

Review Article



Pan Cyclin-Dependent Kinase Inhibitors for the Treatment of Breast Cancer

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Article History:

Received: January 23, 2023

Accepted: February 12, 2023

ePublished: March 15, 2023

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Abstract

Background: Despite extensive attempts for the treatment of breast cancer (BC), it is the most prevalent cancer type among women, and its treatment remains elusive, particularly in patients with advanced disease. Although there are several therapeutic options, none of them is effective for complete relief, especially in metastatic patients. Cancer cells exhibit a high proliferation rate, which is usually associated with the dysregulation of cell cycle progress. Various proteins such as cyclin-dependent kinases (CDKs) and cyclins are involved in cell cycle modulation.

Methods: Databases including PubMed, Scopus, Web of Science and Google Scholar were used to extract information. Articles published in English until 2022 were used.

Results: Regarding the dysregulation of various CDKs in several cancer types, the pharmacological inhibitors of CDKs have extensively been evaluated to treat several cancer types such as BC. The blockade of CDKs strongly suppresses tumor growth through cell cycle arrest. Moreover, the combination of CDK inhibitors and other anti-cancer therapeutics has demonstrated potent synergistic effects on the treatment of various cancers.

Conclusion: Therefore, various CDK inhibitors have been designed and evaluated as anti-proliferative therapeutics to suppress the proliferation of cancer cells. Pan CDK inhibitors, including flavopiridol, dinaciclib, purvalanol A, SNS-032, and roscovitine, are the most effective CDK inhibitors investigated in several studies. They inhibit various CDKs such as the CDK1, 2, 4, 5, 6, 7, and 9. In this review, it is attempted to discuss the efficacy of Pan CDK inhibitors as an anti-cancer therapy in BC.

Keywords: CDK inhibitor, Cyclin-dependent kinases, Breast cancer

Introduction

Breast cancer (BC), the most prevalent women-related cancer, is characterized by the rapid and uncontrolled proliferation of mammary epithelial cells. It involves about 30% of new cancer diagnoses and 15% of cancer-triggered deaths globally. In addition, disease occurrence is significantly correlated with age.¹ It should be noted that early diagnosis by advanced tools has potentially improved survival time. Despite rigorous advances in identifying the immunopathogenesis of BC, the precise molecular mechanisms responsible for disease onset and progression remain elusive. Although several therapeutic approaches have been evaluated to treat BC, none of them was associated with complete remission, particularly in the advanced stages of the disease. Currently, chemotherapy, hormone therapy, surgery, and radiation therapy are the most common therapeutic approaches used to treat BC. Although cytotoxic drugs are an important part of the treatment regimen, the majority

of patients with BC exhibit chemoresistance and cancer recurrence up to two years post-treatment. Therefore, the identification of novel efficient therapeutic approaches is a critical issue for the treatment of patients with BC. The precise identification of molecular mechanisms responsible for disease progression may help in the development of novel therapeutic approaches based on new molecular targets.² Since cancer is characterized by the rapid and uncontrolled proliferation of malignant cells, it is assumed that molecules involved in cell cycle progress may be dysregulated during the tumorigenesis process. Cyclins and cyclin-dependent kinases (CDKs) are two critical types of molecules involved in cell cycle modulation in mammalian cells. The family of CDK proteins contains 13 members, including CDK1 to CDK13.³ They are classified into members responsible for the regulation of proliferation, including CDK1, 2, 3, 4, and 6, as well as those involved in the transcription process, including CDK7, 8, 9, 11, 12, and 13.^{4,5} Regarding



the important role of these factors in the regulation of cancer cell proliferation, it is suggested that the blockade of these molecules can be considered a potent anti-cancer therapeutic approach in BC.⁶ Accordingly, several pharmacological CDK inhibitors such as CDK4/6 (e.g., palbociclib, ribociclib, and abemaciclib) and Pan CDK (e.g., flavopiridol, dinaciclib, purvalanol A, and roscovitine) inhibitors have been examined in several preclinical and clinical studies.^{7,8} Pan CDK inhibitors (i.e., flavopiridol, dinaciclib, purvalanol A, SNS-032, and roscovitine) are potent CDK inhibitors that can suppress various CDKs such as CDK1, CDK2, CDK5, CDK7, and CDK9.⁹ The pro-apoptotic and anti-proliferative effects of these CDK inhibitors are also demonstrated in BC cells, implying their potential as novel promising therapeutics alone or in combination with other anti-cancer drugs to treat BC patients.¹⁰ In this review, we intend to discuss studies performed to investigate the efficacy of Pan CDK inhibitors in the treatment of BC.

Breast Cancer

BC, the second cause of cancer-triggered deaths in women, is the most prevalent non-epidermal cancer which involves women worldwide.¹¹ Patients may be afflicted with BC at different ages, but the average age of disease is 65 years in women. Intriguingly, men constitute about 1% of patients with BC.¹² Despite several attempts to control the disease, an accumulating number of new cases is diagnosed each year.¹³ Due to advancements in early diagnosis and treatment strategies, the survival rate of BC patients is increased and mortality is decreased; nonetheless, high incidence and cancer recurrence are of the main challenges during the last three decades. The high prevalence of disease, particularly in developed countries, is partly due to increased risk factors, including physical inactivity, obesity, bad food behaviors, and modified reproductive patterns. It is also shown that an increased death rate after the age of 65 in women is related to modified fertility patterns.¹⁴ There are several risk factors affecting disease incidence; however, a few preventive strategies such as genetic screening and chemoprophylaxis by tamoxifen are available.¹⁵

Based on clinical and pathological characteristics, it is possible to classify patients with BC into five subtypes, including the HER2-enriched (HER2⁺, ER⁻, and PR⁻), luminal A (HER2⁻, PR⁺, ER⁺, and Ki67 < 14%), luminal B (HER2^{-/+}, PR⁺, ER⁺, and Ki67 ≥ 14%), normal-like, and basal-like (HER2⁻, PR⁻, and ER⁻).^{16,17} The majority of estrogen receptor (ER)-α-expressing patients (about 70%) exhibit luminal A or B phenotype.¹⁸ The expression of this receptor is critical for disease progression, thus the blockade of its signaling by endocrine therapy has been proposed as an effective therapeutic strategy. However, resistance against endocrine therapy is the main challenge in this type of treatment.¹⁹ The expression of HER2 is also detected in nearly 25% of patients and is correlated with poor prognosis.²⁰ Chemotherapy in association with

radiation therapy is normally used to treat patients with advanced ER⁺/Her2⁺ phenotype.²¹ Triple-negative BC (TNBC) constitutes 15% of BC patients and is the most progressive disease which is associated with high tumor size and tumor grade, cancer recurrence, and metastasis. Surgery and heavy chemotherapy are two available treatment options for these patients.²²

Pharmacological CDK Inhibitors

Increased CDK activity potently enhances the proliferation of cancer cells, implying the use of CDK inhibitors as a novel promising anti-cancer therapeutic approach.²³ Several CDK inhibitors have been designed and investigated in different cancer types, including non-selective (olomoucine, flavopiridol, kenpaullone, roscovitine, AT7519, AG-024322, SNS-032, and R547), selective (ryuvudine, fascaplysin, NU2058, purvalanol A, SU 9516, BML-259, P-276-00, and PD 0332991), and third-generation (dinaciclib and CR8) inhibitors. The majority of these drugs are entered into clinical trials, and some of them are also evaluated in patients with BC.²⁴⁻²⁶ This group of therapeutics exerts potent anti-proliferative effects on cancerous cells. The structure of CDK inhibitors is related to anion transporter polypeptides (ATP), and thereby they compete with kinases for binding to ATP.²⁷ Selective inhibition and low toxicity have made CDK inhibitors novel and promising alternative therapeutics to current chemotherapies²⁸ (Table 1).

CDK4/6 Inhibitors

Palbociclib

Palbociclib, which is also known as PD0332991, Ibrance, commercialized by Pfizer; NY, USA, was developed as an oral selective CDK4/6 inhibitor for cancer therapy.^{29,30} It prevents the binding of ATP with CDK6/cyclin-D2, CDK4/cyclin-D1, and CDK4/cyclin-D3 complexes.³¹ Palbociclib was an efficient CDK inhibitor as evaluated in various preclinical studies on sarcomas, glioblastoma, neuroblastoma, bladder cancer, colorectal carcinoma, and gastric cancer.³²⁻³⁷ Moreover, ER⁺ and HER2⁺ patients with BC exhibited high responses to treatment with this drug.^{38,39} Moreover, retinoblastoma (Rb)-deficient BC patients had no response to treatment with this inhibitor^{29,40} because the CDK4/6-cyclin D complex promotes cell division through the phosphorylation of Rb. In fact, palbociclib prevents Rb phosphorylation by the blockade of CDK4/6. The combination therapy of patients with ER⁺HER2⁻ advanced BC in phase III clinical trial (PALOMA-2) by palbociclib and Letrozole led to the Food and Agriculture Organization (FDA) approval of this CDK inhibitor in March 2017.⁴¹

Ribociclib

Ribociclib (LEE-011, Kisqali developed by Novartis and Astex Pharmaceuticals) is a blocker of cyclin D1/CDK4 and CDK6 that inhibits Rb phosphorylation.⁴² The efficacy of this CDK inhibitor has been confirmed in the

Table 1. CDK Inhibitors in Cancer Therapy

CDK Inhibitor	Administration Route	IC ₅₀	Cancer Type	Clinical Trial Phase
Palbociclib	Oral	CDK4: 11 nM, CDK6: 16 nM	BC and GI tumors	III
Ribociclib	Oral	CDK4, 6: N/A	GI cancers and BC	III
Abemaciclib	Oral	CDK4, 6: N/A	Lung cancer and BC	I/(III)
Flavopiridol	Intravenous	CDK9: 10 nM CDK7: 10 nM CDK4: 20 nM CDK1: 30 nM CDK6: 60 nM CDK2: 100 nM	Prostate cancer, non-Hodgkin's lymphoma, Renal cancer, colon cancer, and BC	I/II
Dinaciclib	Intravenous	CDK5: 1 nM CDK2: 1 nM CDK1: 3 nM CDK9: 4 nM	BC	III
Purvalanol A	-	CDK2/cyclin B:4 nM CDK2/cyclin E:35 nM CDK2/cyclin A:70 nM CDK5-p35:75 nM CDK4/cyclin D1:850 nM	Breast cancer	-
SNS-032	Intravenous	CDK9: 4 nM CDK2: 38 nM CDK7: 62 nM	BC, multiple myeloma, and chronic lymphocytic leukemia	I
Roscovitine	Oral	CDK2: 0.1 μM CDK7: 0.5 μM CDK9: 0.8 μM CDK1: 2.7 μM	Advanced malignancies	II

Note. CDK: Cyclin-dependent kinase; IC₅₀: The half maximal inhibitory concentration; GI: Gastrointestinal; BC: Breast cancer.

treatment of BC and other drug-resistant cancers.⁴³ Based on positive results in clinical trials, ribociclib was approved by the US FDA in March 2017 for use in combination with aromatase inhibitors (AI), including fulvestrant or letrozole, to treat patients with ER⁺/HER2⁻ advanced or metastatic BC. The administration of ribociclib to patients in a clinical trial could significantly improve progression-free survival in BC patients (25 months) compared to the placebo group (16 months).⁴⁴⁻⁴⁷

Abemaciclib

Abemaciclib, which is also known as LY2835219, trade names Verzenio and Verzenios developed by Eli Lilly, is a selective inhibitor of CDK4/6. Its affinity to CDK4 is significantly (14 times) higher than CDK6.⁴⁸ This CDK inhibitor can also block the function of several other kinases at low concentrations (lower than 100 nM).⁴⁹ Although ribociclib and palbociclib are structurally based on the pyrido [2,3-d] pyrimidin-7-one scaffold, the structure of abemaciclib is related to the 2-anilino2,4-pyrimidine-[5-benzimidazole].⁴⁹ Several preclinical studies on various cancer models such as glioblastoma, head, and neck squamous cell carcinoma, melanoma, and BC have shown the anti-cancer activity of this drug.⁵⁰⁻⁵³ The anti-cancer potential of abemaciclib has been demonstrated in various cancer types such as bladder cancer, melanoma, and BC.^{37,50,54} Regarding these anti-tumor functions, it was considered a breakthrough therapy for BC by the FDA in October 2015 and was approved for the treatment of hormone receptor-positive, HER2⁻ advanced, or metastatic BC on September 28, 2017.

