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Review Article

Endogenous and Pharmacological Inhibitors of Cyclin Dependent Kinases in Cell Cycle Regulation of Normal and Cancer Cells

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Abstract

Cell cycle dysregulation has been demonstrated to play an important role in the development of cancer cell growth. Cyclin Dependent Kinases (CDK) are essential proteins required for the control and completion of the cell cycle. Aberrant CDK regulation has been implicated in the development of many types of human cancers. This observation has led to the investigation and development of various Cyclin Dependent Kinase Inhibitors (CDKI) as potential antineoplastic agents. Early attempts of developing an effective CDKI were met with toxic side effects and decreased efficacy due to lack of specificity and off-target effects. However, recent clinical trials have demonstrated the efficacy of newer CDK inhibitors such as Palbociclib. While CDK inhibitors can also be used to treat various diseases such as neurodegenerative conditions, we have mainly focused here on the therapeutic potential of these drugs in neoplastic disease. In this paper, we briefly described the role of endogenous CDK inhibitors such as members of Cip/Kip and INK4 families. Subsequently, we highlighted several pharmacological CDK inhibitors that are currently undergoing clinical evaluation in cancer patients and offer some perspectives for future research in this field.

Keywords: Cell cycle regulation; Cyclins; CDKs; CDK inhibitors; Cancer; Clinical trials

Abbreviations

AURK: Aurora Kinase; CDK: Cyclin Dependent Kinase; CDKI: CDK Inhibitor; Cyc: Cyclin; CRS: Cytokine Release Syndrome; DLT: Dose Limiting Toxic Effect; FACS: Fluorescence Activated Cell Sorting; IC: Inhibitory Concentration; MTD: Maximum Tolerated Dose; PFS: Progression Free Survival; PLK: Polo-Like Kinase; TLS: Tumor Lysis Syndrome

Introduction

Dysregulation of cell division has been demonstrated to play a crucial role in cancerous cell growth. Given that mammalian cells have many fail proof mechanisms to prevent cellular transformation, it is rare for a single mutation in tumor-suppressor genes or proto-oncogenes to cause cancer cell growth. Only upon multiple synergistic mutations do cells gain the ability to divide uncontrollably. To ensure proper duplication of genetic material and creation of two identical daughter cells, normal mammalian cells complete the cell cycle in a controlled stepwise process which is tightly regulated by multiple mechanisms [1].

The cell cycle is subdivided into 4 distinct phases: G1, S, G2 and M phases. "The S-phase refers to the DNA synthesis phase where the genetic material is duplicated, and the M-phase (mitosis) is the phase where duplicated chromosomes are divided equally between two daughter cells. Finally, the physical division of two genetically identical daughter cells is completed by cytokinesis [2,3]. The G1 and G2 phases refer to the gap times preceding the S- and M-phases respectively. During both the G1 and G2 phases, important regulatory events necessary for successful cell division take place. Cells which are not actively dividing enter a quiescent or resting stage termed G0. Similarly, cells which do not receive either proliferation signals or anti-mitogenic signals can effectively withdraw from the cell cycle and enter G0 [1].

The transition from one cell cycle phase to another is tightly regulated by a number of cellular proteins [1,4]. Cyclin dependent kinases (CDKs) are a family of serine/threonine protein kinases which are activated at different stages of the cell cycle and play a key role in cell cycle progression. CDKs are activated by binding to regulatory subunits named cyclins (cyc). In mammals, there are at least 15 cyclins, however, not all members of the cyclin family play a direct role in cell cycle regulation [5]. Cyclins C, K, L and T play a role in the regulation of transcription, whereas cyclin A, B, D and E play a direct role in CDK regulation. Cyclin H plays a role in both transcriptional and CDK regulation. Transcriptional events as well as ubiquitin mediated degradation of cyclins regulate their cyclic appearance and disappearance during various phases of the cell cycle. To date, eleven CDKs and nine CDK-like proteins have been reported. Of the eleven CDKs, five have been shown to play a direct role in cell cycle progression (CDK1, 2, 3, 4 and 6), five in transcriptional control (CDK 7, 8, 9, 10 and 11), and one in neuronal cell function (CDK5) [6]. CDK4/cycD and CDK6/cycD complexes are responsible for G0/G1 and G1/S transition. CDK2/cycE complexes are responsible for G1/S transition. CDK2 can also bind cycA, and CDK2/cycA complexes are necessary for S-phase entry and completion. CycA binds CDK1 and plays a role in S/G2 transition. CDK1 binding to

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cycB is required for G2/M transition [1,4,5]. CDK4/cycD complex has been demonstrated to be essential for the proliferation of most cells [7, 8] while CDK6/cycD has been identified to be important for the proliferation of hematopoietic cells [9].

