic therapeutic approaches. Numerous cell surface and intracellular markers have been employed to define and sort out BCSCs, including CD44+ / CD24-, aldehyde dehydrohydrogenase 1 (ALDH1), and epithelial cell adhesion molecule (EpCAM). These markers are stemness markers and are also associated with the self-renewal capacity and tumorigenic potential of the cells.

The most well-known BCSC marker to date is the CD44+ / CD24- phenotype (Romaniuk-Drapała *et al.*, 2024). CD44 is a

transmembrane glycoprotein that is involved in cell adhesion, migration, and interaction with the tumor microenvironment, whereas CD24 is a surface protein that is mainly found in differentiated cells. BCSCs usually have high levels of CD44 and low levels of CD24 (CD44+ / CD24-), which has been connected to increased invasiveness, apoptosis resistance, and increased tumorigenicity. CD44+ / CD24- cells, which were isolated from breast tumors, have been shown to have a higher tumor-initiating capacity in xenograft models than non-CSCs. The CD44 receptor is also involved in the maintenance of CSC plasticity by binding to hyaluronic acid and activating key signaling pathways such as PI3K/AKT, Wnt/beta-catenin, and Hedgehog, thus making it a possible therapeutic target (Xu *et al.*, 2022).

The other marker that is critical for BCSCs is Aldehyde Dehydrogenase 1 (ALDH1), an intracellular enzyme that is involved in detoxification, oxidative stress regulation, and the metabolism of retinoic acid. Elevated ALDH1 activity has been characterized as a feature of stem-like cancer cells that possess increased self-renewal capacity, enhanced resistance to chemotherapy, and tumorigenicity. In initial experiments, ALDH1-positive BCSCs have been found to form tumors more effectively than ALDH1-negative cells (Elbaiomy *et al.*, 2020). Of particular importance, ALDH1 expression is also related to worse prognosis and more frequent relapse in breast cancer patients. CD44+ / CD24-, which mainly marks mesenchymal-like BCSCs, is different from CD44. ALDH1 expression is often linked to epithelial-like CSC populations, which shows the heterogeneity of BCSCs (Elbaiomy *et al.*, 2020).

Epithelial Cell Adhesion Molecule (EpCAM) is another surface marker that is frequently employed in the characterization of BCSCs. EpCAM participates in cell adhesion and signaling and its level is increased in cells with high proliferative potential, stemness and tumorigenicity (Conde *et al.*, 2022). EpCAM+ BCSCs have been found to participate in tumor formation and metastasis and their presence is related to a worse prognosis (Wu *et al.*, 2019). Also, EpCAM is involved in the maintenance of epithelial phenotype, which allows to distinguish between epithelial-like BCSCs and their mesenchymal counterparts. In addition to these primary markers, the CD133, Sox2, Oct4, and Nanog proteins have been implicated in the biology of BCSCs (Song *et al.*, 2021). These markers are linked to pluripotency, self-renewal, and therapy resistance. The complexity of BCSCs indicates that a single marker cannot capture the entire population; rather, a set of markers is usually employed to sort and classify these cells.

Role in tumor progression, metastasis and resistance

Metastasis is a complicated multi-step process that involves the shedding of tumor cells from the parent tumor, their penetration through the surrounding tissues, their penetration into the blood stream, and their lodgement in other organs including the lungs, liver, bones and brain. Due to their ability to undergo EMT (Epithelial-Mesenchymal Transition), BCSCs are well positioned for this process since they are known to possess the marked capacity to undergo EMT, a phenotypic change that enhances cell motility, invasiveness, and resistance to anoikis (apoptosis induced by the lack of cell adhesion). EMF reduces the expression of E-cadherin, a typical epithelial marker, and increases the levels of N-cadherin and vimentin, markers of mesenchymal cells, which enable the cells to migrate and invade (Fiori *et al.*, 2019). When the BCSCs arrive at a distant organ, they can return to an epithelial-like state through the reverse process known as the Mesenchymal-Epithelial Transition (MET), and form new tumor colonies and promote metastatic growth.

BCSCs are also very resistant to conventional therapies including chemotherapy, radiotherapy, and targeted therapies, and are a major determinant of treatment failure and disease relapse. One important mechanism of resistance is their quiescent or slow-cycling phenotype that renders them relatively insensitive to the chemotherapeutic agents that are designed to target mitotically active cells. Moreover, the BCSCs possess increased levels of ATP-Binding Cassette (ABC) transporters, including ABCG2 and P-glycoprotein that efflux the chemotherapeutic drugs from the inside of the cell, thus decreasing the intracellular concentration of the drugs and the effectiveness of treatment (Modi *et al.*, 2022). Furthermore, BCSCs have increased capacity to repair DNA and are thus able to survive the genotoxic stress associated with radiation and chemotherapy. This is further augmented by their apoptosis resistance, which is sustained by the overexpression of anti-apoptotic factors, including BCL-2 and survivin.

There is another major factor that is responsible for the therapy resistance and that is the interaction of BCSC with the TME (Tumor Microenvironment). The TME is composed of stromal cells, immune cells, extracellular matrix components and secreted cytokines and it creates a protective environment that allows BCSCs to survive and self-renew. The Cancer-Associated Fibroblasts (CAFs) and TAMs produce cytokines that support the stemness and therapy resistance of CSC (Gaggianesi *et al.*, 2021). Moreover, the hypoxic areas of the tumors stimulate the HIFs, which in turn activate the survival pathways of BCSCs such as Notch and Wnt signaling. The protective interactions in the TME are therefore a major problem in the attempt to eliminate BCSCs with conventional treatments.

Signaling pathways regulating BCSCs

Several signaling pathways are there that regulate the BCSCs and their differentiation. The Wnt signaling pathway is necessary for the preservation of the BCSCs' stemness and self-renewal (Weerackoon *et al.*, 2021). The canonical Wnt signaling pathway is triggered when Wnt ligands interact with Frizzled receptors and low-density Lipoprotein Receptor-Related Protein (LRP)

co-receptors, which result in the stabilization and nuclear localization of β-catenin (Hu *et al.*, 2024). Once in the nucleus, β-catenin interacts with transcription factors to activate genes that are involved in stemness, proliferation, and survival. The Wnt pathway is therefore dysregulated in BCSCs, which in turn increases the propensity of forming tumors, becoming therapy resistant, and metastasizing. Nevertheless, non-canonical Wnt signaling, which occurs independently of β-catenin, has been found to regulate BCSC plasticity and migration. Blocking Wnt signaling with small molecule inhibitors or monoclonal antibodies seems like a good way to deplete BCSCs and thus improve the overall treatment results.

Another important pathway that regulates the BCSCs and their differentiation is the Notch signaling pathway. Notch signaling is switched on by the binding of Delta-like or Jagged ligands to Notch receptors, which results in cleavage of the Notch Intracellular Domain (NICD) (Wang *et al.*, 2022). NICD is released into the nucleus where it triggers the expression of genes that are involved in stemness, proliferation and apoptosis resistance. Aberrant Notch signaling in BCSCs leads to tumor growth, metastatic spread, and resistance to chemotherapy and radiotherapy. Notch signaling also participates in the crosstalk between BCSCs and the TME by facilitating immune suppression and angiogenesis. *In vitro* studies have shown that inhibitors of γ-secretase, an enzyme required for the activation of Notch, can eliminate BCSCs and improve the efficacy of standard therapies (Ray *et al.*, 2024).

The Hh signaling pathway is required during embryonic development and tissue regeneration, but it is overactivated in different cancers, including breast cancer. The Hh pathway is activated when one of the Hh ligands (Sonic Hedgehog, Indian Hedgehog, or Desert Hedgehog) binds to the patched receptor, which in turn removes the inhibition of transmembrane protein Smoothened (SMO) (Jing *et al.*, 2023). This results in the activation of Gli transcription factors that induce the expression of genes that support the self-renewal, cell survival, and chemoresistance phenotype (Citarella *et al.,* 2023). Hedgehog signaling pathway is responsible for the aggressive nature of the tumor and therapy resistance through the maintenance of stemlike properties and EMT. Small molecule inhibitors of SMO or Gli transcription factors are potentially useful for the treatment of BCSC-driven tumor growth.

The PI3K/AKT signaling pathway is involved in the regulation of cell survival, metabolism, and proliferation of breast cancer, including BCSCs. The activation of Phosphoinositide 3-Kinase (PI3K) is auto- or paracrine through Receptor Tyrosine Kinases (RTKs) or G protein-coupled receptors, which results in the activation of AKT through phosphorylation. This cascade brings about survival by suppressing apoptosis and increasing the expression of stemness markers. The PI3K/AKT pathway is cross talked with Wnt and Notch pathways to sustain BCSCs and to make them resistant to targeted therapies (Jan *et al.*, 2025). Mutations in components of the PI3K pathway, including PIK3CA, are common in breast cancer and confer resistance to therapy. Current studies are ongoing on the use of PI3K and AKT inhibitors to kill BCSCs and make the tumors sensitive to conventional treatments.