Pan CDK Inhibitors

Flavopiridol (Alvociclib)

Flavopiridol or alvociclib (also known as L86-8275 or NSC 649890) is a semisynthetic flavonoid alkaloid and inhibits CDK9. The drug is a semisynthetic analog of rohitukine extracted from *Amoora rohituka* and *Dysoxylum binectariferum*. The initial evaluation of flavopiridol showed its anti-cancer potential in 60 different human cancer cell lines, representing its high and non-tumor type-specific function.⁵⁵ It could block CDK9 activity, suppress transcription, induce apoptosis, and prevent angiogenesis.⁵⁶ Flavopiridol suppresses the function of positive transcription elongation factor b (P-TEFb) which leads to the blockade of mRNA production.⁵⁷ This CDK inhibitor is currently under clinical evaluation by Tolero Pharmaceuticals, Inc for the treatment of acute myeloid leukemia. Flavopiridol is also evaluated for the treatment of atherosclerotic plaque formation and arthritis.⁵⁸ Recently, the FDA has donated an orphan drug topic for flavopiridol for the treatment of patients with acute myeloid leukemia; however, little is known regarding its efficacy for the treatment of BC, and this issue needs to be further investigated in future studies.

Dinaciclib

Dinaciclib, also known as SCH727965 or MK-7965, is another potent CDK inhibitor, which interacts with the acetyl-lysine recognition site of bromodomains and blocks the function of various CDKs such as CDK1, 2, 5, and 9. Dinaciclib could significantly induce apoptosis in cancer cells in vitro and suppresses tumor growth in both tumor-bearing animal models and human cancers.⁵⁹⁻⁶² Accordingly, dinaciclib could suppress the

progression of melanoma in a p53-dependent manner.⁶³ It also prevented the expansion of chronic lymphocytic leukemia⁶⁴ and osteosarcoma cells⁶⁵ through the induction of apoptosis. Anti-cancer effects were also observed in the murine xenograft models of pancreatic cancer.⁶⁶ The anti-cancer activity of this drug has also been demonstrated in BC cell lines and xenograft BC models.^{61,62,67} In vitro studies indicated the high activity of dinaciclib in TNBC.⁶⁸ Dinaciclib also showed higher activity and lower adverse effects compared to flavopiridol in clinical trials.⁵⁹ Potent anti-cancer function observed in preclinical studies led to the entrance of dinaciclib into the initial phases of clinical trials in various cancer types such as BC.⁶⁹⁻⁷¹ It is now under consideration in clinical trials for the treatment of various cancer types, including advanced BC (phase II),⁷² non-small cell lung cancer (NSCLC, phase II),⁷³ multiple myeloma (phase II), advanced melanoma (phase II), and chronic lymphocytic leukemia (phase III). It was developed by Merck et al and assigned as an orphan drug by the FDA.⁷⁴

Purvalanol A

Purvalanol A is another CDK inhibitor that selectively suppresses CDK1 and CDK2 and arrests the cell cycle at G1/M and G2/S phases.⁷⁵ Purvalanol A has shown synergism with chemotherapeutics such as paclitaxel to suppress the growth of Hela cells in part through reducing the expression of *Bcl-2* anti-apoptotic proteins.⁷⁶⁻⁷⁸ The anti-cancer function of this CDK inhibitor is demonstrated in vitro by inducing apoptosis in MDA-MB-231 and MCF-7 BC cell lines.⁷⁵

SNS-032

SNS-032, also known as BMS 387032 and developed by Sunesis, has a thiazole unit and selectively inhibits CDK2, 7, and 9.⁷⁹ The comparison of this CDK inhibitor with flavopiridol represented its lower toxicity, higher suppression of transcription, and higher apoptosis induction.⁸⁰ Treatment of BC cells such as MDA-MB-435, MCF-7, and MDA-MB-435 led to the apoptosis of cancer cells partly through the downregulation of Mcl-1 and XIAP proteins. Moreover, the administration of this CDK inhibitor into MDA-MB-435 nude murine xenografts was associated with tumor regression, implying the efficacy of SNS032 in the treatment of BC.⁸¹

Roscovitin

Roscovitin, also known as seliciclib, CYC202, and developed by Cyclacel, was first generated by De Azevedo et al as a potent member of CDK inhibitors.^{82,83} The oxidative reaction of the hydroxymethyl group of the C₂ amino alcohol at the purine core led to the generation of this compound, which is soluble in DMSO and HCl (up to 50 mM).⁸⁴ Rapid metabolism of roscovitin by CYP2B6 and CYP3A4 leads to the generation of carboxylate PMF30-128 and M1/M2 glucuronides.⁸⁵ This purine analog can potentially suppress various CDKs such as

CDK1, 2, 5, 7, and 9 (with an IC₅₀ of 0.2-0.7 μM), whereas affects the function of CDK4, 6, and 8 (with an IC₅₀ of higher than 100 μM). It can also inhibit several kinases, including IRAK4, FAK, ERK2, ERK1, EPHB2, DYRK1A, CK1δ, CK1α, and CaMK2 in the concentrations of 1 to 40 μM.^{82,86,87} It is indicated that roscovitin can arrest cell cycle progression at all stages, including G0, G1, S, or G2/M, which relies on cancer type, treatment time, and administrated dose. This wide spectrum impact on the cell cycle can disrupt several signaling pathways, including JAK-STAT,⁸⁸ ER,⁸⁹ p53,⁹⁰ NF-κβ,⁹¹ and Ras-MAPK.⁹² Consequently, it can reduce the expression of several genes involved in the survival of cancer cells (e.g., XIAP,⁹³ Mcl-1,⁹⁴ survivin,⁹³ and Bcl-2⁹⁵) and increase Bcl-x,⁹⁶ p53,⁹⁷ and p53AIP1.⁹⁸ The pro-apoptotic effects of this CDK inhibitor have synergistic impacts when it is combined with chemotherapeutics.⁹⁹⁻¹⁰² Accordingly, its synergism has been shown with several chemotherapeutics and anti-cancer drugs such as doxorubicin, tamoxifen, etoposide, irinotecan, cisplatin, trastuzumab, alemtuzumab, vinblastine, 5-fluorouracil, and paclitaxel.^{100,103} It has been reported that roscovitin exerts potent anti-tumor activity partly through the upregulation of the pro-apoptotic Bcl-x protein. Roscovitin also suppresses the expansion of various cancer cells such as MCF-7 BC, lung cancer, HeLa cervical cancer, and HCT116 colon cancer cells through cell cycle arrest at G1/S and G2/M phases. It is proposed that roscovitin can be an effective anti-cancer therapeutic for hormonal therapy-sensitive patients with BC. Moreover, the anti-proliferative effects of roscovitin are through decreasing the expression of cyclin D1 via the MAPK pathway and depend on ER-α expression status.^{92,104} Interestingly, roscovitin also enhances the autophagy process.^{87,95,97}

In addition to cancer, the efficacy of roscovitin as a strong CDK/cyclin E inhibitor has been confirmed in various pathologic conditions such as glomerulonephritis, viral infections, and neurodegenerative disorders. Since it can induce apoptosis in neutrophils, it is proposed that roscovitin could be used as a potential treatment for chronic inflammatory diseases.¹⁰⁵ As an anti-cancer therapeutic, it was effective in several cancer cell lines in vitro. Furthermore, several preclinical studies have confirmed its anti-cancer potential in various cancer models. For instance, the intraperitoneal administration of roscovitin into LoVo human colorectal cancer-bearing CD1 nude mice led to a significant reduction in the tumor size. Similar results were observed following the oral administration of roscovitin into the tumor xenografts of MESSA-DX5 human uterine carcinoma cells.¹⁰⁶ Orally treated HCT116 human colon tumor-bearing nude mice with roscovitin demonstrated significantly smaller tumors compared to control mice.¹⁰⁷ Intraperitoneal administered roscovitin also potently arrested tumor growth in the A4573 Ewing's sarcoma and PC-3 prostate cancer xenografts.^{108,109} Similar anti-tumor functions were detected in the osteosarcoma tumor xenograft in B6D2F1

mice¹¹⁰ and HT29 human colon cancer xenografts in nude mice¹¹¹ following treatment with roscovitine. Additionally, the treatment of RAMOS or HBL-2 tumor-bearing immunodeficient mice with roscovitine led to potent tumor regression.¹¹² Likewise, other preclinical studies performed in BC animal models will be discussed in the next section.^{113,114}

Roscovitine is now under clinical evaluation for the treatment of various cancers. Investigating the efficacy of this drug in patients with NSCLC showed some adverse effects such as vomiting, nausea, transient hypokalemia, and transient elevations in serum creatinine and liver function. Its assessment in patients with B-cell lymphomas was associated with the downregulation of MCL1 protein.⁹⁴ Roscovitine is also used in phase I trial against glomerulonephritis and phase II trials against lung cancer and BC.¹¹⁵

Pan CDK Inhibitors in Breast Cancer

Flavopiridol

This section reviews studies on the effectiveness of this CDK inhibitor in the treatment of BC. Schwartz et al found that flavopiridol enhances the cellular toxicity of mitomycin-C (MMC), a chemotherapeutic agent, by stimulating drug-induced apoptosis in MDA-MB-468 and KN-74 tumor cell lines. Apoptosis enhancement caused by flavopiridol may somewhat depend on PKC and is not associated with a specific area of the cell cycle.¹¹⁶ Furthermore, Motwani et al examined events related to apoptosis and the cell cycle associated with the combined treatment of MKN-74 and MCF-7 cell lines with paclitaxel and flavopiridol. They reported that flavopiridol enhances apoptosis caused by paclitaxel via increasing the activation of caspases and poly (ADP-ribose) polymerase (PARP) cleavage. The cell treatment with flavopiridol inactivates Cdc-2 kinase, which inhibits paclitaxel mitotic arrest.¹¹⁷ Flavopiridol also inhibits the growth of MDA-MB-435 and 435.eB cells; regardless of excessive c-erbB-2 expression, it causes apoptosis and suppresses the expression of MMPs and invasion of BC cells.¹¹⁸ Carlson et al concluded that the flavopiridol-induced reduction in cyclin D1 due to the suppression of cyclin D1 promoter transcription is a specific primary event in MCF-7 and U2OS human cell lines.¹¹⁹ Similarly, Nahta et al that the activity of CDK4/6 is particularly essential for erbB2-mediated transformation and mutually regulates erbB2 expression. They reduced the level of erbB2 and suppressed the cell proliferation using the combination therapy of (erbB2 +) BC cells with flavopiridol and herceptin against cyclin D1 and erbB2.¹²⁰ In another study, they showed that the expression of epidermal growth factor receptor (EGFR) and erbB2 proteins in BT474, MDA-MB-453, and SKBR was rapidly downregulated after the combination therapy of trastuzumab and flavopiridol.¹²¹ It has also been shown that the combination of flavopiridol and trastuzumab synergistically inhibits the Ras-MAPK and/or PI3K/Akt pathway and reduces the levels of Cdks and/or cyclin D1,

as well as the S-phase transition in the HBL-100, MDA-MB-231/453, and MCF7.¹²² Wall et al found that Thr34 phosphorylation is necessary to retain the expression of survivin in tumor cells, and p34cdc2 kinase inhibition in the arrested cells of the mitotic phase using flavopiridol leads to the downregulation of survivin and the increased apoptosis in MCF-7 and HeLa cells.¹²³ Further, the treatment of SKBR-3 and MB-468 with flavopiridol and epothilone B altered the Bax structure and greatly reduced Bcl-xL, cIAP-2, XIAP, and Mcl-1 expression, and increased apoptosis. However, MB-468/Bcl-2 cells were less sensitive to Epo B and FP.¹²⁴ It has also been represented that flavopiridol reduces the expression of the $\alpha 5 \beta 1$ integrins and the adherence to fibronectin, inhibits Akt phosphorylation induced by fibroblast growth factor 2 (FGF-2), and leads to the survival of T-47D and MCF-7 cells.¹²⁵ According to the report of another study, the combined therapy of MCF-7 and MDA-MB-231 cells using flavopiridol and gemcitabine resulted in apoptosis induction, cell growth inhibition, and cell accumulation in the S phase.¹²⁶ Meyer et al evaluated the effect of flavopiridol on germ cell tumors-derived cell lines 2102 EP, NCCIT, and NT2 compared to cell lines derived from MCF7, HeLa, and SKOV. They found that GCT cells were more sensitive to flavopiridol than the other cell lines. Depending on the correlation of RB and E2F1 transcription factor, flavopiridol induces apoptosis or cell cycle arrest.¹²⁷ Another study represented that flavopiridol promotes the degradation of the c-FLIP anti-apoptotic protein, abrogates the NF- κ B activity, downregulates the XIAP, induces TRAIL signaling, and increases the activation of caspase-8 and apoptosis in breast tumor cells.¹²⁸ Flavopiridol decreases proapoptotic proteins (Bax, Bak, and PUMA- α) and FLIPL expression and promotes TRAIL-induced apoptosis in BC and myeloma cancer cells. Moreover, the knockdown of the *FLIPL* gene with small interfering RNA (siRNA) sensitizes MM1S cells to TRAIL.¹²⁹ Data from another study confirmed that flavopiridol in combination with vorinostat inhibits AKT and ERK1/2 function, decreases the expression of various inhibitors of internal and external apoptosis pathways (XIAP, BCL-xL, and c-FLIP-l/s), activates cathepsin-dependent protease pathways, and leads to increased apoptosis in BC cells.¹³⁰ Fornier et al concluded that flavopiridol enhances the docetaxel-dependent apoptosis in patients with ovarian, breast, and pancreatic carcinoma in a time- and sequence-dependent manner. To induce this effect, docetaxel should be administrated at least 4 hours before flavopiridol in vivo.¹³¹ It was also represented that the suppression of CDK4/6 and cyclin D1 by flavopiridol leads to decreased or increased stem-like cell function and migration in (ER⁺) and (ER⁻) BC cells, respectively.¹³² In addition, the simultaneous targeting of Ras-MAPK pathways and CDK with sorafenib (SFN) and flavopiridol enhances the cellular toxicity and lethality induced by SFN in the cell lines with the KRAS-BRAF mutation or EGFR/HER-2 overexpression, respectively.