Aberrant CDK regulation has been implicated in many types of human cancers. Overexpression of CDK4 or its gene amplification has been reported in ovarian [10], urinary bladder [11], endometrial [12] and oral [13] cancers. A point mutation discovered in CDK4, Arg24Cys, which renders it resistant to INK4 regulation has been reported to play a role in melanoma [14]. CDK6 overexpression was found to play a role in oral cancers [13]. CDK7 and CDK5 have been shown to play a role in breast [15] and lung [16] cancers, respectively. Loss or inhibition of certain CDK activity has been reported to interfere with the ability of both normal and cancerous cells to proliferate. For example, combined depletion of CDK1 and CDK2 activities can lead to either G2/M arrest or apoptosis depending on the type of cancer cells [17].

With the understanding that dysregulation of CDKs plays a role in a variety of cancer cells, the prospect of targeting these kinases for possible therapeutic benefit has emerged. In this review, we briefly described the role of endogenous CDK inhibitors in normal cell cycle regulation. Subsequently, we provided a survey of several important pharmacological inhibitors of CDKs and discuss the efficacy of these compounds in cancer treatment.

Endogenous Cyclin Dependent Kinase Inhibitors (CDKI)

Endogenous cyclin dependent kinase inhibitors (CDKI) are divided into two families: Cip/Kip family and the INK4 family.

Cip/Kip Family

The Cip/Kip family is composed of three proteins: p21, $p27^{Kip1}$ (p27) and $p57^{Kip2}$ (p57). Members of the Cip/Kip family share a homologous inhibitory domain which allows for these proteins to both bind and inhibit CDK4 and CDK2 containing complexes.

P21

The first member of the Cip/Kip family to be isolated was p21 also known as Cip1, WAF1, SD11, CAP20, PIC1 and mda-6. P21 was isolated through its ability to interact with CDK2 by Harper *et al.* using a two-hybrid system [18]. P21 was found to be upregulated in wild type cells in response to DNA damaging agents indicating that p21 might play a role in p53-activated cells. As part of the DNA damage response, p53 is stabilized and activated as a transcription factor. Once activated, p53 binds to a p53-binding site in the p21 gene promoter region upregulating p21 gene expression. Upon expression, p21 is thought to inhibit the cell cycle by binding to CDK complexes necessary for G1 cell cycle progression and inhibiting their kinase activity [19,20]. In addition to playing a role in p53 dependent cell cycle inhibition, p21 was also observed to play a role in cell cycle exit mediated by cellular senescence. It was observed that p21 levels accumulated in ageing cells as they approach senescence [21].

An alternate mechanism by which p21 inhibits cell cycle is by interacting with PCNA, an elongation factor for DNA polymerase δ which is also part of the DNA repair machinery. Binding of p21 to PCNA was reported to inhibit the DNA replication but not repair function of PCNA. Thus, p21 cell cycle inhibition is thought to be mediated by two independent mechanisms: 1) binding to and inhibiting the activity of various CDK/cyclin complexes and 2) by inhibiting PCNA activity. P21 function is not limited to inhibiting cell cycle but it has also been implicated in promoting cell cycle by binding to and assisting with the assembly of CDK/cyclin complexes. Currently, p21 function is thought to be controlled by phosphorylation. Unphosphylated p21 has been reported to localize to the nucleus where it functions as an anti-proliferative protein by binding to CDK/cyclin complexes and PCNA and inhibiting their functions. Upon phosphorylation on T145 and S146 residues, p21 loses its ability to interact with PCNA and is transported to the cytoplasm [22,23]. Once in the cytoplasm, p21 can interact with cytoplasmic proteins where it plays a role in alternate cellular processes [24].

