Targeting Forkhead Box M1 Transcription Factor in Breast Cancer

Ruth M. O'Regan and Rita Nahta

1University of Wisconsin Carbone Cancer Center
2Departments of Pharmacology and Hematology & Medical Oncology, Emory University School of Medicine and Winship Cancer Institute

Abstract

Breast cancer continues to be the most commonly diagnosed malignancy and second most common cause of cancer-related deaths among women in the United States. Improved understanding of the molecular heterogeneity of breast tumors and the approval of multiple targeted therapies have revolutionized the treatment landscape and long-term survival rates for patients with breast cancer. Despite the development of highly effective targeted agents, drug resistance and disease progression remain major clinical concerns. Improved understanding of the molecular mechanisms mediating drug resistance will allow new treatments to be developed. The forkhead box M1 (FoxM1) transcription factor is overexpressed in breast cancer and strongly associated with resistance to targeted therapies and chemotherapy. FoxM1 regulates all hallmarks of cancer, including proliferation, mitosis, EMT, invasion, and metastasis. Inhibition of FoxM1 transcription factor function is a potential strategy for overcoming breast cancer progression. In this research update, we review the role of FoxM1 in breast cancer and pharmacological approaches for blocking FoxM1 transcription factor function. Future preclinical studies should evaluate combination drug strategies to inhibit FoxM1 function and upstream kinase signaling pathways as potential strategies to treat resistant and metastatic breast cancers.

Graphical abstract

Corresponding author: Rita Nahta, PhD; RNahta@emory.edu; Suite 5001, 1510 Clifton Rd, Atlanta, GA 30322.

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1. Introduction

Breast cancer remains the most frequently encountered malignancy among women in the United States, and one of the most deadly forms of cancer upon relapse and metastatic spread. Breast tumors exhibit substantial heterogeneity, with multiple molecular subtypes identified. Targeted molecular treatments exist for luminal and HER2 subtypes, although disease recurrence after acquired drug resistance remains a significant clinical concern. By contrast, the triple-negative subgroup lacking estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) overexpression has no approved targeted treatments. Thus, identification of new molecular targets in recurrent luminal and HER2-positive and all triple-negative tumors is an important area of investigation.

The forkhead box M1 (FoxM1) transcription factor plays a central role in drug resistance, epithelial to mesenchymal transition (EMT), invasion and metastasis, with expression observed in all subtypes of breast cancer. We review recent studies of FoxM1 expression and function in breast cancer according to molecular subtype. We also review potential pharmacological strategies for inhibiting FoxM1 transcription factor function and upstream signaling pathways. These novel FoxM1-targeted strategies may represent new therapeutic approaches for drug-resistant, metastatic breast cancers.

2. Breast Cancer Subtypes and Treatments

Breast cancer is the most common tumor type diagnosed among women in the United States, accounting for 30% of new cancer diagnoses and approximately 15% of cancer-related deaths in female patients [1]. The lifetime risk of developing breast cancer is one in eight, with more than 250,000 new cases and 40,000 deaths estimated to occur in 2018 in the United States [1]. Most breast cancers are diagnosed as localized disease, which carries an 80% or higher five-year relative survival rate depending on stage [1]. Approximately 6% of breast cancers are diagnosed as metastatic [1], with another 30% of women who were initially diagnosed with localized or regional disease eventually developing metastatic...
disease [2]. Survival outcomes for patients with metastatic breast cancer is substantially lower than localized disease, with a five-year relative survival rate of only 27% [1].

Breast tumors demonstrate significant intra- and inter-tumor heterogeneity at the molecular and histological levels, contributing to variable responses to therapy and differences in risk of disease progression [3]. Major intrinsic molecular subgroups of breast cancer are stratified based on gene expression profile and hormone receptor (HR) or HER2 expression status (Table 1). However, intrinsic subtyping does not always correlate with HR and HER2 detection by immunohistochemistry (IHC). Approximately 75% of breast cancers are luminal, which is generally defined by positive expression of ER and PR [4]. The luminal A subgroup consists of grade 1-2 tumors that are negative for HER2 and have low expression of the Ki67 proliferation marker. By contrast, luminal B breast tumors carry a worse prognosis and are generally higher grade, express Ki67 at high levels, and exhibit variable expression of HER2 [4]. As many as 10%-15% of luminal B tumors overexpress HER2. Another 10% of breast cancers overexpress HER2, defined as IHC 3+ or fluorescent in situ hybridization ≥2.0, in the absence of hormone receptor expression [4]. Basal breast cancers account for 10%-15% of breast cancers and are generally negative for ER, PR, and HER2 but may express high levels of basal cytokeratin markers or epidermal growth factor (EGFR) [4].