Other pathways, such as JAK/STAT, NF-κB, and Hippo/YAP, also participate in the maintenance of BCSCs and resistance (Shabna *et al.*, 2023). These pathways are interlinked in a combinatorial manner to support the stemness and adaptive outcome of BCSCs during the stress of therapy. It is, therefore, important to know the detailed mechanisms of crosstalk between these signaling pathways in order to design effective combination therapies that can home in on BCSCs and prevent tumor recurrence.

Therapeutic strategies targeting BCSCs

The therapeutic approaches aimed at BCSCs aim at the elimination of their stemness, that is their ability to self-renew and to resist conventional treatments. Inhibitors of small molecules that target Wnt, Notch and Hedgehog pathways disrupt the signaling networks that are critical in maintaining BCSCs. Monoclonal antibodies, checkpoint inhibitors of the immune system, and CAR T/NK cell therapies help the immune system to recognize and kill the BCSCs. Also, therapeutic approaches like siRNA, miRNA and CRISPR/Cas9 based gene editing are used to modulate the stemness associated genes precisely. Nanoparticle based drug delivery and combination therapy with chemotherapy or radiation therapy increases the treatment outcome and also decreases the probability of relapse (Figure 1).

Small molecule inhibitors

Using small molecule inhibitors of self-renewal pathways of BCSCs has been proposed to target therapy resistant tumor cells and prevent recurrence. The Wnt, Notch and Hedgehog signaling pathways are crucial to the self-renewal, survival and plasticity of BCSCs and their aberrant activation is involved in tumor progression, metastasis and resistance to standard treatment. The pathways are currently under investigation in preclinical and clinical studies for the development of small molecule inhibitors as novel therapeutic agents for breast cancer.

Wnt pathway inhibitors are meant to prevent the hyperactivation of β-catenin signaling that is required for the stem like properties of BCSCs. Among them, small molecules that target Wnt ligands, Frizzled receptors or downstream β-catenin activity have been developed. LGK974 is a porcupine inhibitor and has the potential to decrease BCSC populations in breast cancer models through the inhibition of Wnt ligand secretion (Romaniuk-Drapała *et al.*, 2024). XAV939 is a tankyrase inhibitor that enhances the degradation of β-catenin and suppresses Wnt driven tumor growth (Das *et al.*, 2025). Furthermore, PRI-724, a CBP/β-catenin interaction inhibitor, prevents the transcription of Wnt target genes and decreases the formation of BCSCs that lead to tumor

initiation (Peri *et al*., 2023). However, like other Wnt inhibitors, these compounds have toxicity issues and also, the Wnt pathway is important in the normal tissue homeostasis, and thus, has a complex redundancy.

Notch signaling pathway is also a crucial therapeutic target in BCSCs, their maintenance, survival and therapy resistance. γ Secretase Inhibitors (GSIs), such as DAPT and RO4929097, inhibit cleavage of Notch receptors, which prevents the release of NICD and subsequent gene activation (Wang *et al.*, 2024). These inhibitors have shown the capacity to affect the self-renewal of BCSC and to render tumors more sensitive to chemotherapy and radiotherapy. Nevertheless, owing to the important role of Notch signaling in normal tissue development and immune function, systemic toxicity is still a major problem. Newer, more specific Notch inhibitors are being explored, such as CB-103, which specifically targets NICD transcriptional activity. Monoclonal antibodies against Notch receptors like Brontictuzumab (against Notch1) are also being investigated to increase the specificity and decrease the side effects.

The Hh signaling pathway is also a crucial determinant of the BCSC self-renewal and drug resistance. Many Hedgehog inhibitors have been created to inhibit SMO, a key activator of the pathway. Vismodegib (GDC-0449) and Sonidegib (LDE225) are SMO inhibitors approved for basal cell carcinoma and are being investigated in breast cancer (Lear *et al.*, 2023). These inhibitors act on BCSCs through the suppression of Gli transcription factors to prevent tumor growth. However, SMO inhibitors are known to develop resistance through mutations in the downstream signaling effectors, such as SUFU and Gli proteins. To overcome this, direct Gli inhibitors such as GANT61 are being investigated as an alternative strategy (Wang *et al.*, 2024).

Beside small molecules, epigenetic regulation is also involved in the sustainment of the plasticity, self-renewal, and therapy resistance of BCSCs. While genetic mutations result in changes in the DNA sequence, epigenetic modifications involve reversible changes in gene expression through DNA methylation, histone modifications, and chromatin remodelling. These epigenetic mechanisms are utilized by BCSCs to sustain their stem-like properties and escape standard regimens. Hence, inhibition of epigenetic modifiers, including Histone Deacetylase (HDAC) and DNA Methyltransferase (DNMT) inhibitors, is a promising concept to target BCSCs and treat therapy resistance. HDAC inhibitors are under consideration as anti-BCSC agents because they can reverse the abnormal epigenetic silencing and induce the differentiation of CSCs to non-tumorigenic cells (Landeros *et al.*, 2023). HDACs deacetylate histones, thereby causing chromatin compaction and suppression of the expression of tumor suppressor genes. HDAC activity is inhibited, thereby allowing the expression of differentiation-associated genes and the sensitization of BCSCs to chemotherapy. Vorinostat (SAHA) and Panobinostat have been found to display preclinical efficacy in decreasing BCSCs through the inhibition of stemness pathways such as Wnt, Notch, and Hedgehog in preclinical studies (Alalhareth *et al.,* 2025; Jung *et al.,* 2024). Entinostat, a selective HDAC1/HDAC3 inhibitor, has been found to have potential to suppress the self-renewal of BCSCs and improve the response to immune checkpoint inhibitors in breast cancer models (Jung *et al.,* 2024). Further, the use of DNMT inhibitors to target aberrant DNA hypermethylation, a mechanism by which BCSCs keep tumor suppressor genes silenced and thus undifferentiated, has been explored. DNMT inhibitors such as AZA (5-azacytidine) and DAC (decitabine) are incorporated into DNA and counteract the function of DNMT, resulting in the reactivation of genes that are involved in differentiation, apoptosis and immune recognition (Zhou *et al.*, 2023). DNMT inhibitors have been found to decrease the BCSC fractions and make the tumors more sensitive to the standard treatments in breast cancer. Moreover, the combination of DNMT and HDAC inhibitors has been found to have synergistic effects in the reversal of epigenetic reprogramming of BCSCs, thus increasing the curative potential.

Beyond HDAC and DNMT inhibitors, other epigenetic modulators, including Bromodomain and Extra-Terminal (BET) inhibitors, and EZH2 inhibitors are investigated for their role in regulating BCSC (Kim *et al.*, 2023; Mahendran *et al.*, 2024). BET inhibitors such as JQ1 block the binding of BET proteins to acetylated histones, which blocks the transcriptional programs that support CSC propagation (Gargano *et al.*, 2023). EZH2 is a key component of the Polycomb Repressive Complex 2 (PRC2), which catalyses histone methylation and suppresses the expression of differentiation-related genes. EZH2 inhibitors such as Tazemetostat have been found to reverse CSC associated epigenetic alterations and decrease tumor propagating capacity. Epigenetic modulators are therefore a new class of targets for the elimination of BCSCs, by erasing the genes that enable stemness and treatment resistance. However, issues including, side effects, toxicities, and the dynamic nature of epigenetic modifications are still a concern (Masoudi *et al.,* 2024). Current studies and clinical investigations are aimed at enhancing these agents and finding ways to combine them with other therapies to enhance the overall outcomes of breast cancer treatment.

BCSCs have specific metabolic changes that enable them to sustain themselves, proliferate and tolerate therapy. Not like the general tumor cells which have their metabolism based on the glycolytic pathway (Warburg effect), BCSCs are able to switch between the glycolytic and Oxidative Phosphorylation (OXPHOS) depending on the conditions (Ali *et al.*, 2024). This metabolic flexibility allows BCSCs to endure stress, resist standard treatments, and cause tumor recurrence. Metabolic pathways such as mitochondrial metabolism, glycolysis and lipid metabolism have been identified as possible targets in the efforts to eliminate BCSCs. The application of mitochondrial inhibitors to block OXPHOS and ATP production in BCSCs is also being investigated.