P27

$P27^{Kip1}$ mediated cell cycle inhibition was first detected in contact inhibited cells and TGF-beta treated cells. Its cell cycle inhibitory function was reported to be due to its ability to bind and inhibit CDK2 [20,25]. P27 was also reported to interact with CDK4/cyclin D complexes by a tri-hybrid screen. Like p21, p27 cell cycle mediated inhibition has been demonstrated to be mostly due to inhibiting CDK/cyclin kinase activity preventing the phosphorylation of pRb. This inhibits the cells from transitioning from the G1 to S phase of the cell cycle [19,20]. However, p27 has also been reported to play other roles within the cell namely apoptosis and cell motility. Since p27 has 6-8 phosphorylation sites, it is thought that based on the phosphorylation pattern of p27, the cellular localization of the protein, folding and metabolism are affected dictating the protein's cellular function. When p27 is localized to the nucleus it is believed to mediate cell cycle inhibition by binding to CDK/cyclin complexes and deactivating them. Similar to the localization changes observed for p21, p27 phosphorylation is also thought to promote the transport of p27 into the cytoplasm where it can participate in other cellular functions such as assisting in cellular motility [26].

P57

As part of the Cip/Kip family, p57 also has the ability to bind and inhibit CDKs. p57 can bind and regulate the function of the following CDK/cyclin complexes: CDK2-cyclin E, CDK2-cyclin A, CDK3-cyclin E, CDK4-cyclin D1, CDK4-cyclin D2, CKD1-cyclin B and CDK6-cyclin D2 [27-29]. The amino terminus of p57 contains three sites, a cyclin binding region, a CDK binding site, and a 3_{10} helix, which are necessary for the inhibition of CDK-cyclin activity [20]. P57 inhibits CDK/cyclin complexes by binding to them and inactivating their kinase activity. Similar to p21, p57 is thought to have the ability to assist with the assembly of CDK-cyclin complexes [20]. However, the assembly process is thought to be controlled by the levels of p57 within the cell rather than phosphorylation events [20]. At low levels, p57 assists with assembly of CDK-cyclin complexes and helps them translocate to the nucleus, whereas at higher levels it binds to the CDK-cyclin complexes inhibiting their kinase function [30]. The effect of phosphorylation on p57 function within the cell has not yet been reported, however, it is known that p57 degradation is regulated by phosphorylation [31].

Ink4 Family

The Ink4 family is composed of four proteins: $p16^{INK4a}$ (p16), $p15^{INK4b}$ (p15), $p18^{INK4c}$ (p18) and $p19^{INK4d}$ (p19). These proteins share a common repeating ankyrin motif which is necessary for CDK4 or CDK6 inhibition. Ink4 family members bind specifically to either CDK4 or CDK6 competing with the association of D-type cyclins [32]. As a result Ink4 cell cycle inhibition depends on the presence of pRb in the cell. In cells with wild type pRb, pRb binds to the transcription factor E2F maintaining it in an inactive form. Once D-type cyclins bind to CDK4 and/or CDK6, the activated kinase complex phosphorylates pRb causing the release of E2F. Once released, the activated E2F complex translocates to the nucleus where it promotes the transcription of genes involved in cell cycle progression. One of those genes is cyclin E. Once expressed, cyclin E binds to CDK2 promoting S phase entry. In cells lacking pRb, there is an elevated level of cyclin E which allows the cell to overcome cell cycle regulation mediated by Ink4 proteins [19,20].

