Targeting breast cancer stem cells: mechanisms of resistance and emerging therapeutic strategies

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Summery

One of the primary causes of cancer-related death for women is breast cancer, and two of the biggest obstacles to treatment are tumor recurrence and resistance to traditional treatments. One of the main causes of these results is the existence of breast cancer stem cells (BCSCs), a subpopulation of tumor cells that have traits similar to those of tissue stem cells, including the potential to self-renew, differentiate, and resistance to therapy. BCSCs, which are recognised by markers such ALDH1 and CD44+/CD24−, are linked to the development, spread, and recurrence of tumors. The aim of the research is to investigate the contribution of breast cancer stem cells to tumor recurrence and resistance to conventional therapies, and to evaluate emerging therapeutic strategies designed to specifically target these cells to enhance treatment efficacy and patient outcomes. Through the utilisation of strategies including drug efflux, improved DNA repair mechanisms, quiescence, and the ability to resist apoptosis, BCSCs successfully circumvent standard therapies. Moreover, their participation in epithelial-to-mesenchymal transition (EMT) and the activation of essential signaling pathways Notch, Wnt/β-catenin, Hedgehog, and TGF-β further enhances metastasis and resistance to treatment. Immune checkpoint inhibitors, monoclonal antibodies (such as anti-CD44 or anti-EpCAM), pathway-specific inhibitors, and CAR T-cell treatments that target CD133 are examples of promising therapeutic strategies. However, the heterogeneity and plasticity of BCSCs, along with immunosuppressive tumor microenvironments and off-target effects, limit current clinical efficacy. These results highlight the significance of BCSCs in the development of breast cancer and the failure of therapy. Targeting BCSCs effectively, especially with combination strategies of targeted therapies and including radiation, presents a viable way to surpass resistance, reduce recurrence, and enhance long-term treatment success.

Introduction

Breast cancer (BC) is the most common cancer diagnosed in women worldwide and continues to be a serious public health concern. It makes a substantial contribution to cancer-related morbidity and mortality, with around 2.3 million new cases and 685,000 deaths reported in 2020 alone (Sung et al., 2021). Breast cancer constitutes 25% of cancer cases and 16% of cancer fatalities, ranking first in incidence in most nations (Sung et al., 2021). BC development is complex, resulting from the interaction of genetic predispositions, hormonal factors, environmental exposures, and lifestyle decisions. Principal risk factors encompass growing age, familial history—especially mutations in the BRCA1 and BRCA2 genes—early menarche, delayed menopause, nulliparity, obesity, and alcohol intake (Arun et al., 2024; Sung et al., 2021). The BRCA1 and BRCA2 proteins are key constituents of the cell's DNA damage response (DDR), in which they perform an essential role in the faithful repair of DNA double-strand breaks through the homologous recombination repair (HRR) pathway (Q. Li et al., 2023; Tung & Garber, 2018). When there are loss-of-function mutations in these genes, their ability to mediate homologous recombination is impaired and thereby leading to so-called homologous recombination deficiency (HRD) (Tung & Garber, 2018). As a result, the cells damaged by MRX have to rely on error-prone repair mechanisms, thereby accumulating DNA double-strand breaks, genomic instability, and potential for malignant transformation (Q. Li et al., 2023; Tung & Garber, 2018).

BC can be categorized into distinct subtypes based on the expression patterns of certain biomarkers, which are typically assessed through immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) techniques (X. Zhang et al., 2020). These biomarkers are the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) (Toss & Cristofanilli, 2015; X. Zhang et al., 2020). Depending on their presence or absence, breast cancer is generally divided into three major molecular subtypes (X. Zhang et al., 2020).

