Cyclin-dependent kinase inhibitors as targeted therapy in breast cancer: Review

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Abstract: Breast cancer is the most common cancer in women worldwide. Understanding the biology of this tumor is a prerequisite for selecting an appropriate treatment. Cell cycle alterations are seen in many cancers such as breast cancer. Newly popular targeted agent in breast cancer are cyclin-dependent kinase inhibitors (CDKIs) which are agents inhibiting the function of cyclin-dependent kinases (CDKs) is a member of protein kinase family. It plays an important role in regulating various events of eukaryotic cell division cycle. Accumulated evidences indicated that over expression of CDKs should cause the abnormal regulation of cell-cycle, which would be directly associated with hyper proliferation in cancer cells. They are categorized as selective and non-selective inhibitors of CDK. CDKIs have been tried as monotherapy andcombination therapy. In this review, we present the structure, functions and activation of CDKs by cyclin binding with special focus on recent advances in the development of different CDKI in breast cancer.

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1. Introduction

Cancer is a collective term used to describe a group of different diseases that are characterized by the loss of control of cell growth and division, leading to a primary tumor that invades and destroys adjacent tissues. It may also spread to other regions of the body through a process known as metastasis, which is the cause of 90% of cancer deaths. Cancer remains one of the most difficult diseases to treat and is responsible for approximately 14.5% of all deaths worldwide [1]. This incidence is increasing due to the aging of the population in most countries, including those under development. Indeed, against a widely held belief, more than two-thirds of all cancer deaths occur in low- and middle-income countries, and the estimated increase in cancer incidence by 2030, compared with 2008, will be greater in low- (82%) and lower-middle-income countries (70%) compared with the upper-middle- (58%) and high-income countries (40%) [2].

Cell division and cell death are the two predominant physiological processes that regulate normal tissue homeostasis. Alteration of these two physiological processes has a pivotal role in the pathogenesis of cancer [3]. Great efforts to ascertain components of the cell cycle are guiding to novel approaches for the treatment of cancer. Genes encoding components of the cell cycle such as cyclin, CDKs and their endogenous inhibitors which are found in normal conditions are often impaired in many human cancers [4]. For example, CDKs are overactive in some cancers depending on cyclin overexpression or downregulation of endogenous CDKIs [5]. According to this data, researchers focus on whether the strategy of CDK inhibition is able to render cancer treatment more successful. Some studies suggest that inhibiting CDKs may be an effective therapy in many cancers including breast cancer [6]. There were many pieces of evidence to support that the inhibition of CDKs could play vital role in suppressing cancer [7]. Frequent misregulation of CDKs in cancerous cell has made them to be striking targets for cancer therapy [8].

2. Cyclin-Dependent Kinases (CDKs)

The human protein kinases set (kinome), is constituted of 518 identified proteins, divided in seven families [9]. Cyclin-Dependent Kinases (CDKs) are part of the CMGC family named after the members: Cyclin-dependent kinases (CDKs), Mitogen-activated protein kinases (MAPKs), Glycogensynthase kinases (GSKs) and CDK-like kinases (CLKs). For their discovery, Hartwell, Nurse and Hunt received the Nobel Prize in 2001 [10]. The number of CDKs increased during development and was marked by a greater expansion of the cell-cycle related group; human cells have 20 CDKs and 29 cyclins [11]. The crucial role of CDKs is that control of cell cycle, in spite of that only several of them have been shown to have direct role in the cell cycle progression such as CDK1, CDK2 and CDK4[12-14].

Cell cycle is regulated by cyclins, CDKs, and CDKIs. These three key classes of regulatory molecules determine a cell's progress through the cell cycle [15]. Cell cycle is divided into 4 distinct phases (G□, S, G□, and M). G□ represents exit from the cell cycle. Specific cyclin and CDKs complexes conduct

cell cycle progression by regulating transition through G□-G□-S-G□-M phases **(Figure 1)**. Cell cycle is driven by CDKs, which are positively and negatively regulated by cyclins and CDKIs, respectively [16]. Cyclins form the regulatory subunits and CDKs the catalytic subunits of an activated heterodimer; cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin [17]. When activated by a bound cyclin, CDKs perform acommon biochemical reaction called phosphorylationthat activates or inactivates target proteinsto orchestrate coordinated entry into the nextphase of the cell cycle. Cyclin-CDK complexes inearlier cell-cycle phase help activate cyclin-CDKcomplexes in later phases [18].