Most BCSCs, especially in the hypoxic regions of the tumor, depend on mitochondria for energy production. Metformin, a commonly used oral diabetes drug, has been found to have a strong effect on targeting BCSCs by attacking mitochondrial complex I, decreasing ATP production and inducing oxidative stress apoptosis (Ali *et al.*, 2024). Other OXPHOS inhibitors such as IACS-010759 that targets mitochondrial complex I has also been found to be effective in targeting BCSCs by disturbing their metabolic homeostasis (Jiao *et al.*, 2023). Moreover, MitoQ, a mitochondria-targeted antioxidant, induces ROS imbalance in BCSCs, leading to their selective elimination (Rondeau *et al.*, 2024). Inhibitors of glycolysis aim at the increased glucose metabolism which is a characteristic of BCSCs and prevents these cells from meeting their energy requirements. 2-Deoxy-D-glucose (2-DG) is a glucose analog that acts as a competitive inhibitor of hexokinase, the first enzyme of glycolysis, resulting in ATP depletion and death of the glycolysis-dependent BCSCs (Zhuo *et al.*, 2020). Lonidamine is also a glycolysis inhibitor that interferes with hexokinase and mitochondrial functions, that leads to energy deprivation and reduction in tumorigenicity (Orlovskiy *et al.*, 2024). Furthermore, Dichloroacetate (DCA) acts as PDH activator, thereby switching the metabolism from glycolysis to OXPHOS and hence increases the sensitivity of BCSCs to apoptosis (Schoenmann *et al.*, 2023). Besides the two major metabolic pathways of glycolysis and OXPHOS, BCSCs also depend on lipid metabolism for their survival and therapy resistance. Enhanced Fatty Acid Oxidation (FAO) has been associated with the propagation of BCSCs and their metastasis. Etomoxir, a CPT1 (Carnitine Palmitoyltransferase) inhibitor, blocks FAO and affects the energy equilibrium in BCSCs, thus rendering them more vulnerable to chemotherapy (Du *et al.*, 2024). That, in turn, opens new possibilities for using statins, inhibitors of cholesterol synthesis, for the suppression of BCSC proliferation and self-renewal. Metabolic targeting strategies are particularly effective when used in conjunction with conventional therapies, and BCSCs often evade treatment by altering their metabolic state. These inhibitors significantly hold promise to overcome therapy resistance and decrease tumor recurrence in breast cancer by disrupting key metabolic pathways. However, challenges like systemic toxicity, metabolic compensation, and patient heterogeneity must be addressed to enhance their clinical effectiveness to the maximum.

Immunotherapy approaches

Antibodies to BCSC markers are attached to monoclonal antibodies, which are now coming out as a potential means of selectively killing BCSCs with minimal effect on normal cells. BCSCs are characterized by the expression of specific surface markers, such as CD44+CD24-, ALDH1, and EpCAM, which differentiate them from general tumor cells and are required for their self-renewal, survival, and metastasis. Monoclonal antibodies act by recognizing these markers, blocking their signaling, and inducing immune-mediated cytotoxicity and the efficacy of conventional therapies. Antibodies to CD44 have been studied in detail because CD44 is involved in the regulation of BCSC stemness, tumor progression, and chemotherapy resistance (Saha *et al.*, 2022). CD44 interacts with Hyaluronic Acid (HA) present in the tumor microenvironment and triggers signaling pathways such as PI3K/AKT and MAPK. The CD44 monoclonal antibody RG7356 exhibits anti-tumor activity by interfering with CD44 signaling and promoting ADCC (Antibody-Dependent Cellular Cytotoxicity) (MacLean *et al.*, 2024). Moreover, the existence of bispecific antibodies that recognize CD44 and immune checkpoint molecules is under investigation to improve immune attacks on BCSCs.

Epithelial Cell Adhesion Molecule (EpCAM) a known marker for BCSC plays a role in cell adhesion and tumor progression processes in the body. Researchers have been exploring the use of Catumaxomab - an antibody that targets both EpCAM and CD3 for treating EpCAM positive cancers by activating T cells to eliminate BCSCs (Gamage *et al.*, 2023). Another antibody known as Edrecolomab has also been tested in trials as a treatment option for EpCAM related conditions (Dogbey *et al.*, 2024.).; however, its effectiveness varies among individuals. Innovative Antibody Drug Conjugates (ADCs) that focus on targeting EpCAM are currently, under development to improve the destruction of BCSC specifically. Antibodies that target ALDH (Aldehyde Dehydrogenase 4 Family Member A Protein) known for its Role in detoxifying Oxygen Species (ROS) and supporting the survival of BCSC are currently under investigation in early-stage development efforts [51] as therapies when used in combination with chemotherapy or immune checkpoint inhibitors to improve treatment outcomes for patients with breast cancer. In addition to the focus on ALDH targeting antibodies and their potential benefits for survival under stress conditions and ROS detoxification processes is being explored further through investigating monoclonally targeting other proteins associated with BCSC like CD133 and CD47 along with integrins to broaden the scope of treatments available, for breast cancer patients (Ali *et al.*, 2024). CD133 targeting antibodies have displayed potential, in animal studies by reducing the ability of tumors to grow and spread; on the hand antiCD47 antibodies are designed to boost the ability of cells called macrophages to eliminate breast cancer stem cells through phagocytosis. Volociximab which targets integrin like aβ integrin disrupt cell adhesion and invasion thus limiting the spread of cancer to other parts of the body (Teoh *et al.*, 2025; Ranga *et al.*, 2025). Immune Checkpoint Inhibitors (ICIs) have greatly changed cancer therapy by enhancing anti-tumor immunity and there is growing interest in their use in the management of BCSCs. BCSCs possess immune-evasive capabilities that include the expression of immune checkpoint molecules such as PD-1/PD-L1 (programmed death-1) and CTLA-4, which enable them to evade immune surveillance and promote tumor growth and therapy resistance. ICIs work by

reversing this suppression, thereby enabling the immune system to act against BCSCs. PD-1/PD-L1 Inhibitors have shown promising results in breast cancer, especially in TNBC where BCSCs are enriched. PD-1 is an inhibitory receptor on T cells; its ligand, PD-L1, is overexpressed on BCSCs and tumor-associated immune cells. This interaction checks T cell activation, thereby enabling BCSCs to escape immune surveillance. The FDA-approved PD-1/PD-L1 inhibitors, including Pembrolizumab (anti-PD-1) and Atezolizumab (anti-PD-L1), have demonstrated clinical benefit in TNBC by reactivating T cells against tumors. Preclinical studies suggest that BCSC populations are particularly sensitive to PD-1/PD-L1 blockade, especially when combined with chemotherapy or radiation therapy, which increases PD-L1 expression on cancer cells.

CTLA-4 (Cytotoxic T-Lymphocyte Associated Protein 4) Inhibitors act on still one more immune checkpoint that controls T cell activation. CTLA-4 is an inhibitory receptor on T cells that acts by competing with CD28 for B7 ligands binding and thus down modulating the immune responses. BCSCs use CTLA-4 signaling to sustain an immunosuppressive microenvironment. Ipilimumab, a CTLA-4 blocking antibody, has been investigated in breast cancer and has shown potential in improving T cell infiltration into the tumors. However, owing to its systemic immune activating properties, CTLA-4 blockade is usually accompanied by higher immune related toxicities (Zhang *et al.*, 2023). Combination of immune checkpoint inhibitors with other BCSC targeting therapies is believed to be a promising way of circumventing resistance. Epigenetic modulators, such as DNMT and HDAC inhibitors, are believed to increase the expression of immune checkpoint ligands on BCSCs thus making them amenable to ICIs. Also, metabolic reprogramming strategies, including OXPHOS inhibitors, may remove the energy advantage of BCSCs and thus make them more vulnerable to immune attack.

Cancer vaccines

Cancer vaccines are a novel type of immunotherapy that seek to induce an immune response against the BCSCs. This is because conventional therapies are known to fail to eliminate BCSCs due to their immune evasion but cancer vaccines improve on the durability of immune memory to decrease the likelihood of tumor return or spread. Dendritic Cell (DC)-based vaccines, peptide-based vaccines, and whole-cell vaccines are under investigation for the possibility of targeting BCSCs in breast cancer (Keshavarz *et al.*, 2023). In addition to the challenges associated with CSCs, the management of breast cancer is also hindered by the ineffectiveness of current therapeutic approaches that result in Normal Stem Cell (NSC) damage.

Dendritic Cells (DCs) are Antigen Presenting Cells (APC) of professional type, which are involved in the initiation of antitumor immunity. Anti-tumor immunity is primed by DC based vaccines by recognizing BCSC specific antigens and stimulating T cells to induce cytotoxic responses. These vaccines are made by extracting patient's DCs, presenting them with Tumor Associated Antigens (TAAs) or BCSC markers (CD44, EpCAM, ALDH1) then returning them to the patient to stimulate an immune response against the tumor. Sipuleucel-T is one of

Figure 1: Hypothetical model showing integrated multi-targeted therapy for BCSC eradication.

the most investigated DC based vaccines in breast cancer despite being initially developed for prostate cancer and showing a way for BCSC targeted immunotherapy (Gul *et al.*, 2021). The previous studies have demonstrated that BCSC specific DC vaccines are effective in inducing T cells to recognize and kill BCSCs, and this combination may be particularly effective when used with immune checkpoint inhibitors or chemotherapy (Vasileiou *et al.*, 2024).