Furthermore, the combination of flavopiridol and SFN inhibits the Mcl-1, Rb-E2F signaling, Erk, tumor growth, and pulmonary metastatic burden in vivo.¹³³ Hicks et al found that JUNB plays an important role in the survival of BC cells treated with kinase inhibitors. Thus, they showed that FP treatment initially lowers Pol IIser2p levels, as well as MCL1, JUNB, MYC, and CEBPD expression, in MDA-MB-231/468, and SKBR3 BC cells.¹³⁴ Brenner et al revealed that organic anion-transporting polypeptides (OATP1B1/B3 and OATP2B1) play a substantial role in the uptake of flavopiridol to cancer cells. They indicated that the use of OATP inhibitors such as rifampicin inhibits the flavopiridol uptake in OATP1B3/B1- and OATP2B1-transfected CHO cells. Moreover, after flavopiridol treatment, wild-type ZR-75-1 cells were blocked in the G1 phase and G2/M, while OATP1B1 knockdown ZR-75-1 cells were only inhibited in the G1 stage.¹³⁵ Another study reported that the treatment of MCF-7 cells with flavopiridol induces oxidative stress, alters multiple metabolic pathways, including glutathione metabolism, glycerophospholipid metabolism, and polyamine metabolism, and finally leads to cell death. Additionally, spermine and spermidine, which exacerbate cancer progression, are downregulated after treatment due to the G1 phase arrest.¹³⁶ Other studies represented that autophagy increases the survival of cancer cells after radiation and chemotherapy. Accordingly, the findings of Okada et al revealed that treatment with flavopiridol/CDK4 inhibitor, along with autophagy inhibition using chloroquine or BECN1/ATG5 knockdown, inhibits stress-induced autophagy, arrests the cell cycle, and causes apoptosis in MDA-MB435S, BT474, SW480, A431, and SKBr3 cells.¹³⁷ It has also been shown that the treatment of MCF-7 cells with flavopiridol reduces the expression of anti-apoptotic proteins in the BCL-xl and IAP family, and increases the expression of BAX pro-apoptotic proteins independent of the ER status.¹³⁸ Flavopiridol also inhibits the growth of CD44⁺/CD24⁻ BC stem cells, ribosome biogenesis, and translation¹³⁹ (Table 2, Figure 1).

Dinaciclib

Studies on BC treatment with this inhibitor will be discussed in this section. Mita et al evaluated the safety and efficacy of dinaciclib, compared with capecitabine, in patients with advanced BC who have previously undergone treatment. They demonstrated that dinaciclib treatment has a poor antitumor function as capecitabine, with 1 verified partial response (PR), 1 unverified PR, an overall response rate of 8%, and a time to disease progression lower than capecitabine treatment in patients with (ER⁺/HER2⁻) BC. Additionally, dinaciclib therapy has anti-tumor activity and is commonly safe and tolerable, but its efficacy is less than capecitabine.⁷² The findings of another study represented that the combination of dinaciclib and epirubicin is not an effective treatment selection for TNBC and is associated with considerable toxicity.¹⁴⁰ It has also been reported that CDK9, cyclin B1,

and MYC can be used as potential therapeutic targets in TNBC. Dinaciclib decreases cyclin B1 and MYC expression through CDK9 inhibition, arrests the G2/M phase of the cell cycle, and induces apoptosis in TNBC patient-derived xenograft (PDX) both in vitro and in vivo. Furthermore, CDK1/cyclin B1 knockdown using siRNA in MDA-MB-231 and BT549 cells greatly inhibits cell proliferation and induces apoptosis. In addition, the knockdown of the P-TEFb subunit of CDK9 by siRNA leads to decreased levels of MYC and cyclin B1 in TNBC cell lines.⁶⁸ Recent studies showed that ER phosphorylation at the S294 site (pS294) is essential for the transcription of the ER-dependent gene and is mediated by an unknown CDK. Scott et al also reported that CDK2 is involved in the initial formation of pS294 as a mediator, and the use of dinaciclib, a selective CDK2 inhibitor, together with the anti-pS294 mAb, inhibits pS294 formation and suppresses ER-dependent gene expression in wild type (ER⁺) or (ER mutant⁺) BC. They created ER mutations Ermut (D538G) and (Y537S) in MCF7 cells and illustrated their capacity to trigger tamoxifen-resistant ligand-independent growth, along with the expression of the CDK2-dependent pS294. After the growth of E2-independent and tamoxifen (TAM) resistant MCF7 ERmut (Y537S) tumors in the nude mice, treatment was initiated with different concentrations of dinaciclib or palbociclib. Scott et al concluded that the combined treatment with TAM and palbociclib suppresses tumor growth without any effect on pS294 formation, whereas the TAM/Dinaciclib combination arrests tumor growth and inhibits pS294 expression.¹⁴¹ Dinaciclib is a CDK1 and 2 inhibitor that greatly sensitizes the wild-type BRCA and primary/resistance-mutated BRCA model of TNBC for PARP inhibition. Moreover, dinaciclib increases the response to PARP inhibition in homologous recombination (HR)-deficient cancer.¹⁴² Further, dinaciclib treatment leads to an increase in the cleaved caspase 3 and cleaved PARP, downregulates the *Mcl1* gene, induces apoptosis, and decreases the number of inflammatory BC cells in a dose-dependent manner. Similarly, dinaciclib enhances the activity of DNA-damaging chemotherapeutic agents, while paclitaxel lacks this ability.¹⁴³ Another study found that 3-phosphoinositide-dependent protein kinase 1 (PDK1) modulates ribociclib sensitivity in (ER⁺) MCF-7 cells. Therefore, PDK1 inhibition with GSK2334470 or siRNA in combination with CDK4/6 inhibitor ribociclib or palbociclib suppresses the proliferation and promotes apoptosis in (ER⁺) BC cells. On the other hand, Ribociclib-resistant cells upregulate the expression of phospho CDK2, cyclins D1, A, E, and phospho-S477/T479 AKT. Therefore, treatment with CDK2 inhibitor dinaciclib or GSK2334470 restores the susceptibility of ribociclib-resistant cells to CDK4/6 inhibition. The combination of ribociclib, GSK2334470, and PI3K α inhibitor alpelisib decreases tumor growth in the xenograft model. According to research, the PI3K/PDK1 signaling pathway

Table 2. Studies Related to the Role of Flavopiridol in Breast Cancer

Cell Lines	Drug/SiRNA	Human/Animal Model	In vivo/In vitro	Study Result	Reference
MKN-74 MDA-MB-468	Flavopiridol, MMC	-	In vitro	Flavopiridol and MMC induce apoptosis in MDA-MB-468 and MKN-74 cells	116
MKN-74 MCF-7	Flavopiridol, Paclitaxel (Taxol)	-	In vitro	Flavopiridol induces caspase activation and apoptosis in gastric and BC cells treated with Paclitaxel	117
MDA-MB-435 435.eB1 435.eB4	Flavopiridol	-	In vitro	Flavopiridol inhibits the MMPs expression, growth, and metastasis of BC cells	118
MCF-7 U2OS	Flavopiridol	-	In vitro	Flavopiridol leads to a fast decrease in cyclin D1 levels	119
NIH3T3 MCF-7 SKBR3 MDA-MB-453	Flavopiridol, trastuzumab (Herceptin)	-	In vitro	Combination therapy of (erbB2 ⁺) BC cells with flavopiridol and trastuzumab reduces erbB2 levels and suppresses cell proliferation	120
MDA-MB-453 BT474 SKBR3	Flavopiridol, trastuzumab, Z-VAD-FMK (Caspase inhibitor), AG99, AG1478	-	In vitro	The combination of flavopiridol and trastuzumab reduces EGFR and erbB2 expression	121
MCF7 MDA-MB-453 MDA-MB-231 HBL-100	Flavopiridol, trastuzumab, PD98059 (MEK1 inhibitor), LY 294002 (PI3K inhibitor)	-	In vitro	Flavopiridol and trastuzumab inhibit the PI3K/Akt and/or Ras-MAPK pathway, DNA synthesis, and cell proliferation	122
MCF-7 HeLa	Flavopiridol	-	In vitro	Inhibition of p34cdc2 kinase in arrested cells using Flavopiridol leads to decreased Survivin levels and increased apoptosis in MCF-7 and HeLa cells	123
MB-468 SKBR-3	Flavopiridol, Taxotere (docetaxel), Epothilone B (Epo B), Proteasome inhibitor ALLnL	-	In vitro	FP reduces the IAP and Bcl-2 family levels, alters the BAX structure, and induces apoptosis in BC cells	124
MCF-7 T-47D	Flavopiridol, MEK inhibitors: UO126, PD98059, P38 inhibitors: SB203580, D169316	-	In vitro	Flavopiridol suppresses the expression of $\alpha 2$, $\alpha 5$, $\beta 3$, and $\beta 4$ integrins upregulated by FGF-2 and declines the survival of BC cells	125
MCF-7 (ER ⁺) MDAMB-231 (ER ⁻)	Flavopiridol, gemcitabine	-	In vitro	Flavopiridol and gemcitabine lead to apoptosis by upregulating P16 and p21WAF1 cell cycle inhibitors	126
NTer2, 2102 EP NCCIT MCF-7 SKOV, HeLa	Flavopiridol, cisplatin, paclitaxel	-	In vitro	Flavopiridol induces cell death in cancer cells, especially in GCT cell lines	127
MCF-7, MDA-MB-231, EVSA-T Jurkat, MDAMB-468 SKBR-3, and BT474 MDA-MB-435S	Flavopiridol, proteasome inhibitor: MG-132, caspases inhibitor: Z-VAD-FMK, recombinant human TRAIL (residues 95-281)	-	In vitro	Flavopiridol induces TRAIL signaling and apoptosis and promotes the degradation of c-FLIP anti-apoptotic protein in BC cells	128
MM1S MDA-MB-468	Flavopiridol, c-FLIP-siRNA	-	In vitro	Treatment with flavopiridol through suppressing transcription and reducing FLIPL expression causes the apoptosis of breast and myeloma cancer cells	129
MDAMB-231 MCF7	Flavopiridol, vorinostat	-	In vitro	Flavopiridol in combination with vorinostat inhibits AKT and ERK1/2 function, minimizes the expression of XIAP, BCL-xL, and c-FLIP-l/s anti-apoptotic proteins, and leads to increased apoptosis in BC cells	130
-	Flavopiridol, docetaxel	Human	In vivo	Flavopiridol enhances the docetaxel-dependent apoptosis in patients with ovarian, breast, and pancreatic carcinoma in a time- and sequence-dependent manner	131
MDA-MB-231 MDA-MB-468 (ER ⁻) MCF7, T47D (ER ⁺)	Flavopiridol, PD0332991	-	In vitro	Flavopiridol leads to a decrease or an increase in the migration in (ER ⁺) and (ER ⁻) BC cells, respectively	132
MDA-MB-468, MDA-MB-231 SK-BR3, MCF-7	Flavopiridol (FPD), sorafenib (SFN) lapatinib, erlotinib, U0126, LY294002, U73122, and AZD5363 PD0332991	BalbC-RAG2 ^{-/-} /JIL2R ^{yc} mice	In vivo In vitro	FPD/SFN combinations cause the inhibition of tumor growth and the reduction of pulmonary metastatic burden in mutant RAS/RAF and (EGFR/HER-2 ⁺) BC model	133
MDA-MB-231 MDA-MB-468 MCF10A ^{met} SKBR3	Flavopiridol, JUNB siRNA, Actinomycin D, U0126 (MEK1 inhibitor), sorafenib, doxorubicin	-	In vitro	FPV treatment is used to delay the expression of the JUNB gene in BC cells	134
ZR-75-1 CHO	Flavopiridol, OATP inhibitors: bromsulphophthalein (BSP), and rifampicin	-	In vitro	OATP1B1/B3 and OATP2B1 act as transporters for the cellular uptake of flavopiridol, and the use of OATP inhibitors rifampicin inhibits the flavopiridol uptake in OATP1B3/B1- and OATP2B1-transfected CHO cells	135

Table 2. Continued.

