



# Cyclin-dependent kinases in breast cancer: expression pattern and therapeutic implications

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## Abstract

Presently, breast cancer (BC) is one of the most common malignancies diagnosed and the leading cause of tumor-related deaths among women worldwide. Cell cycle dysregulation is one of the hallmarks of cancer, resulting in uncontrolled cell proliferation. Cyclin-dependent kinases (CDKs) are central to the cell cycle control system, and deregulation of these kinases leads to the development of malignancies, including breast cancer. CDKs and cyclins have been reported as crucial components involved in tumor cell proliferation and metastasis. Given the aggressive nature, tumor heterogeneity, and chemoresistance, there is an urgent need to explore novel targets and therapeutics to manage breast cancer effectively. Inhibitors targeting CDKs modulate the cell cycle, thus throwing light upon their therapeutic aspect where the progression of tumor cells could be inhibited. This article gives a comprehensive account of CDKs in breast cancer progression and metastasis and recent developments in the modulation of CDKs in treating malignancies. We have also explored the expression pattern and prognostic significance of CDKs in breast cancer patients. The article will also shed light on the Implications of CDK inhibition and TGF- $\beta$  signaling in breast cancer.

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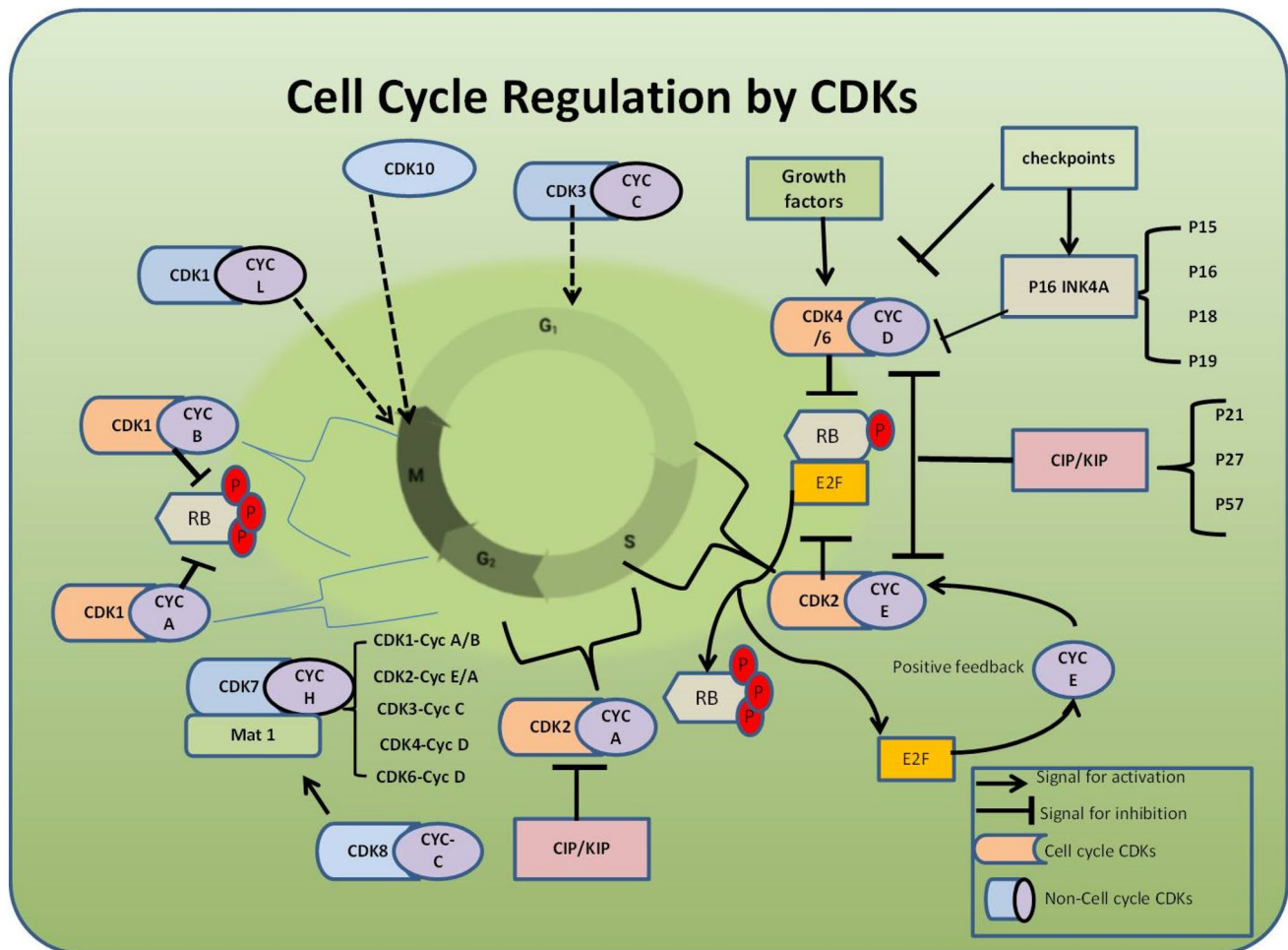
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## Graphical abstract



**Keywords** Cyclin-dependent kinases · Cyclins · Breast cancer · Cell cycle · CDK inhibitors · Prognosis

### Abbreviations

BC	Breast cancer
Lum A	Luminal A
HER2 <sup>+</sup>	Human epidermal growth factor-2
Lum B	Luminal B
CDK	Cyclin-dependent kinase
OS	Overall survival
EMT	Epithelial-mesenchymal transition

### Introduction

Breast cancer is the most prevalent cancer diagnosed worldwide [1–5]. According to the GLOBOCAN 2020 data, the incidence rate of BC is highest compared to the other ten most common types of cancer. Globally, the no of new cancer cases has reached a total count of 19.3 in

2020. With an anticipated 2.3 million new cases (11.7%), female breast cancer has surpassed lung cancer as the most often diagnosed malignancy, followed by lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach (5.6%). With a projected 1.8 million fatalities (18%), lung cancer remained the top cause of cancer death, followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%) cancers. In transitioned vs transitional countries, the overall incidence was 2–3 fold greater for both sexes, although mortality varied twofold for males and slightly for women. Female breast and cervical cancer death rates, on the other hand, were much higher in transitioning nations than in transitioned countries (15.0 vs 12.8 per 100,000 and 12.4 vs 5.2 per 100,000, respectively) [6]. The overall survival of BC patients having early-stage, non-metastatic BC stands at 70–80% [7]. Due to its heterogeneity, BC possesses various morphological