Improved understanding of the molecular composition of individual tumors and the advent of molecular targeted therapies has revolutionized the treatment landscape and survival outcomes for patients with breast cancer. ER-positive or HER2-overexpressing tumors are candidates for targeted endocrine or hormone therapies, including selective ER modulators (SERMs) and aromatase inhibitors (AI), or HER2 monoclonal antibodies and tyrosine kinase inhibitors (TKIs), respectively. Combination AI plus cyclin-dependent kinase (CDK)4/6 inhibitor therapy is approved in the first-line setting for ER-positive metastatic breast cancer tumors based on results from several randomized trials demonstrating a doubling in progression-free survival (PFS) in patients treated with an AI plus palbociclib vs AI alone [5–8]. First-line treatment for HER2-overexpressing tumors consists of the HER2 monoclonal antibodies trastuzumab (Herceptin) and pertuzumab (Perjeta) combined with docetaxel chemotherapy based on results from the CLEOPATRA trial, which demonstrated improved PFS in patients treated with the antibody combination vs trastuzumab and docetaxel (median PFS, 18.5 months vs 12.4 months; p<0.001) [9]. Despite the development of highly effective targeted agents, resistance to endocrine and HER2 therapies remain major clinical concerns. Additional AIs and endocrine agents and HER2 antibody-conjugate T-DM1 and TKIs, including lapatinib, are available for later-line treatment of ER-positive and metastatic HER2-positive cancers, respectively.

In contrast to other breast tumor subgroups, triple-negative cancers are not candidates for currently approved targeted treatments. Triple-negative breast cancers (TNBCs) comprise approximately 80% of basal breast cancers [10], and are genetically diverse, with six distinct subtypes identified, two basal-like, an immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor subtype [11]. Basal-like TNBCs exhibit genomic instability and a subset carry BRCA1/2 mutations or are enriched for expression of DNA damage or cell cycle control genes and may be candidates for PARP inhibition [12]. The
immunomodulatory subgroup demonstrates expression of genes involved in the immune response, whereas the luminal androgen receptor subtype expresses androgen receptor (AR) [12]. The mesenchymal and mesenchymal stem-like subtypes exhibit EMT changes, including expression of mesenchymal markers and transcription factors [12]. Clinical approaches for treating TNBC consist almost exclusively of cytotoxic chemotherapy. Pathologic complete response rates to neoadjuvant chemotherapy are higher for TNBCs vs luminal and HER2-positive tumor subgroups, ranging from 28% to 67% depending on regimen [12]. However, recurrence rates are almost 3-fold higher for TNBCs compared with non-TNBC tumors, particularly among those who present with node-positive disease. Recurrence peaks approximately three years post-therapy [13], and the five-year overall survival (OS) rate for patients with TNBC is lower than for patients of other subtypes [14]. Improved understanding of the underlying molecular mechanisms driving the development of drug resistance and disease progression will facilitate the development of targeted treatments for metastatic breast cancers.

3. FoxM1 Transcription Factor

3.1. Overview

Several outstanding recent reviews demonstrate a role for the FoxM1 transcription factor in the development of drug resistance in many types of solid tumors, including breast cancers [15–17]. FoxM1 is a member of the forkhead family of transcription factors, which includes more than 100 helix-turn-helix transcription factors [18]. Under normal physiological conditions, FoxM1 expression varies with cell cycle phase, increasing during S phase and peaking at G2-M. FoxM1 regulates the transcription of genes involved in cell cycle regulation at the G1-S and G2-M transitions, such as p27kip1, cyclin D1, and cdc25, and is required for proliferation and mitosis in preclinical models of breast cancer [19]. FoxM1 knockdown inhibits proliferation without inducing apoptosis of breast cancer cell lines independent of ER status [19]. The p53, retinoblastoma, and p19ARF tumor suppressor proteins repress expression of FoxM1 [20–22] and may function as important regulators of FoxM1 function at the G1-S transition. Mutation of p53, which occurs in approximately 80% of TNBCs, can lead to upregulation of FoxM1 [23].