Cell Lines	Drug/SiRNA	Human/Animal Model	In vivo/In vitro	Study Result	Reference
MCF-7	Flavopiridol	-	In vitro	Treatment of MCF-7 cells with Flavopiridol induces the G1 phase arrest and oxidative stress, as well as lipid oxidation and mitochondrial lesions, and finally leads to cell death	136
SW480, A431 KMST-6 NCI-N87 BT474 MDA-MB231 MDA-MB435S SKBr3 MCF7	Flavopiridol CDK4 inhibitor: 2-bromo-12 and 13-dihydro-5 H-indolo-dione [2 and 3-a] pyrrolo [3 and 4-c] carbazole-5 and 7 (6H)-dione Autophagy inhibitor: Chloroquine (CQ), ATG5 siRNA, BECN1 siRNA	-	In vitro	Flavopiridol, or CDK4 inhibitor, along with autophagy inhibition inhibited stress-induced autophagy, arrests the cell cycle, and causes apoptosis in some cell lines	137
MCF-7	Flavopiridol (FP) ATG5 siRNA	-	In vitro	Treatment of MCF-7 cells with Flavopiridol reduces the expression of the BCL-xl and IAP family, while it increases the BAX expression	138
MCF7 (CD44 ⁺ /CD24 ⁻)	Flavopiridol	-	In vitro	Flavopiridol inhibits the growth of CD44 ⁺ /CD24 ⁻ BC stem cells	139

Note. SiRNA: Small interfering RNA; MMC: Mitomycin-C; FGF: Fibroblast growth factor; BC: Breast cancer; ER: Estrogen receptor; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; c-FLIP: Cellular FLICE (FADD-like IL-1 β -converting enzyme)-inhibitory protein; CDK: Cyclin-dependent kinase; GCT: Giant cell tumor; FPV: Feline panleukopenia virus; OATP: Organic anion transporter polypeptide; CHO: Chinese Hamster Ovary; EGFR: Epidermal growth factor receptor.

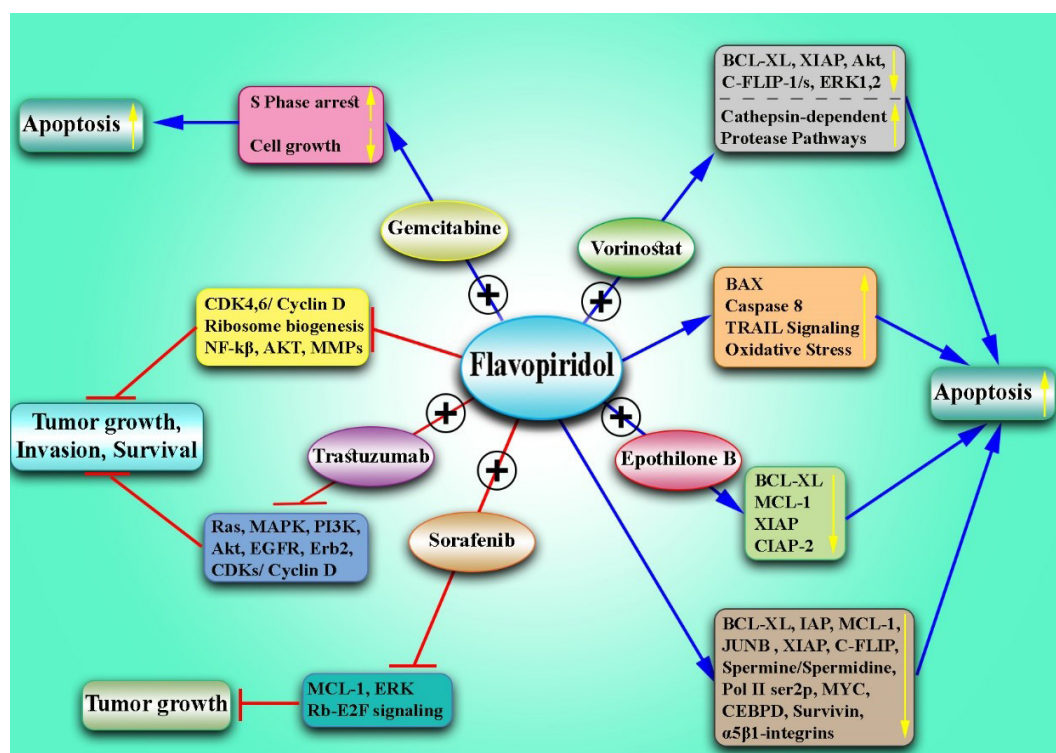


Figure 1. The Effects of Flavopiridol Alone and in Combination With Other Drugs for the Treatment of Breast Cancer. Note. The data illustrate that Flavopiridol inhibits the growth of CD44⁺/CD24⁻ BC stem cells, ribosome biogenesis, and translation. Moreover, it reduces the expression of the BCL-xl and IAP family, increases the expression of BAX pro-apoptotic proteins, down-regulates the spermine and spermidine, induces oxidative stress, lowers Pol IIser2p levels and MCL1, JUNB, MYC, and CEBPD expression, and suppresses the CDK4/6 and cyclin D1. Flavopiridol decreases proapoptotic proteins (e.g., Bax, Bak, and PUMA- α) and promotes TRAIL-induced apoptosis. Flavopiridol promotes the degradation of the c-FLIP anti-apoptotic protein, abrogates the NF- κ B activity, down-regulates XIAP, induces TRAIL signaling, and increases the activation of caspase-8 and apoptosis. In addition, Flavopiridol declines the expression of the α 5 β 1 integrins and the adherence to fibronectin and inhibits Akt phosphorylation induced by FGF-2, and survival. It also leads to a drop in survivin surface and inhibits the MMP expression and invasion in the BC cells. The combination of Flavopiridol and Sorafenib inhibits the Mcl-1, Rb-E2F signaling (Erk), and tumor growth. Moreover, Flavopiridol in combination with vorinostat inhibits AKT and ERK1/2 function, decreases the expression of XIAP, BCL-xL, and c-FLIP-l/s, activates cathepsin-dependent protease pathways, and leads to increased apoptosis in BC. Flavopiridol and Gemcitabine resulted in apoptosis induction, cell growth inhibition, and cell accumulation in the S phase. Flavopiridol and Epothilone B altered Bax's structure and greatly reduced Bcl-xL, cIAP-2, XIAP, and Mcl-1 expression and increased apoptosis. The combination of Flavopiridol and trastuzumab synergistically inhibits the Ras-MAPK and/or PI3K/Akt pathway, reducing the levels of EGFR, erbB2, Cdk, and cyclin D1. BC: Breast cancer; NF- κ B: Nuclear factor kappa β ; XIAP: X-linked inhibitor of apoptosis protein; Bcl-xl: B-cell lymphoma-extra-large; IAP: Inhibitors of apoptosis proteins; BAX: Bcl-2-associated X protein; MCL1: Myeloid cell leukemia 1; CEBPD: CCAAT Enhancer binding protein delta; BAK: Bcl-2 homologous antagonist/killer, PUMA: p53 upregulated modulator of apoptosis; TRAIL: TNF-related apoptosis-inducing ligand; c-FLIP: FLICE-like inhibitory protein; NF- κ B: Nuclear factor kappa light chain enhancer of activated B cells; XIAP: X-linked inhibitor of apoptosis protein; FGF-2: Basic fibroblast growth factor (bFGF); MMP: Matrix metalloproteinase; cIAP-2: Cellular inhibitor of apoptosis 1; PI3K: Phosphoinositide 3-kinases; EGFR: Epidermal growth factor receptor.

leads to resistance to the CDK4/6 inhibitor through the upregulation of CDKs and S-phase cyclins. Adding dinaciclib to GSK2334470/ribociclib does not increase cell proliferation inhibition.¹⁴⁴ Most patients with BC are positive for the hormone receptor and need anti-hormonal treatments such as AI. Reports indicate that low molecular weight (LMW)-E-dependent cell cycle deregulation causes AI resistance, and a combination of CDK2 inhibitors and letrozole can be used to treat patients with (HR⁺) tumors expressing LMW-E. MCF7 xenografts containing LMW-E are resistant to letrozole, and the tumor volume increases after treatment with AI. Therefore, CDK2 inhibitor dinaciclib reversed LMW-E-dependent resistance, but treatment with CDK4/6 inhibitor Palbociclib does not cause any sensitization.¹⁴⁵ Zhu et al reported that the expression of *BRCA1* and *BARD1* in tamoxifen-resistant mammary tumor cells dramatically increases and leads to resistance to DNA-damaging chemotherapeutic agents such as adriamycin and cisplatin. Hence, the knockdown of the *BRCA1* or *BARD1* gene or the phosphorylation inhibition of *BRCA1* by dinaciclib enhances sensitivity to cisplatin in tamoxifen-resistant cells. Further, the activity of the PI3K/AKT pathway upregulates the expression of *BRCA1* and *BARD1* genes. Thus, the use of PI3K inhibitors reduces the expression of *BRCA1* and *BARD1* in tamoxifen-resistant cells and re-sensitizes them to cisplatin.¹⁴⁶ It has also been indicated that MAPK signaling plays a key role in regulating cell proliferation, drug resistance, and oncogenic transformation. Trametinib, dabrafenib, and lapatinib participate in the positive regulation of the MAPK pathway, while dinaciclib and rigosertib negatively regulate the MAPK pathway and arrest the cell cycle. Dinaciclib and rigosertib also enhance the phosphorylation of MEK1/2, ERK1/2, and MAPK pathways in BC cells.¹⁴⁷ The finding of another study revealed that treatment with AZD1775 results in cleaved PARP and G1 phase accumulation in cyclin E-induced 76NE6 and MDA231 cells. The overexpression of cyclin E causes the stress of DNA replicative and increases DNA double-strand breaks, which can be repaired by activating the Wee1 kinase and ATR-CHK1 pathway. However, after the inhibition of Wee1 kinase by AZD1775 in the cyclin E-high cells, DNA damage repair is not performed enough and cell death occurs due to the accumulation of abnormal nuclei. The combination therapy of AZD1775 and dinaciclib sensitizes cyclin E-low TNBC cells for the inhibition of Wee1 kinase.¹⁴⁸ Carey et al showed that MYC-positive tumors were involved in the upregulation of the HR DNA and resistance to DNA-damaging agents such as PARP inhibitors. MYC regulates HR through DNA repair proteins, including RAD51, and inhibition of MYC expression causes susceptibility to PARPi independent of BRCA status. Therefore, the pharmacological inhibition of PARP along with the siRNA knockdown of RAD51 and MYC decreases cell proliferation and increases apoptosis in SUM149 and

MB231 cells. The use of dinaciclib and PARP inhibitor niraparib increases DNA damage and decreases the HR and epithelial-mesenchymal transition. Furthermore, Dinaciclib downregulates the expression of the *MYC* gene and re-sensitizes niraparib-resistant TNBC cells.¹⁴⁹ The inhibition of CDK2 and the enhancer of zeste homolog 2 (EZH2) by CDK2i and EZH2i at a lower dose than recommended phase 2 dose (RP2D) induces the expression of ER- α in TNBC cells. Studies have shown that cytokine/chemokine-coding genes such as CCL20, CXCL2, and TNF α , which are upregulated by CDK2 and EZH2i, affect the tumor microenvironment. Likewise, IL-36 cytokine is upregulated by CDK2i/EZH2i and has wide anti-tumor effects. CDK2i/EZH2i also downregulates the *RPL39* gene and decreases metastasis and TNBC expansion. CDK2 or EZH2 inhibition leads to re-expressed ER- α and converts TNBC to luminal ER- α -positive, increasing the sensitivity of TNBC cells to tamoxifen. Moreover, the combination therapy with the EZH2 or CDK2 inhibitor and tamoxifen effectively inhibits tumor growth and increases the survival of the mice with TNBC tumors¹⁵⁰ (Table 3, Figure 2).