The first is ER-positive breast cancer, indicated by expression of the estrogen receptor, and which is generally associated with expression of the PR, and is generally hormone-sensitive (X. Zhang et al., 2020). The second of these subtypes is HER2-positive breast cancer, with overexpression or gene amplification of the HER2 gene, that is more aggressive in its tumor behavior but that can be treated with certain targeted therapies against HER2 (X. Zhang et al., 2020). The third most common category is triple-negative breast cancer (TNBC), that does not express ER, PR, or HER2. TNBC is more virulent and has fewer targeted treatment options, thus it is harder to treat (Toss & Cristofanilli, 2015; X. Zhang et al., 2020). This classification system is crucial in establishing the treatment strategy and the prognosis of clinical outcomes in patients with breast cancer (X. Zhang et al., 2020).

The aim of the research is to investigate the contribution of breast cancer stem cells to tumor recurrence and resistance to conventional therapies, and to evaluate emerging therapeutic strategies designed to specifically target these cells to enhance treatment efficacy and patient outcomes.

Breast cancer stem cells: characteristics and identification

Given the intricate biological mechanisms driving breast cancer development, increasing attention has turned to the role of cancer stem cells (CSCs) in its pathogenesis. CSCs, subset of tumor cells, have the same characterizing properties as normal stem cells, their ability to self-renew and differentiate into multiple cell types (Chu et al., 2024). In breast cancer, CSCs are believed to originate either through the transformation of normal mammary stem/progenitor cells or from more differentiated cells that acquire stem-like properties through genetic or epigenetic alterations (Batlle & Clevers, 2017; Chu et al., 2024). In addition, BCSCs can produce diverse cell lines inside the tumor through differentiation,

which contributes to the variety of phenotypes (Phi et al., 2018; X. Zhang et al., 2020). Intrinsic factors (such the transcriptional regulators SOX2, NANOG, and OCT4) and extrinsic signals from the tumor microenvironment (TME) (including cytokines, extracellular matrix elements, and hypoxia) can both affect this plasticity (Kreso & Dick, 2014; Phi et al., 2018). The dynamic and reversible process of plasticity, or the capacity to transition between stem-like and non-stem-like states, allows cells to adjust to changing environmental factors and therapeutic demands (Q. Li et al., 2023).

Aldehyde dehydrogenase (ALDH1) activity is enhanced and the surface marker profiles $CD133^+$, $CD44^+$, $CD24^-$, and $EpCAM^+$ are commonly used to identify breast cancer stem cells (BCSCs) (Chu et al., 2024; X. Zhang et al., 2020). These markers help to distinguish BCSCs from the bulk of tumor cells and are used to isolate and study them both in vitro and in vivo (X. Zhang et al., 2020). Functionally, BCSCs are thought to reside at the apex of a cellular hierarchy within the tumor, driving not only primary tumor growth but also seeding distant metastases through their enhanced motility and adaptability (X. Zhang et al., 2020).

One of the most clinically significant features of BCSCs is their inherent drug resistance to conventional treatments, including chemotherapy, radiotherapy, and targeted therapies (Arnold et al., 2020; Saha & Lukong, 2022; Q. Zheng et al., 2021). They are resistant because of numerous reasons, such as reduced cell cycling, efficient DNA repair, drug efflux capacity (e.g., through ATP-binding cassette transporters), and shielding in the TME (Grindem et al., 2018; Saha & Lukong, 2022). Consequently, traditional therapies often fail to remove the BCSC population, which can lead to treatment failure and tumor recurrence, even if they may reduce tumor growth by targeting rapidly growing cells.

Role of BCSCs in invasion and metastasis

The main cause of death from breast cancer is metastasis, which is largely caused by BCSCs (Park et al., 2022). BCSCs exhibit more metastatic potential than other breast cancer cell types, as evidenced by the upregulation of proteins associated with cell metastasis and movement, along with a marked decrease in adhesion protein levels (Gallardo-Pérez et al., 2017). Oliphant et al., 2019 study indicates that Six homeobox 2 (Six2), which is essential in metastatic colonisation, enhances the expression of SRY-box transcription factor 2 (SOX2) (Oliphant et al., 2019). SOX2 regulates multiple features of cancer cells, including proliferation, epithelial-to-mesenchymal transition (EMT), migration, invasion, metastasis, sphere and colony formation, tumor initiation, cancer stem cell development, and resistance to apoptosis and therapy, effectively inducing stem cell characteristics and exacerbating TNBC metastasis (Novak et al., 2020; Oliphant et al., 2019; Park et al., 2022).