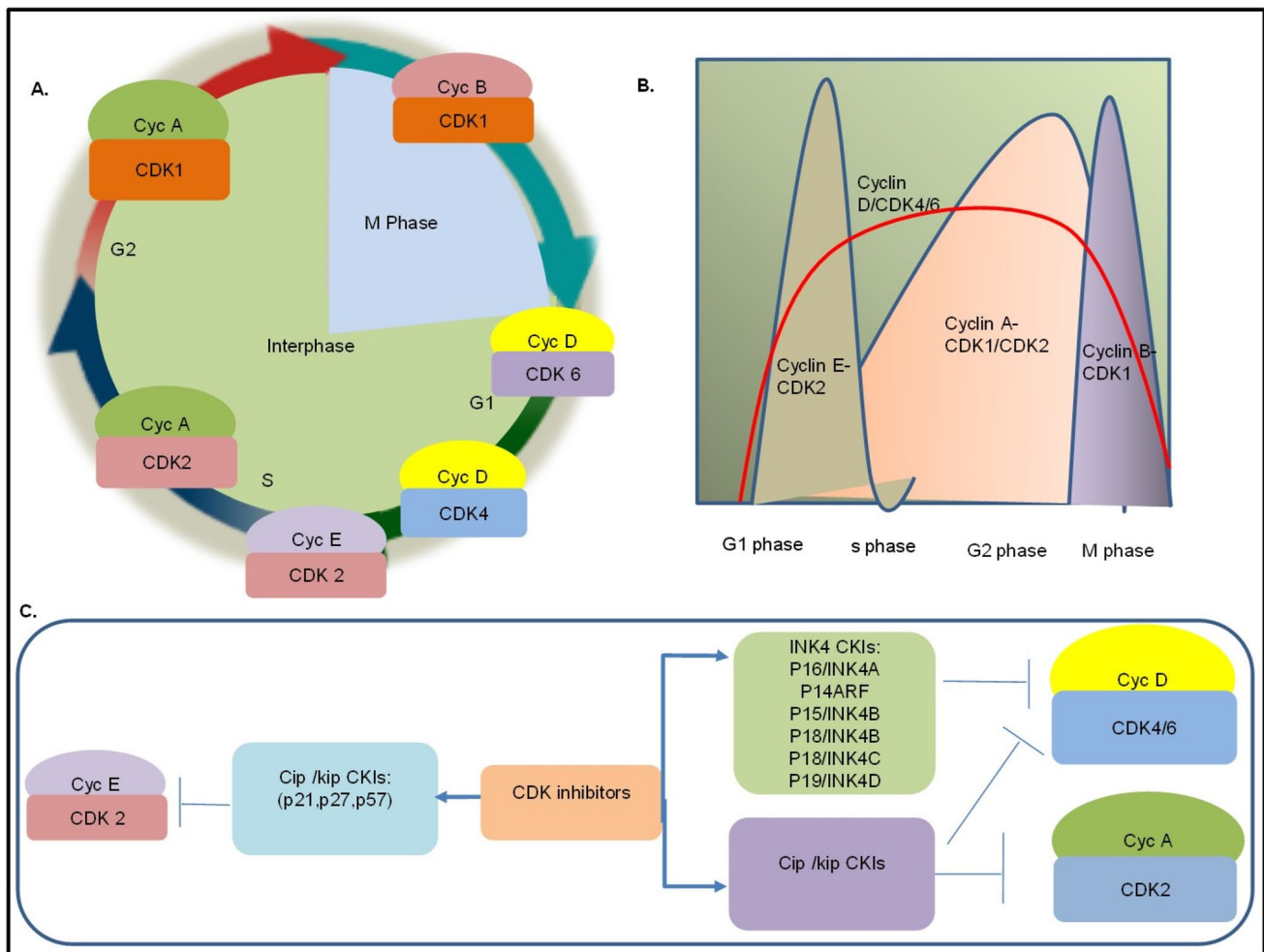
descriptions, differences in their profiles concerning their immune-histology, and a specific pattern of distinct molecular subgroups [8]. Based on the gene expression patterns and molecular portraits, BC has been classified into four subtypes: luminal A (Lum A), Luminal B (Lum B), HER2-enriched (HER2<sup>+</sup>), and triple-negative breast cancer (TNBC) [9, 10]. Among these subtypes, TNBC is the most aggressive one due to the loss of all three receptors, i.e., ER, PR, and HER2 receptors [8, 11]. This heterogeneity of breast tumors has to be considered for any treatment approach. The treatment strategies presently available for BC patients include radiotherapy, chemotherapy, surgery, targeted therapy, and a combinatorial approach [10, 12, 13]. Recent advances in diagnosis and therapeutic strategies have significantly increased the overall survival of BC patients [14, 15]. However, breast tumors demonstrate tumor relapse and therapeutic resistance due to the inherent genetic instability of tumor cells and tumor-stroma cross talks [16–18]. Thus, there is an urgent need to explore novel therapeutic targets and treatment strategies for effective breast cancer treatment.

Among the various hallmarks of cancer, increased cell proliferation is one of the most critical aspects that need to be considered [19, 20]. The proliferation of cells is regulated by the cell cycle, and there are well-defined regulatory mechanisms that regulate the cell cycle. The cell cycle is controlled by various cyclins and cyclin Dependent Kinases (CDKs). The importance of cyclins and CDKs in the cell cycle was revealed in a study of fission yeast, which demonstrated the significance of complex Cdc2 (CDK1) [21]. CDKs belong to the serine-threonine kinases regulated by cyclins and phosphorylate several downstream targets during cell cycle progression [21]. Cell division during an individual's life span and development occurs only at specific places and at a specific time and divides the cell's content accurately [22]. The cell cycle checkpoints maintain the coherence, integrity, and maintenance of every step in the cell cycle [22]. Breast cancer, like many other cancers, involves increased proliferation of cells, which results from the disruption in the cell cycle regulation by dysregulated expression and activation of CDKs [23, 24]. CDK dysregulation during BC leads to the uncontrolled proliferation of cancer cells, thus maintaining BC's progression and other factors [21]. Studies have revealed that BC is associated with dysregulation of several Cyclin/CDKs, and their dysregulation has also been observed in breast tumor heterogeneity [25]. Targeting CDKs in BC has been explored, demonstrating significant inhibition of tumor growth with several therapeutic regimens at the clinical stage. In this review, we have shed light on the role of CDKs in the cell cycle, their importance in BC progression and metastasis, their prognostic significance, and the therapeutic implication of CDK inhibition in BC.

## Cyclin-dependent kinases and cell cycle

The expansion and division of cells are regulated by a series of events known as the cell cycle. The cell cycle comprises of mainly four phases, namely the G1 phase (growth phase-1), S phase (DNA replication phase), G2 phase (growth phase-2), and M phase (mitotic phase). All these phases are regulated by a set of CDK/cyclin complexes that assist the cell's progress through these phases. CDKs throughout the process of cell division remain the same while the cell regulates the expression of different cyclins depending upon the stage of the cell cycle [26]. The structure of CDKs consists of two parts: cyclin and kinase. Protein kinase, an enzyme, possesses a highly conserved structure [22]. Cyclin binding to the CDKs triggers the activation of the kinases, and their expression is highly regulated in the multi-step process of the cell cycle. Crystallographic investigations have shed light on the transition of CDKs from inactive to an active form and vice versa [27]. The binding of cyclins induces a conformational change in the catalytic cleft of the kinase with the inherent conformational flexibility. Because of this flexibility, CDKs can exhibit a diverse range of responses in the presence of various growth regulatory signals [27]. CDKs are serine/threonine kinases that phosphorylate several proteins in response to the intrinsic and extrinsic signal, regulating several aspects of cell growth and division [28]. The specificity of CDKs is revealed through the association of specific cyclin with specific CDK (Fig. 1). For instance, CDK2 can form a complex with both cyclin E and cyclin A and acts distinctly upon binding to different cyclins [29]. This demonstrates that the specific CDK having a specific structure possesses a specific purpose in the cell cycle [21]. Besides, the CDK-cyclin complex regulates the stabilization, phosphorylation, and activation of CDKs in distinct cell cycle phases [22]. The importance of this complex lies in the fact that these complexes are crucial for the progression of the cell cycle in terms of phosphorylation of various target genes, like retinoblastoma protein, a tumor suppressor protein. A switch occurs between the inhibition and activation of these cyclins/CDKs in response to the mitogenic signals and checkpoints, respectively [22]. The CKIs (Cyclin-dependent kinase inhibitors), such as INK4 proteins and CIP/KIP (CDK-interacting protein/kinase inhibitory proteins), negatively regulate cyclin/CDKs (Fig. 1) [30]. Also, the cell cycle transition phases are controlled by various mitotic proteins like APC/C (anaphase-promoting complex/cyclosome) and SCF (Skp1–Cul1–F-box-protein) [31–33]. The disruption in any one of the above mechanisms can elevate the proliferation of cells by dysregulating the cell cycle, which is a hallmark of several cancers, including BC [9].