Peptide based vaccines employ short sections of tumor antigens to trigger reactions, against BCSCC cells in the body. They commonly target antigens like Mucin. 11 HER 22 and Survivin that are often found in amounts in BCSCC cells (Pallerla *et al.*, 2021). The E75 peptide vaccine, known as Nelipepimut S hails from the HER 22 protein has demonstrated potential in diminishing breast cancer recurrence rates by boosting HER 22. CTL responses (Liu *et al.*, 2023). On the other hand, whole cell vaccines such, as GVAX utilize engineered tumor cells to elicit a broad immune reaction (Swathi *et al.*, 2025). These vaccines have the potential to improve their effectiveness, in eradicating cancer cells that're resistant to treatment by incorporating BCSC related markers into their design. The use of CAR T cell therapy has shown promise in combating BCSCs (Yang *et al.*, 2022). This involves modifying T cells to identify and remove these cell populations effectively.

Chimeric antigen receptor CAR T cells are altered at the level to produce receptors that can recognize specific BCSC related markers, like CD44 EpCAM and HER2 (Manian *et al.*, 2025). CAR T cells differ from T cell responses as they can identify tumor antigens without relying on histocompatibility complex which enhances their ability to target immune evading BCSC cells effectively.

The current literature suggests that CD44 directed CAR-T cells can effectively eliminate BCSCs and suppress tumor growth in breast cancer preclinical models. As CD44 is a crucial determinant of BCSCs' self-renewal and metastasis, CAR-T cell therapy aimed at this marker may hold promise in preventing tumor recurrence. Indeed, EpCAM-targeted CAR-T cells have also shown strong antitumor efficacy through recognizing and eliminating EpCAM-positive BCSCs. HER2-directed CAR-T cells are also being investigated in HER2 positive breast cancer settings where BCSCs are known to contribute to therapy resistance. But problems including tumor heterogeneity, antigen escape, and the immunosuppressive tumor microenvironment have been reported to restrict the efficacy of CAR-T therapy in solid tumors such as breast cancer. To address these challenges, researchers are investigating approaches such as dual-target CAR-T cells (CD44/EpCAM or HER2/PD-L1), armed CAR-T cells with cytokine assistance (IL-12, IL-15), and CAR-T cells with immune checkpoint blockade (PD-1 knockout CAR-T cells) to improve persistence and efficacy towards BCSCs.

Immunotherapy with Natural Killer (NK) cells presents another strategy that differs from the CAR-T cell therapy (Figure 2). T cells have a more limited ability to identify and kill tumor cells because they require MHC-dependent recognition. Since BCSCs are resistant to adaptive immunity, NK cells are particularly effective at recognizing and killing them. Cytotoxicity is achieved by NK cells through the release of perforin and granzymes, ADCC, and cytokine production (IFN-γ, TNF-α). CAR-NK cells are genetically engineered to express receptors that recognize BCSC markers such as CD133, EpCAM, or NKG2D ligands to increase their directedness and potency (Maddineni *et al.*, 2022). CAR-NK cells are reported to have reduced likelihood of CRS and GVHD compared to CAR-T cells, which makes them a safer option for BCSC targeted immunotherapy. The source of NK cells for application includes umbilical cord blood and induced Pluripotent Stem Cell (iPSC)-derived NK cells, which are exploitable and universal banked for possible use in the clinic (Albinger *et al.*, 2021).

Gene therapy and RNA-based therapies

CRISPR/Cas9 technology has become a tool, in the fight against BCSC targeting genes essential for their survival and ability to resist treatments effectively. Unlike therapies that struggle to eradicate BCSC due to their adaptability and defense mechanisms, CRISPR/Cas9 allows accurate genetic alterations to hinder activities at a molecular level. CRISPR/Cas9 shows potential in targeting cells by disabling important transcription factors like SOX2 and OCT4 that play a crucial role in sustaining the growth and versatility of these cells (MacLean *et al.*, 2024). Preclinical trials have shown that removing SOX2 using CRISPR significantly decreases the ability of cells to initiate tumors and spread them further (Wu *et al.*, 2021). Targeting NANOG and OCT 3/4 has also been demonstrated to trigger the differentiation of BCSCS into tumorigenic cells which increases their susceptibility to conventional treatments (Mohapatra *et al.*, 2022).

One approach involves disrupting communication pathways that control the survival of BCSC such, as the Wnt and Notch pathways along with Hedgehog and PI3K/AKT pathways. Deactivating β CTNNB a crucial player in the Wnt pathway using CRISPR/Cas9 technology causes a decline in growth and tumor development (Martins-Neves *et al.*, 2023). Similarly targeting NOTCH or SMO (for the Hedgehog pathway) lowers stem cell characteristics boosts sensitivity, to chemotherapy treatments (Nagampalli *et al.*, 2025). These gene editing methods offer a means to halt communication routes that support BCSCs survival. CRISPR/Cas9 can also be applied to make BCSC more responsive, to chemotherapy and immunotherapy by deactivating genes that cause drug resistance like ABCB (MDR) which produces P glycoprotein that plays a role in chemoresistance and has been successfully targeted with CRISPR to boost sensitivity to chemotherapy treatments (Saha *et al.*, 2022). Similarly editing PD L expression, in BCSC can help

immune cells identify them better and boost the effectiveness of checkpoint inhibitors.

RNA interference (RNAi) is a novel treatment strategy that exploits small RNA molecules to knock down specific genes, which is a better targeted way of attacking BCSCs (Kar *et al.*, 2023). BCSCs are responsible for tumor formation, growth, metastasis and treatment resistance; thus, siRNAs and miRNAs have been considered as potential targets in the management of BCSCs. Therefore, RNAi therapy, through selective gene silencing, may be able to address the challenges of current therapies and enhance the overall quality of care for patients.

siRNA-Based Strategies

siRNAs are small double stranded RNA molecules that binds to complementary mRNA leading to degradation of mRNA and inhibition of protein synthesis. In the case of BCSCs, siRNA-based therapies have been developed to knock down genes that are stake holders in stemness, self-renewal, therapy resistance and immune evasion. One important target is SOX2, a transcription factor that is play a crucial role in regulating self-renewal of BCSC. Studies have indicated that SOX2 gene can be turned off through siRNAs, which in turn reduces tumor propagation and increases the tendency of the cells to undergo differentiation, thus making the BCSCs vulnerable to conventional treatment (Serej *et al.*, 2021). Other important transcription factors such as NANOG and OCT4 have also been successfully knocked down with siRNA, which leads to reduced tumorigenicity (Li *et al.*, 2022).

Another promising strategy of siRNA-based therapy is to target signaling pathways that are crucial for the propagation of BCSCs. The Wnt, Notch and Hedgehog pathways are stromal factors that are important for the propagation and therapy resistance of BCSCs. The siRNA mediated depletion of β catenin (CTNNB1) has been found to suppress BCSC proliferation and tumor growth (Kevat *et al.*, 2025; Nogueras Pérez *et al.*, 2023). Likewise, blocking NOTCH1 or JAGGED1 in the Notch pathway reverses the self-renewal ability while inhibiting SMO or GLI1 in the Hedgehog pathway eliminates the stem like properties of BCSCs (Li *et al.*, 2021). and hence can be sensitized to chemotherapy. The efficacy of siRNA therapy in combating chemoresistance in BCSCs has also been investigated. Most of the BCSCs possess the properties of drug efflux pumps such as ABCB1, which transports chemotherapy drugs out of the cells thus decreasing the effectiveness of treatment. By using siRNA to silence the ABCB1 gene, chemosensitivity is reinstated by blocking the efflux of drugs and thus allowing for a more efficient eradication of the BCSCs. In the same manner, knocking down BCL-2, an anti-apoptotic protein increases the rate of apoptosis in BCSCs thus making them sensitive to chemotherapy and radiotherapy.

miRNA-Based Strategies

miRNAs are small, endogenous, non-coding RNAs that translate multiple target mRNAs by inhibiting their translation. In breast cancer, the expression of certain miRNAs is decreased (tumor suppressive miRNAs) or increased (oncogenic miRNAs, also known as oncomiRs) in BCSCs, which in turn affect their proliferation, metastasis and therapy resistance (Cavallari *et al.*, 2021).

Using miRNA mimics to replace lost tumor suppressive miRNAs has the ability to suppress BCSC function. miR-34a is a well-known tumor suppressor that directly targets NOTCH1 and BCL2 to block BCSC self-renewal and induce apoptosis (Ma *et al.*, 2021). The EMT is a key process that allows BCSCs to invade and metastasize, and miR200c is a crucial miRNA in this inhibition. It was reported that ZEB1/2, transcription factors that promote EMT, are suppressed by over expression of miR200c, which in turn reduces the metastatic potential (da Silvia Lima *et al.*, 2024). Another important miRNA, let7, is down regulated in BCSCs and this miRNA targets oncogenes such as HMGA2 and RAS to suppress tumor initiation capacity (Nogueras Pérez *et al.*, 2023).

oncomiRs that support BCSC survival can also be targeted using miRNA inhibitors (antagomiRs). miR21 is one of the most highly expressed oncomiRs in breast cancer and it enhances therapy resistance by repressing tumor suppressors PTEN and PDCD4 (Mir *et al.*, 2024). Using antagomiRs to target miR21 restores PTEN activity, leading to decreased proliferation and increased chemotherapy sensitivity. Similarly, miR155 promotes the BCSC phenotype by activating the PI3K/AKT signaling pathway to increase survival and self-renewal. Reduction of miR155 has been reported to decrease the BCSC population and enhance the efficacy of targeted therapies (Mirchandani *et al.*, 2024).