Pharmacological CDK-inhibitors

Flavopiridol

Flavopiridol (Alvocidib) is a serine-threonine kinase inhibitor. It was one of the first pan-CDK inhibitors to be discovered [33-36]. Flavopiridol has been demonstrated to induce cell cycle arrest in G1 phase and prevent the transition from G1 to S phase of the cell cycle in MCF-7 human breast carcinoma cells. Early studies demonstrated the ability of flavopiridol to induce cell cycle arrest and cell death in both cycling and non cycling cancer cells [33-37]. Mechanistically, the effects of flavopiridol on cell division were demonstrated to be as a result of the inhibition of CDK2 and CDK4. In addition to inhibiting CDK4 and CDK2, flavoripridol also depletes cyclin D1 and cyclin D3 but not cyclin D2 levels [35]. Flavopiridol inhibits the functions of CDK1 (IC_{50} 41nM), CDK2 (IC_{50} 100-170nM), CDK4 (IC_{50} 65nM), CDK7 (IC_{50} 300nM) and CDK9 (IC_{50} 6nM). Additionally, at micromolar concentrations, it has been observed to inhibit the activity of epidermal growth factor receptor tyrosine kinase and protein kinase A [38]. In a kinase assay conducted by Karaman *et al.*, it was observed that flavopiridol possessed low selectivity for CDKs compared with other kinases [39]. It was also observed that the drug was able to inhibit the activity of 25 other kinases and possessed a Kd (dissociation constant) value between 1000 and 5000 nM for another 37 kinases.

Based on the co-crystallized structure of Flavopiridol and CDK9-cyclin T1, Flavopiridol is buried within the ATP-binding pocket with only a small fragment of the molecule protruding. Within the binding site, flavopiridol forms hydrogen bonds using the O4 oxygen and O5 hydroxyl to the amino acid residues Cys106 and Asp104 located in the CDK9 ATP-binding pocket. The protonated N1 atom of the piperidinyl group of Flavopiridol interacts with Asp167 while the O3 hydroxyl group interacts with Lys48 in the ATP-binding pocket [40].

There have been many clinical trials completed for the pan-CDK inhibitor flavopiridol as a single agent or in combination with other drugs. As a single agent, the following phase II clinical trials have been completed: chronic lymphocytic leukemia, multiple myeloma, melanoma, endometrial adenocarcinoma, lymphoma, and relapsed or refractory solid tumors. The majority of the results obtained from the phase II clinical trials using flavopiridol as a single agent have been very discouraging due to high toxicity and poor response. Notably, when flavopiridol was used as a single agent to treat relapsed mantle cell lymphoma or diffuse large B-cell lymphoma in a phase II clinical trial, only two out of 26 analyzed patients had a partial response and no patient had a complete response [41]. Additionally, 100% of analyzed patients experienced a myriad of adverse events of which the most common were myelosuppression, diarrhea, nausea and vomiting. However, phase I studies in chronic lymphocytic leukemia showed some promise where dose and dose regimen adjustment as well as aggressive monitoring and prophylactic measures were adopted to prevent severe toxicities from tumor lysis syndrome (TLS) and cytokine release syndrome (CRS) [42]. Of 52 patients receiving therapy, 21 (40%) achieved partial response with a median progression free survival (PFS) of 12 months. Even though all 21 patients relapsed, out of 6 patients who received retreatment achieved another partial response. As a follow up to this study, a phase II clinical trial was conducted where patients with relapsed refractory chronic lymphocytic leukemia were treated with flavopiridol as a single agent [43]. With aggressive monitoring for TLS and prophylaxis with dexamethasone for CRS, 34 patients (53%) achieved a response of which 30 were partial responses (47%), three were nodular partial responses (5%) and one was a complete response (1.6%). The median PFS among responders was 10 to 12 months. Ultimately, this phase II clinical trial demonstrated a significant clinical activity for flavopiridol in patients with relapsed chronic lymphocytic leukemia. It also became evident that toxic side effects from both TLR and CRS can be controlled with aggressive monitoring and prophylactic treatment [43].