SNS-032

E2F1 and E2F4 play an essential role in cell cycle regulation. Ma et al investigated the role of E2F1/E2F4 in apoptosis resulting from CDK inhibitors (BMS-387032, roscovitine, and flavopiridol) and chemotherapeutic agents (paclitaxel, cisplatin, and VP16). They found that treatment with these CDK inhibitors and chemotherapeutic drugs reduces the level of E2F4. Furthermore, reducing E2F4 through RNAi increases the susceptibility of human cancer cells to the drug.¹⁵¹ In another study, they indicated that treatment with BMS-387032 stabilizes *E2F1* protein by the increase in the expression of p57 protein/mRNA, but suppresses the E2F1 mRNA and some E2F family members in MDA-MB-231 BC cells.¹⁵² Phosphorylated ER- α is considered a biomarker and a predictive/therapeutic value for (ER- α +) BCs. Held et al showed that the Ser294 phosphorylation of ER- α in human BC cells is conducted by the ligand (tamoxifen and estradiol) stimulation of ER- α rather than the growth factor activation (heregulin- β , EGF, and insulin) of ER- α . The use of SNS-032 or roscovitine prevents the Ser294 phosphorylation of ligand-activated without affecting the phosphorylation of other serins (Ser104/106 or Ser118).¹⁵³ It has also been represented that SNS-032 induces apoptosis through both internal and external apoptotic pathways and increases the activation of poly (ADP-ribose) polymerase (PARP) and caspase 8/9 in BC cells. It also promotes RNA polymerase II dephosphorylation in serines 2 and 5 and inhibits RNA synthesis, reduces Mcl-1 and XIAP in BC cells, and represses the mammary tumor growth in mice.¹⁵⁴ ATP-binding cassette transporters cause multidrug resistance (MDR) toward chemotherapeutic/anticancer drugs. Cihlova et al assessed the interaction of CDK inhibitors (SNS-032, AT-7519, and flavopiridol) with ABC transporters (ABCB1,

Table 3. Studies Related to the Effect of Dinaciclib in the Treatment of Breast Cancer

Cell Lines	Drug/SiRNA	Human/Animal Model	In vivo/In vitro	Study Result	Reference
-	Dinaciclib, capecitabine	Humans	In vivo	Dinaciclib therapy has anti-tumor activity and is commonly safe and tolerable, but its efficacy is less than Capecitabine	72
-	Dinaciclib, epirubicin	Humans	In vivo	The combination of dinaciclib and epirubicin has significant toxicity and is not an effective treatment option for TNBC	140
WHIM3 WHIM12, MDA-MB-231 BT549, HCC1806, BT474, MCF7 T47D	Dinaciclib, CDK1 siRNA, CDK2 siRNA, CDK9 siRNA, Cyclin B siRNA,	NOD/SCID mice	In vitro	Dinaciclib targets cyclin B1 via the MYC/CDK9 axis and suppresses tumor growth in the TNBC PDX model in vivo	68
MCF7, SUM44	Dinaciclib, palbociclib, SNS-032, JNJ7706621, BMS-265246, tamoxifen, β -estradiol (E2), CDK1 siRNA, CDK2 siRNA, DK9 siRNA	Nude mice	In vitro/In vivo	The combined treatment with tamoxifen and palbociclib could inhibit tumor growth without any effect on pS294 formation, whereas the TAM/dinaciclib combination arrests tumor growth and inhibits pS294 expression	141
MDA-MB-436, MDA-MB-231, MCF-7, HCC1395, SUM149PT, HCC1937, Hs578T, HCC38, BT549, BT20	Dinaciclib, flavopiridol, veliparib, olaparib, Cisplatin SiBRCA1, SiBRCA2, SiPALB2	NOD-SCID-IL2R γ ^{-/-} mice, NMRI-Foxn1 ^{nu} mice	In vivo/In vitro	The combination of the PARP inhibitor and dinaciclib, and DK12 inhibitor could reverse resistance to PARP inhibition in BRCA-mutated cancer	142
SUM149, HCC1806, HCC1937, MDA-MB-468, BT549, MCF-7, MDA-MB-157, MDA-MB-231, MDA-MB-436, SUM185, KPL4, SKBR3, T47D, MDA-MB-361	Dinaciclib, epirubicin, carboplatin, paclitaxel	Humans	In vivo/In vitro	Treatment with Dinaciclib augments the cleaved PARP/ caspase 3, downregulates the Mcl1 expression, and promotes apoptosis	143
MCF-7, T47D HCC1428, HCC1500	Dinaciclib, abemaciclib, palbociclib, ribociclib, PI3K α inhibitor: alpelisib, GSK2334470, siPDK1	Ovariectomized athymic mice	In vivo/In vitro	Treatment with GSK2334470 or CDK2 inhibitor Dinaciclib restores the susceptibility of Ribociclib-resistant cells to CDK4/6 inhibition	144
Aromatase-overexpressing LMW-E MCF7/T47D	Aromatase inhibitors: letrozole, exemestane, anastrozole, doxycycline	Nude mice Humans	In vivo/In vitro	Dinaciclib reversed LMW-E-dependent AI resistance	145
MCF7, T47D	Dinaciclib, cisplatin, paclitaxel, adriamycin, PI3K inhibitor: BKM120, BRCA1 siRNA, BARD1 siRNA	NOD/SCID mice	In vivo/In vitro	Dinaciclib inhibits the phosphorylation of BRCA1 and re-sensitizes Tamoxifen-resistant cells to cisplatin	146
MCF-10A, MCF-7 MCF12A, BT474, MDA-MB-231, MDA-MB-453, MDA-MB-468	Dinaciclib, rigosertib (polo-like kinase 1 inhibitor), dabrafenib, trametinib	-	In vitro	Dinaciclib and Rigosertib negatively regulate the MAPK pathway and arrest the cell cycle	147
76NE6 cyclin E-inducible, HEK-293T, HCC1806, MDA436, MDA468 MDA231, SUM149, SUM159	Dinaciclib AZD1775 (Wee1 kinase inhibitor), carboplatin	PDX models (mice)	In vivo/In vitro	Combination therapy of AZD1775 and Dinaciclib sensitizes cyclin E-low TNBC cells to the inhibition of Wee1 kinase	148
MDA-MB-231, MDA-MB-468, HCC1937, HCC1806, SUM149, SUM1315, MDA-MB-436, MDA-MB-157, MCF-10A, PC3, DU145 A549, Calu-1, H1299, H1993, OVCAR3, 59M FUOV1, BxPC3, PANC-1, and HCT116	Dinaciclib, Niraparib, MYC siRNA, RAD51 siRNA	Nude mice	In vivo/In vitro	Dinaciclib and PARP inhibitor niraparib increase DNA damage while decreasing the homologous recombination and epithelial-mesenchymal transition. Dinaciclib downregulates the expression of the MYC gene and re-sensitizes niraparib-resistant TNBC cells	149
MDA-MB-231 SUM-149 BT549, T47D	Dinaciclib, EPZ-6438 Tamoxifen	NOD/SCID mice	In vivo/In vitro	A combination of CDK2i Dinaciclib and EZH2 leads to re-expressed ER- α , converts TNBC to luminal ER- α -positive, and increases the sensitivity of TNBC cells to Tamoxifen	150

Note. Small interfering RNA; TNBC: Triple-negative breast cancer; PARP: Poly (ADP-ribose) polymerase; BRCA: Breast cancer gene; LMW: Low molecular weight; AI: Aromatase inhibitor; ER: Estrogen receptor; PDX: Patient-derived xenograft; MAPK: Mitogen-activated protein kinase.

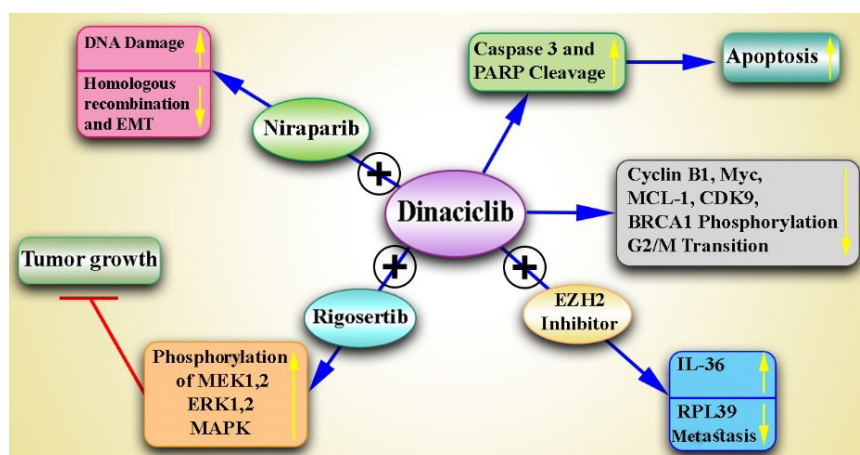


Figure 2. Combination of Dinaciclib With Other Drugs for Breast Cancer Therapy. *Note.* Dinaciclib reduces cyclin B1 and Myc expression through CDK9 inhibition, leads to the arrest of the G2/M phase cell cycle, increases cleaved caspase 3 and cleaved PARP, downregulates the Mcl1 gene, inhibits the phosphorylation of BRCA1, and induces apoptosis. The combination of Dinaciclib and Rigosertib also enhances the phosphorylation of the MEK1/2, ERK1/2, and MAPK pathway in breast cancer cells. Dinaciclib and Niraparib increase DNA damage while decreasing the homologous recombination and epithelial-mesenchymal transition. Dinaciclib and EZH2i upregulate IL-36 cytokine, downregulate the *RPL39* gene, and decrease metastasis. PARP: Poly (ADP-ribose) polymerase; MCL-1: Myeloid cell leukemia 1; MEK: Mitogen-activated protein kinase; ERK: Extracellular-signal-regulated kinase; MAPK: Mitogen-activated protein kinase; EZH2i: Enhancer of zeste homolog 2 inhibitor.

ABCG2, and ABCC1) under laboratory conditions. They reported that SNS-032 decreases ABCG2-dependent efflux. In addition, flavopiridol could inhibit ABCC1 and ABCG2, whereas AT-7519 had no effect on the inhibition of ABCG2, ABCC1, or ABCB1. Additionally, SNS-032 and flavopiridol in combination with the substrates of the ABC transporter such as topotecan and daunorubicin had anti-proliferative effects on cancer cells.¹⁵⁵ It was also revealed that PCTK1, PCTAIRE1, and Cdk16 play a fundamental role in tumor formation and cancer cell resistance to TNF-family cytokines, and their expression in cancer cells represents an increase. The protein kinase PCTAIRE1 takes part in the extrinsic pathway of apoptosis. The knockdown of the *PCTAIRE1* gene using shRNA induces the degradation of RIPK1 and caspase-8 cleavage without any effect on the cell cycle arrest and sensitizes breast and prostate cancer cells to TNF-family cytokines, including TRAIL. Further, SNS-032 suppresses the function of PCTAIRE1 kinase and sensitizes PPC1 prostate cancer cells to TRAIL-mediated apoptosis¹⁵⁶ (Table 4, Figure 3).

Purvalanol A

Villerbu et al found that purvalanol A causes cell cycle arrest and selective phosphorylation inhibition of several CDK substrates in synchronized cells. Prolonged contact of growing cells with this drug causes the stable inhibition of proliferation and cell death. Furthermore, purvalanol A inhibits Rb phosphorylation in the G1 stage, while its inhibitory activity is weak toward cdk4/cyclin D1. Additionally, the drug is unable to inhibit the activity of cdk7, an essential kinase for the activation of Thr172 phosphorylation in cdk4.¹⁵⁷ In another study, it was demonstrated that paclitaxel induces the hyperphosphorylation of the 4E-binding protein (4E-BP1) and decreases its association with eIF-4E in the MDA-

MB231 cancer cells, but it does not affect phosphorylation or expression of eukaryotic initiation factor 4E (eIF-4E). It is noteworthy that 4E-BP1 phosphorylation is associated with the accumulation of G2/M and increased phosphorylation of cdk1 substrates. Combination therapy with purvalanol A, roscovitine, histone deacetylase inhibitor, which indirectly inhibits CDK function, and downregulation of cyclin B using siRNA reduces the hyperphosphorylation of 4E-BP1 in cells treated with paclitaxel. However, monotherapy with paclitaxel escalates the eIF-4E level by raising the hyperphosphorylation of 4E-BP1 via a cdk1-dependent process.¹⁵⁸ A combination of purvalanol A and olomoucine II can strengthen the cytostatic effect of mitoxantrone (ABCG2 substrate) in cells expressing ABCG2. In addition, the concomitant use of purvalanol A or olomoucine II with other anti-cancer drugs, that target ABCG2, can lead to cytostatic effects, which is an effective cancer therapy for predominating MDR.¹⁵⁹ Purvalanol A also promotes apoptosis in a caspase-dependent manner in (ER+) MCF-7 cells, whereas (ER-) MDA-MB-231 cells are less sensitive to the drug. Further, cells that highly express Bcl-2 are more resistant to purvalanol A-induced apoptosis. Moreover, Purvalanol A triggers the upregulation of spermidine/spermine N1-acetyltransferase (SSAT) and polyamine oxidase (PAO) in MCF-7 cells. The use of PAO/spermine oxidase inhibitor (MDL 72,527) impedes purvalanol A-mediated apoptosis. Generally, purvalanol A causes mitochondria-induced apoptosis, reduces cell viability, and modulates anti- and pro-apoptotic proteins and the polyamine catabolic pathway in MDA-MB-231 and MCF-7 cells¹⁶⁰ (Table 4, Figure 4).