One essential process by which BCSCs develop invasive and metastatic characteristics is EMT (Park et al., 2022). During EMT, epithelial cells lose their apical-basal polarity and intercellular adhesion, gaining mesenchymal characteristics such as increased motility, invasiveness, and resistance to apoptosis (Park et al., 2022; C. Wang et al., 2021). In addition, several developmental signalling pathways, such as the TGF-β, Notch, and Wnt/β-catenin pathways, are often abnormally active in BCSCs and govern this transition (Park et al., 2022; C. Wang et al., 2021; L. Zhang et al., 2023a). BCSCs develop mesenchymal characteristics during this transition, which allows them to infiltrate surrounding tissue, get into the circulation, and endure in the circulatory system (L. Zhang et al., 2023a).

In addition to intrinsic signaling alterations, the invasive and metastatic behavior of BCSCs is strongly influenced by the TME (Plaks et al., 2015). The TME provides a specialized niche composed of various cellular and non-cellular components that support BCSC survival, self-renewal, and dissemination (Plaks et al., 2015). Cancer-associated fibroblasts, immune cells such as tumor-associated

macrophages (TAMs), and elements of the extracellular matrix (ECM) collaborate to sustain a pro-metastatic milieu (Basak et al., 2023; Plaks et al., 2015). Cancer-associated fibroblasts secrete growth factors and cytokines that activate EMT signaling pathways, while TAMs produce enzymes and inflammatory mediators that remodel the ECM, facilitating tumor invasion (Plaks et al., 2015). Moreover, hypoxia is another critical factor within the TME that promotes BCSC-mediated metastasis (Plaks et al., 2015). In hypoxic regions of the tumor, stabilization of hypoxia-inducible factors (HIFs), particularly HIF-1α, induces the expression of genes that enhance stemness, EMT, and survival under stress conditions (Plaks et al., 2015).

Therapy resistance mediated by BCSCs

According to the conventional definition of drug resistance, certain tumor cells have genetic alterations that make them resistant to treatment (Saha & Lukong, 2022). Following therapy, these resistant cells eventually outcompete the others and take control of the tumor (Saha & Lukong, 2022). On the other hand, an alternative perspective based on the cancer stem cell theory suggests that because the tumor's stem cells remain inactive, or quiescent, they are naturally resistant to chemotherapy (Saha & Lukong, 2022; Q. Zheng et al., 2021). As a result, some of these cancer stem cells may survive therapy and subsequently promote tumor development (Saha & Lukong, 2022). Multiple investigations have shown that BCSCs ($CD24^{-/low}$ /$CD44^{+}$ cancer-initiating cells) exhibit inherent resistance to widely utilised chemotherapy agents, including paclitaxel and doxorubicin, as well as ionising radiation (Saha & Lukong, 2022). Furthermore, the drug resistance of BCSCs primarily results from the development of transporters facilitating drug efflux and the elevated expression of ALDH1, which functions as a detoxification enzyme to metabolise anticancer agents (Butti et al., 2019). ALDH may generate nicotinamide to perform its antioxidant role, and the drug resistance of BCSCs is also influenced by elevated mitochondrial quality resulting from ALDH activity (Yousefnia et al., 2020). ALDH plays a critical role in BCSCs resistance. In addition, radiotherapy is proficient at causing DNA damage in cells and affects mostly rapidly dividing cells; however, it is significantly less effective against the predominantly quiescent population of BCSCs (Arnold et al., 2020). Furthermore, BCSCs possess enhanced mechanisms for repairing DNA damage, which allows them to endure in tumors that have undergone radiotherapy (Arnold et al., 2020). DNA double-strand breaks can be repaired more effectively by BCSCs because they have elevated DNA damage response (DDR) pathways (Bao et al., 2006; Diehn et al., 2009). BCSCs are more resistant to apoptosis brought on by drugs or radiation because they have activated the phosphatidylinositol 3-kinase (PI3K) and AKT signalling pathways (Bai et al., 2018; Butti et al., 2019; Yousefnia et al., 2020). Forkhead box O3 (FOXO3a) expression levels are downregulated as a result of these pathways, which also increase breast cancer stemness and resistance to treatment (Smit et al., 2016).