Moreover, several enzymes involved in metabolic pathways, including DNA replication or other cell cycle



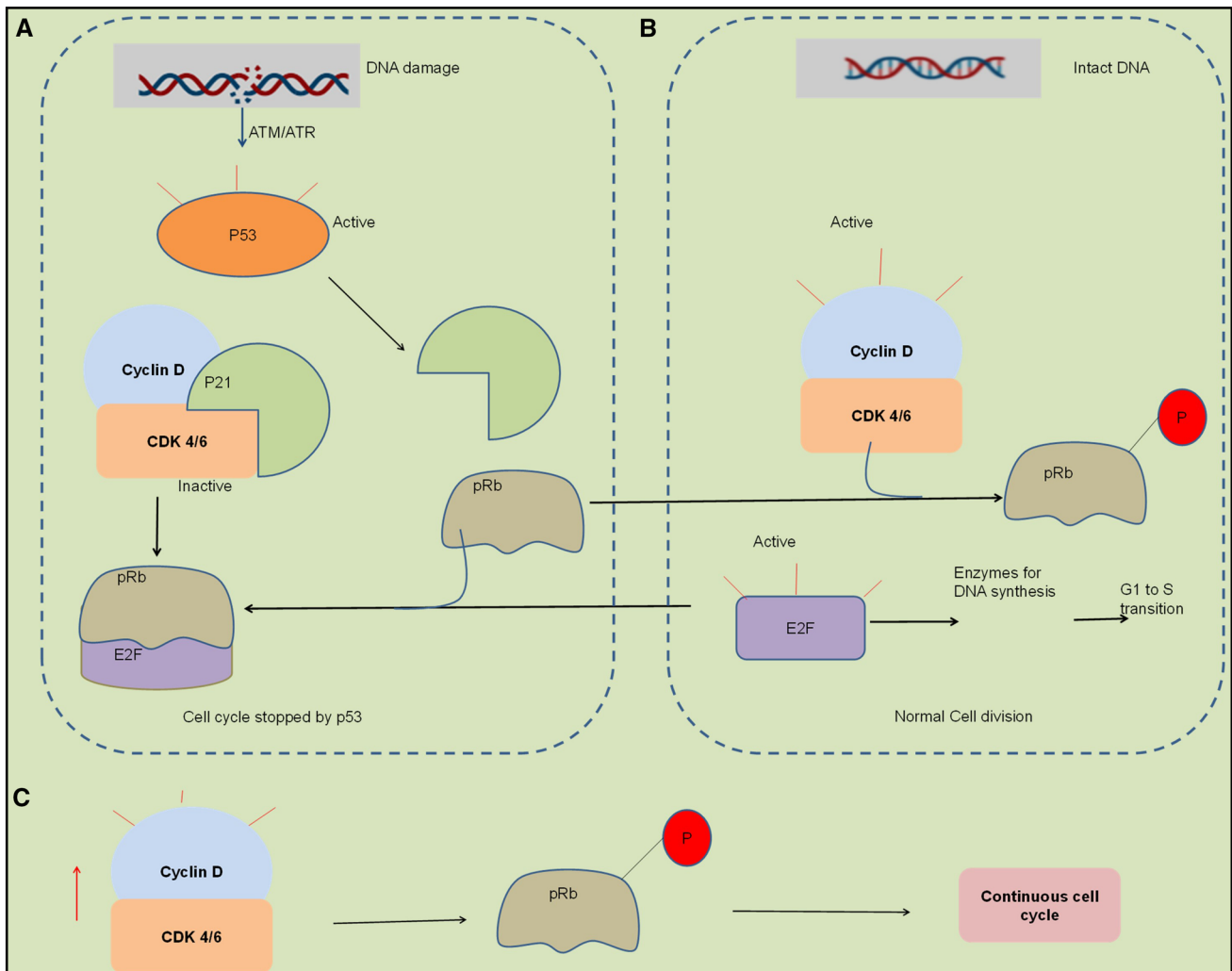
**Fig. 1** CDKs are central to cell cycle regulation: **A** At each phase of the cell cycle, there occurs a specific association between CDKs and their cyclins to proceed further, **B** Cyclic nature of different cyclins, **C** The CDK inhibitors, INK4 and CIP/KIP stop the CDK/cyclin activity

processes, are regulated by CDKs via distinct phosphorylation of crucial enzymes [34]. The association of CDKs with cyclins is of utmost importance, as the latter assists in activating CDKs, and the former phosphorylates the cyclins for the cell cycle phases [35]. CDKs, in association with their cyclin partners, play a significant role in regulating the cell cycle.

### Cell cycle, CDKs, and cancer

Cell cycle deregulation is a classic hallmark of cancers, including BC. In several malignancies, dysregulation of the CDK–cyclin complex disrupts the cell cycle coordination and leads to continuous proliferation of cancer cells [36, 37]. The unsuitable activation of CDKs associated with the amplification and overexpression of cyclin gene, cellular mislocalization or premature cyclin expression, and the inhibition of INK4 or CIP/KIP family leads to the dysregulation in the cell cycle,

thus resulting in cancer [38]. Several studies have revealed that cancer cells mainly lose these inhibitory mechanisms that regulate the cell cycle. The inactivation of the tumor suppressor genes like Rb and TP53 (p53) gene or the upregulation of oncogenes affects the cyclin/CDK upregulation, resulting in an uncontrollable cell cycle progression and proliferation [38]. The cell cycle is negatively regulated by several tumor suppressor genes, such as the Retinoblastoma (Rb) gene, which in its unphosphorylated state acts as a transcription repressor for E2F. The phosphorylation of pRb protein by CDK4/6/cyclin D complex leads to the release of pRb protein from E2F and leads to the transcription of several genes, including DNA polymerase  $\alpha$ , cyclin E, and A responsible for G1/S transition by E2F (Fig. 2). pRb protein in its hypophosphorylated state remains active and arrests the cells in the resting G0 phase by binding to the E2F transcription factor and blocking the expression of genes vital for G1 to S transition. Dysregulation in the CDK/cyclin complex leads to the modulation in the



**Fig. 2** Role of Rb protein in the cell cycle: **A** In its activated state, p53 leads to cell cycle arrest in those cells which are having DNA damage, **B** The phosphorylation of pRb protein by CDK4/6/cyclin D complex leads to the release of pRb protein from E2F and

thereby leads to the transcription of important genes responsible for G1/S transition by E2F, **C** In cancer cells increase in CDK4/6/cyclin D leads to the of Rb protein hyperphosphorylation, thus leading to uncontrolled cell division

phosphorylation state of Rb protein, altering the role of Rb protein and leading to uncontrolled growth of cells [38].

Similarly, p53 is another tumor suppressor gene whose unregulated activation leads to cancer progression [39]. In its activated state, p53 leads to cell cycle arrest in cells with DNA damage. Inactivation of p53 is frequently observed in several types of cancer cells [39]. The inactivation of the tumor suppressor genes like the Rb gene or the upregulation of oncogenes affects the cyclin/CDK upregulation, resulting in an uncontrollable cell cycle progression and proliferation [38, 40].

### CDKs in BC progression

Breast cancer is one of the most common malignancies among women and the leading cause of tumor-related deaths worldwide [7]. Several CDKs have been attributed to the development and regulation of breast tumorigenicity, and inhibiting the overexpressed CDKs reduces tumor burden and metastasis [41]. One of the most crucial CDK/ Cyclin complexes is CDK4/6/Cyclin D which plays a significant role in the initiation and development of various cancers, including BC [42]. Several studies have revealed that cyclin D is overexpressed in early and metastatic BC.

The expression of both cyclin D1 and CDK4 is significantly high in lum B (58% and 25%, respectively) and HER2 BC (38% and 24%, respectively), moderate in lum A (29% and 14%, respectively), and lowest in TNBCs [24].

Moreover, cyclin D1/CDK4/6 is the crucial player contributing to RB protein phosphorylation resulting in the proliferation of cells and thus assisting in the BC tumor progression [7]. Furthermore, several other CDKs have been found deregulated in cancer, including BC. For instance, CDK2 is upregulated in BC, resulting in overexpression of its cognates viz cyclin E and A [43]. The fundamental role of CDK2 in a cell cycle is that it acts as a core regulator in the cell cycle and is functional from late G1 up to the end of the S phase [44]. The activation is done by its cyclin partners, namely cyclins E1 or E2 and A2. CDK2 displays various regulatory functions such as phosphorylating RB protein, SMAD3, and several other proteins that regulate DNA synthesis [45]. The studies have suggested that many cancers have been accompanied by overexpression of CDK2 and are associated with cancer cell proliferation [45]. The study conducted by Xiangming He and co-workers revealed that CDK2 plays a significant role in BC initiation and progression [46]. It was further analyzed that inhibiting CDK2 effectively deaccelerates BC cell proliferation.

