Breast Cancer CDK4/6 Inhibitor Resistance and Immunotherapy Strategies

Qiya Jing [1, *]

[1] School of Life Science and Technology, Shanghai Tech University, Shanghai, China

* Corresponding Author Email: jingqy2023@shanghaitech.edu.cn

Abstract. Breast cancer remains the most common cancer among women worldwide. Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors have emerged as key targeted therapies. They primarily prevent tumor growth in hormone receptor-positive breast cancer by blocking the cell cycle. These drugs not only directly slow down cell growth but also significantly alter the tumor immune microenvironment in important ways. They enhance antigen presentation, facilitate T-cell infiltration into tumors, and reduce the activity of regulatory T cells that suppress immune responses. In clinical practice, drug resistance remains a major issue. This resistance may be present from the outset or develop over time, greatly limiting the long-term efficacy of these drugs. This article reviews the two main ways in which CDK4/6 inhibitors exert their effects. One is to directly block the cell cycle, and the other is to reshape the immune microenvironment. The article also discusses various reasons for drug resistance and explores solutions to this problem. Combination therapy is one option. Its purpose is to provide theoretical support for improving this targeted treatment approach. However, there is still a major issue. The existing CDK4/6 inhibitors are not effective for all subtypes. They have poor efficacy in triple-negative breast cancer. This subtype has the highest mortality rate, grows rapidly, and lacks targeted treatment options. Research is needed to expand the use of these drugs. The research should focus on the forms of resistance and also on under-served subtypes such as triple-negative breast cancer. This work is urgent and will help improve the treatment outcomes for all breast cancer patients.

Keywords: CDK4/6 inhibitors, breast cancer, immunotherapy, drug resistance mechanisms.

1. Introduction

Among women, breast cancer is the cancer that shows up the most often. Out of all breast cancer cases, about 60% to 70% are of the HR-positive, HER2-negative type. This is the most common subtype. Developing precise and effective treatment methods is an urgent need in clinical practice. This high proportion indicates the significance of such work.

Cyclin-dependent kinases 4 and 6 (CDK4/6) are essential proteins that control the transition from G1 to S phase in the cell cycle. CDK4/6 inhibitors are targeted drugs. They block their kinase activity. This stops retinoblastoma protein phosphorylation. The growth of tumor cells will also be slowed down. New research shows these drugs do more. They change the tumor immune environment. They affect how immune cells work. They also alter antigen presentation. These drugs fight tumors in many ways.

CDK4/6 inhibitors have made significant progress in the treatment of HR+/HER2- breast cancer. Drug resistance remains a problem. Some patients do not respond well to the drugs and their conditions may deteriorate. Better biomarkers are needed to help determine which patients are most likely to benefit. This article explores the mechanism of CDK4/6 inhibitors in the immune environment of breast cancer. It analyzes the causes of drug resistance and discusses methods to solve this problem. The aim is to provide theoretical support for improving targeted therapy.

2. The Mechanism of Action of CDK4/6 Inhibitors

CDK4 and CDK6 are key kinases. They trigger cells to move from the G1 phase to the S phase. In normal cells, CDK4/6 binds to cyclin D. This binding causes the retinoblastoma protein (Rb) to be phosphorylated. This phosphorylation enables Rb to release E2 factor (E2F). Once released, E2F

activates the genes necessary for DNA replication. The cell then enters the S phase. After this step, cell division is completed.

CDK4/6 inhibitors target the active sites of CDK4 and CDK6, attaching to them directly. This binding will inhibit their kinase functions. This competitive effect will prevent Rb from being phosphorylated. Rb will combine with E2F. Due to the inactive state of E2F, its activity will also decrease. The cell cannot pass the G1/S checkpoint. The cell cycle will stop at the G1 phase. This is the mechanism of these drugs. They can prevent the abnormal growth of tumor cells.

3. Clinical Research on CDK4/6 Inhibitors

Both Palbociclib and Ribociclib, widely used CDK4/6 inhibitors, have demonstrated clear efficacy in treating patients with HR+/HER2- advanced breast cancer. However, they differ in terms of efficacy strength, long-term survival benefits, and adverse reaction profiles.