Better drug activity has been reported in clinical trials conducted with flavopiridol used in combination with other drugs. Flavopiridol was used in combination in the following phase II clinical trials: acute myeloid leukemia, B-cell chronic lymphocytic leukemia, small lymphocytic lymphoma, refractory metastatic pancreatic cancer, unresectable or metastatic solid tumors. In a phase I trial, flavopiridol showed better activity when used in combination with a proteasome inhibitor bortezomib. Out of 16 patients, two complete responses (12%), and five partial responses (31%) were observed demonstrating that both the dose regimen and the drug combination (flavopiridol and bortezomib) were effective in patients with relapsed and/or refractory multiple myeloma or non-Hodkin's lymphoma [44]. In another phase I clinical trial, flavopiridol administration followed by cytosine arabinoside and mitoxantrone in relapsed and refractory adult acute leukemias achieved clinical effects. Out of the 34 adults receiving therapy, the response rate was 31% in acute myelogenous leukemia and 12.5% in acute lymphoblastic leukemia [45]. Other phase I clinical trials of flavopiridol in combination with doxorubicin demonstrated clinical effects in advanced sarcomas or in combination with cyclophosphamide and rituximab in high risk chronic lymphocytic leukemia. Thus these trials warranted a follow up in further studies [46,47]. In a follow up phase II clinical trial testing flavopiridol in combination with cytosine arabinoside and mitoxantrone, strong evidence for the effectiveness of the drug combination was demonstrated in the treatment of newly diagnosed secondary AML (75% complete remissions) and first relapse after short complete remission (75% complete remission) [48]. However,

not all phase I and II clinical trials using flavopiridol in combination with other anti-neoplastic drugs report strong clinical efficacy. In patients with relapsed, refractory, or poor prognosis acute leukemia, flavopiridol treatment in combination with vorinostat did not yield objective responses [49]. In a phase II study using flavopiridol in combination with docetaxel in patients with refractory, metastatic pancreatic cancer, three patients (33%) achieved transient stable disease with one of these patients achieving a 20% reduction in tumor size. However, the median survival was 4.2% with no patients alive at the time the study data was analyzed [50].

Seliciclib (Roscovitine)

Seliciclib is a purine derivative with the ability to bind and inhibit CDK1, 2, 5, 7 and 9 at micromolar concentrations [51]. Seliciclib binds to the ATP binding pocket of CDK where by the purine ring of seliciclib competitively occupies the site where the ATP purine ring binds. In addition to inhibiting the activity of CDK2-cyclin E and CDK1-cyclin B complexes, seleciclib inhibits the activation of RNA pol II by inhibiting CDK7 and 9 [52,53]. Additionally, seliciclib has been shown to decrease the expression of many cell cycle regulatory genes such as *AURKA* and *AURKB* (aurora kinase A and B), *PLK* (polo-like kinase), *WEE1* and *CDC25C* (cell-division cycle 25 homolog C) [54]. Seliciclib has also been shown to decrease the levels of many important survival factors, such as survivin and XIAP and these findings suggest that the drug may contribute to the amplification of caspase cascades and apoptosis [55]. Seliciclib has been demonstrated to inhibit cell proliferation at an IC_{50} of less than 17 μM and induce apoptosis through inhibition of transcription of essential survival factors [56,57].

Both phase I and II clinical trials have been conducted for seliciclib (roscovitine). In a phase I clinical trial, it was observed that roscovitine did not cause any dose related toxicity up to a 200 mg dose. The maximum tolerated dose was determined to be 800 mg/m^2 twice daily given every 3 weeks for 7 days. At the maximum dosing, the common observed side effects were hypokalemia, hyponatremia, hyperglycemia, elevated gamma-glutamyl transpeptidase and skin rash. Currently no objective responses have been reported for the treatment with roscovitine but disease stabilization has been observed in eight patients [58]. The only phase II clinical trial using roscovitine (seliciclib) as a single agent has been terminated with no results provided. The study was to be completed in patients with non-small cell lung cancer.

SNS 032 (BMS-387032)

SNS-032 is a thiazole derivative with the ability to bind and inhibit CDK1, 2, 4, 7 and 9 at concentrations lower than 1000 nM. This compound is most effective in inhibiting CDK9 with an IC_{50} at 4 nM. Unfortunately, like other ATP-competitive inhibitors it has been shown to inhibit off target kinases such as GSK3α and GSKβ (IC_{50} lower than 1000 nM). In the study conducted by Karaman *et al.* SNS-032 was found to interact with another 20 kinases with a binding affinity at the nanomolar range [39,59].