In addition to the above, the most critical CDK for maintaining the regulation of cell cycle development in a large number of mammalian cells is CDK1 [47]. CDK1 is a universal master kinase and is an essential regulatory CDK of mitosis. Ligation of CDK1 with cyclins A and B mediates the S-G2 and G2-M transition. CDK1 acts as a crucial CDK regulating the cell cycle through phosphorylation and dephosphorylation. Activation of CDK1 plays a significant role in apoptosis induction [48]. Dysregulation of CDK1 can lead to chromosomal instability, vigorous tumor growth, and a high proliferation rate of cancerous cells [49]. CDK1 is also overexpressed in several cancers, including BC [50]. The studies have revealed that inhibiting CDK1 either with a single drug or with a combination

of drugs is associated with practical anti-cancer effects, thus throwing light on the prognostic significance of CDK1 in BC. Also, CDK1 inhibition has been proposed as a promising therapeutic strategy for MYC-dependent BC patients [50].

While CDK1, CDK2, and CDK4/6 directly influence the modulation in the cell cycle progression, other CDKs like CDK7, CDK8, and CDK9 play their role in transcription (Table 1) [50]. CDK7 acts both as a kinase and a subpart of the transcription factor TFIIH, which regulates transcription and DNA repair [21]. The activation of CDK7 is done by MAT1 and cyclin H, and it shows its role in the cell cycle by phosphorylating other CDKs in the cell [51]. CDK7 has a more significant correlation with the TNBC subtype than other BC subtypes [52]. Another research has also revealed that inhibition of CDK7 in HER2<sup>+</sup> will show much sensitivity compared to the ER<sup>+</sup> BC [52]. The study done by Li Tang and co-workers revealed that CDK7 is upregulated in BC. It was further analyzed that CDK7 expression levels are considerably higher in lobular BC, ductal BC, and invasive ductal BC. The study showed a significant correlation between high CDK7 levels and poor prognosis [53]. Besides CDK7, dysregulation of CDK8 has been associated with various cancers, including HER2-enriched BC [54, 55]. Furthermore, CDK9 is also showing its dysregulation in BC. The studies have pointed out that CDK9 inhibitors retard the growth of BC cells, thus highlighting the role of CDK9 in BC progression [56–58].

From the above discussion, it is clear that BC progression is associated with the dysregulation of various CDKs that play a significant role in the cell cycle progression. Thus, these CDKs can be used as biomarkers for detecting BC. Targeting CDKs can be a potent anti-cancer marker for the treatment of BC, thus throwing light on the prognostic value of CDKs in BC.

**Table 1** Role of some CDK/Cyclin complexes in normal cells and BC cells [10]

CDKs	Cyclin/CDK complex	Role in normal cells	Role in BC Cells
CDK1	Cyclin A/B	Assists in M phase of cell cycle	Plays role in apoptosis of MYC-driven TNBC
CDK2	Cyclin A/E	Related with G1/S phase of cell cycle	Plays role in developing BC or TNBC phenotype
CDK4	Cyclin D	Helps G1/S phase shift of the cell cycle	Helps in BC initiation and regulation of tumorigenesis
CDK6	Cyclin D	Assists in the G1/S phase progression of the cell cycle	Associated with the initiation of BC and regulation of tumorigenesis
CDK7	Cyclin H	Associates with transcription of CAK and RNAPII	Regulates transcriptional addiction to a vital cluster of genes in TNBC
CDK8	Cyclin C	Helps to initiate transcription	Responds to adjuvant therapy in BC; related with tumor progression
CDK9	Cyclin T	RNAPII transcription; regulates elongation of transcription	A prognostic biomarker in BC patients following NACT

### CDKs in breast cancer metastasis

Breast cancer metastasis significantly impacts BC patients' mortality and survival rates. BC metastasis is the leading cause of mortality among BC patients [17]. Almost 20–30% of early-stage BC patients develop distant metastasis, and approximately 90% of BC deaths occur due to the issues of metastatic BC [59]. Thus, it becomes crucial to throw light on the role of CDKs in BC metastasis. BC metastasis often expands to the organs like the brain, liver, lungs, and bones. The aberrant alterations in cyclins and their CDKs lead to continuous cell division and the migration of tumor cells to other organs [60]. The BC cells could be slothful. However, some carcinogens, such as cyclin D1 and CDK4/6, could lead to a systemic response, resulting in the metastasis of BC cells [61]. This depicts the dual function of cyclins that

can interact with the CDKs and create a more aggressive nature of this disease. The studies have revealed the role of CDK4/6 in Epithelial-Mesenchymal Transition (EMT), which is a characteristic feature of cancer metastasis [62, 63]. The research was done by Zhen and co-workers and revealed the role of CDK4/6 in BC metastasis [64]. CDK5 has been studied for its role in TGF-β1 induced EMT in BC progression [65]. Similarly, Adrian and co-workers also revealed the role of cyclin F in EMT [63]. Thus, CDKs show their significant role in BC metastasis, thereby enhancing the aggressiveness of this disease among BC patients.

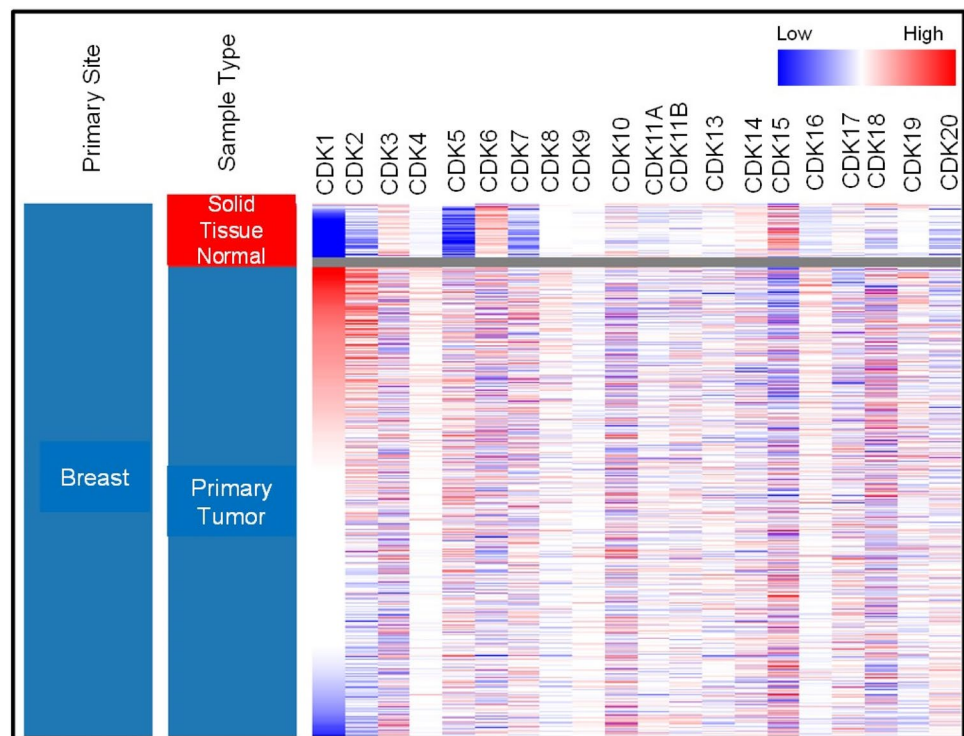
### Expression pattern and prognostic significance of CDKs in BC

The bioinformatics analysis of CDK expression in BC patients had revealed that the CDKs are highly deregulated in BC patients [66–68] (Table 2). Additionally, the expression pattern of various CDKs in BC was analyzed using UCSC XENA online database [69]. We utilized the TCGA dataset of breast cancer and grouped patients into normal, solid tissue, and primary tumors. The expression pattern was analyzed, and a heat map of the given CDKs was generated. It was concluded that CDK1, CDK2, CDK4, CDK7, and CDK8 show high overexpression in primary tumors as compared to the CDK6 and CDK9 which shows low expression in primary tumors (Fig. 3). Also, various other studies have