In addition to regulating proliferation, FoxM1 regulates mitosis and EMT of breast cancers. Depletion of FoxM1 promotes chromosomal instability and polyploidy. Stable knockdown of FoxM1 may induce mitotic catastrophe due to faulty cytokinesis leading to centrosomal amplification and the formation of multipolar spindles [19]. FoxM1-mediated EMT occurs in part through upregulation of EMT transcription factors, such as Slug [24]. Importantly, FoxM1 appears to play a critical role in EMT driven by constitutive cell surface receptor kinase signaling, such as insulin-like-growth factor-1 receptor signaling [25, 26]. FoxM1 overexpression promotes resistance to kinase-targeting strategies, with FoxM1 inhibition blocking EMT and invasion of kinase-driven breast cancer cells [25, 26].

The 25-kb foxm1 gene contains 10 exons and is located at chromosomal position 12p13.33 [18]. Alternative splicing of exons Va and/or VIIa yields at least four human FoxM1 isoforms, FoxM1a, FoxM1b, FoxM1c, and FoxM1d [18, 27], each containing an N-terminal repressor domain, a highly conserved DNA-binding domain, and a C-terminal
transactivation domain (Figure 1). Other published articles provide detailed information about FoxM1 isoforms and functions, as well as the roles that other Fox family members play in regulating FoxM1 function [15, 18, 28–35]. FoxM1b and FoxM1c are the main isoforms studied in the context of cancer research, as both are expressed at high levels in multiple tumor models [36]. FoxM1b lacks exons Va and VIIa, whereas FoxM1c includes exon Va. Both isoforms bind consensus DNA sequence, 5‘-A-C/T-AAA-C/T-AA-3‘, in the promoter of target genes, activating transcription [18]. FoxM1b overexpression occurs in many solid tumors, including ovarian, brain, gastric, pancreatic, skin, and lung tumors, in association with poor prognosis and high tumor grade [37–43]. FoxM1b exhibited a 10-fold higher transforming activity than FoxM1c in ovarian cancer soft agar colony formation assays [36]. Further, overexpression of FoxM1b significantly increased lung metastasis of hepatocellular carcinoma in a p19ARF-null background and promoted EMT prior to cellular invasion [44]. FoxM1b overexpression also induced tumor growth and metastasis of orthotopic gastric cancer xenografts, which was suppressed by FoxM1b knockdown [39].

3.2. FoxM1 expression in breast cancer

The foxM1 transcript is upregulated in breast tumors compared with normal breast tissue [19, 45–47]. Overexpression of FoxM1 is associated with resistance to targeted therapy and chemotherapy in multiple subtypes of breast cancer, although the majority of studies do not specify which isoform is being evaluated [48–56]. Analysis of three data cohorts (TCGA, Richardson Breast 2, and Curtis) in the Oncomine database indicates that foxM1 is overexpressed by 2- to 18-fold in breast tumors versus normal breast [57, 58]. Transcriptional profiling demonstrates upregulation of foxm1 in infiltrating ductal breast carcinomas (n=194) compared with normal breast tissues (n=14), fibrocystic breast tissue (n=17), or fibroadenomas (n=7) [19]. Further, real-time PCR analysis of matched pairs of breast tumor tissue and normal breast tissue shows up to 116-fold increased foxm1 transcript levels in tumors, with stage III carcinomas showing higher foxM1 than stage II carcinomas [19]. Immunohistochemical (IHC) staining also demonstrates higher expression of nuclear FoxM1 in breast cancers versus normal breast tissues [45].