There are only a couple of phase I clinical trials reported for this compound. The first trial tested the safety and tolerability of SNS-032 in patients with advanced chronic lymphocytic leukemia and multiple myeloma [60]. Pharmacokinetic data from the study demonstrated that target plasma levels were exceeded and sustained for 6 hours after the administration of doses of 50 mg/m^2 or greater of SNS-032. This study recruited 19 patients with chronic lymphocytic leukemia and 18 patients with multiple myeloma. In chronic lymphocytic leukemia patients, the dose limiting toxicity was due to TLS with a maximum tolerated dose (MTD) of 75 mg/m^2. Generally SNS-032 was well tolerated with the most common toxicities being neutropenia and thrombocytopenia. Only one patient, in a cohort of 4 receiving 75 mg/m^2, with chronic lymphocytic leukemia had a greater than 50% reduction in disease however, there were no improvements in hematologic parameters. Another patient in the same cohort with low tumor burden had stable disease for four courses. Dose limiting toxic effects (DLT) and TLS were not reported for multiple myeloma patients because the study was closed early. Only one patient with multiple myeloma in a cohort 3 receiving 33mg /m^2 of SNS-032 had stable disease after three courses [60]. The second phase I clinical trial tested safety and tolerability of SNS-032 when administered as a weekly 1-h infusion in patients with metastatic refractory solid tumors. Due to a change in portfolio priorities from the sponsor this study was discontinued. However, prior to discontinuation, the trial showed that SNS-0932 was well tolerated with minor side effects like fatigue and nausea [61]. No clinical efficacy has yet been reported with this drug.

AT7519

AT7519 is a compound which inhibits the activity of CDK1, 2, 4, 5, 6 and 9 with an IC_{50} below 300 nM. This drug was first discovered by X-ray crystallographic screening of drugs which interact with CDK2. AT7519 can also bind to and inhibit 23 off target kinases with an IC_{50} greater than 1000 nM. AT7519 inhibits cellular proliferation in a p53 and pRb-dependent manner and has been shown to inhibit the proliferation of 26 tumor cells at concentrations ranging from 40 to 920 nM. This compound has also been shown to have anti-tumor activity in early and late stages of HCT116 colon carcinoma tumor xenograft mouse model. Compared with other ATP competitive inhibitors like flavopiridol, this compound has been shown to cause the regression of subcutaneous tumors in mice [62]. Experiments using fluorescensce activated cell sorting (FACS) analysis, tunel and colony formation assays have implicated the compound in causing apoptosis.

There is one phase I clinical trial completed with reported results for AT7519 as a single agent in patients with refractory solid tumors. Twenty-eight patients were treated at seven dose levels ranging from 1.8 to 40 mg/m^2 per day. At the highest dose one patient developed hypotension and ST segment elevation. DLTs at 34 mg/^{2} per day were QTc prolongation with one death, fatigue and mucositis. Out of the twenty eight patients, four patients exhibited stable disease for greater than 6 months and one patient had a prolonged partial response. The authors reported inhibition of markers of CDK activity at all the tested doses and antiproliferative activity at a dose of 29 mg/m^2. Currently, there are three ongoing phase II studies for AT7519 [63]. Two of the studies are testing the effects of AT7519 as a single agent in patients with relapsed mantle cell lymphoma and relapsed and/or refractory chronic lymphocytic leukemia and one study is testing the effects of AT7519 as a combination with bortezomib in patients with previously treated multiple myeloma.