Moreover, BCSCs express more anti-apoptotic proteins (such Bcl-2 and Survivin), which helps them resist therapy-induced cell death (Diehn et al., 2009; Safa, 2022). Additionally, since these cells produce more antioxidant enzymes, they maintain lower intracellular ROS levels, which reduces oxidative damage induced on by radiation or chemotherapy (Diehn et al., 2009; Safa, 2022). Together, these mechanisms enable BCSCs to persist through therapy, repopulate the tumor, and potentially seed distant metastases.

Targeting Strategies Against BCSCs

BCSCs demonstrate abnormal activation of many critical developmental signalling pathways, including Wnt, Notch, and Hedgehog, which are essential for regulating self-renewal, maintenance, and differentiation (Takebe et al., 2011; L. Zhang et al., 2023b). Of these, the Wnt/β-catenin pathway is frequently shown to be increased in BCSCs and is linked to improved stem cell-like characteristics as

well as poor clinical outcomes for patients with breast cancer (Takebe et al., 2011). Preclinical studies have shown that blocking this route with monoclonal antibodies or small molecule drugs may have therapeutic benefits (Takebe et al., 2011).

Furthermore, the Notch signalling system is essential for sustaining BCSCs and controlling mammary stem cells, especially through its receptors Notch1 and Notch4 (Takebe et al., 2011; Zhou et al., 2020). For mesenchymal-like BCSCs, Notch4 in particular has been identified as a critical regulator that supports their quiescence and epithelial-to-mesenchymal transition (EMT) via upregulating transcription factors such as SLUG and GAS1 (Zhou et al., 2020). Moreover, Hedgehog (Hh) signalling, which includes ligands such as Sonic hedgehog (Shh), promotes carcinogenesis via the activation of GLI transcription factors (S. Liu et al., 2006). Hh pathway inhibitors, including vismodegib, have shown the ability to target BCSCs in experimental breast cancer models (S. Liu et al., 2006).

Additionally, TGF-β signaling enhances stemness by activating canonical Smad-dependent transcriptional programs and non-canonical pathways such as PI3K/Akt, MAPK, and Rho GTPases (L. Li et al., 2015). Smad2/3 phosphorylated and form a complex with Smad4 then translocate into the nucleus, where they regulate the expression of genes involved in self-renewal (Nanog, Oct4, Sox2), EMT (Snail, Slug, ZEB1), and drug resistance (L. Li et al., 2015). These pathways not only regulate stemness but also interact with EMT and survival pathways, making them attractive targets for combinational therapies, see the overview in figure 1.