3.1. Comparison of Efficacy and Survival Benefits

Palbociclib: Its key Phase III study confirmed that, in combination with letrozole, it significantly extended the median progression-free survival (mPFS) to 20.2 months (compared to 10.2 months in the control group). This result established its position in the first-line treatment of advanced disease[1].

Ribociclib: Its Phase III study also extended the mPFS to 20.2 months (compared to 12.8 months in the control group). Its standout advantage lies in demonstrating a clear overall survival benefit during long-term follow-up, with a median overall survival extended by 12.5 months compared to the control group. It is currently the only CDK4/6 inhibitor proven to have significant overall survival (OS) advantages in long-term follow-up [2].

3.2. Adverse Reactions and Safety Management

The most common adverse reaction for both is neutropenia, requiring routine monitoring of blood counts during treatment [3]. Palbociclib can cause some major side effects. Neutropenia is quite common. Fatigue is often experienced. Joint pain can also occur. Doctors will closely monitor the patient's blood cell count when using this drug. Ribociclib can also cause blood problems. It can also cause more gastrointestinal discomfort symptoms. Nausea is one example. Diarrhea is another. The impact of Ribociclib on the liver is more severe than that of Palbociclib. Liver function needs to be monitored when using this drug [4]. Ribociclib also requires electrocardiogram monitoring. Doctors need to check the QT interval. This is listed in the prescribing information [5].

3.3. Clinical Selection Considerations

Palbociclib was the first approved drug. It has complete data on both efficacy and safety. Doctors have been using it for many years. Ribociclib can also delay the progression of the disease. In this regard, its effect is comparable to that of Palbociclib. In long-term studies, Ribociclib shows a stronger survival advantage. This is supported by the existing evidence. Ribociclib causes more types of side effects. Doctors need to pay close attention to these side effects. They need to conduct appropriate monitoring. Treatment options should vary according to individual patients. Doctors must consider each patient's specific situation. Other diseases are also important. How the patient tolerates specific side effects is also important. The patient's emphasis on long-term survival is also very important. All these factors will guide the final choice.

4. CDK4/6 Inhibitors Regulate the Immune Microenvironment and Strategies for Combined Immunotherapy

4.1. Regulation of the Immune Microenvironment

The CDK4/6 inhibitors have three major functions. They can prevent the growth of harmful cells. They can promote the growth of beneficial cells. They can also improve the presentation of tumor antigens. Harmful cells include tumor cells. They also include regulatory T cells. These T cells prevent the immune system from attacking tumors. CDK4/6 inhibitors reduce the activity of DNA methyltransferase 1. This enables genes with tumor suppressor functions to be activated. The growth rate of tumor cells will slow down. Regulatory T cells also cannot function properly and survive normally. New research points to another reason for CDK4/6 inhibitors inhibiting the proliferation of regulatory T cells. Regulatory T cells express more CDK6 than other cells do. This high expression may explain why CDK4/6 inhibitors stop these cells from growing. Cluster of Differentiation 8 Positive T cells (CD8+ T cells) kill cancer cells directly. However, after prolonged activity, their surface produces immune checkpoints. This causes the T cells to stop functioning and leads to immune exhaustion.

The low activity of DNA methyltransferase 1 has another function. It reduces the expression of genes related to immune checkpoints. As a result, CD8+ T cells can kill tumor cells more effectively. CDK4/6 inhibitors can also keep the Rb protein in a non-phosphorylated state. The E2F transcription factor cannot be released. This further reduces the activity of DNA methyltransferase 1 in breast cancer cells. Eventually, the production of type III interferons increases. The presentation ability of tumor antigens is also improved [6].

4.2. Combined Immunotherapy

Studies have shown that CDK4/6 binds to p73, which belongs to the p53 family. This binding causes p73 to be phosphorylated and remain in the cytoplasm. Blocking CDK4/6 removes this phosphate group. When p73 enters the nucleus, it activates death receptor 5 (DR5). This makes cancer cells more sensitive to death signals. They are more likely to undergo programmed cell death. Researchers later removed the DR5 gene from cancer cells. Without DR5, CDK4/6 inhibitors were unable to enhance the effects of TNF-Related Apoptosis-Inducing Ligand (an immune factor that activates DR5 to induce apoptosis), 5-fluorouracil (a chemotherapy drug), and anti-PD-1 immunotherapy [7]. This indicates that CDK4/6 inhibitors rely on inducing death receptor 5 (DR5) expression to enhance the efficacy of other therapies, including chemotherapy and immunotherapy.