Roscovitine

As mentioned in the previous section, roscovitine can be considered a potent anti-cancer therapeutic when

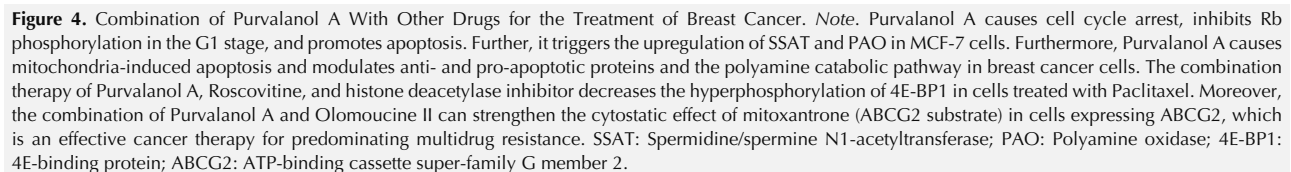
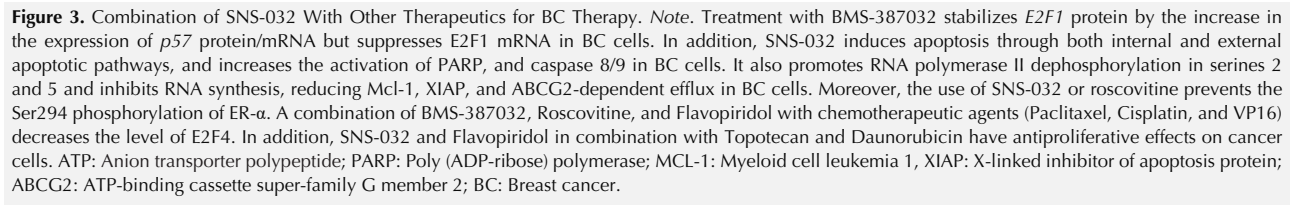
Table 4. Studies Related to the Role of SNS-032 and Purvalanol A in Breast Cancer

Cell Lines	Drug/SiRNA	Human/Animal Model	In vivo/In vitro	Study Result	Reference
H1299, MDA-MB-231 MCF7, 3T3	Flavopiridol, roscovitine, BMS-387032, cisplatin, VP16, paclitaxel, E2F1 RNAi	-	In vitro	Treatment with CDK inhibitors and chemotherapeutic drugs decreases the level of E2F4	151
H1299, MCF-7 MDA-MB-231 T98G (wt Rb, p53, and mut p16)	BMS-387032, p57RNAi	-	In vitro	Treatment with BMS-387032 stabilizes the E2F1 protein but suppresses E2F1 mRNA in the MDA-MB-231 breast cancer cells	152
MCF-7, BT474, T47D	SNS-032, β -estradiol, tamoxifen, okadaic acid, forskolin, insulin, heregulin- β , IGEPAL CA-630	-	In vitro	The use of SNS-032 or roscovitine prevents from the Ser294 phosphorylation of ligand-activated in ER- α	153
MCF-7, MDA-MB-435	SNS-032	BALB/c-nu mice	In vivo/In vitro	SNS-032 induces apoptosis through both internal and external apoptotic pathways in breast cancer cells	154
MDCKII (MDCKII-ABCB1, MDCKII-ABCG2, and MDCKII-ABCC1), HepG2, T47D	SNS-032 AT-7519, Flavopiridol, Daunorubicin, Mitoxantrone, MK-571 (ABCC1 inhibitor), LY335979 (ABCB1 inhibitor), Ko143, (ABCG2 inhibitor)	-	In vitro	SNS-032 decreases ABCG2-dependent efflux SNS-032 and flavopiridol in combination with the substrates of the ABC transporter such as topotecan and daunorubicin have antiproliferative effects on cancer cells	155
PPC1, Du145, MDA-MB-468, T47D, MCF7 IMR-90, HeLa HEK293T, 267B1, 267B1/K-ras	SNS-032, SiPCTAIRE1 SiPCTAIRE2 SiPCTAIRE3, Sicaspase-8 SiRIPK1	-	In vitro	SNS-032 suppresses the acting of PCTAIRE1 kinase and sensitizes PPC1 prostate cancer cells to TRAIL-mediated apoptosis	156
MCF7, MELN (MCF-7 ERE-beta-globin Luciferase Neo), MCF-7/mdr1, HT-29, A31, BP-A31	Purvalanol A	-	In vitro	Porvalvanol A inhibits Rb phosphorylation in the G1 stage, while its inhibitory activity is weak toward cdk4/cyclin D1	157
MDA MB 231, HeLa, A2780, SKOV3, DRO90-1, ARO81-1, HCT116, HCT116 p21 ^{-/-}	Purvalanol A, roscovitine, paclitaxel, cyclin B siRNA, Histone deacetylase inhibitor	-	In vitro	Combination therapy with Purvalanol A, Roscovitine, histone deacetylase inhibitor, and downregulation of cyclin B using siRNA decreases the hyperphosphorylation of 4E-BP1 in cell treated with PTX	158
MDCKII (MDCKII-ABCG2)	Purvalanol A, lomoucine II, roscovitine, bohemin,olomoucine, mitoxantrone, fumitremorgin C (ABCG2 inhibitor)	Wistar rats	In vivo/In vitro	A combination of Purvalanol A and Olomoucine II can strengthen the cytostatic effect of mitoxantrone (ABCG2 substrate) in cells expressing ABCG2	159
MCF-7, MDA-MB 231	Purvalanol A, MDL72,527 (PAO/SMO inhibitor)	-	In vitro	Purvalanol A causes mitochondria-induced apoptosis and modulates anti- and pro-apoptotic proteins and the polyamine catabolic pathway in MDA-MB-231 and MCF-7 cells	160

Note. PAO: Polyamine oxidase; SMO: *Spermine oxidase*; CDK: Cyclin-dependent kinase; SiRNA: Small interfering RNA; ER: Estrogen receptor; PTX: Paclitaxel; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand.

administrated alone or in combination with other anti-tumor therapeutics. Several studies have confirmed the effectiveness of this CDK inhibitor in the treatment of BC, which will be discussed in this section. Mgbonyebi et al first reported the pro-apoptotic and anti-proliferative impact of roscovitine on MDA-MB-231 and MCF-7 BC cells in vitro.^{161,162} Wojciechowski et al attempted to clarify the pro-apoptotic and anti-proliferative effects of roscovitine in MCF-7 cells. They showed that treatment of cancer cells with roscovitine modifies the pattern of the argyrophilic nucleolar organizer regions and nucleolar RNA in cancer cells in a time-dependent manner. This was associated with the increased number of nucleolar fragments, redistribution of the nucleolin, and reduced histone phosphorylation and acetylation. These changes

arrested cancer cells at the G2/M phase, increased p53 levels, and induced apoptosis, which was related to the upregulation of caspases. Therefore, it was proposed that roscovitine promotes the apoptosis of cancer cells through the primary disintegration of nucleoli and cell cycle arrest.¹⁶³ Roscovitine upregulates p53 partly through the repression of its negative regulator (MDM2). It was hypothesized that the degradation of p53 by proteasome inhibitors may mimic the effects of roscovitine. However, the examination of this hypothesis in MCF-7 cells revealed that while roscovitine arrests the cell cycle at the G2 phase, proteasomes do it at the S phase. Thus, although proteasome inhibitors could increase p53 expression, they did not mimic roscovitine effects.¹⁶⁴ Wesierska-Gadek et al also demonstrated the mechanism by which roscovitine



phosphorylation of p53 at Ser-46, thereby stabilizing wild-type p53 and enhancing roscovitine-mediated apoptosis in cancer cells.¹⁶⁵

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cancer cells depends on the cell cycle status in vitro. This claim was proved by the exposure of G1 phase-arrested (through serum withdrawal) and S phase-arrested (by hydroxyurea) cells to roscovitine. The treatment of G1-synchronized cancer cells with roscovitine led to increased cell cycle arrest and decreased number of S-phase cells. A lesser outcome was obtained following the treatment of MCF-7 cells rescued from G1 phase arrest for 4 hours with roscovitine. On the other hand, the treatment of cells rescued from G1 arrest for 24 hours with roscovitine increased the frequency of G1 cells. Roscovitine could also increase S phase cells in MCF-7 cells rescued from S phase arrest. Therefore, based on the cell cycle status, roscovitine differentially affects the proliferation of cancer cells.¹⁶⁶ Similarly, it was found that the outcome of the treatment of BC cells (MCF-7) with roscovitine critically relies on the cell cycle status and drug dose. Although roscovitine mainly suppresses CDKs at low doses, which leads to cell cycle arrest, it induces apoptosis at high doses.¹⁶⁷

In another attempt, the anti-proliferative effect of roscovitine was evaluated in MCF-7 cells in vitro. The treatment of cancer cells with roscovitine led to cell cycle arrest at the G2/M phase after 6 hours, which was associated with the upregulation of p53. Roscovitine induced apoptosis in MCF-7 cells through the mitochondrial pathway.⁹⁹ Furthermore, the blockade of farnesyl protein transferase enzyme in MCF-7 cells increased the anti-proliferative impact of roscovitine in these cells in vitro. The overexpressed c-Ha-Ras protein in MCF-7 cells needs to be farnesylated by the farnesyl protein transferase to achieve full biological function. The exposure of MCF-7 cells to roscovitine in combination with L-744,832, an inhibitor of farnesyl protein transferase, markedly induced higher apoptosis in cancer cells compared to monotherapy with either roscovitine or L-744,832. Similarly, combined therapy potently reduced cancer cell proliferation in comparison to monotherapy.¹⁶⁸ Downregulation of phosphatase nuclear targeting subunit (PNUTS) also enhanced roscovitine-mediated apoptosis in MCF-7 cells. The phosphorylation status of Rb plays an important role in the modulation of apoptosis and proliferation in cancer cells. CDKs promote Rb phosphorylation following growth signals, while protein phosphatase 1 (PP1) enhances Rb dephosphorylation through E2F1 following pro-apoptotic and anti-proliferative signals. The knockdown of PNUTS (PP1 interacting protein) promotes the dephosphorylation of Rb and apoptosis in MCF-7 cells. Accordingly, the dephosphorylation of Rb by roscovitine or siRNA mediated the knockdown of PNUTS led to increased apoptosis in cancer cells up to two folds. Combined treatment by roscovitine and PNTUS-specific siRNA further enhanced apoptosis up to four folds.¹⁶⁹

It has been reported that the anti-proliferative and pro-apoptotic effects of roscovitine are mainly through targeting CDK5 activity in highly invasive MDA-MB231 cells in vitro. Goodyear et al demonstrated that bFGF

increases CDK5 expression and CDK5 regulates the proliferation of MDA-MB231 cells. They indicated that roscovitine selectively suppresses CDK5, resulting in the induction of apoptosis and prevention of cancer cell proliferation.¹⁷⁰ Regarding the importance of CDK5 activity in tumor-initiating cells, it is also assumed that the blockade of this CDK can suppress the development of these cells. Accordingly, the blockade of CDK5 by roscovitine or siRNA technology suppressed sphere formation and tumor establishment in vivo. Mandl et al showed that the blockade of CDK5 suppressed tumorsphere formation through inducing the Foxo1 transcription factor and thereby upregulating Bim pro-apoptotic protein.¹⁷¹

It is proposed that roscovitine can promote the apoptosis process in MCF-7 cells through different pathways, relying on the caspase 3 status in these cells. Roscovitine promoted the mitochondrial pathway of apoptosis in caspase 3-deficient MCF-7 cells through p53 phosphorylation at Ser46, leading to the upregulation of p53AIP1 protein and thus the depolarization of mitochondrial potential. On the other hand, roscovitine could not promote p53 Ser46 phosphorylation in caspase-3-expressing cells.⁹⁸ However, the forced expression of caspase 3 in MCF-7 cells did not affect their sensitization to roscovitine-induced apoptosis.¹⁷² It is also reported that the blockade of ornithine decarboxylase, which is overexpressed in BC cells, can affect roscovitine-mediated apoptosis through the mitochondrial pathway in MCF-7 cells. Ornithine decarboxylase regulates the concentration of polyamines in cells. It has been demonstrated that the blockade of ornithine decarboxylase by its inhibitor (α -difluoromethylornithine) suppresses the proliferation of BC cells. The exposure of MCF-7 cells to α -difluoromethylornithine changed the pro-apoptotic effects of roscovitine through reducing polyamine levels, inducing reactive oxygen species, and regulating the expression of *Bcl-2* family proteins.¹⁷³ Interestingly, exogenous polyamines play a pivotal role in the induction of apoptosis or autophagy by roscovitine in MCF-7 (ER⁺) and MDA-MB-231 (ER⁻) cells. MDA-MB-231 cells represented higher resistance to roscovitine compared to MCF-7 cells, which was partly due to the regulation of the autophagy pathway in ER⁻ cells.¹⁷⁴

In addition to pro-apoptotic action, it is found that roscovitine can sensitize cancer cells against radiation both in vitro and in vivo. The treatment of MDA-MB 231 cells with roscovitine in combination with ionizing radiation led to a robust increase in micronuclei frequency and impairment in DNA-double-strand break repair compared to cells treated with ionizing radiation alone. A similar defect in the nonhomologous end-joining pathways was detected in BALB murine cells compared to DNA-PKcs-deficient SCID murine cells. Roscovitine-induced radiosensitization was also associated with the blockade of the DNA-dependent protein kinase function triggered via a reduced Ku-DNA binding affinity.