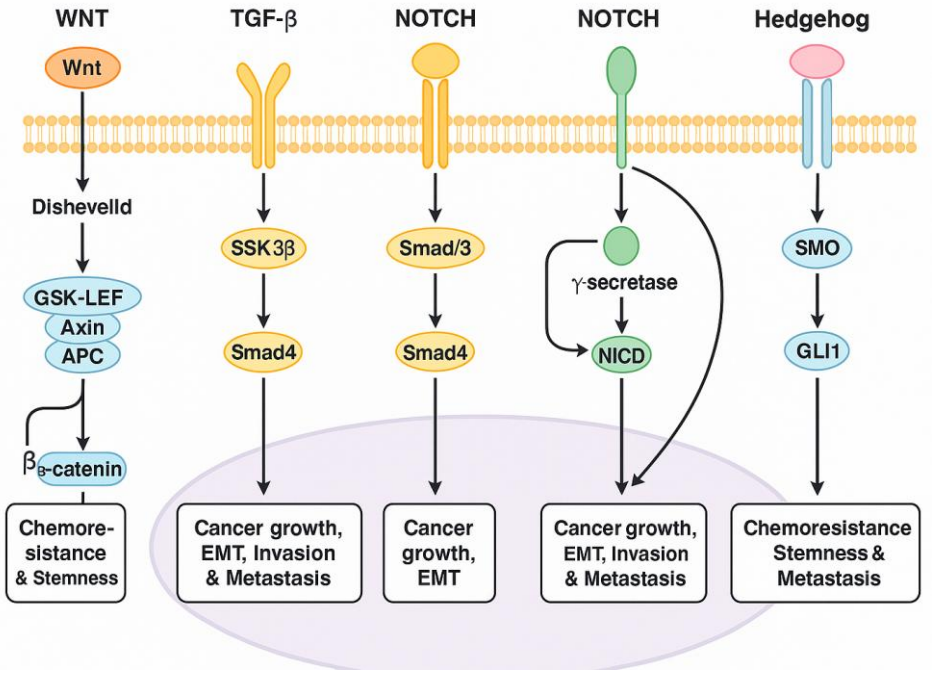

Figure 1 *Simplified signaling pathways (Wnt, TGF-β, Notch, and Hedgehog) involved in the regulation of breast cancer stem cells, highlighting their roles in chemoresistance, epithelial–mesenchymal transition (EMT), invasion, metastasis, and the preservation of stemness, created by BioRender: Ammar Akeleya.*

Immunotherapy and targeted drug delivery

For immune surveillance, the immunosuppressive phenotype of BCSCs presents a major obstacle. Recent research, however, indicates that BCSCs may be specifically targeted by immunotherapeutic approaches. One of the targets is monoclonal antibodies directed against BCSC markers such as CD44

and EpCAM have shown efficacy in reducing BCSC populations and impairing tumor growth (Ginestier et al., 2007; J. Zheng et al., 2017). Furthermore, the potential of immune checkpoint inhibitors (e.g., anti-PD-1/PD-L1) to boost T-cell responses against BCSCs is being investigated, especially in triple-negative breast cancer (TNBC), where BCSCs are more common (Mittendorf et al., 2014).

A potential approach to treating cancer is chimeric antigen receptor (CAR) T-cell therapy, which targets CD133 and other antigens linked to BCSCs (Cui et al., 2021). This is especially true for solid tumors and cancers with a large concentration of cancer stem cells (CSCs) (Cui et al., 2021; Maalej et al., 2023). In this regard, CD133, a surface marker often expressed on BCSCs, has become a crucial target (Maalej et al., 2023). By being engineered to specifically recognize and eliminate cancer cells that express CD133, CAR T-cells provide a focused and possibly successful treatment strategy, see figure 2 (Cui et al., 2021; Maalej et al., 2023).