Nanotechnology-based targeting

One of the most promising strategies to effectively eradicate CSCs is nanoparticle-based drug delivery, which enables the precise, targeted and sustained release of drugs to CSC populations with minimal impact on normal cells (Beach *et al.*, 2024). Nanoparticles have several advantages including increasing the solubility of the drug, increasing the circulation time of the drug, site specific drug delivery and controlled drug release and the ability to cross biological barriers which are otherwise inaccessible to conventional drug delivery systems. The different nanocarrier systems developed to target breast cancer stem cells include lipid-based nanoparticles, polymeric nanoparticles, metallic nanoparticles and extracellular vesicles. Lipid nanoparticles such as liposomes and SLN (solid lipid nanoparticles) have been most commonly used because they are biocompatible and can encapsulate hydrophobic drugs (Tang *et al.*, 2021). Selective targeting of CSCs with liposomes loaded with agents such as doxorubicin, paclitaxel or salinomycin has also been found to be effective. Polymeric nanoparticles such as those prepared

from materials like PLGA or chitosan enable the controlled and sustained release of drugs, which reduces the frequency of dosing (Montazersaheb *et al.*, 2023). Metallic nanoparticles including gold, silver and iron oxide nanoparticles have emerged due to their surface properties and potential to use in theranostics (combination of therapy and imaging). Gold nanoparticles conjugated with siRNA or small molecule inhibitors of CSC signaling pathways (Wnt, Notch, Hedgehog, etc.,) has been found to be effective in suppressing CSCs self-renewal (Fatima *et al.*, 2024). Hyperthermia therapy is another potential application of magnetic nanoparticles, especially iron oxide-based formulations, which can be used to induce localized heating to kill BCSCs (Safa *et al.,* 2022).

To increase the nanoparticle drug delivery to CSCs specificity, ligand targeting has been considered. CSCs of breast cancer can be distinguished by surface markers including CD44, CD133, EpCAM and ALDH1 (Wu *et al.*, 2021). Antibody or ligand-functionalized nanoparticles selectively accumulate in CSCs through their attachment to these markers. For instance, BCSCs-targeting hyaluronic acid-coated nanoparticles have been created to transport chemotherapeutic agents and small-molecule inhibitors to CSC populations using CD44 ligand, leading to a reduced tumor recurrence (Giuli *et al.*, 2021). In addition, nanoparticles conjugated with anti-EpCAM antibodies have shown promising results in the targeting and elimination of BCSCs in preclinical studies. Stimuli-responsive nanoparticles have also been used which get activated to release the drug in response to change in pH, temperature, enzymes or redox in the tumor environment (Yang *et al.*, 2020). Because CSC niches are usually acidic and hypoxic, pH-sensitive nanoparticles have been created to deliver drugs within CSC-rich areas with less impact on other tissues. Enzyme-responsive nanoparticles that are degraded by tumor-associated proteases also enhance the specificity of drug delivery and reduce the side effects systemically.

Combination strategies with conventional therapies

Chemotherapy and radiation therapy are commonly used to treat breast cancer as the methods of treatment; however, their effectiveness can be limited due, to the presence of BCSC. Breast Cancer Stem Cells which naturally resist these treatments well by having DNA repair mechanisms and high levels of drug transporters that reduce the effects of standard treatments by going into a dormant state as a way to evade them. Bringing together chemotherapy and radiation therapy, with inhibitors that target BCSC has shown promise in improving treatment outcomes and reducing the chances of cancer returning in the future (Table 1).

Chemotherapy is effective, in fighting tumor cells. Sometimes doesn't completely eliminate BCSCs leading to cancer recurrence and spreading to other parts of the body (metastasis). When chemotherapy drugs are given along with inhibitors targeting pathways such as those related to the Notch and Hedgehog

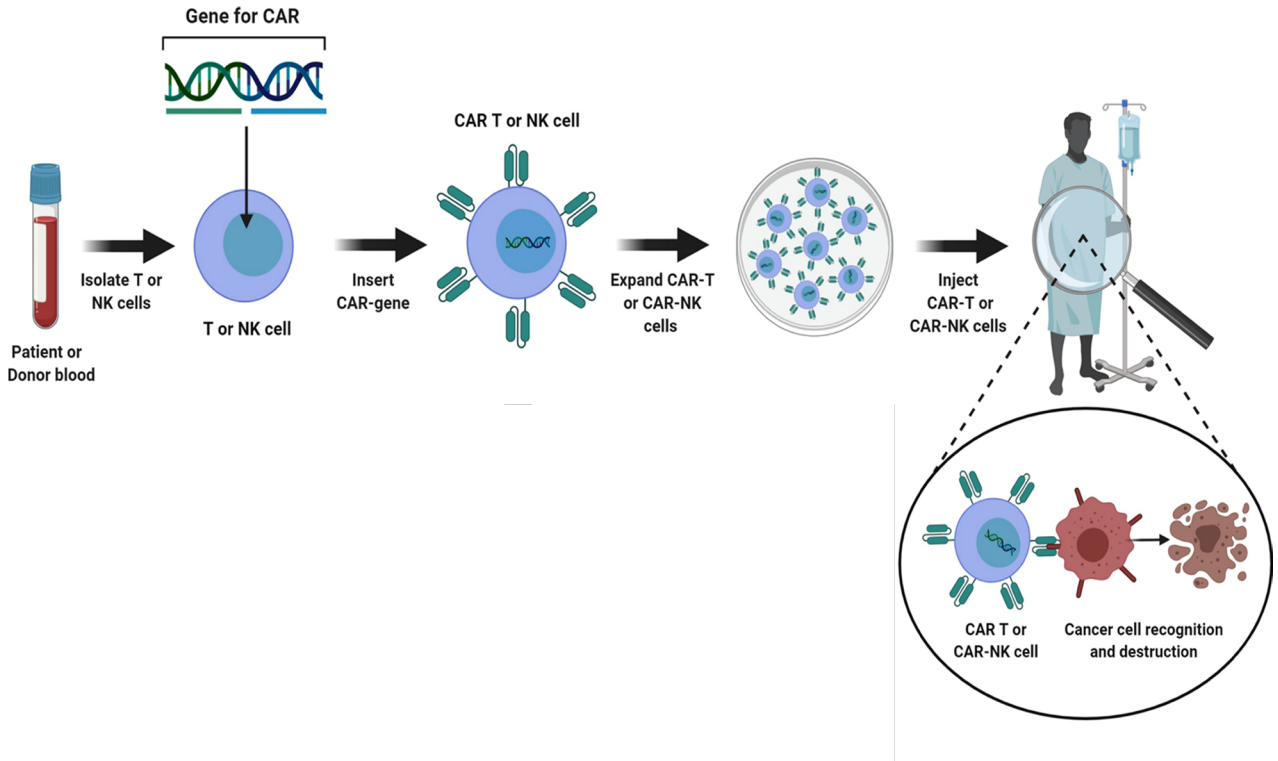

Figure 2: CAR-T and NK cell therapy in cancer treatment (From the patient or the donor blood, T or NK cells are harvested. Then the cells are genetically engineered to express Chimeric Antigen Receptors (CARs). Expanding CAR-T or CAR-NK cells until there are enough for injection back into the patient, where they can help fight cancer cells) (Saenz-Antoñanzas *et al.*, 2021).

Table 1: Summary table of chemotherapy and radiotherapy with CSC inhibitors for the treatment of breast cancer.

Therapeutic Approach	CSC Inhibitor	Targeted Pathway	Chemotherapy/ Radiotherapy Agent	Mechanism of Action	Key Findings	References
Chemotherapy + Notch Inhibitor	γ-Secretase Inhibitors (GSIs)	Notch Signaling	Paclitaxel, Doxorubicin	Inhibits BCSC self-renewal and sensitizes cells to chemotherapy.	Enhanced cytotoxicity & reduced tumor recurrence	(Wu *et al.*, 2021).
Chemotherapy + Wnt Inhibitor	LGK974	Wnt/β-Catenin Pathway	Doxorubicin, Cisplatin	Blocks Wnt signaling to prevent BCSC maintenance and enhances chemotherapy response.	Increased apoptosis and decreased BCSC population	(Babikian *et al.*, 2020).
Chemotherapy + Hedgehog Inhibitor	Vismodegib, Sonidegib	Hedgehog Pathway	5-Fluorouracil (5-FU), Paclitaxel	Suppresses Hedgehog-driven CSC survival and enhances chemotherapy efficacy.	Improved tumor shrinkage and lower recurrence rates	(Gounder *et al.*, 2022).
Radiotherapy + Notch Inhibitor	RO4929097	Notch Signaling	Ionizing Radiation	Reduces BCSC radioresistance by impairing DNA repair and survival signaling.	Enhanced radiosensitivity and tumor control	(Maddineni *et al.*, 2022).
Radiotherapy + Wnt Inhibitor	PRI-724	Wnt/β-Catenin Pathway	Ionizing Radiation	Disrupts BCSC stemness and improves radiotherapy response.	Increased radiation-induced apoptosis in BCSCs	(Maddineni *et al.*, 2022).
Combination Therapy with Dual Inhibitors	Bortezomib + GSIs	Notch & Proteasome Pathway	Cisplatin, Radiation	Targets multiple resistance pathways to eliminate BCSCs.	Greater tumor reduction and prolonged survival	(Moore *et al.*, 2020).

genes among others like the Wonder gene (Wnt) there have been noticeable improvements in treatment results observed.