**Table 2** Log2 fold change of some CDKs in breast cancer

Gene	Log 2 median fold change	Status
CDK1	2.842	Highly Upregulated
CDK2	0.352	Upregulated
CDK4	0.635	Upregulated
CDK6	− 0.423	Down regulated
CDK7	0.877	Upregulated
CDK8	0.181	Upregulated
CDK9	− 0.341	Down regulated

**Fig. 3** Heat map of CDKs in Breast cancer; CDK1, CDK2, CDK4, CDK7, and CDK8 show high overexpression in primary tumors as compared to the CDK6 and CDK9 which shows low expression in primary tumors

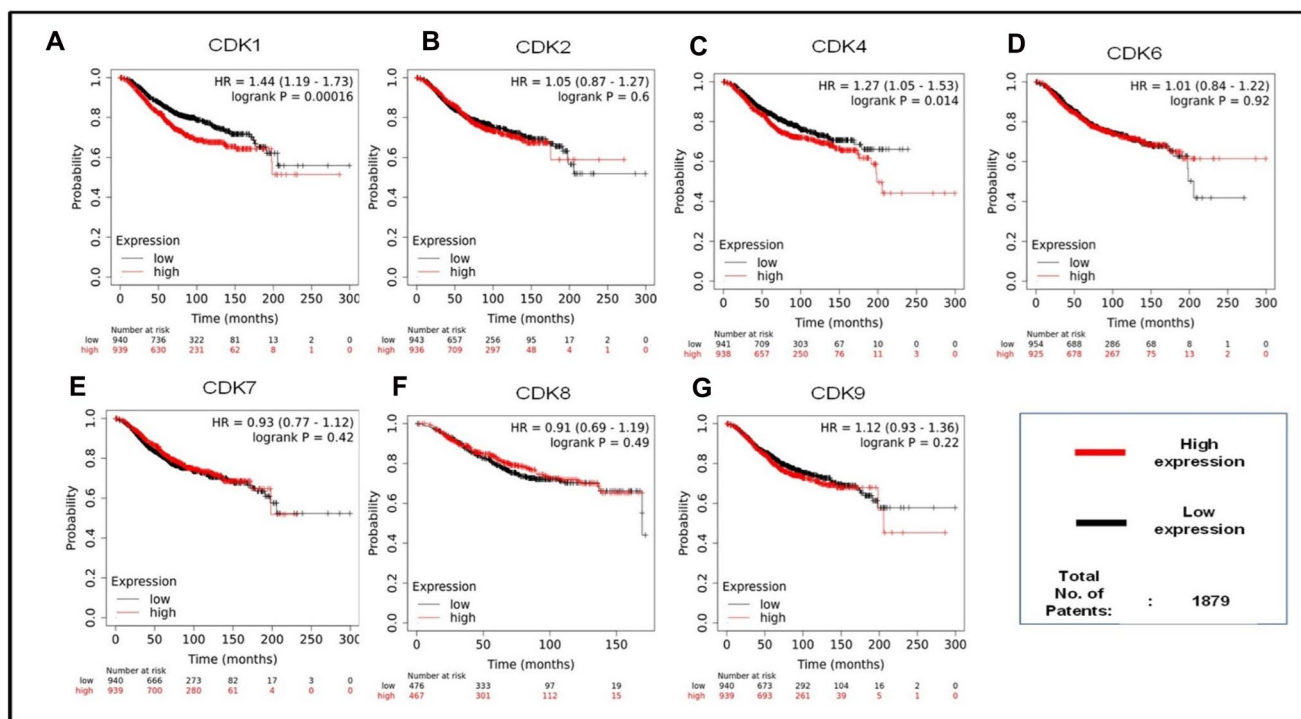


revealed the dysregulation of many CDKs that contribute to the progression of BC [21].

As discussed above, several elegant studies have established the role of CDKs in BC progression, stemness, and metastasis [70]. Additionally, the expression pattern of CDKs in BC is related to the prognosis of BC patients. We analyzed the prognostic significance of highly deregulated CDKs in BRCA patients using a Kaplan–Meier plotter. The Kaplan–Meier plotter (<https://kmplot.com/>) is a web resource with gene expression data and information regarding the survival of BC patients [71]. Breast cancer patients were divided into two cohorts based on the median expression of CDKs, defined as high and low expression groups. The results revealed that BC patients with low mRNA levels of CDK1 and CDK4, exhibited better Overall Survival (OS) (Fig. 4). The other deregulated CDKs did not show significant association with OS. These results demonstrate that targeting of CDK1 and CDK4 in BC can potentially bear fruitful results and also their modulation in combination with conventional therapies may be a promising approach in effective management of breast cancer patients.

## CDK inhibition in BC: therapeutic implications

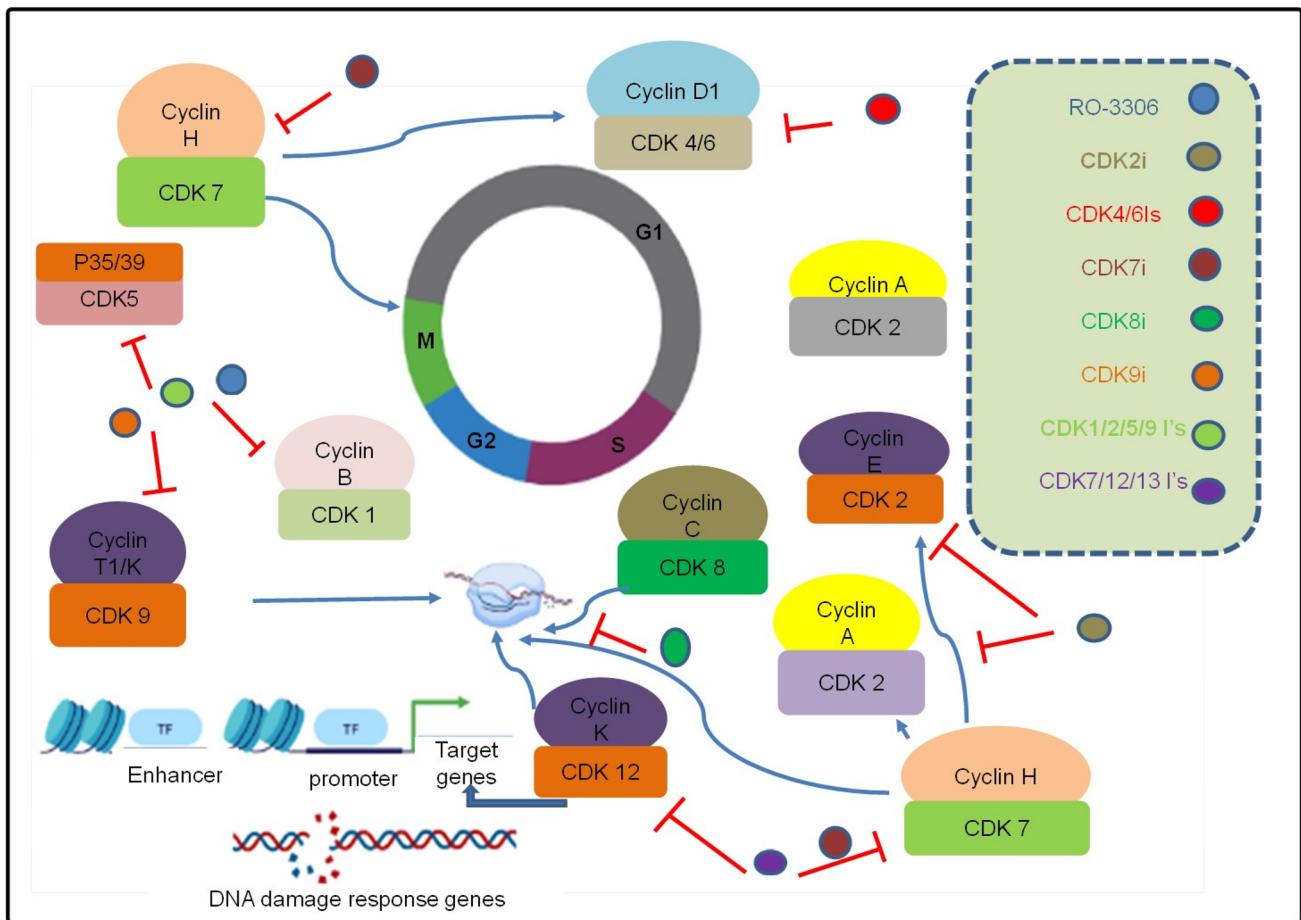
CDKs and their cyclins are promising targets for treating BC due to their function of supporting tumor cell proliferation. There has been significant progress in BC treatment because of the innovations and efficient medications during the last decade. In terms of the above CDKs, various studies have analyzed the role of certain drugs used as inhibitors against specific CDK. CDK inhibitors have been categorized into non-selective pan-inhibitors and selective inhibitors based upon their specificity toward a particular CDK [42]. These inhibitors have been analyzed during various clinical studies of BC. CDK inhibitors target the malignant cells by interfering with their cell cycle regulators (Fig. 5), thus throwing light upon their therapeutic aspect where the progression of tumor cells could be retarded with an enduring impact on the normal cells [21].