FoxM1 association with breast cancer subtype varies according to individual studies. In luminal subtypes, FoxM1 regulates ER alpha expression and transcriptional activity, with forkhead binding domains identified in the ESRI (ER alpha) promoter region [59, 60]. ER beta1 appears to repress foxM1 transcription by displacing ER alpha from the foxM1 promoter in ER-positive breast cancers, with a significant inverse correlation found between foxM1 transcript or FoxM1 protein and ER beta1 expression levels in patient samples [61]. High foxM1 transcript levels significantly correlate with shorter distant metastasis-free, relapse-free, or overall survival rates particularly in luminal subtypes and in patients receiving adjuvant chemotherapy alone or tamoxifen alone, suggesting potential association with resistance to chemotherapy or endocrine therapy [57, 60, 62]. Recent data also suggest that upregulation of FoxM1 is significantly associated with large tumor size, high tumor grade, lymph node metastasis, high Ki67, chemo- and endocrine resistance, and reduced disease-free and overall survival in male breast cancer patients [63, 64].
Another study suggests that nuclear FoxM1 IHC score does not correlate with stage, lymph node status, or histological grade, but does significantly correlate with high HER2 expression level [45, 47]. Correlations between HER2 subtype and FoxM1 overexpression may reflect the published effects of HER2 signaling on FoxM1 transcription and function. Transfection of a HER2 expression plasmid increases FoxM1 reporter-promoter activity and FoxM1 protein expression; conversely, HER2 knockdown or kinase inhibition using lapatinib reduces FoxM1 reporter-promoter activity and FoxM1 expression in her2-amplified breast cancer cell lines [47]. Transgenic MMTV-c-neu mice also show increased FoxM1 by IHC in mammary epithelial cells compared with control wild-type mice [47]. Recent data mining analyses confirm that FoxM1 transcript levels are significantly higher in the HER2 subtype, but also demonstrate significant FoxM1 overexpression in the basal-like subtype compared with luminal subtypes of breast cancer [57]. Stratification of patient samples into basal-like versus non-basal-like subtypes or TNBC versus non-TNBC confirm significantly higher FoxM1 transcript levels in basal-like and TNBC subgroups [57, 62]. Gene set enrichment analyses of TCGA and METABRIC patient groups indicate that FoxM1 is the most highly upregulated gene in TNBC [65].

Despite strong data implicating FoxM1 in the progression of multiple subtypes of breast cancer, published studies have generally not differentiated among isoforms. Future research is needed to determine which isoforms are overexpressed in each subtype of breast cancer, and which isoforms should be therapeutically targeted in drug-resistant and metastatic breast cancers.

3.3. Upstream kinase signaling regulation of FoxM1 function in breast cancer

As transcription factor to more than 200 target genes [17], FoxM1 plays a critical role regulating each of the classical hallmarks of cancer [30]. Upstream kinase signaling pathways that induce phosphorylation of FoxM1 directly regulate its transcription factor function and subsequent biological effects in cancer cells (Figure 2). MEK-Erk1/2 induces phosphorylation of S331 and S704 on FoxM1 [66], and is constitutively activated in some HER2-positive tumors and TNBCs in association with disease progression and mortality [67–69]. Sustained MEK signaling drives resistance to HER2-targeted agents via FoxM1 phosphorylation, nuclear re-localization and increased transcription factor function [70]. Further, preclinical data demonstrate that MEK inhibition improves response to HER2-targeted agents in trastuzumab-resistant breast cancer via modulation of FoxM1 expression [70]. These data provide rationale for pursuing combination MEK and HER2 inhibition as a strategy for suppressing FoxM1 activity in drug-resistant HER2-positive breast cancers.