CONCLUSION

Breast cancer stem cell is a crucial target to overcome therapy resistance, metastasis and tumor recurrence. However, while much is now known about BCSC biology, phenomena such as plasticity, tumor heterogeneity, and microenvironmental interactions continue to pose a challenge to treatment efficacy. Combination strategies of chemotherapy, radiotherapy, small molecule inhibitors, immunotherapy, and nanocarrier-based drug delivery seem to hold promise in eradication of both CSCs as well as bulk tumor cells. However, these approaches cannot be simply translated to clinical success, and this needs better preclinical models, biomarker driven therapies and personalized treatment strategies. There is therefore going to be a continued need for research and innovation in the field of CSC targeted therapies in order to obtain durable responses and improve survival rates in breast cancer patients.

ABBREVIATIONS

BCSCs: Breast Cancer Stem Cells; **TNBC:** Triple-negative breast cancer; **CSCs:** Cancer stem cells; **MBC:** Metastatic breast cancer; **ABC:** ATP-binding cassette; **ALDH1:** Aldehyde dehydrohydrogenase 1; **MET:** Mesenchymal-epithelial transition; **EMT:** Epithelial-Mesenchymal Transition; **TME:** Tumor microenvironment; **CAFs:** Cancer-associated fibroblasts; **NICD:** Notch intracellular domain; **PI3K:** Phosphoinositide 3-kinase; **RTKs:** Receptor tyrosine kinases; **GSIs:** γ secretase inhibitors; **HDAC:** Histone deacetylase; **DNMT:** DNA methyltransferase; **DCA:** Dichloroacetate; **FAO:** Fatty acid oxidation; **ADCC:** Antibody-dependent cellular cytotoxicity; **EpCAM:** Epithelial Cell Adhesion Molecule; **ADCs:** antibody drug conjugates; **ICIs:** Immune checkpoint inhibitors; **CTLA-4:** Cytotoxic T-Lymphocyte Associated Protein 4; **DC:** Dendritic cell; **NSC:** Normal stem cell; **APC:** Antigen presenting cells; **TAAs:** Tumor associated antigens; **CAR T cells:** Chimeric antigen receptor CAR T cells; **NK:** Natural killer; **iPSC:** Induced pluripotent stem cell; **CARs:** Chimeric antigen receptors; **RNAi:** RNA interference; **SLN:** Solid lipid nanoparticles.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

GENERATIVE AI STATEMENT

The author declares that Generative AI tools, including, Grammarly, and QuillBot, was used to enhance the language & clarity of this work. I take full responsibility for the accuracy & integrity of the content.

SUMMARY

Targeting breast cancer stem cells is key to overcoming therapy resistance, metastasis, and tumor recurrence. While our understanding of these cells has grown, issues like plasticity, tumor diversity, and the tumor environment still make treatment difficult. Using a mix of chemotherapy, radiotherapy, small molecule inhibitors, immunotherapy, and nanocarrier-based drug delivery can help address both cancer stem cells and the main tumor, but these methods do not always lead to success in patients. To improve results, better preclinical models, therapies based on biomarkers, and more personalized treatment plans are needed. Ongoing research and new approaches focused on cancer stem cells are crucial for lasting progress and better survival rates for people with breast cancer.

REFERENCES

Alalhareth, I. S., Alyami, S. M., Alshareef, A. H., Ajeibi, A. O., Al Munjem, M. F., Elfifi, A. A., *et al.* (2025). Cellular Epigenetic Targets and Epidrugs in Breast Cancer Therapy: Mechanisms, Challenges, and Future Perspectives. Pharmaceuticals, 18(2), 207.

Ali, K., Nabeel, M., Mohsin, F., Iqtedar, M., Islam, M., Rasool, M. F., *et al.* (2024). Recent developments in targeting breast cancer stem cells (BCSCs): a descriptive review of therapeutic strategies and emerging therapies. Medical Oncology, 41(5), 112.

Albinger, N., Hartmann, J., & Ullrich, E. (2021). Current status and perspective of CAR-T and CAR-NK cell therapy trials in Germany. Gene therapy, 28(9), 513-527.

Bai, X., Ni, J., Beretov, J., Graham, P., & Li, Y. (2018). Cancer stem cell in breast cancer therapeutic resistance. Cancer treatment reviews, 69, 152-163.

Beach, M. A., Nayanathara, U., Gao, Y., Zhang, C., Xiong, Y., Wang, Y., & Such, G. K. (2024). Polymeric nanoparticles for drug delivery. Chemical Reviews, 124(9), 5505-5616.

Babikian, P. (2020). Anti-neoplastic Effect of Adjuvant Epigenetic Therapy of Decitabine and Suberoylanilide Hydroxamic Acid on U937 Acute Myeloid Leukemia Cell Line (Doctoral dissertation, Lebanese American University).

Citarella, A., Catanzaro, G., Besharat, Z. M., Trocchianesi, S., Barbagallo, F., Gosti, G., *et al.* (2023). Hedgehog-GLI and notch pathways sustain chemoresistance and invasiveness in colorectal cancer and their inhibition restores chemotherapy efficacy. Cancers, 15(5), 1471.

Conde, I., Ribeiro, A. S., & Paredes, J. (2022). Breast cancer stem cell membrane biomarkers: Therapy targeting and clinical implications. Cells, 11(6), 934.

Cavallari, I., Ciccarese, F., Sharova, E., Urso, L., Raimondi, V., Silic-Benussi, M., *et al.* (2021). The miR-200 family of microRNAs: fine tuners of epithelial-mesenchymal transition and circulating cancer biomarkers. Cancers, 13(23), 5874.

da Silvia Lima, R. Q., Vasconcelos, C. F. M., Gomes, J. P. A., de Menezes, E. D. S. B., de Oliveira Silva, B., Junior, C. C. M., *et al.* (2024). miRNA-21, an oncomiR that regulates cell proliferation, migration, invasion and therapy response in lung cancer. Pathology-Research and Practice, 155601.

Das, S., Byran, G., Biswas, K., & Rajagopal, K. (2025). Understanding Tankyrase Inhibitors and Their Role in the Management of Different Cancer. Current Cancer Drug Targets.

Du, J., & Qin, H. (2024). Lipid metabolism dynamics in cancer stem cells: potential targets for cancers. Frontiers in Pharmacology, 15, 1367981.

Dogbey, D. M., Andong-Koung-Edzidzi, U. C., Molope, G. A., Singh, J., Bvudzijena, T. L., Naran, K., & Barth, S. (2024). EpCAM-targeting cancer immunotherapies: Evidence from clinical studies and the way forward. Tumor Discovery, 4926.

Elbaiomy, M. A., Akl, T., Atwan, N., Elsayed, A. A., Elzaafarany, M., & Shamaa, S. (2020). Clinical impact of breast cancer stem cells in metastatic breast cancer patients. Journal of oncology, 2020(1), 2561726.

Fiori, M. E., Di Franco, S., Villanova, L., Bianca, P., Stassi, G., & De Maria, R. (2019). Cancer-associated fibroblasts as abettors of tumor progression at the crossroads of EMT and therapy resistance. Molecular cancer, 18, 1-16.

Fatima, N., Umar, M., Ambreen, S., Shaququzzaman, M., Alam, M. M., & Ali, R. (2024). Targeted cancer stem cell therapeutics: an update. Current Topics in Medicinal Chemistry.

Gaggianesi, M., Di Franco, S., Pantina, V. D., Porcelli, G., D'Accardo, C., Verona, F., ... & Stassi, G. (2021). Messing up the cancer stem cell chemoresistance mechanisms supported by tumor microenvironment. Frontiers in Oncology, 11, 702642.

Gargano, D., Segatto, M., & Di Bartolomeo, S. (2023). Regulation of cell plasticity by bromodomain and extra terminal domain (BET) proteins: A new perspective in glioblastoma therapy. International Journal of Molecular Sciences, 24(6), 5665.

Gamage, S. K., Ranaweera, C. D., Maduwage, K. P., Cheng, T., Islam, F., & Gopalan, V. (2023). Novel Therapeutics Targeting Cancer Stem Cell Surface Markers. In Cancer Stem Cells: Basic Concept and Therapeutic Implications (pp. 167-198). Singapore: Springer Nature Singapore.