5. Mechanisms of Resistance to CDK4/6 Inhibitors and Exploration of Subsequent Treatment Strategies

5.1. PI3K/AKT/mTOR Signaling Pathway

The PI3K/AKT/mTOR pathway regulates cell growth. It is a core signaling cascade reaction. Approximately 35% of HR+/HER2- breast cancer metastasis cases have mutations in this pathway. These mutations activate the pathway, thereby increasing the levels of various cyclins. As a result, the activity of CDK2 also increases. The effect of CDK4/6 inhibitors will fail. The effect of cell cycle arrest will be weakened. Drug resistance will also form[8]. Drugs targeting this pathway have shown clinical efficacy. For example, combination regimens involving the PI3Kα inhibitor alpelisib, the AKT inhibitor capivasertib, or the mTOR inhibitor everolimus have significant effects. They can help patients who have already used CDK4/6 inhibitors to extend the mPFS and increase the clinical benefit rate. This proves that this pathway can lead to drug resistance. It also proves that this pathway is a good target for treatment.

The PI3K/AKT/mTOR pathway regulates cell survival. It also controls cell growth. It also regulates metabolism. This is a core pathway. Approximately 35% of HR+/HER2- breast cancers

have mutations in this pathway. These mutations activate the pathway. This is one of the main ways in which cancer cells evade CDK4/6 inhibitors.

The PI3K/AKT/mTOR signaling pathway may be abnormally activated, thereby increasing the production of cyclins. Cyclin D1 is one of them, and cyclin E is another. This is achieved through downstream effectors, with mTORC1 being one such effector. The activity of CDK2 is also enhanced. This shifts the driving signals for tumor cell proliferation from the CDK4/6-Cyclin D1 axis partially or entirely to alternative cyclin-kinase complexes, such as Cyclin E-CDK2. Even when CDK4/6 is blocked, cells can still pass the G1/S checkpoint and continue to grow. Resistance then follows.

Targeting this mechanism, combining PI3K/AKT/mTOR pathway inhibitors represents a core strategy to overcome resistance.

5.1.1. PI3Kα Inhibitor

Some patients have PIK3CA mutations. These patients are now being treated with alpelisib, which can inhibit PI3Kα. Patients took it with fulvestrant. This combination therapy has become the standard treatment protocol. It is used after the failure of CDK4/6 inhibitors. A key III-stage study tested this. The study included patients who had used CDK4/6 inhibitors. The mPFS of this combination therapy was 11.0 months. The effect of using fulvestrant alone was much worse. This combination therapy is significantly superior to monotherapy[8].

5.1.2. AKT Inhibitor

A Phase III study tested capivasertib, which can inhibit AKT. Patients were also given fulvestrant in combination with this drug. The mPFS of the patients doubled, from 3.6 months to 7.2 months. This result was applicable to all the patients participating in the study. In one subgroup, namely those who had already used CDK4/6 inhibitors, this benefit was more significant. Overall, the side effects were manageable [8].

5.1.3. mTOR Inhibitor

The mTOR inhibitor everolimus combined with endocrine therapy (such as exemestane) is an earlier approved regimen. Recent studies indicate that the triple combination of everolimus with the CDK4/6 inhibitor ribociclib and exemestane showed a certain clinical benefit rate (41.1%) in pretreated patients, providing a potential option for highly resistant cases [8].

5.2. Compensation and Dysregulation of Cell Cycle Checkpoints

In addition to bypass pathway activation, abnormalities in other checkpoint proteins within the cell cycle can directly lead to the failure of CDK4/6 inhibitors.