Another critical regulator of FoxM1 phosphorylation and activation is the mitotic kinase Polo-like kinase 1 (PLK1), which is significantly associated with metastasis, drug resistance, and p53 mutation in breast cancer cells [71–74]. Preclinical genetic or pharmacological PLK1 inhibition induces mitotic catastrophe and apoptosis, reduces survival of tumor-initiating cells, suppresses tumor growth and metastasis, and demonstrates synergy with radiation, taxanes, and HER2-targeted agents in various models and subtypes of breast cancer [71, 73–79]. Unfortunately, PLK1 inhibitor monotherapy demonstrated limited anti-tumor activity among patients with multiple types of tumors, including breast cancer [80–
However, targeting PLK1 in specific molecular subgroups, including p53-mutant cancers, was beneficial in another tumor type [84]. PLK1 and FoxM1 exhibit coordinate expression and positive feedback. In fact, the ability of PLK1 to promote mitotic progression depends on FoxM1 function, which, in turn, upregulates PLK1 expression [85–88]. PLK1 binds the C-terminus of FoxM1 after Cdk1-dependent phosphorylation and directly phosphorylates multiple residues on FoxM1 [86]. PLK1 drives invasion of basal-like breast cancer, in part by regulating vimentin and beta 1 integrin [89]. Similarly, FoxM1 induces expression of beta 1 integrin with increased Src-FAK signaling in TNBC [90]. Increased integrin signaling through Src-FAK then reduces PLK1 degradation, facilitating mitotic progression [91]. Thus, FoxM1 and PLK1 appear to form a feedback loop, driving mitosis and invasion of breast cancer cells, and supporting co-targeted therapeutic strategies.

### 3.4. Targeting FoxM1 expression and transcription factor function

Based on data demonstrating upregulation of FoxM1 in breast cancer, and correlations between FoxM1 expression level and clinical outcomes, preclinical studies have evaluated FoxM1 as a potential therapeutic target in breast cancer. FoxM1 is expressed in proliferating embryonic cells, including cardiac myocytes, but expression is almost undetectable in most normal adult tissues [19, 92], making it an attractive tumor-specific target. Several small-molecule chemical inhibitors of FoxM1 are described in the literature and may prove to be suitable lead compounds for further drug development. Preclinical studies should evaluate direct chemical inhibition of FoxM1 in combination with established upstream signaling inhibitors as a potential strategy for treating breast cancers.

A potential strategy for reducing FoxM1-mediated cancer progression is disruption of specific FoxM1 protein-protein interactions. One example of this strategy uses a peptide mimic of p19ARF amino acids 22-44, which is the sequence that binds FoxM1b, resulting in inhibition of metastasis of FoxM1b-overexpressing hepatocellular cancer [44] and reduced U2OS osteosarcoma colony growth in soft agar [20]. Tumor tissues in xenograft models of hepatocellular carcinoma treated with the p19ARF peptide inhibitor exhibit reduced proliferation and increased apoptosis with reduced expression of the FoxM1 targets PLK1, Aurora B kinase and survivin [93]. As illustrated by the p19ARF peptide, peptide mimics that block interactions of FoxM1 with critical mediators of cancer-related processes may be developed as therapeutics. Understanding the mechanisms through which FoxM1 promotes breast cancer invasion and metastasis will provide key information to design these types of therapies.

Pharmacological disruption of FoxM1-DNA interactions or knockdown of FoxM1 expression are additional strategies for blocking FoxM1-regulated transcription. These strategies are illustrated by the thiazole antibiotics siomycin A and thiostrepton, which selectively down-regulate FoxM1 expression and activity without affecting other forkhead proteins [94, 95]. FoxM1 overexpression reduces thiazole-stimulated apoptosis, indicating that the anti-cancer activity of thiazole antibiotics relates in part to their regulation of FoxM1 expression [94]. Thiostrepton interacts directly with the DNA-binding domain of the FoxM1 protein, impeding FoxM1 binding to target promoter sites and suppressing expression of FoxM1 target genes in ER-positive MCF7 breast cancer cells [60, 96]. These FoxM1 target
genes (n=38), which include **CCNB1, LMNB1, CDC20, AURKA**, and multiple genes encoding kinesin and centromere or centrosome proteins, correlate with poor clinical outcome of patients with ER-positive breast cancer [60]. Thus, thiazole antibiotics should be further developed as drugs that disrupt FoxM1-DNA interactions, potentially leading to improved clinical outcomes for patients with breast cancer.