Therefore, roscovitine enhances radiosensitization in BC cells lacking a functional p53 partly through an effect on the DNA repair system.¹⁷⁵ Interestingly, this combination therapy could also inhibit the angiogenesis process both in vitro and in vivo. This combination approach could significantly reduce microvascular density and angiogenic hot spots in MDA-MB 231 in comparison with either roscovitine or ionizing radiation. Reduced angiogenesis was associated with increased Ang-2/Tie-2, reduced vascular endothelial growth factor (VEGF) levels, and thereby vessel destabilization. Ionizing radiation also enhanced roscovitine-mediated anti-proliferation without triggering apoptosis in endothelial cells. On the other hand, roscovitine suppressed the radiation-induced secretion of VEGF by endothelial and MDA-MB 231 cells. Furthermore, the combination of roscovitine and ionizing radiation robustly inhibited the invasion of endothelial cells and the capillary-like tube formation. The lack of toxicity against normal cells further substantiated the efficacy of this combinatorial treatment.¹⁷⁶

Naturally occurring compounds can also enhance the anti-cancer potential of roscovitine.¹⁷⁷ Considering that polyphenols such as resveratrol and epicatechins, which are rich in food, exert pro-apoptotic and anti-proliferative effects on cancer cells as anti-oxidant agents, it is assumed that they may improve the anti-cancer effects of roscovitine. Therefore, MCF-7 cells were treated with the combination of roscovitine and resveratrol which was associated with higher pro-apoptotic and anti-proliferative effects compared to cells treated with roscovitine or resveratrol, implying their synergistic effect.¹⁷⁸ In addition to the impact of other anti-cancer therapeutics on the efficacy of roscovitine, it can also enhance the anti-cancer activity of some drugs. For instance, it is demonstrated that roscovitine facilitates the apoptosis of BT-474, MDA-MB435-S, SKBr-3, and MDA-MB468 cancer cells by the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). It is reported that BC cells are resistant to TRAIL-mediated apoptosis. Interestingly, the addition of roscovitine to the culture of TRAIL-treated cancer cells resulted in TRAIL-mediated apoptosis in TRAIL-resistant cells. Roscovitine-induced TRAIL sensitivity, partly through the downregulation of FLICE-inhibitory proteins, cFLIP(S), and cFLIP(L), enhances the formation of TRAIL death-inducing signaling complex, activates caspase-8, and downregulates Mcl-1 and E2F1.¹⁷⁹ The modulation of Mcl-1 expression by roscovitine also sensitizes BT474 BC cells to BAX/BAK-dependent mitochondrial apoptosis both in vitro and in vivo.¹⁸⁰

Moreover, it has been shown that roscovitine can enhance the anti-cancer activity of doxorubicin in MCF-7 cells and MCF7 xenograft-bearing nude mice mainly through increasing cell cycle arrest and to a lesser extent, by inducing apoptosis. Appleyard et al found that combined therapy by roscovitine and doxorubicin significantly reduced the proliferation of cancer cells as assayed by

MIB1 (ki67) immunohistochemistry and increased the expression of p21 and p27. However, it did not affect the expression of p53 and survivin and p53 phosphorylation (Ser15).¹⁰⁰ This combination strategy could also kill LMV cyclin E-expressing MCF-7 cells in vitro. LMW-E is only expressed in cancer cells, binds to CDK2, and promotes BC progression.^{181,182} The expression of LMW-E in BC cells triggers resistance to letrozole. Treatment with roscovitine prevented resistance to letrozole in LMW-E-expressing MCF-7 cells.¹⁸³ Additionally, Jabbour-Leung et al concluded that sequential combined therapy by roscovitine and Doxorubicin is potentially lethal in TNBC with p53 mutation. The p53 mutations in TNBC help in the G1 bypass and promote cell cycle progress even in the lack of DNA repair. Thus, it was expected that TNBCs to be sensitive to cell cycle arrest by roscovitine in combination with chemotherapy. Accordingly, the sequential treatment of TNBC cells with doxorubicin after roscovitine resulted in significant apoptosis of cancer cells. Roscovitine arrests the cell cycle in the G2/M phase, leading to DNA damage in TNBC cells. Combined therapy increased DNA damage, which was associated with reduced function of the DNA repair system. Moreover, the sequential administration of roscovitine before doxorubicin into MDA-MB-468 breast tumor-bearing nude mice led to reduced tumor size and increased survival rate, representing that the disruption of p53 signaling is needed to induce cytotoxicity by combination therapy in TNBC, making p53 a prognosticator factor of response to combination treatment.¹⁸⁴ Given the relationship between HER2 and cyclin E, it was anticipated that the combination of anti-HER-2 and roscovitine might be an effective strategy for BC therapy.¹⁸⁵ Accordingly, a synergistic effect was observed following the treatment of SKBR3 and MCF-7 BC cells with the combination of roscovitine and the anti-HER-2 antibody both in vitro and in vivo.¹⁸⁶

Evidence also indicates the role of roscovitine in the induction of apoptosis in drug-resistant P-glycoprotein (P-gp)-overexpressing MCF-7 BC cells both in vitro and in vivo. Drug resistance is usually associated with the upregulation of the *MDR1* gene, encoding the P-gp transporter. Caspase 3, which is defective in MCF-7 BC cells, can cleave P-gp. The treatment of P-gp-overexpressing MCF-7 cells with recombinant caspases, 3, 6, and 7 led to the cleavage of P-gp in vitro. On the other hand, the apoptosis of cancer cells by roscovitine was not associated with P-gp cleavage even in the presence of active caspases 6 and 7 in vivo. Surprisingly, P-gp overexpressing cancer cells showed higher sensitivity to roscovitine compared to wild-type cells, highlighting the impact of this CDK inhibitor on P-gp mediated drug resistance.¹⁸⁷

It is demonstrated that the apoptotic effects of roscovitine in MCF-7 cells can be potentially reduced in the presence of estrogen analogs. Therefore, it is assumed that roscovitine might affect the phosphorylation status of ER- α in these cells. The treatment of MCF-7 cells

with roscovitine resulted in the blockade of CDK2 and 7, inhibited RNA polymerase II phosphorylation by CDK7, and decreased basal ER- α phosphorylation (Ser118) in non-stimulated MCF-7 cells. The stimulation of MCF-7 cells with estrogen potently stimulated ER- α by phosphorylation at Ser118, which could be prevented by roscovitine. Tamoxifen (anti-estrogen) could further enhance roscovitine-induced cell cycle arrest at G1 or G2 phases. Hence, roscovitine can inhibit the activating phosphorylation of ER- α and exhibit synergistic effects in combination with anti-estrogen therapy.⁸⁹ Consistently, the treatment of various ER- α positive or negative BC cells with the combination of roscovitine and tamoxifen synergistically suppressed their proliferation and induced apoptosis in vitro.¹⁸⁸⁻¹⁹⁰ In addition, roscovitine can suppress the growth and development of hormonal therapy-resistant BC cells through the blockade of

CDK2 both in vitro and in vivo. Nair et al concluded that the activation of the CDK2 pathway is aberrant in three hormonal therapy-resistant cells, including the MCF-7-HER2, MCF-7-LTLTca, and MCF-7-TamR. The treatment of these cells with roscovitine led to cell cycle arrest at the G2 phase, reduced CDK2 activity, downregulated cyclin D1, and reduced the expression levels of ER- α and its coregulators such as PELP1 and AIB1. The administration of roscovitine into xenograft tumor models further substantiated the efficacy of this CDK inhibitor in the suppression of hormone therapy-resistant tumors.¹¹⁴

The PR/cyclin D1 pathway promotes BC progression through cell cycle-dependent transcriptional signals, thus it is assumed that roscovitine can modulate this program. Accordingly, the inhibition of CDK2 by roscovitine in T47D cells blocked the interaction of PR/cyclin D1

Table 5. Studies Related to the Role of Roscovitine in Breast Cancer

Cell Lines	Drug/SiRNA	Animal Model	In vivo/In vitro	Study Result	Reference
MDA-MB-231	Roscovitine	-	In vitro	Roscovitine enhances apoptosis in the ER-MDAMB-231 BC cells	161, 162
MCF-7	Roscovitine	-	In vitro	Roscovitine induces apoptosis in cancer cells through the primary disintegration of nucleoli and cell cycle arrest	163
MCF-7	Roscovitine, Proteasome inhibitors: MG-132 and MG-262	-	In vitro	Proteasome inhibitors did not mimic roscovitine effects for increasing p53 expression	164
MCF-7	ROSC, olomoucine, nocodazole	-	In vitro	Roscovitine-mediated phosphorylation of p53 at Ser-46 stabilizes wild-type p53, leading to apoptosis in cancer cells	165
MCF-7	Roscovitine	-	In vitro	The effect of roscovitine on the proliferation and apoptosis of cancer cells depends on the cell cycle status	166
MCF-7, HeLaS3	Roscovitine	-	In vitro	The outcome of treatment with roscovitine relies on cell cycle status and drug dose	167
MCF-7	Roscovitine, Olomoucine	-	In vitro	Roscovitine induces apoptosis in MCF-7 cells through the mitochondrial pathway	99
MCF-7	Roscovitine, farnesyltransferase (FPTase) inhibitor: L-774,832	-	In vitro	Blockade of farnesyl protein transferase in MCF-7 cells enhances the anti-proliferative impact of roscovitine	168
MCF7 HCT116 p53+/+ HCT116 p53-/-	Roscovitine, PNUTS-siRNA	-	In vitro	Downregulation of PNUTS increases roscovitine-induced apoptosis in cancer cells	169
MDA-MB231	Roscovitine, olomoucine	-	In vitro	Roscovitine suppresses the proliferation of MDA-MB231 cells through targeting cdk5	170
MDA-MB231, MCF10A, MCF-7, T24	Roscovitine, Cdk5 shRNA	Balb/c nude mice	In vitro/In vivo	Knockdown of Cdk5 or its inhibition by roscovitine decreased tumor formation and induced cell death in tumor-initiating cells	171
MCF-7	Roscovitine	-	In vitro	Roscovitine promotes apoptosis through the upregulation of p53AIP1 protein in MCF-7 BC cells	98
MCF-7, MCF-7.0.3, MCF-7.3.28	Roscovitine, olomoucine, DRB, etoposide (VP16), staurosporine	-	In vitro	Forced expression of caspase-3 in MCF-7 cells sensitizes these cells to the function of DNA-damaging proteins but not to roscovitine	172
MCF-7	Roscovitine, ornithine decarboxylase inhibitor: DFMO	-	In vitro	Inhibition of ornithine decarboxylase can affect the Roscovitine-mediated apoptosis through the mitochondrial pathway	173
MCF-7, MDA-MB-231	Roscovitine	-	In vitro	Exogenous polyamines play a critical role in the induction of apoptosis or autophagy by roscovitine	174
MDA-MB-231 (p53 mutated)	Roscovitine	SCID (DNA-PKcs deficient) and BALB athymic nude mice	In vitro/In vivo	Roscovitine can sensitize cancer cells against radiation	175

Table 5. Continued.