**Fig. 4** Expression pattern of cyclin-dependent kinases is associated with overall survival (OS). **A** BC patients with low mRNA levels of CDK1 show better Overall Survival (OS) with HR=1.44 and log-rank *p*-value of 0.00016. **B** Breast cancer patients with low mRNA levels of CDK2 does not show significant association with Overall Survival (OS) (HR=1.05 and log-rank *p*-value of 0.6). **C** Breast cancer patients with low mRNA levels of CDK4 show better Overall Survival (OS) (HR=1.27 and log-rank *p*-value of 0.014). **D** Breast cancer patients with low mRNA levels of CDK6 does not show sig-

nificant association with Overall Survival (OS) (HR=1.01 and log-rank *p*-value of 0.92). **E** Breast cancer patients with low mRNA levels of CDK7 does not show significant association with Overall Survival (OS) (HR=0.93 and log-rank *p*-value of 0.42). **F** Breast cancer patients with low mRNA levels of CDK8 does not show significant association with Overall Survival (OS) (HR=0.91 and log-rank *p*-value of 0.49). **G** Breast cancer patients with low mRNA levels of CDK9 does not show significant association with Overall Survival (OS) (HR=1.12 and log-rank *p*-value of 0.22)





**Fig. 5** Mechanism of action of CDK inhibitors; these inhibitors specifically bind a particular CDK and inhibit them in a very specific manner; thus, helping to control the cell cycle dysregulation

**CDK inhibitors**

**1st generation CDK inhibitors**

Generally, first-generation inhibitors belong to Pan-CDK inhibitors, with most of them non-specific toward CDKs. One of the classic pan-CDK inhibitors highly studied among 1<sup>st</sup> generation CDK inhibitors is Flavopiridol. Phase I and phase II studies have demonstrated that Flavopiridol exhibits the most negligible single-agent potency and displays specific toxicities like neutropenia [72]. For instance, Flavopiridol in MBC exhibit significantly greater rates of neutropenia [73]. Other Pan-CDK inhibitors are UCN 01 and R-Rescovitine (Cyclacel, seliciclib) (Table 3). UCN-01 is involved in arresting cells at the G1/S phase, activation of P21, and pRb hypo phosphorylation. Besides this, UCN 01 exhibits several toxicities like arrhythmia, hyperglycemia, and pulmonary dysfunction in phase 1 clinical studies [74, 75].

**Table 3** Pan-CDK inhibitors in clinical trials [70]

Name	Alternate name	Clinical phase
UCN-01	7-Hydroxystaurosporine	Phase I
Dinacliclib	SCH727965	Phase III
Flavopiridol	L868275	Phase II
Roniciclib	BAY1000394	Phase II
R-roscovitine	CYC202, Seliciclib	Phase II
TG02	SB1317	Phase II
AT7519	AT519	Phase II
Indirubin	Indigopurin	Phase IV

**2nd generation CDK inhibitors**

The problem with the 1<sup>st</sup> generation of CDK inhibitors lies with their potency, safety, and durability. They are not specific against their target CDKs, thus resulting in various side effects. In terms of clinical potency, safety, and tolerance, CDK inhibitors have mainly shown disappointing results. One of the significant drawbacks of 1<sup>st</sup>

generation inhibitors is that they lack particularity toward the target kinases, which are responsible for their unpredictable and severe side effect profiles. Also, 2nd generation CDK inhibitors were discovered in the late 1990s and early 2000s that exhibited specific inhibition toward a particular CDK [70].

### CDK4/6 specific inhibitors

There are many inhibitors of CDK4/6 that have been studied in clinical trials. One such inhibitor is Palbociclib. Palbociclib was approved by US FDA on 3 February 2015, being used as 1st line treatment in advanced post-menopausal ER<sup>+</sup> and HER2<sup>-</sup> BC. This CDK4/6 inhibitor is taken orally, possesses IC<sub>50</sub> of 0.01 μM in vitro conditions, and exhibits high specificity when examining other 36 kinases, including CDK2 (IC<sub>50</sub> > 5 μM) (Table 4) [76]. Studies revealed that Palbociclib acts specifically to target CDK4/6 only. It shows a well-defined inhibition of BC cell proliferation, associated with complete G1 arrest and pRb protein dephosphorylation and retards the E2F-dependent gene expression [77]. Phase 1st clinical trials of Palbociclib displayed excellent bioavailability, usually mild to average unfavorable event profile, and significant dose-limiting toxicities associated mainly with myelosuppression [78]. One study demonstrated the inhibitory effect on the growth of various BC cell lines with different molecular characteristics. It was observed that Lum, HER2<sup>+</sup>, or ER<sup>+</sup> featured BC cell lines showed greater sensitivity than the non-luminal ones that showed more excellent resistance toward Palbociclib [79]. This study also revealed the synergistic effect of Palbociclib in combination with tamoxifen or trastuzumab in ER<sup>+</sup> and HER2<sup>+</sup> cell lines, respectively. Also, Palbociclib exhibited activity in those cell lines resistant to tamoxifen, thus revealing that it could be clinically effective in ER<sup>+</sup> BC, which is hormone-resistant. Besides Palbociclib, other CDK4/6 inhibitors present in early clinical trials include Abemaciclib and Ribociclib, both of which have entered phase III clinical trials based on the palbociclib experience [42].

**Table 4** CDK4/6 inhibitors in clinical trials [42]

Inhibitor	Alternate names	Phase	In-vitro inhibition profile (IC <sub>50</sub> , nM)
Palbociclib	(PD-0332991)	Approved	CDK4 (cyclinD1): 11 CDK4 (cyclinD3): 9 CDK6 (cyclinD2): 15
Abemaciclib	(LY2835219)	III	CDK6 (cyclinD1): 9.9 CDK4 (cyclinD1): 2
Ribociclib	(LEE011)	III	CDK6 (cyclinD2): 40 CDK4 (cyclinD1): 10

### CDK1/2 specific inhibitors

CDK1/2 inhibitors which mainly target these CDKs include Roscovitine, Ro-3306, and Dinaciclib [80]. It should be noted that none of the three has been approved by FDA. Roscovitine is an inhibitor of various CDKs like CDK1, 2, 5, 7, and 9 and inhibits CDKs by competing directly at ATP binding sites [81]. The inhibitory effect of Roscovitine has been studied on a diverse range of cancer cell lines, and it was evaluated that this particular inhibitor inhibits the growth of all tested cancer cell lines by arresting their cell cycle with an average IC<sub>50</sub> of approximately 15 μM [82]. Based upon the time, dose, or cell line, Roscovitine arrests the cells in G0, G1, S, or G2/M phase by inhibiting CDKs directly [82]. Roscovitine has entered phase I clinical trial along with sapacitabine in BC, Pancreatic cancer, and ovarian cancer patients (NCT00999401). The adverse impacts of Roscovitine include increased levels of transaminase and bilirubin, abdominal pain, hyperglycemia, and neutropenia [80]. Another CDK1/2 inhibitor is Ro-3306 which inhibits the transition from G2 to M and leads to the apoptosis of tumor cells with prolonged exposure [83, 84]. One of the CDK inhibitors that shows dual nature in terms of inhibition is Dinaciclib (SCH727965). The studies have revealed that Dinaciclib inhibits HR repair and sensitizes multiple myeloma cells toward the veliparib, a PARP inhibitor [80]. Clinical trials involving only dinaciclib have displayed adverse impacts like diarrhea, nausea, hypotension, fatigue, and vomiting [80].