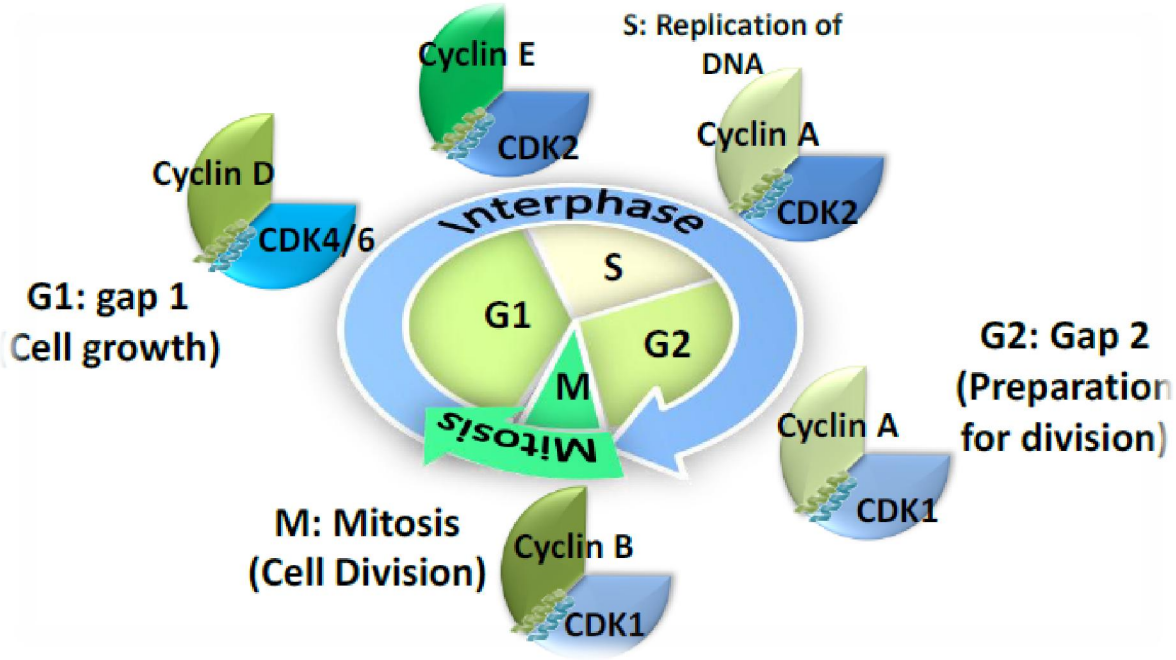


Figure 1: Cycline-dependent kinase with cyclin in cell-cycle[16].

In addition, a secondgroup of CDKs are responsible for the regulationof cellular transcription. They have role ofmaintenance for cancer cells' survival. This groupof CDKs consists of CDK7, CDK8, CDK9, CDK10, and CDK11.

A CDKI protein is an endogenous protein that interacts with a cyclin-CDK complex to block kinase activity, usually during G□ or in response to signals from the environment or from damaged DNA. In the human body, there are two major CDKI protein families: the INK4a/ARF family and the Cip/Kip family. The INK4 family proteins are strictly inhibitory and bind CDK monomers. Crystal structures of CDK6-INK4 complexes show that INK4 binding twists the CDK to distort cyclin binding and kinase activity. The Cip/Kip family proteins bind both the cyclin and the CDK of a complex and can be inhibitory or activating. The Cip/Kip family proteins activate cyclin D and CDK4 or CDK6 complexes by enhancing complex formation [19]. To push the cell from G□ to S phase the phosphorylation of retinoblastoma (Rb) protein byCDK4 or CDK6 in complex with their activating subunits, cyclin D1, D2 and D3 is necessary. The hyperphosphorylated Rb protein dissociates from the E2F/DP1/Rb complex to activate E2F. Activation of E2F results in transcription of various genes such as cyclin E, cyclin A, DNA polymerase, and thymidine kinase. For instance cyclin E thus produced binds to CDK2, forming the cyclin E-CDK2 complex that keeps up the progression through G□-S phase. CDK2-cyclin A and CDK1-cyclin A regulate the completion of S phase. Then G□/S progression initiates the G□/M transition[20].