Similar to thiazole antibiotics, FDI-6, a small molecule identified from a library of more than 54,000 chemical compounds, directly binds to the DNA-binding domain of FoxM1, impeding interactions with target gene promoters in ER-positive MCF7 breast cancer cells [97]. *In silico* modeling demonstrates that thiostrepton, FD-6, and the thiazolidinedione troglitazone [98], each employ an electron-deficient sulfur atom to bind His287 in the DNA-binding domain of the FoxM1 protein [99]. Interestingly, FoxM1 small-molecule inhibitors FDI-6 and thiostrepton also inhibit AR signaling through a FoxM1-dependent mechanism involving beta catenin [100]. These data suggest that His287 may be a critical target site against which drugs should be developed to effectively inhibit FoxM1 transcription factor function. Further, small-molecule FoxM1 inhibitors may have benefit against specific AR-driven cancers, including the AR subtype of TNBCs.

Another class of pharmacological agents that reduces FoxM1 expression is the bromodomain and extraterminal domain (BET) inhibitors. BET protein BRD4 is an epigenetic reader that binds acetylated histones to regulate gene transcription. BRD4 expression correlates with FoxM1 expression in ovarian cancer [101]. BRD4 induces expression of ER alpha target genes, mediates proliferation of ER-positive breast cancer cells, and stimulates TNBC migration and invasion [102–104]. BET bromodomain inhibitors, including JQ1, competitively displace BRD4 from chromatin, blocking transcription of target genes [105]. TNBCs are particularly sensitive to bromodomain inhibitors, possibly due to suppression of Aurora kinases and subsequent inhibition of mitosis [104, 105]. Bromodomain inhibitors reduce expression of FoxM1 and target genes, including those encoding Aurora kinase B and PLK1, in ovarian cancer [101]. Thus, it is possible that FoxM1 inhibition is one mechanism through which bromodomain inhibitors block TNBC growth and progression. Dual inhibition of BRD4 and FoxM1 should be evaluated in preclinical studies to determine efficacy against breast cancers, particularly drug-resistant ER-positive and triple-negative breast cancers.

### 4. Summary and Conclusions

FoxM1 overexpression occurs in many drug-resistant breast cancers, including those of the HER2 and TNBC subgroups. Past pharmacological efforts targeting transcription factors focused primarily on strategies to alter expression, DNA-binding activity, or protein-protein interactions [106]. Examples of these strategies include antisense, peptide mimics that impede protein-protein interactions, and small-molecule chemical disruptors of protein interactions with DNA or other proteins [106]. The latter approach is currently the most commonly employed for experimental manipulation of FoxM1 function and offers several potential leads for drug development. Although the area encompassing a protein-protein interaction may be rather sizeable, structural biochemistry may facilitate identification of a minimal region required for a specific interaction. The risk of altering multiple protein-
protein interactions unrelated to the target transcription factor may be high, however, as the binding ability of the partner protein to other proteins may also be affected. Most recently identified small-molecule inhibitors of FoxM1 target its DNA-binding region to disrupt transcription factor activity. Alteration of FoxM1 protein-protein or protein-DNA interactions is likely to affect dozens of target genes and biological pathways. Further, because FoxM1 represents the convergence of multiple signaling pathways, blockade of FoxM1 function would ultimately inhibit numerous upstream kinase cascades. Although this may increase the anti-cancer activity of FoxM1-targeted approaches, potential toxicities must be carefully considered. Finally, expression of isoforms b and c are documented in many solid tumors, with isoform d only recently being described. Questions remain regarding which isoforms must be targeted for optimal inhibition of FoxM1 function in breast cancer. Future studies should investigate isoform-specific functions and differential anticancer activity of isoform-specific inhibitors in breast cancer. In addition, studies should determine which FoxM1-targeted combination regimens are likely to benefit patients with progressive disease, and which subtypes are most likely to benefit from FoxM1-targeted therapy.