Giuli, M. V., Mancusi, A., Giuliani, E., Screpanti, I., & Checquolo, S. (2021). Notch signaling in female cancers: A multifaceted node to overcome drug resistance. Cancer Drug Resistance, 4(4), 805.

Gounder, M. M., Rosenbaum, E., Wu, N., Dickson, M. A., Sheikh, T. N., D'Angelo, S. P., ... & Schwartz, G. K. (2022). A phase Ib/II randomized study of RO4929097, a gamma-secretase or Notch inhibitor with or without vismodegib, a hedgehog inhibitor, in advanced sarcoma. Clinical Cancer Research, 28(8), 1586-1594.

Gul, A., Alak, S. E., Gül, C., Karakavuk, T., Can, H., Karakavuk, M., *et al.* (2021). Breast Cancer Vaccines: Current Status and Future Approach. In Frontiers in Clinical Drug Research-Anti-Cancer Agents: Volume 8 (pp. 108-141). Bentham Science Publishers.

Hu, L., Chen, W., Qian, A., & Li, Y. P. (2024). Wnt/β-catenin signaling components and mechanisms in bone formation, homeostasis, and disease. Bone Research, 12(1), 39.

Jing, J., Wu, Z., Wang, J., Luo, G., Lin, H., Fan, Y., & Zhou, C. (2023). Hedgehog signaling in tissue homeostasis, cancers and targeted therapies. Signal transduction and targeted therapy, 8(1), 315.

Jan, A., Sofi, S., Jan, N., & Mir, M. A. (2025). An update on cancer stem cell survival pathways involved in chemoresistance in triple-negative breast cancer. Future Oncology, 1-21.

Jiao, Z., Pan, Y., & Chen, F. (2023). The metabolic landscape of breast cancer and its therapeutic implications. Molecular Diagnosis & Therapy, 27(3), 349-369.

Jung, M., Nicholas, N., Grindrod, S., & Dritschilo, A. (2024). Dual-targeting class I HDAC inhibitor and ATM activator, SP-1-303, preferentially inhibits estrogen receptor positive breast cancer cell growth. Plos one, 19(7), e0306168.

Kai, K., Arima, Y., Kamiya, T., & Saya, H. (2010). Breast cancer stem cells. Breast Cancer, 17, 80-85.

Kim, S. L., Choi, H. S., & Lee, D. S. (2023). BRD4/nuclear PD-L1/RelB circuit is involved in the stemness of breast cancer cells. Cell Communication and Signaling, 21(1), 315.

Keshavarz, S., Wall, J. R., Keshavarz, S., Vojoudi, E., & Jafari-Shakib, R. (2023). Breast cancer immunotherapy: A comprehensive review. Clinical and Experimental Medicine, 23(8), 4431-4447.

Kar, S., Niharika, N., Roy, A., & Patra, S. K. (2023). Overexpression of SOX2 gene by histone modifications: SOX2 enhances human prostate and breast cancer progression by prevention of apoptosis and enhancing cell proliferation. Oncology, 101(9), 591-608.

Kevat, S., Mistry, A., Oza, N., Majmudar, M., Patel, N., Shah, R., ... & Parashar, G. (2025). Cancer Stem Cell Regulation as a Target of Therapeutic Intervention: Insights into Breast, Cervical and Lung Cancer. Cell Biochemistry and Biophysics, 1-15.

Landeros, N., Castillo, I., & Pérez-Castro, R. (2023). Preclinical and clinical trials of new treatment strategies targeting cancer stem cells in subtypes of breast cancer. Cells, 12(5), 720.

Lear, J. T., Morris, L. M., Ness, D. B., & Lewis, L. D. (2023). Pharmacokinetics and pharmacodynamics of Hedgehog pathway inhibitors used in the treatment of advanced or treatment-refractory basal cell carcinoma. Expert Review of Clinical Pharmacology, 16(12), 1211-1220.

Liu, D., Che, X., Wang, X., Ma, C., & Wu, G. (2023). Tumor vaccines: unleashing the power of the immune system to fight cancer. Pharmaceuticals, 16(10), 1384.

Li, X., Yang, J., Ni, R., Chen, J., Zhou, Y., Song, H., ... & Pan, Y. (2022). Hypoxia-induced lncRNA RBM5-AS1 promotes tumorigenesis via activating Wnt/β-catenin signaling in breast cancer. Cell death & disease, 13(2), 95.

Li, W., Wang, Y., Liu, R., Kasinski, A. L., Shen, H., Slack, F. J., & Tang, D. G. (2021). MicroRNA-34a: potent tumor suppressor, cancer stem cell inhibitor, and potential anticancer therapeutic. Frontiers in cell and developmental biology, 9, 640587.

Modi, A., Roy, D., Sharma, S., Vishnoi, J. R., Pareek, P., Elhence, P., *et al.* (2022). ABC transporters in breast cancer: their roles in multidrug resistance and beyond. Journal of drug targeting, 30(9), 927-947.

Montagna, E., & Colleoni, M. (2019). Hormonal treatment combined with targeted therapies in endocrine-responsive and HER2-positive metastatic breast cancer. Therapeutic Advances in Medical Oncology, 11, 1758835919894105.

Mahendran, G., Shangaradas, A. D., Romero-Moreno, R., Dona, N. W., Sarasija, S. S., Perera, S., & Silva, G. N. (2024). Unlocking the epigenetic code: new insights into triple-negative breast cancer. Frontiers in Oncology, 14, 1499950.

Masoudi, M., Moti, D., Masoudi, R., Auwal, A., Hossain, M. M., Pronoy, T. U. H., *et al.* (2024). Metabolic adaptations in cancer stem cells: A key to therapy resistance. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 167164.

MacLean, M. R., Walker, O. L., Arun, R. P., Fernando, W., & Marcato, P. (2024). Informed by cancer stem cells of solid tumors: advances in treatments targeting tumor-promoting factors and pathways. International journal of molecular sciences, 25(7), 4102.

Manian, M., Taherian, M., Nickho, H., Emami Nejad, A., & Shaverdi, S. (2025). Hypoxia, Stem Cells and Cancer Stem Cells. In Cancer Stem Cells and Cancer Therapy (pp. 29-114). Cham: Springer Nature Switzerland.

Maddineni, S., Silberstein, J. L., & Sunwoo, J. B. (2022). Emerging NK cell therapies for cancer and the promise of next generation engineering of iPSC-derived NK cells. Journal for ImmunoTherapy of Cancer, 10(5), e004693.

MacLean, M. R., Walker, O. L., Arun, R. P., Fernando, W., & Marcato, P. (2024). Informed by cancer stem cells of solid tumors: advances in treatments targeting tumor-promoting factors and pathways. International journal of molecular sciences, 25(7), 4102.

Ma, Y., Shen, N., Wicha, M. S., & Luo, M. (2021). The roles of the Let-7 family of MicroRNAs in the regulation of cancer stemness. Cells, 10(9), 2415.

Mir, G. J., Manzoor, I., Islam, A. U., Ganie, S. A., & Hamid, R. (2024). Cancer Stem Cells as Promising Nanotherapeutic Targets. In Personalized and Precision Nanomedicine for Cancer Treatment (pp. 27-68). Singapore: Springer Nature Singapore.

Mirchandani, Y., Patravale, V. B., & Brijesh, S. (2021). Solid lipid nanoparticles for hydrophilic drugs. Journal of controlled release, 335, 457-464.

Montazersaheb, P., Pishgahzadeh, E., Jahani, V. B., Farahzadi, R., & Montazersaheb, S. (2023). Magnetic nanoparticle-based hyperthermia: A prospect in cancer stem cell tracking and therapy. Life Sciences, 323, 121714.

Moore, G., Annett, S., McClements, L., & Robson, T. (2020). Top notch targeting strategies in cancer: a detailed overview of recent insights and current perspectives. Cells, 9(6), 1503.

Mohapatra, P., & Chandrasekaran, N. (2022). Wnt/β-catenin targeting in liver carcinoma through nanotechnology-based drug repurposing: A review. Biomedicine & Pharmacotherapy, 155, 113713.

Martins-Neves, S. R., Sampaio-Ribeiro, G., & Gomes, C. M. (2023). Self-renewal and pluripotency in osteosarcoma stem cells' chemoresistance: notch, hedgehog, and wnt/β-catenin interplay with embryonic markers. International journal of molecular sciences, 24(9), 8401.

Nogueras Pérez, R., Heredia-Nicolás, N., de Lara-Peña, L., López de Andrés, J., Marchal, J. A., Jiménez, G., & Griñán-Lisón, C. (2023). Unraveling the potential of miRNAs from CSCs as an emerging clinical tool for breast cancer diagnosis and prognosis. International Journal of Molecular Sciences, 24(21), 16010.

Nagampalli, R. S. K., Vadla, G. P., & Nadendla, E. K. (2025). Emerging Strategies to Overcome Chemoresistance: Structural Insights and Therapeutic Targeting of Multidrug Resistance-Linked ATP-Binding Cassette Transporters. International Journal of Translational Medicine, 5(1), 6.