Finally, the cell cycle is completed and cell is going to divide. All cancers activate cell cycle to sustain their survival. Selecting an appropriate agent for the appropriate tumor type is very hard, because, first of all, it should be identified which regulator of the cell cycle is responsible for the cell cycle downstream of an oncogenic event. Therefore, mouse models have been used to understand what kind of the cell cycle inhibitor is against which cancer type. In many cancers CDKs are overactive or CDK-inhibiting proteins are dysfunctional. For example, upregulation of CDK4 or downregulation of a naturally occurring inhibitor of CDK4, called p16INK4A, lead to loss of proliferative control of cell through enhanced CDK4 activity, resulting in hyperphosphorylation of Rb

protein and in carcinogenesis [21]. According to this information, it is rational to target CDK function to prevent overproliferation of cancer cells and to use CDKIs to treat human cancers.

3. CDKs in breast cancer

Breast cancer affects millions of women annually worldwide and remains the most common cancer diagnosis in women. Although treatment options for patients with breast cancer have improved over time, metastatic breast cancer remains incurable. Hormone receptor positive (HR+) disease is the most common subtype of both early and late stage breast cancer. With scientific advances, new targeted therapies are emerging to address specific areas of tumor biology [22]. Checkpoint deregulations play a key role in some breast cancers. Alterations of pathways that include cyclin, CDK, endogenous CDKI and Rb protein are seen in nearly all cancers, including breast cancer. Cyclin D1 and cyclin E overexpression, decreased expression of CDKI p27Kip1 are some of them in human breast cancer [23,24]. Cyclin D1 amplification is seen in nearly 60% of breast cancers. Estrogen uses cyclin D1 as one of its target genes to mediate its mitogenic effects. Some studies suggested that among patients with high tumor expression of cyclin D1, overexpression of HER2 was associated with reduced recurrence- free survival and tamoxifen responsiveness[25]. Overexpression of cyclin D1 changes the antagonistic effect of tamoxifen to an agonistic effect. Therefore tamoxifen resistance might be predicted with cyclin D1 overexpression [26]. However, this data has not been exactly verified and the prognostic significance of cyclin D1 overexpression is not completely understood.

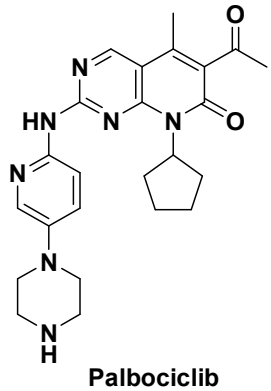
In breast cancer cells, the process of cell division becomes unregulated, resulting in uncontrolled growth that leads to the development of a tumor. A number of mechanisms contribute to the dysregulation of the cell cycle in malignant cells including the amplification and hyperactivity of CDK 4/6, or their genomic instability, which may cause CDK 4/6 to become oncogenic drivers of cell replication [27]. Usurping these mechanisms, cancer cells can continue to replicate by triggering the G1 to S phase transition [28]. This process appears to be facilitated by a shortening of the G1 phase. In a cancer cell, CDK 4/6 antagonizes intrinsic tumor suppression mechanisms including cell senescence and apoptosis, which further augments the growth of a tumor [27]. Cyclin-dependent-kinase (CDK) 4/6 inhibitors, which affect cell cycle progression to halt tumor growth, are an exciting new direction for the treatment of HR+ breast cancer. This class of drugs is being investigated extensively in both pre-clinical and clinical studies.

4. CDK 4/6 Inhibitorsin breast cancer

There are a lot of CDKIs that have gone through or are currently tested in ongoing clinical trials in cancer treatment [29]. Most of them are targeting multiple CDKs, but some are targeting specific for CDK 4/6. Selective inhibition of CDKs is much better than non-selective, because more adverse and toxic effects have been seen with non-selective inhibitors[30-32].