P276-00

P276-00 binds to and inhibits CDK4-cyclin D1, CDK1-cyclin B, CDK2-cyclin A, CDK6-cyclin D3 and CDK9-cyclin T1 enzyme activity with concentrations ranging from 20 to 396 nM (IC_{50}) [64]. It was also shown to inhibit the enzymatic activity of CDK7-cyclin H and GSK3β in the low micromolar concentrations. *In vitro* studies have shown that P276-00 is able to inhibit the proliferation of 12 tumor cell lines in the 300-800 nM range. P276-00 has also been able to cause 70% tumor regression in MM1.S plasmacytomas, CA-51, Lewis lung sarcoma, HCT116 and H-460 tumor xenograft mouse models [65,66]. There have been many clinical trials completed for P276-00 as a single agent or in combination with other drugs. As a single agent, three phase I clinical trials have been completed of which one was terminated without results [67-69]. The clinical trials were conducted in patients with refractory multiple myeloma and advanced refractory neoplasms of which the former was completed and the latter was terminated [69]. The study completed on patients with refractory multipole myeloma went on to complete phase II [67]. As an agent used as part of a combination therapy, two phase I clinical trials have been completed [70,71].The clinical trials were conducted in patients with advanced pancreatic cancer and advanced head and neck cancer. Both of these studies have gone on to complete phase II [70,71]. In patients with advanced pancreatic cancer, P276-00 was tested in combination with gemcitabine whereas patients with advanced head and neck cancer were treated with P276-00 in combination with radiation. In addition to these studies, there was an ongoing phase I clinical trial testing the effects of P276-00 in combination with gemcitabine and carboplatin in patients with metastatic triple negative breast cancer that has just recently been terminated with no reported results [72]. As a single agent, a total of 4 phase II clinical trials have been completed and one of which, in patients with refractory multiple myeloma, has been mentioned above [67]. The other three studies were conducted on patients with malignant melanoma, relapsed and/or refractory mantle cell lymphoma (terminated) and recurrent and/or locally advanced head and neck cancer [73-75]. Of all the completed and active phase I and II clinical trials there are only results for the terminated phase II clinical trial testing the effects of P276-00 as a single agent in relapsed and/or refractory mantle cell lymphoma. The study recruited 13 patients where patients received 185 mg/m2/day as intravenous infusions over 30 minutes from day 1 to day 5 in each 21 days cycle for a minimum of 6 cycles and a maxim of 12 cycles. The study was terminated based on interim results and no data or analysis was provided, however, toxicity results were provided. Based on toxicity data provided, the most common adverse events were hypotension and diarrhea [74].

Palbociclib (PD 0332991)

Palbociclib is a highly selective inhibitor of CDK4 and CDK6 at concentrations with an IC_{50} below 15 nM. The activity of this compound is very selective showing low or no activity against an additional 36 protein kinases including CDK1, 2, 5 and a variety of tyrosine and serine threonine kinases [76]. Palbociclib effectively inhibits cell proliferation by preventing the progression of the cell cycle from G1 to into S phase. As expected, due to the selectivity of palbociclib to CDK4 and CDK6, cell proliferation is only inhibited in cells which are positive for pRb. Cells negative for pRb do not require CDK4 or CDK6 kinase activity for proliferation due to increased cyclin E levels in these cells. In tumor xenograft mouse models, palbociclib demonstrated tumor regression with no signs of resistance in different tumor types including breast cancer xenografts [76]. Interestingly, even though palbociclib only demonstrated cyotostatic effects with no cytotoxic effects leading to apoptosis in cancer cell lines *in vitro*, it has caused tumor regression *in vivo*. This phenomenon could be attributed to the idea that Cdk4/6 activity is required for the survival of certain tumors. Additionally, another possibility could be attributed to the fact that in most solid tumors there is a fraction of spontaneously dying cells with a rapidly dividing population. Inhibition of cell proliferation in the actively dividing population might tip the balance in favor of the dying cell population [76].

Palbociclib has undergone multiple phase I and II clinical trials and is currently undergoing a phase III clinical trial. Based on the results of phase I studies testing palbociclib in patients with pRb-positive solid tumors or non-Hodgkin's lymphoma refractory to standard therapy [77,78], a phase II single-arm study of palbociclib at 125 mg/day was conducted in breast cancer patients with tumors positive for pRb. Based on an abstract from the 2013 ASCO Annual meeting, thirty six patients were enrolled, of whom 28 completed cycle one (18 were HR+/Her2-, 2 were HR+/Her2+ and 8 were HR-/Her2-). Of the 28 patients, two (7%) demonstrated a partial response and 14% showed disease stabilization from which 4 lasting longer than 3 months. PFS was 4.1 months (95% CI 2.3 to 7.7) in ER+/Her2- patients, 18.8 months (95% CI 5.1 to ∞) in ER+/Her+ patients and 1.8 months (95% CI 0.9 to ∞) in ER-/Her- patients. Grade 3/4 toxicities were limited to transient neutropenia and thrombocytopenia. These results demonstrated that palbociclib as a single agent is well tolerated and demonstrated response or prolonged stable disease in patients with advanced breast cancer. Additionally, this trial demonstrated HR+/Her+ positive breast cancers respond more readily to treatment with palbociclib compared to HR-/Her- breast cancers [79].