Nikolaou, M., Pavlopoulou, A., Georgakilas, A. G., & Kyrodimos, E. (2018). The challenge of drug resistance in cancer treatment: a current overview. Clinical & Experimental Metastasis, 35, 309-318.

Orlovskiy, S., Gupta, P. K., Roman, J., Arias-Mendoza, F., Nelson, D. S., Koch, C. J., *et al.* (2024). Lonidamine induced selective acidification and De-energization of prostate cancer xenografts: enhanced tumor response to radiation therapy. Cancers, 16(7), 1384.

Peri, S. S., Narayanaa Y, K., Hubert, T. D., Rajaraman, R., Arfuso, F., Sundaram, S., *et al.* (2023). Navigating tumour microenvironment and Wnt signalling crosstalk: implications for advanced cancer therapeutics. Cancers, 15(24), 5847.

Pallerla, S., Abdul, A. U. R. M., Comeau, J., & Jois, S. (2021). Cancer vaccines, treatment of the future: with emphasis on HER2-positive breast cancer. International journal of molecular sciences, 22(2), 779.

Romaniuk-Drapała, A., Totoń, E., Taube, M., Idzik, M., Rubiś, B., & Lisiak, N. (2024). Breast Cancer Stem Cells and Tumor Heterogeneity: Characteristics and Therapeutic Strategies. Cancers, 16(13), 2481.

Ray, S. K., & Mukherjee, S. (2024). Breast cancer stem cells as novel biomarkers. Clinica Chimica Acta, 117855.

Romaniuk-Drapała, A., Totoń, E., Taube, M., Idzik, M., Rubiś, B., & Lisiak, N. (2024). Breast Cancer Stem Cells and Tumor Heterogeneity: Characteristics and Therapeutic Strategies. Cancers, 16(13), 2481.

Ranga, V., Dakal, T. C., Maurya, P. K., Johnson, M. S., Sharma, N. K., & Kumar, A. (2025). Role of RGD-binding Integrins in ovarian cancer progression, metastasis and response to therapy. Integrative Biology, 17, zyaf003.

Rondeau, J. D., Lipari, S., Mathieu, B., Beckers, C., Van de Velde, J. A., Mignion, L., *et al.* (2024). Mitochondria-targeted antioxidant MitoQ radiosensitizes tumors by decreasing mitochondrial oxygen consumption. Cell Death Discovery, 10(1), 514.

Schoenmann, N., Tannenbaum, N., Hodgeman, R. M., & Raju, R. P. (2023). Regulating mitochondrial metabolism by targeting pyruvate dehydrogenase with dichloroacetate, a metabolic messenger. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1869(7), 166769.

Song, K., & Farzaneh, M. (2021). Signaling pathways governing breast cancer stem cells behavior. Stem Cell Research & Therapy, 12(1), 245.

Shabna, A., Bindhya, S., Sidhanth, C., Garg, M., & Ganesan, T. S. (2023). Long non-coding RNAs: fundamental regulators and emerging targets of cancer stem cells. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1878(3), 188899.

Swathi, N. L., Basavan, I., Odoh, E. C., Eze, S. C., Swaraj, J. C. S., & Sharma, N. (2025). Breast Cancer Stem Cells Targeted Strategies. In Spatially Variable Genes in Cancer: Development, Progression, and Treatment Response (pp. 151-176). IGI Global Scientific Publishing.

Saha, T., & Lukong, K. E. (2022). Breast cancer stem-like cells in drug resistance: a review of mechanisms and novel therapeutic strategies to overcome drug resistance. Frontiers in oncology, 12, 856974.

Saenz-Antoñanzas, A., Moncho-Amor, V., Auzmendi-Iriarte, J., Elua-Pinin, A., Rizzoti, K., Lovell-Badge, R., & Matheu, A. (2021). CRISPR/Cas9 deletion of SOX2 regulatory region 2 (SRR2) decreases SOX2 malignant activity in glioblastoma. Cancers, 13(7), 1574.

Safa, A. R. (2022). Drug and apoptosis resistance in cancer stem cells: a puzzle with many pieces. Cancer Drug Resistance, 5(4), 850.

Serej, Z. A., Ebrahimi, A., Kazemi, T., Najafi, S., Amini, M., Nastarin, P., *et al.* (2021). NANOG gene suppression and replacement of let-7 modulate the stemness, invasion, and apoptosis in breast cancer. Gene, 801, 145844.

Tang, Y., Chen, Y., Zhang, Z., Tang, B., Zhou, Z., & Chen, H. (2021). Nanoparticle-based RNAi therapeutics targeting cancer stem cells: Update and prospective. Pharmaceutics, 13(12), 2116.

Teoh, P. L., & Saini, N. (2025). Biomarkers, isolation methods, and therapeutic implications of breast cancer stem cells. Cancer Pathogenesis and Therapy, 3, E33-E83.

Testa, U., Castelli, G., & Pelosi, E. (2020). Breast cancer: a molecularly heterogenous disease needing subtype-specific treatments. Medical Sciences, 8(1), 18.

Vasileiou, M., Diamantoudis, S. C., Tsianava, C., & Nguyen, N. P. (2024). Immunotherapeutic strategies targeting breast cancer stem cells. Current Oncology, 31(6), 3040-3063.

Wu, Q., Wang, J., Liu, Y., & Gong, X. (2019). Epithelial cell adhesion molecule and epithelial-mesenchymal transition are associated with vasculogenic mimicry, poor prognosis, and metastasis of triple negative breast cancer. International journal of clinical and experimental pathology, 12(5), 1678.

Weerackoon, N., Gunawardhana, K. L., & Mani, A. (2021). Wnt signaling cascades and their role in coronary artery health and disease. Journal of cellular signaling, 2(1), 52.

Wang, L. L., Wan, X. Y., Liu, C. Q., & Zheng, F. M. (2022). NDR1 increases NOTCH1 signaling activity by impairing Fbw7 mediated NICD degradation to enhance breast cancer stem cell properties. Molecular Medicine, 28(1), 49.

Wang, X., Wang, T., Song, X., Gao, J., Xu, G., Ma, Y., & Song, G. (2024). Current status of hedgehog signaling inhibitors. Current Topics in Medicinal Chemistry, 24(3), 243-258.

Wang, S., Gu, S., Chen, J., Yuan, Z., Liang, P., & Cui, H. (2024). Mechanism of notch signaling pathway in malignant progression of glioblastoma and targeted therapy. Biomolecules, 14(4), 480.

Wu, F., Qiu, F., Wai-Keong, S. A., & Diao, Y. (2021). The smart dual-stimuli responsive nanoparticles for controlled anti-tumor drug release and cancer therapy. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 21(10), 1202-1215.

Wu, H. J., & Chu, P. Y. (2021). Epigenetic regulation of breast cancer stem cells contributing to carcinogenesis and therapeutic implications. International journal of molecular sciences, 22(15), 8113.

Xu, H., Zhang, F., Gao, X., Zhou, Q., & Zhu, L. (2022). Fate decisions of breast cancer stem cells in cancer progression. Frontiers in Oncology, 12, 968306.

Yadav, A. K., & Desai, N. S. (2019). Cancer stem cells: acquisition, characteristics, therapeutic implications, targeting strategies and future prospects. Stem Cell Reviews and Reports, 15, 331-355.

Yang, Y. H., Liu, J. W., Lu, C., & Wei, J. F. (2022). CAR-T cell therapy for breast cancer: from basic research to clinical application. International journal of biological sciences, 18(6), 2609.

Yang, Y., Li, X., Wang, T., Guo, Q., Xi, T., & Zheng, L. (2020). Emerging agents that target signaling pathways in cancer stem cells. Journal of hematology & oncology, 13, 1-18.

Zhuo, D. (2020). Transcriptional Regulation of Warburg Effect in 3D Cultured Breast Cancer Cells by Orai1 (Doctoral dissertation, The Chinese University of Hong Kong (Hong Kong).

Zhang, X., Powell, K., & Li, L. (2020). Breast cancer stem cells: biomarkers, identification and isolation methods, regulating mechanisms, cellular origin, and beyond. Cancers, 12(12), 3765.

Zhang, H., Mi, J., Xin, Q., Cao, W., Song, C., Zhang, N., & Yuan, C. (2023). Recent research and clinical progress of CTLA-4-based immunotherapy for breast cancer. Frontiers in Oncology, 13, 1256360.

Zhou, S., Ou, H., Wu, Y., Qi, D., Pei, X., Yu, X., *et al.* (2023). Targeting tumor endothelial cells with methyltransferase inhibitors: mechanisms of action and the potential of combination therapy. Pharmacology & Therapeutics, 247, 108434.

Cite this article: Alam Q, Alazragi RS, Al-Kathiri A, Albaz AA, Alshehri JM, Alsaffar FA, *et al*. Cancer Stem Cells in Breast Cancer: Unlocking New Frontiers in Therapeutic Strategies. Int. J. Pharm. Investigation. 2026;16(2):523-35.