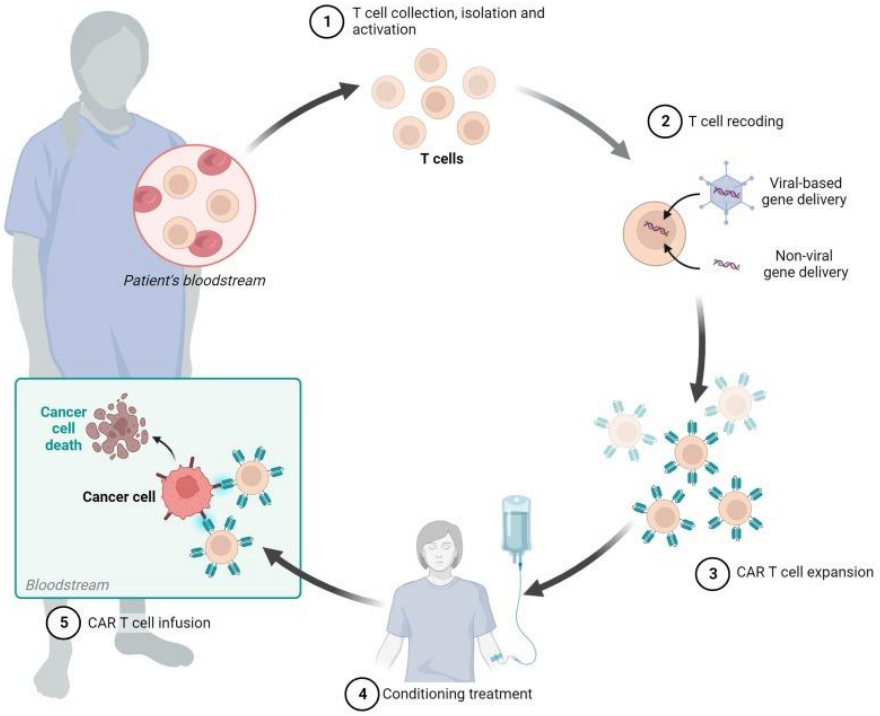

Figure 2 *Primary phases of autologous CAR-T cell production. (1) Activation, isolation, and collection of T cells. (2) Recoding of T cells. (3) proliferation of CAR-T cells. (4) Treatment for conditioning. (5) Infusion of CAR-T cells* ***(Buono et al., 2025)****.*

The safety and viability of CD133-targeted CAR T-cell therapy in patients with advanced hepatocellular carcinoma (HCC) and other solid tumors have been demonstrated by clinical trials (Cui et al., 2021). In one trial, which included 21 evaluable patients with advanced HCC, six patients saw disease progression after receiving CAR T-cells, 14 patients showed stable disease for 2–16.3 months, and one patient reported a partial response (Cui et al., 2021). Given that BCSCs also express CD133, these results imply that CD133-targeted CAR T-cell therapy may find use in targeting BCSCs (Buono et al., 2025; Maalej et al., 2023). However, there is still research being done on the application of CAR T-cell therapy for breast cancer, and there is currently little available clinical data (Buono et al., 2025). Its safety and effectiveness in the context of breast cancer are being investigated in ongoing studies; conclusive findings are still pending (Buono et al., 2025). According to previous CAR T-cell treatments, potential side effects including cytokine release syndrome (CRS), a systemic inflammatory response that may vary from mild flu like symptoms to severe, life threatening responses (Gatto et al., 2023; Siegler & Kenderian, 2020).

Furthermore, gamma-secretase inhibitors (GSIs), like MK-0752, have been studied for their ability to reduce BCSC populations in patients with breast cancer and to induce cell growth inhibition, G2/M phase cell cycle arrest, and apoptosis along with the down-regulation of Notch1 (Jia et al., 2021; Schott et al., 2013). Notch signaling plays a crucial role in the maintenance and self-renewal of BCSCs, making it a promising therapeutic target, figure 1 (Jia et al., 2021). Krop et al., 2012 conducted a phase I clinical research to evaluate the safety of MK-0752 in patients with advanced solid tumors, including breast cancer. The study showed that MK-0752 modulated the expression of Notch target genes, therefore effectively inhibiting Notch signalling (Krop et al., 2012). However, the monotherapy showed limited clinical efficacy, with only one complete response observed among patients with high-grade gliomas (Krop et al., 2012). Further research explored the combination of MK-0752 with chemotherapy agents; Tocilizumab (D. Wang et al., 2018). Both the bulk tumor cells and the BCSC population were targeted in order to increase the anti-tumor effects (D. Wang et al., 2018). The results indicated that the combination was generally well-tolerated and showed significantly decreases in BCSCs, and inhibits tumor growth with some patients achieving stable disease (D. Wang et al., 2018).