### Novel CDK inhibitors

The present investigation of new CDK inhibitors against the CDKs and better understanding of different subgroups of BC and their adverse impacts have led to more interest in discovering some novel CDK inhibitors. In this regard, various researchers have analyzed many inhibitors against CDKs. Jeong and co-workers revealed the inhibitory role of piperlongumine (PL) against the cell proliferation and migration of ER<sup>+</sup> BC cells and thereby retards the tumor progression [85]. SR-4835, analyzed by Quereda and co-workers, can inhibit specifically CDK13 and CDK12 and retards the cell proliferation in TNBC [86]. Pandurate A (PA), possessing many properties, including anti-cancer activity, arrests the cell cycle during the G0/G1 phase by explicitly inhibiting the dose-dependent CDK4/cyclin D1 complex [87]. Vanicoside B, besides possessing many inhibitory roles, inhibits selectively CDK8 and arrests the cell cycle in HCC38 and MDA-MB-231 cells [88]. The Fomes fomentarius ethanol extract (FFE) specifically inhibits CDK2, SKP2, and cyclin A/E, resulting in apoptosis and retardation in MDA-MB-231 cell migration [89]. Recent studies have demonstrated that MTH-3 (a water-soluble bis(hydroxymethyl) alkanooate

curcuminoid derivative) arrests the G2/M phase in MDA-MB-231 cells by inhibiting the CDK1 expression [90]. Furthermore, (5, 7, 8-trihydroxyflavone (NOR-wogonin) can specifically inhibit the growth of TNBC cell lines such as MDA-MB-231 in comparison to the non-tumorigenic BC lines like MCF-10A by inhibiting CDK1 expression [91]. In addition to this, a plant compound, namely Galangi, possesses anti-cancer properties and retards the progression of MCF-7 cells, and leads to apoptosis by inhibiting CDK4,2 and 1, resulting in cell cycle arrest [92]. Resveratrol is another inhibitor that can lead to cell cycle arrest by inhibiting specific CDKs like CDK2,4 and 6 [93]. One more inhibitor, namely Icariin, has been shown to retard the expression of CDK2 and CDK4 in those MCF7/TAM cell lines which are resistant to tamoxifen-resistant [94]. AZD1775, Tyrosine kinase WEE1 inhibitor, combined with AZD6738, results in cell death [95]. One specific CDK1 inhibitor, namely, methyl2-(-5-fluoro-2-hydroxyphenyl)-1H-benzoimidazole-5-carboxylate (MBIC) is associated with p53 expression and can result in cell death [96].

Summing up the contention, BC is a challenging disease that can be treated with the CDK mentioned above inhibitors in combination with other drugs resulting in a synergistic effect in terms of treatment and good results. For instance, the studies have revealed that the synergistic effect of palbociclib/paclitaxel in inhibiting cell growth and enhancing cell death is much better than the monotherapy treatments [97]. Thus, the combined effect of both CDK inhibitors and other drugs will be more effective in cell cycle arrest, cell death, and slowing down cell proliferation.

## CDK inhibition and TGF- $\beta$ signaling in breast cancer

TGF- $\beta$  signaling is one of the central pathways involved in Breast Cancer pathogenesis. This signaling pathway plays a dual role in Breast Cancer, as it assists in tumor progression and tumor suppression [98]. The well-known fact about the TGF- $\beta$  signaling pathway is that it switches its function according to the stages of BC; it acts as a tumor promoter in the late stages of BC and tumor suppressor in the early stages of BC [98]. All the essential metabolic pathways, i.e. cell proliferation, growth, and metastasis, are influenced by the TGF- $\beta$  signaling pathway in BC [99]. An overexpression of TGF- $\beta$  receptors usually characterizes breast tumors. These receptors need to get suppressed for treating BC patients [100]. TGF- $\beta$  signaling pathway maintains its tumor-suppressive state in healthy cells by promoting several regulatory processes, such as cell cycle regulation and apoptosis [101]. Canonical TGF- $\beta$  signaling involves the transcription factor SMAD3, phosphorylated to promote tumor suppression. This tumor-suppressive state can

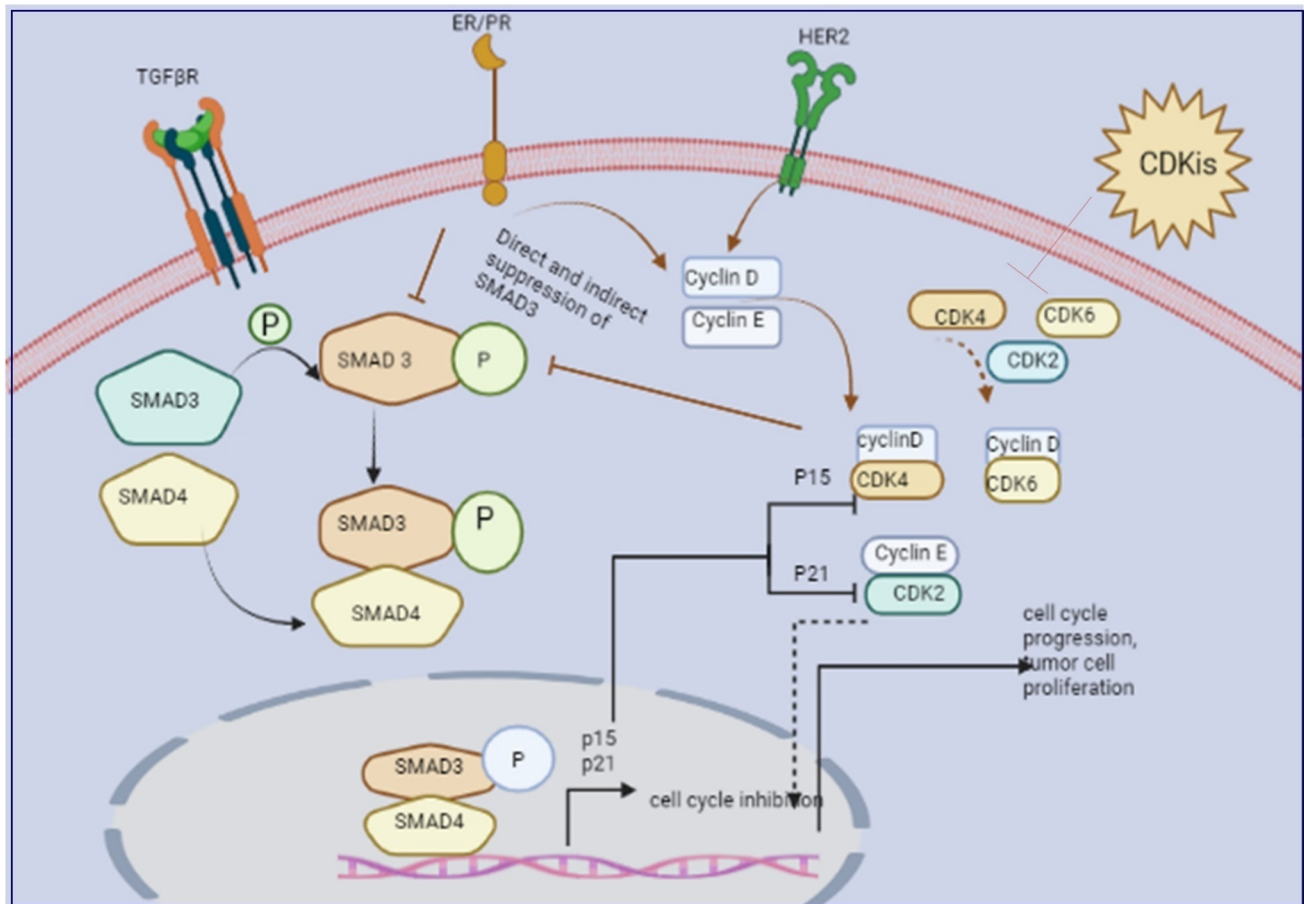
be inhibited during BC progression (Fig. 6). The overexpression of CDK2/4 in BC inhibits the tumor-suppressive nature of SMAD3, thus leading to tumor progression. This throws light on CDK-mediated SMAD 3 phosphorylation leading to BC progression. The CDKi, such as CDK2/4 inhibitors, play a decisive role in reversing this CDK-mediated protumorigenic function of TGF- $\beta$  signaling, thus serving as a brake in Breast tumor progression [98].