5.2.1. Loss or Inactivation of Rb Protein

The Rb protein is the direct downstream target of CDK4/6 inhibitors. If tumor cells exhibit RB1 gene deletion or functional loss mutations, the key step of phosphorylating Rb and releasing the E2F transcription factor by the CDK4/6-Cyclin D complex is bypassed. The continuous activation of E2F drives cell cycle progression, rendering CDK4/6 inhibitors completely ineffective. This mechanism is often observed in acquired resistance after long-term treatment.

Patients with Rb functional loss exhibit primary or secondary resistance to CDK4/6 inhibitors. Currently, there are no drugs directly targeting Rb deficiency, and treatment must shift to other strategies that do not rely on the Rb pathway.

5.2.2. Overactivation of the Cyclin E-CDK2 Pathway

Overexpression of Cyclin E and enhanced CDK2 activity represent another common resistance mechanism. Studies have shown that CCNE1 gene amplification is frequently detected in cell lines and patient samples resistant to CDK4/6 inhibitors [9]. The Cyclin E-CDK2 complex can directly phosphorylate and inhibit Rb or independently drive S-phase entry by phosphorylating other substrates, forming a positive feedback loop independent of CDK4/6.

Small-molecule inhibitors directly targeting CDK2 are currently under clinical development and are being tested in combination trials with endocrine therapy or PI3K inhibitors to precisely target this resistance pathway. Additionally, multi-target inhibitors of CDK2/4/6 are also under exploration.

5.2.3. Dysregulation of G2/M Checkpoint (WEE1/AURKA)

WEE1 kinases (WEE1) and Aurora Kinase A(AURKA) are key proteins regulating the G2/M checkpoint. Some tumor cells upregulate WEE1 to cope with DNA replication stress, ensuring cell division even in the presence of genomic damage. Inhibiting WEE1 leads to the accumulation of DNA damage, triggering "mitotic catastrophe." AURKA, responsible for spindle assembly, is overexpressed in cases of chromosomal instability and drug resistance. When the G1 phase is blocked by CDK4/6 inhibitors, tumor cells may rely more heavily on the integrity of the G2/M checkpoint for survival. Inhibiting WEE1 or AURKA can synergistically induce cell death.

Clinical studies of the AURKA inhibitor alisertib have shown single-agent antitumor activity (Objective Response Rate 20.0%) in breast cancer patients resistant to CDK4/6 inhibitors. The WEE1 inhibitor adavosertib and other drugs are also undergoing clinical trials in combination with chemotherapy or other targeted therapies for resistant patients [10].

5.3. Other Potential Resistance Mechanisms and Emerging Targets

5.3.1. Abnormal Growth Factor Receptor Signaling

Some receptors tend to overexpress. Fibroblast growth factor receptor (FGFR) is an example of this. These receptors activate downstream signaling pathways. As a result, multiple cell cycle proteins increase. Cell cycle inhibition is relieved. Drug resistance subsequently emerges. Drugs that can block receptor tyrosine kinases may be helpful. FGFR inhibitors are one such type. They may work well when combined with CDK4/6 inhibitors. They may also work well when combined with endocrine therapy. Currently, clinical trials of these combination therapies are underway.

5.3.2. Epigenetic Alterations and Tumor Microenvironment Adaptation

Tumor cells utilize epigenetic modifications to alter the expression of key genes. This enables them to adapt to drugs and develop drug resistance. Simultaneously, immune cells, fibroblasts, and other components in the tumor microenvironment can provide paracrine growth signals by secreting factors, aiding tumor cell survival. Targeting the tumor microenvironment or epigenetic regulators (e.g., Histone Deacetylase inhibitors) represents a promising direction for combination therapy.

6. Conclusion

This article examines the effects of CDK4/6 inhibitors on immune cells. It analyzes how they alter the presentation of tumor antigens. The reasons for developing drug resistance are also discussed. Additionally, the subsequent treatment steps are outlined. New research indicates that the combined use of immunotherapy yields significant results. The aim of this article is to assist doctors in making more informed decisions, with the hope of improving the treatment outcomes for patients.

CDK4/6 inhibitors do not treat all breast cancer types yet. Triple-negative breast cancer (TNBC) has the highest death rate. This subtype remains a big challenge. More research is needed here. It will help improve treatment plans. Future research will refine how we use these drugs. CDK4/6 inhibitors will play a bigger role in breast cancer care.

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