Given the role CDKs and estrogen play in breast cancer cell division, there has been a lot of interest to investigate the effects of drugs that target the estrogen synthesis and signaling pathways as well as CDK inhibitors [80-84]. Phase I and II clinical trials were conducted to test the safety and efficacy of the aromatase inhibitor letrozole and the CDK4/CDK6 inhibitor palbociclib in combination in patients with advanced breast cancer. In phase I trials, 12 post-menopausal ER-/Her+ patients treated with palbociclib (125 mg/day on a schedule 3/1) and letrozole (2.5 mg/day) well tolerated continuous drug treatment with leuko-neutropenia and fatigue as the most common side effects [85]. Additionally, out of 9 patients with measurable disease, 3 patients had a partial response.

Phase II of the clinical trial PALOMA-1 assessed the efficacy and safety of letrozole (2.5 mg/day) in combination with palbociclib (125 mg/day schedule 3/1) or letrozole (2.5 mg/day) alone for the first line treatment of patients with ER+/Her2- advanced breast cancer. Phase II was divided into two parts where the first part recruited patients based on ER and HER2 status and the second part recruited patients based on CCND1 (cyclin D1) amplification and/or p16 loss. For part 1, 66 patients were enrolled where patients treated with letrozole in combination with palbociclib showed a significant improvement in progression-free survival compared to patients treated with letrozole

alone (HR 0.35, 95% CI 0.17 to 0.72) [86]. Patients treated with the combination drug also demonstrated an improvement in objective response rate (27% combination vs 23% single treatment) and in partial responses plus stable disease (59% combination vs 44% single treatment) [86]. The most common adverse events reported in the combination treatment arm were neutropenia, leukopenia and fatigue. In part 2 of the study 99 patients were recruited. Data from both parts were combined and presented at the 2012 San Antonio Breast Cancer Symposium. Authors of the study reported a median PFS of 26.1 months with letrozole plus palbociclib compared with 5.7 months for letrozole alone. The combination therapy resulted in a response rate of 45% compared with 31% for the single therapy with an overall clinical benefit rate (partial response plus stable disease) of 70% with combination therapy compared to 44% single therapy [86]. Based on the dramatic improvements in progression-free survival with the combination therapy compared with the single therapy, the FDA granted palbociclib a Breakthrough Therapy designation for the treatment of patients with breast cancer in April 2013.

Final results of the study were presented at the American Association for Cancer Research (AARC) in April of 2014. The final analysis reported a significant improvement in PFS of 20.2 months in the combination arm vs 10.2 months for the single treatment arm. Best overall response rate was 43% for the combination treatment vs 33% for the letrozole single treatment. Additionally, clinical benefit rate (partial response and stable disease) was 81% for the combination arm vs 58% for the single treatment arm [87]. Based on the success of this trial, a phase III study of palbociclib in combination with letrozole in the same metastatic breast cancer population is ongoing (PALOMA-2). In addition, other phase III clinical trials are also designed to investigate the effects of palbociclib with fulvestrant (PALOMA-3) in metastatic breast cancer.

Perspectives

Activities of different CDK/cyc complexes are shown to be terminated by the actions of several endogenous CDK inhibitors (CDKI) such as members of Cip/Kip and INK4 families. Given the prominence of CDK deregulation in many forms of human cancers, several research groups have embarked on development of potent CDK inhibitors. A clear understanding of protein-protein interactions between CDKs and cyclins combined with crystal structure studies has facilitated development of several inhibitory compounds specially targeted to the ATP binding pocket of CDKs. Unfortunately, many of these inhibitors lacked specificity or selectivity as they also served as effective inhibitors for several cellular kinases that are not involved in cell cycle regulation. Although cancer patients treated with first generation CDK inhibitors experienced several adverse events such as toxicity and poor response, combination treatment of CDK inhibitors with other drugs has proven to be effective in improving progression free survival. Notably, recent development of highly selective CDK inhibitors such as Palcbociclib has indeed increased the hope for new discoveries in this field. Since the efficacy of Palcbociclib seems to be limited to cells expressing pRb, development of highly specific CDK inhibitors for pRb negative cancers is urgently needed.

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