Acknowledgments

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References


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Figure 1. Schematic of FoxM1 protein isoforms
The figure illustrates differences in exons Va and VIIa among FoxM1 isoforms a-d. FoxM1a contains both exons, lacks transcription factor function, and retains DNA-binding activity, resulting in potential transcriptional repressor activity [27, 107]. FoxM1b lacks exons Va and VIIa, whereas FoxM1c includes exon Va. FoxM1d was only recently identified [27], and contains exon VIIa. Expression of FoxM1d promoted EMT in colorectal cancer cells through an interaction with and activation of Rho-associated kinase 2 (ROCK2), leading to subsequent actin polymerization [27]. FoxM1d promoted metastasis in a xenograft model of colorectal cancer, and high expression levels of FoxM1d correlated with metastasis among patients with colorectal cancer [27].
Figure 2. Signaling regulation of FoxM1 phosphorylation
The schematic illustrates the roles of MEK-Erk1/2 and PI3K-Akt-mTOR as upstream regulators of FoxM1 phosphorylation and nuclear localization, activating transcription factor function. The figure also shows specific phosphorylation sites and kinases directly or indirectly regulating phosphorylation of those sites. We highlight the roles of MEK-Erk1/2 and PLK1 in FoxM1 phosphorylation within the text, and the potential for combination targeting of FoxM1 with MEK or PLK1. Another upstream signaling regulator of FoxM1 function is CDK4/6 due to its interaction with hypophosphorylated retinoblastoma (Rb) protein, which leads to repression of the transactivation function of FoxM1 [22]. Upon phosphorylation by CDK4/6-cyclin D, Rb no longer binds FoxM1, increasing protein stability [108] through an indirect mechanism involving phosphorylation and release of Rb [109]. Dual targeting of CDK4/6 and FoxM1 is a potential combination strategy to evaluate in preclinical models of FoxM1-overexpressing breast cancer.
# Table 1

Molecular Subtypes of Breast Cancer

<table>
<thead>
<tr>
<th>Molecular Subtype</th>
<th>Molecular Characteristics</th>
<th>Prevalence</th>
<th>Standard Treatments</th>
<th>References</th>
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<tbody>
<tr>
<td>Luminal A</td>
<td>ER+/PR+ HER2- Ki67 low</td>
<td>65%-75%</td>
<td>AIs, SERMs, SERDs, CDK4/6 inhibitors, mTOR inhibitor</td>
<td>[4–8]</td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+/PR+ HER2- or HER2+ Ki67 high</td>
<td></td>
<td>AIs, SERMs, SERDs, CDK4/6 inhibitors, mTOR inhibitor</td>
<td></td>
</tr>
<tr>
<td>HER2-enriched</td>
<td>ER-/PR- Usually HER2+ Proliferation genes</td>
<td>10%-15%</td>
<td>HER2 mAbs, HER2 TKIs</td>
<td>[4, 9]</td>
</tr>
<tr>
<td>Basal-like</td>
<td>ER-/PR- Usually HER2- (80% of basal-like are TNBC) Basal cytokeratins</td>
<td>15%-20%</td>
<td>Chemotherapy</td>
<td>[10–14]</td>
</tr>
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Table 2

FoxM1-Targeting Strategies

<table>
<thead>
<tr>
<th>Type of Inhibitor/Strategy</th>
<th>Example</th>
<th>Mechanism</th>
<th>References</th>
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<tbody>
<tr>
<td>Peptide mimics against FoxM1 or binding partners</td>
<td>p19ARF peptide mimic</td>
<td>Directly bind FoxM1 or binding partner, disrupting specific FoxM1 protein-protein interactions</td>
<td>[20, 44, 93]</td>
</tr>
<tr>
<td>Thiazole antibiotics</td>
<td>Siomycin A, thiostrepton</td>
<td>Selectively down-regulate FoxM1 expression and activity</td>
<td>[60, 94–96]</td>
</tr>
<tr>
<td>Forkhead domain inhibitor</td>
<td>FDI-6</td>
<td>Small molecule that directly binds DNA-binding domain of FoxM1, impeding FoxM1 function</td>
<td>[97–100]</td>
</tr>
<tr>
<td>Bromodomain and extraterminal domain (BET) inhibitors</td>
<td>BRD4 inhibitor, JQ1</td>
<td>Competitively displace BRD4 from chromatin, blocking transcription of target genes, indirectly reducing FoxM1 expression</td>
<td>[102–105]</td>
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