Cell Lines	Drug/SiRNA	Animal Model	In vivo/In vitro	Study Result	Reference
MDA-MB-231 HMVECs, HMEC-1	Roscovitine	-	In vitro	The combination of roscovitine and radiation inhibits the angiogenesis process	176
MCF-7	Roscovitine	-	In vitro	Naturally occurring compounds such as phenol red can enhance the anti-cancer potential of roscovitine	177
MCF-7	Roscovitine, resveratrol	-	In vitro	Resveratrol modulates roscovitine-mediated cell cycle arrest	178
MDA-MB-231 MCF-7, BT 474, SKBr-3, EVSA-T, MDA-MB-435-S	Roscovitine, TRAIL, Pancaspase inhibitor, (z-VAD.FMK), Cycloheximide (CHX), cFLIP-siRNA Mcl-1- siRNA, E2F1-siRNA	-	In vitro	Roscovitine enhances the apoptosis of cancer cells by TRAIL	179
MCF-7 4T1, BT474, SKBR3	Roscovitine, Flavopiridol, ERBB1/ ERBB2 inhibitor (Lapatinib), BH3 domain inhibitor (Obatoclax), Si-BCL-2, Si-BCL-X, Si-MCL-1, Si-BAX, Si-BAK	-	In vitro	Modulation of Mcl-1 expression by roscovitine sensitizes BC cells to BAX/BAK-dependent mitochondrial apoptosis	180
MCF-7	Seliciclib, doxorubicin	Nude (nu/nu) mice	In vitro/In vivo	Roscovitine increases the antitumor effect of doxorubicin	100
MCF7, MDA-MB-436, HCC1806, HCC1569, ZR75-1, UACC812, MCF-7	Roscovitine, doxorubicin, roscovitine, meriolin 5, Cdk2 inhibitor: CVT-313	Nulliparous mice	In vitro In vitro/In vivo	Roscovitine in combination with doxorubicin synergistically enhanced apoptosis in LMW-E-expressing cells Silencing of Cdk2 enhances apoptosis in LMW-E overexpressing cell lines	181, 182
MCF-7, Ac1	Roscovitine, aromatase inhibitors: letrozole and exemestane anastrozole	-	In vitro	Roscovitine reverses resistance to letrozole due to LMW-E expression	183
HMEC, MCF-10A, 76NE6, 76NF2V, MDA-MB-157, MDA-MB-231, HCC1806, MCF-7, ZR75-1, T47D, MDAMB-468, HCT116 p53 ^{+/+} , HCT116 p53 ^{-/-} , HEK-293T	Roscovitine, doxorubicine	Balb/c Nu/nu mice	In vitro/In vivo	Sequential combination treatment of roscovitine and doxorubicin synergistically inhibits p53-mutant TNBC cells	184
MCF-7 SKBr3 BT474	Roscovitine, HER2/neu siRNA, trastuzumab	Nude mice	In vitro/In vivo	HER2-mediated signalling affects LMW cyclin E expression and cell cycle	185
MCF7, A549, H460, SkBr3, H1650, H292, H358	Seliciclib, AG1478, trastuzumab	(Nu/nu) mice	In vitro/In vivo	Roscovitine and the anti-HER-2 antibody synergistically suppress tumor growth	186
MCF-7, MCF7 MDR	Roscovitine, Sangivamycin, Caspase-6 inhibitor (Z-VEID-FMK), Caspase-3/7 inhibitor I	-	In vitro	Roscovitine induces apoptosis in drug-resistant P-gp-overexpressing BC cells	187
MCF-7, BT-20, SKBr-3	Roscovitine, DRB, tamoxifen, 4-HOT	-	In vitro	Roscovitine can inhibit the activating phosphorylation of ER- α and exhibit synergistic effects in combination with anti-estrogen therapy	89
MCF-7, MCF-7-E6, BT-20, SKBr-3	Roscovitine, tamoxifen, Staurosporine, DRB, Estradiol (E2), 4-HOT	-	In vitro	Tamoxifen increases the anti-proliferative function of roscovitine	188-190
MCF7-LTLTca, CF7-TamR	Roscovitine	Nude mice	In vitro/In vivo	Roscovitine suppresses tumor growth in hormone therapy-resistant tumors	114
T47D(T47D-YB, T47D PR-null, T47D wt PR-B, T47D S345A PR), MCF-7, COS-1, BT-474 cells	Roscovitine, MAPK inhibitor (U0126)	-	In vitro	Blockade of CDK2 by roscovitine inhibits the interaction of PR/cyclin D1 with DNA and reduces the expression of HSPB8	191

Note. SiRNA: Small interfering RNA; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; PNUTS: Phosphatase nuclear targeting subunit; LMW: Low molecular weight; TNBC: Triple-negative breast cancer; CDK: Cyclin-dependent kinase; MAPK: Mitogen-activated protein kinase; ER: Estrogen receptor; PR: Partial response; DRB: 5,6-dichlorobenzimidazole 1-b-D-ribofuranoside; 4-HOT: 4-hydroxytamoxifen; P-gp: P-glycoprotein

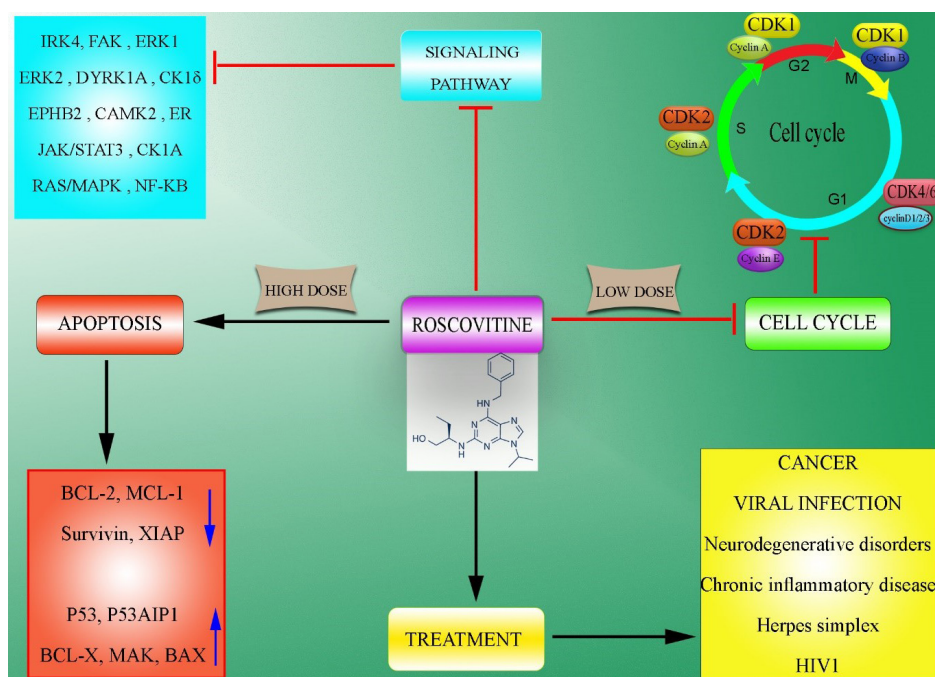


Figure 5. Action Mechanisms of Roscovitine. *Note.* Roscovitine can arrest the cell cycle in G0, G1, S, or G2/M phases in the dose-, time-, and cell-type-dependent manner by suppressing CDKs. Although Roscovitine suppresses cell cycle progression at low doses through direct inhibition of CDKs, it induces apoptosis at high doses, which is partly through the downregulation of anti-apoptotic factors such as BCL-2, MCL-1, Survivin, and XIAP, as well as the upregulation of pro-apoptotic factors such as P53, BAK, and BAX. It has also been demonstrated that Roscovitine can modulate several signaling pathways such as Ras-MAPK, NF- κ B, p53, estrogen receptor, JAK-STAT, and the like. In addition to cancer diseases, Roscovitine can be an effective therapeutic option for the treatment of several pathological conditions such as viral infections, glomerulonephritis, chronic inflammatory disease, neurodegenerative disorders, HIV, and Herpes simplex infections. BCL-2: B-cell lymphoma 2; MCL-1: Myeloid cell leukemia 1; XIAP: X-linked inhibitor of apoptosis protein; BAK: BCL2-antagonist/killer 1; BAX: *Bcl*-2-associated X protein, MAPK: Mitogen-activated protein kinase; NF- κ B: Nuclear factor kappa light chain enhancer of activated B cells; JAK-STAT: Janus kinase/signal transducers and activators of transcription.

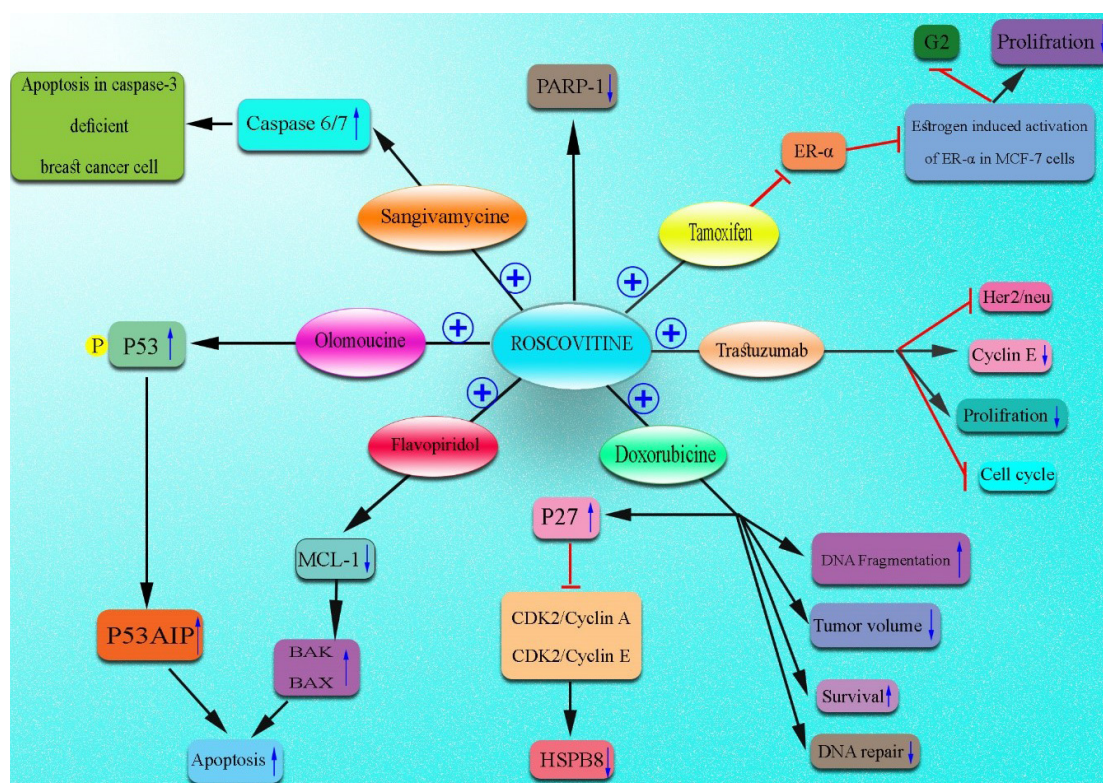


Figure 6. Combination of Roscovitine With Other Therapeutics for Breast Cancer Therapy. *Note.* Several anti-cancer therapeutics have been used in combination with Roscovitine to achieve synergistic ameliorative effects. It has been demonstrated that Roscovitine in combination with olomoucine induces apoptosis in BC cells through the upregulation of the *p53AIP1* protein. A combination of Roscovitine and sangivamycin also induces apoptosis in breast cancer cells via the upregulation of caspase 6/7. Additionally, Roscovitine and Doxorubicin synergistically exerted anti-cancer effects. Tamoxifen is another therapeutic which has been successfully employed in combination with Roscovitine. Trastuzumab and Flavopiridol also represented synergism when combined with Roscovitine for the treatment of BC. *p53AIP1*: p53-regulated apoptosis-inducing protein 1; BC: Breast cancer.

with DNA and reduced the expression of HSPB8. As a heat-shock protein, HSPB8 is one of the PR target genes whose upregulation leads to tamoxifen resistance¹⁹¹ (Table 5, Figures 5 and 6).

Conclusion

Although several outstanding discoveries regarding the treatment of BC resulted in the low mortality of patients with BC during the last three decades, its treatment is yet one of the main health-related challenges. Currently available anti-BC therapeutics are usually safe, well-tolerated, and efficient; however, cancer recurrence following the emergence of drug-resistant cancer cells often leads to the appearance of more advanced, progressive, and metastatic tumors. Therefore, the identification of novel effective therapeutic approaches is a critical issue on this topic. Accordingly, new therapeutic targets need to be identified to overcome current treatment limitations.¹⁹² Cancer cells show rapid proliferation, implying the presence of dysregulated cell cycle modulators. CDKs are the main molecules involved in the regulation of the cell cycle and transcription process. Thus, several pharmacological inhibitors of CDKs have been developed in recent years as a novel promising treatment strategy against several cancer types, including BC. However, the unknown precise action mechanism of CDK inhibitors has limited their use, alone or in combination with other anti-cancer therapeutics, in the clinic.¹⁹³ Pan CDK inhibitors are the most important CDK inhibitors that enable the suppression of various CDKs, including the CDK1, 2, 5, 7, and 9.¹⁹⁴ Several in vitro studies have reported that they can exert cell cycle arrest and apoptosis in a wide variety of BC cell lines. Although preclinical studies using Pan CDK inhibitors, alone or in combination with other therapeutics, were associated with potent breast tumor growth arrest, clinical trials were not hopeful. However, it should be noted that some trials were performed to evaluate the efficacy of these CDK inhibitors in cancer therapy (<https://www.clinicaltrials.gov/>). Hence, further trials are crucial to assign the safety and efficacy of Pan CDK inhibitors in patients with BC. Based on the results of preclinical and clinical studies, it seems that combination therapies with Pan CDK inhibitors may be more effective compared to monotherapy; nonetheless, the identification of the most effective combined strategy is a crucial issue, which should be addressed in future studies. Another proposal for future studies is the design, development, and evaluation of new derivatives of Pan CDK inhibitors.^{195,196}

Ethics statement

Not applicable.

Disclosure of funding source

This study was supported by grant from Tabriz University of Medical Sciences (grant number: 69964).

Conflict of interests declaration

All authors declare no conflict of interests.

Data availability statement

Not applicable.

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Consent for publication

Not applicable.

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