Palbociclib:

Among the CDK 4/6 inhibitors undergoing clinical trial investigation, palbociclib (Pfizer) is the farthest in development.



Palbociclib (PD-0332991)

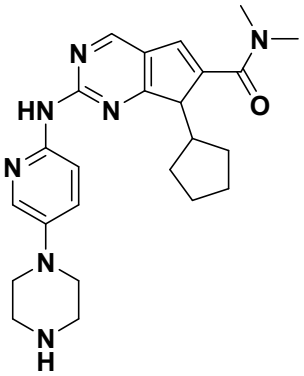
Pharmacokinetics and Dosing

Palbociclib selectively inhibits CDK 4 and CDK 6 with half maximal inhibitory concentrations(IC50) of 0.011 μmol/L and 0.016 μmol/L, respectively [33].

In clinical trials, palbociclib has been given at doses of 125 mg daily for 21 days out of a 28 daycycle, or 200 mg daily for 14 days out of a 21 day cycle [33,34]. In a phase I study, 33 patients with refractory Non-Hodgkin's Lymphoma or Rb positive advanced tumors were given 200 mg of palbociclib daily for 2 out of 3 weeks. This trial established the safety of this dose, with the major toxicity being uncomplicated myelosuppression. Nine out of 31 patients experienced disease stability with treatment. A phase I dose escalation study of 41 patients with Rb positive advanced malignancies investigated palbociclib given once daily at doses of 75, 125, or 150 mgfor 21 of 28 days, and determined the maximum tolerated dose as 125 mg once daily. The dose limiting toxicity in this study was also neutropenia. Eleven of 41 patients experienced stable disease with 4 or more cycles of treatment [33].

Ribociclib:

Ribociclib (Novartis) is another CDK 4/6 inhibitor undergoing clinical development.



Ribociclib (LEE-011)

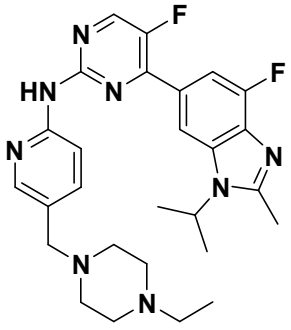
Pharmacokinetics and Dosing

Ribociclib selectively inhibits CDK 4/6 at the nanomolar level [35].

A phase I trial treated 70 patients with advanced lymphomas or solid malignancies with ribociclib, using doses from 70 mg to 1200 mg daily for 21 out of 28 days, and noted drug activity at doses of 600 mg or above, with 900 mg being the maximum tolerated dose. In this study, 10 out of 70 patients had stable disease while on treatment, and a partial response was noted in 1 patient with HR+ breast cancer. The main toxicities identified were myelosuppression, diarrhea, nausea, and asymptomatic QTc prolongation [35].

Abemaciclib

Abemaciclib (Eli Lilly) is the third CDK 4/6 inhibitor being studied in clinical trials of breast cancer, with very promising results.



Abemaciclib (LY2835219)

Pharmacokinetics and Dosing

Abemaciclib is an inhibitor of CDK 4/6 at the nanomolar level, inhibiting CDK 4/cyclin D1 with an IC50 of 2.0 nM, and CDK 6/cyclin D1 with an IC50 of 9.9 nM[36].

Unlike the other two CDK 4/6 inhibitors described above, abemaciclib is unique in that it can be dosed continuously without interruption and it appears to have clinically relevant single agent activity. A recent phase I trial investigated abemaciclib in patients with various solid tumors, with an emphasis on metastatic breast cancer, using 150 mg to 200 mg of abemaciclib twice daily in a 28 day cycle [37]. The dose limiting toxicity with this agent is diarrhea, different from the bone marrow toxicity seen with the other CDK 4/6 inhibitors undergoing clinical development. The diarrhea appears to be fairly easy to manage with prophylactic antidiarrheal medications, and by interruptions or reductions in the dose of abemaciclib as needed. Other toxicities include neutropenia, nausea, vomiting, and fatigue, with uncomplicated neutropenia being the most commonly noted grade 3 toxicity [37].

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