In addition, monoclonal antibodies (mAbs) is other type of targeting therapy used specific against BCSCs. Targeting, CD44 and epithelial cell adhesion molecule (EpCAM), have demonstrated considerable promise in preclinical studies (Münz et al., 2004; van Pham et al., 2012). By specifically targeting BCSCs, these targeted medicines aim to address a major factor contributing to tumor metastasis, resistance, and recurrence (van Pham et al., 2012). It has been demonstrated that anti-CD44 monoclonal antibodies, in particular, inhibit BCSC self-renewal and proliferation, trigger apoptosis, and increase the cells' susceptibility to traditional chemotherapeutic treatments(van Pham et al., 2012). Similarly, EpCAM-targeting antibodies have been reported to suppress tumor growth and inhibit metastatic potential by disrupting the epithelial characteristics and signaling pathways essential for BCSC survival (Münz et al., 2004). Patients with metastatic breast cancer received two doses of adecatumumab, a completely human IgG1 monoclonal antibody that targets EpCAM, in order to evaluate the antibody's safety and effectiveness (Schmidt et al., 2010). Adecatumumab was generally well tolerated, according to the results, and some patients experienced stable disease. Endpoints showed that individuals receiving high-dose anti EpCAM (adecatumumab) had a reduced chance of tumor progression than those receiving low-dose, see figure 3 (Schmidt et al., 2010).

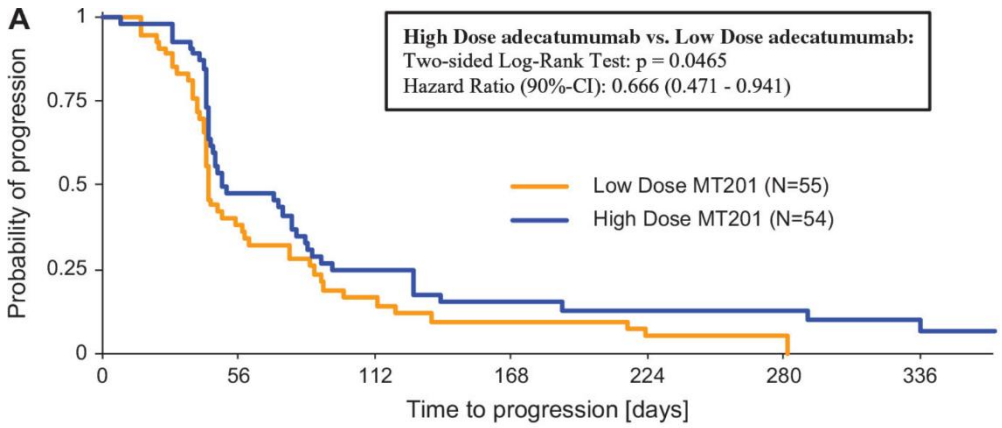

Figure 3 *The results of Schmidt et al., 2010 the curve of time to progression by treatment group.*

In a different research, the combination of adecatumumab and docetaxel was assessed in extensively pretreated patients with advanced-stage breast cancer (Schmidt et al., 2012). The combination therapy was deemed safe, practical, and possibly effective, with clinical benefit noted in 44% of patients administered adecatumumab and docetaxel every three weeks (Schmidt et al., 2012). The benefit increased to 63% in patients with BC with significant levels of EpCAM expression (Schmidt et al., 2012).

Limitations and Challenges in Targeting BCSCs with Immunotherapy

Clinical translation and efficacy of immunotherapeutic approaches against BCSCs are being faced with several challenges despite encouraging preclinical and early clinical reports. One of the significant challenges is the high degree of phenotypic plasticity and heterogeneity of BCSCs (S. Liu et al., 2011). In response to therapeutic pressure or microenvironmental signals, these cells can dynamically transition between stem-like and non-stem-like states, avoiding immune surveillance and targeted treatments (S. Liu et al., 2011).

Furthermore, breast cancers that are TNBC frequently have a highly immunosuppressive TME (Z. Liu et al., 2018). Through expressing immune checkpoint ligands (like PD-L1) and secreting cytokines (such TGF-β and IL-6) that prevent T-cell activation and proliferation, BCSCs help to achieve this immunosuppression (Burugu et al., 2018; Guha et al., 2023).