## Role of CDK inhibition and TGF- $\beta$ signaling pathway in breast tumor microenvironment

Breast Tumor Environment is associated with a wide range of cells, including Cancer-Associated Fibroblasts that potentially lead to the EMT in BC cells. This EMT transition is generally regulated by the TGF- $\beta$  signaling pathway [102]. TGF- $\beta$  signaling also activates several immune cells that switch from anti-tumorigenic phenotype to pro-tumorigenic phenotype, thus leading to an increase in BC aggressiveness [103]. The inhibition of CDKs influences the tumor immune microenvironment so that the pro-tumor environment reverses into an antitumor environment [98]. The studies have revealed that CDK inhibition can enhance the anti-cancerous immune response and effectively overcome the immunosuppressive functions of the TGF- $\beta$  signaling pathway [98]. Several studies have observed that blockade of TGF- $\beta$  signaling through CDK inhibition can augment the potency of checkpoint blockade therapy, thus showing the synergistic effect of CDK inhibitor therapy and immune checkpoint blockade therapy [98]. For example, the antitumor effect of CDK4/6 inhibitors has potentially affected the efficacy of anti-PD1 immunotherapy synergistically. Thus CDKi shows its significance in cancer immunotherapy by inducing the immunogenic effects that ultimately result in immune surveillance (Fig. 7) [104].

## Conclusion and future prospects

Breast cancer is one of the most threatened types of cancer and is associated with the dysregulation of many CDKs. The dysregulation of many CDKs and their cyclins leads to an increase in the progression of BC. These dysregulated CDKs can be targeted by specific CDK inhibitors, which will inhibit a particular CDK and thus will help in retarding the progression of the disease. In this aspect, various CDK inhibitors have been discovered, some of them have been approved by FDA, and some are still in clinical trials. CDK inhibitors have also been used in the combinatorial approach to have much more potency and efficacy than monotherapy approaches. CDK4/6 have been explored very much, and their combination with other drugs can become promising anticancer agents. With this understanding of

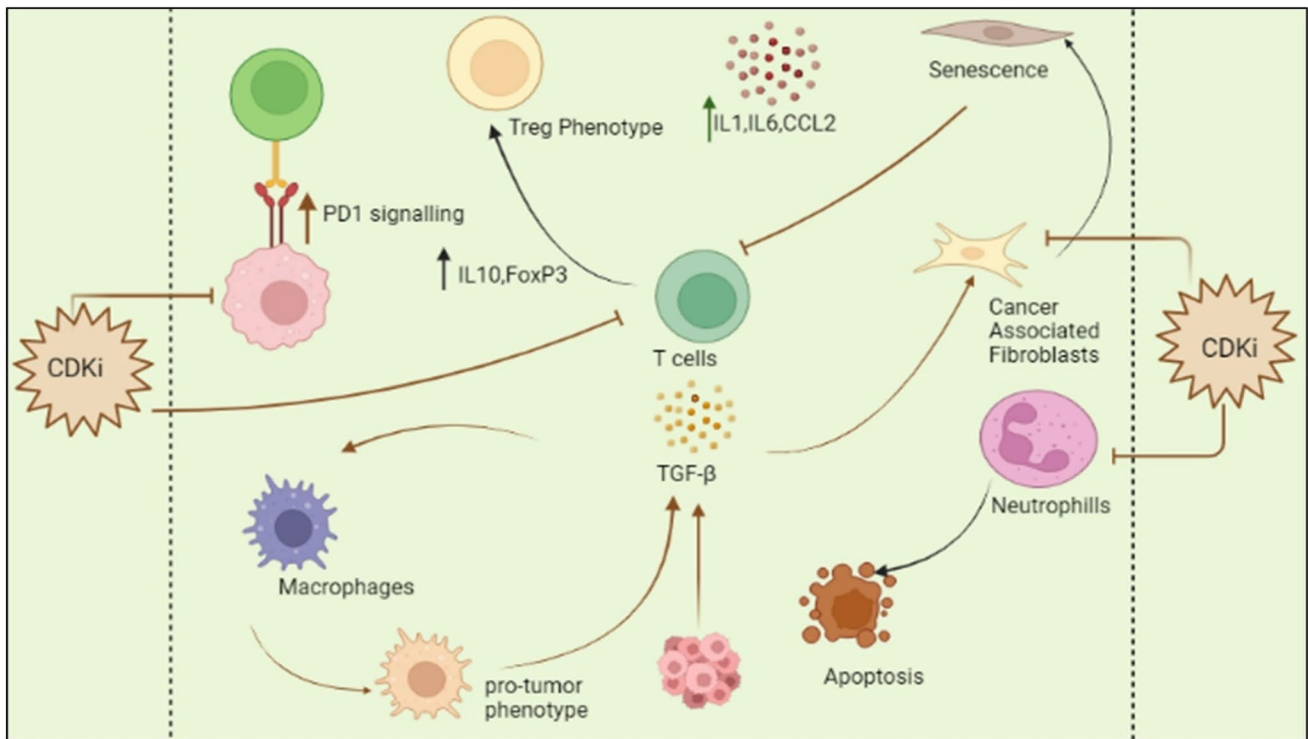


**Fig. 6** Oncogenic activation of CDKs and TGF- $\beta$  in Breast Cancer. SMAD3 phosphorylation leads to antitumor response. This tumor-suppressive state can be inhibited during BC progression. The over-

expression of CDK2/4 in BC inhibits the tumor-suppressive nature of SMAD3, thus leading to tumor progression

CDKs, advances in CDK inhibition therapy have been developed. Ribociclib, Palbociclib, and abemaciclib are three FDA-approved CDK 4/6 inhibitors that have been evaluated as monotherapies and in combination with an adjuvant endocrine therapy like letrozole and fulvestrant. The positive results promote using these drugs to treat ER<sup>+</sup>/HER2<sup>-</sup> metastatic breast cancer in the future. CDK4/6 inhibition has been well-tolerated and effective in a clinical environment, with enhanced overall survival relative to placebo groups and higher PFS and CBR. While the results of using these treatments on ER<sup>+</sup> BC patients indicate clear advantages over more harmful treatments such as chemotherapy and radiation, there are still challenges, such as endocrine therapy resistance. Neutropenia and weariness, typical side effects in patients taking these medications, can often be addressed symptomatically and without further

complications. Developing a unique combinatorial approach, including CDK inhibitors, can significantly change BC treatment strategy. The novel CDK inhibitors have made an immense contribution to this field. However, the complaints of resistance and higher costs need to be addressed. Many novel approaches need to be evolved that may inhibit various targets in the common signal pathways, thereby highlighting the therapeutic significance of BC. For instance, MRX34 (MiR-34 mimic) inhibits transcripts of numerous cell cycle genes and has come into phase I clinical trial. Further studies are required to target CDKs uniquely, considering all the unfavorable events and using them with other therapeutic drugs to treat BC best.



**Fig. 7** The interaction between TGFβ signaling and CDK inhibition leads to the change in Breast Tumor microenvironment. The inhibition of CDKs influences the tumor immune microenvironment in such a way that the protumor environment reverses into antitu-

mor environment. CDK inhibition can enhance the anti-cancerous immune response and effectively overcome the immunosuppressive functions of TGF-β signaling pathway

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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