Aside from this, mAbs therapies against surface antigens like CD44 and EpCAM substantially decrease BCSC (Schmidt et al., 2010, 2012). However, these antigens are not expressed selectively on BCSCs; they also occur on normal epithelial tissues and non-malignant stem cells, raising questions on toxicity and off-target effects (it was toxic in phase I but the combination reduced the toxicity) (Schmidt et al., 2010, 2012). Clinical trials using EpCAM-targeting agents like adecatumumab have shown only modest clinical benefits and limited efficacy in metastatic breast cancer, suggesting that such approaches might not be sufficient as monotherapy (Schmidt et al., 2010, 2012).

Likewise, whereas CAR T-cell treatments target antigens linked to BCSCs, such CD133, their use in breast cancer is still very experimental not clinical (Maalej et al., 2023). Immune escape mechanisms, on-target off-tumor toxicity, and restricted tumor invasion remain significant hurdles (Buono et al., 2025; Maalej et al., 2023). Additionally, clinical data on CAR T-cell efficacy in breast cancer are not yet established, and prospective trials have not yet produced ultimate conclusions regarding safety or therapeutic impact (Buono et al., 2025).

In addition, GSIs monotherapy has been proven to have minimal clinical effects and is typically followed by gastrointestinal toxicity (Krop et al., 2012). Even when combined with chemotherapy or IL-6 inhibitors like Tocilizumab, its therapeutic effects are minimal, and long-term consequences are unknown (D. Wang et al., 2018).

Conclusion

The aim of the research was to investigate the contribution of breast cancer stem cells to tumor recurrence and resistance to conventional therapies, and to evaluate emerging therapeutic strategies designed to specifically target these cells to enhance treatment efficacy and patient outcomes.

BCSCs play a pivotal role in tumor initiation, progression, drug resistance, and recurrence. BCSCs are characterized by particular surface antigens $CD24^{-/low}$ /$CD44^{+}$ and ALDH1 activity and possess high plasticity and drug resistance. BCSCs can escape conventional treatments like chemotherapy and radiation therapy. Through mechanisms like enhanced DNA repair, drug efflux, anti-apoptotic signaling, and maintenance of quiescence, BCSCs are resistant to cytotoxic insults and can repopulate the tumor, and this is a significant role in relapse and metastasis.

Importantly, BCSCs take use of important developmental pathways that promote their self-renewal and stem-like behaviour, including Notch, Wnt/β-catenin, Hedgehog, and TGF-β. In addition to promoting resistance, these pathways also promote metastasis and the epithelial-to-mesenchymal transition (EMT), particularly in aggressive subtypes such as TNBC.

Emerging therapeutic strategies, including pathway-targeting inhibitors (e.g., GSIs, Wnt inhibitors), immunotherapies (e.g., anti-CD44/EpCAM monoclonal antibodies, checkpoint inhibitors), and adoptive therapies such as CAR T-cells in the shape of CD133-targeting CAR T-cells, can be expected to eradicate BCSCs. While these approaches have demonstrated preclinical activity and limited clinical effects, significant hurdles exist. These are the heterogeneous and plastic nature of BCSCs, immunosuppressive TME, toxic off-target effects, and constrained clinical efficacy in breast cancer patients.

In conclusion, BCSC targeting will be a crucial frontier for the improvement of long-term outcomes in patients with breast cancer. Due to limited access to recent experimental studies, not all references cited were from the most current literature. Future studies must concentrate on improving these treatments' specificity, reducing their toxicity, and confirming their effectiveness in well planned clinical trials. In particular, combination of radiotherapy with BCSC-targeting agents holds significant potential. Since BCSCs are inherently radioresistant, combining molecular inhibitors or immunotherapies with radiation may sensitise these cells to treatment, enhancing tumor control and reducing recurrence. While exploring optimal timing, dosing, and sequencing of such combinations will be vital.

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- ***Grammar and spelling were reviewed and refined using ChatGPT and Google Gemini.***