

Review Article

CDK inhibitors in cancer therapy, an overview of recent development

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Abstract: Dysregulated cell division, which leads to aberrant cell proliferation, is one of the key hallmarks of cancer. Therefore, therapeutic targets that block cell division would be effective for cancer treatment. Cell division is mainly controlled by a complex composed of cyclin and cyclin dependent kinases (CDKs). To date, the CDK inhibitors (CDKIs), specifically the ones that block the enzyme activity of CDK4 and CDK6 (CDK4/6), have been approved by FDA for the treatment of metastatic hormone receptor positive breast cancer. However, due to the non-selectivity and significant toxicity, most of the first generation CDK inhibitors (so called pan-CDK inhibitors that target several CDKs), have not been approved for clinical application. Despite this, great efforts and progress have been made to enable pan-CDK inhibitors application in the clinical setting. Notably, the development of combination therapy strategies in recent years has made it possible to reduce the toxicity and side effects of pan-CDK inhibitors. Thus, as a combination therapy approach, pan-CDK inhibitors regain great potential in clinical application. In this review, we introduced the CDK family members and discussed their major functions in cell cycle controlling. Then, we summarized the research progress regarding CDK inhibitors, especially those other than CDK4/6 inhibitors. We reviewed first-generation pan-CDKIs Flavopiridol and Roscovitine, and second-generation CDKIs Dinaciclib, P276-00, AT7519, TG02, Roniciclib, RGB-286638 by focusing on their developing stages, clinical trials and targeting cancers. The specific CDKIs, which targets to increase specificity and decrease the side effects, were also discussed. These CDKIs include CDK4/6, CDK7, CDK9, and CDK12/13 inhibitors. Finally, the efficacy and discrepancy of combination therapy with CDK inhibitors and PD1/PDL1 antibodies were analyzed, which might give insights into the development of promising strategy for cancer treatment.

Keywords: CDK, pan-CDK inhibitors, specific CDK inhibitors, cancer, combination therapy

Introduction

Cell division is one of the fundamental biological activities, occurring in various physiological processes such as individual development, organ homeostasis, tissue regeneration, as well as in pathological process of tumorigenesis. The sequence of stages in cell division is known as the cell cycle, and is divided into a synthesis phase, a mitotic segregation phase and two intervenient phases G1 and G2 (**Figure 1**). Cell enlarges itself in the G1 phase to prepare for the DNA synthesis, which is regulated by a

“restriction point” in mammals. Whether a cell can enter into the cell cycle is determined by both intrinsic factors (such as protein synthesis) and extrinsic factors (such as growth factors). The absence of these essential factors causes the cell to end its cell cycle and enter into a dormancy period, known as G0 phase. Cell cycle regulation involves three “checkpoints”: the G1/S, G2/M, and mitotic spindle checkpoints. Growing evidence has demonstrated that the eukaryotic cell cycle is driven by a conserved central mechanism, including cyclin-dependent kinases (CDKs), which pro-

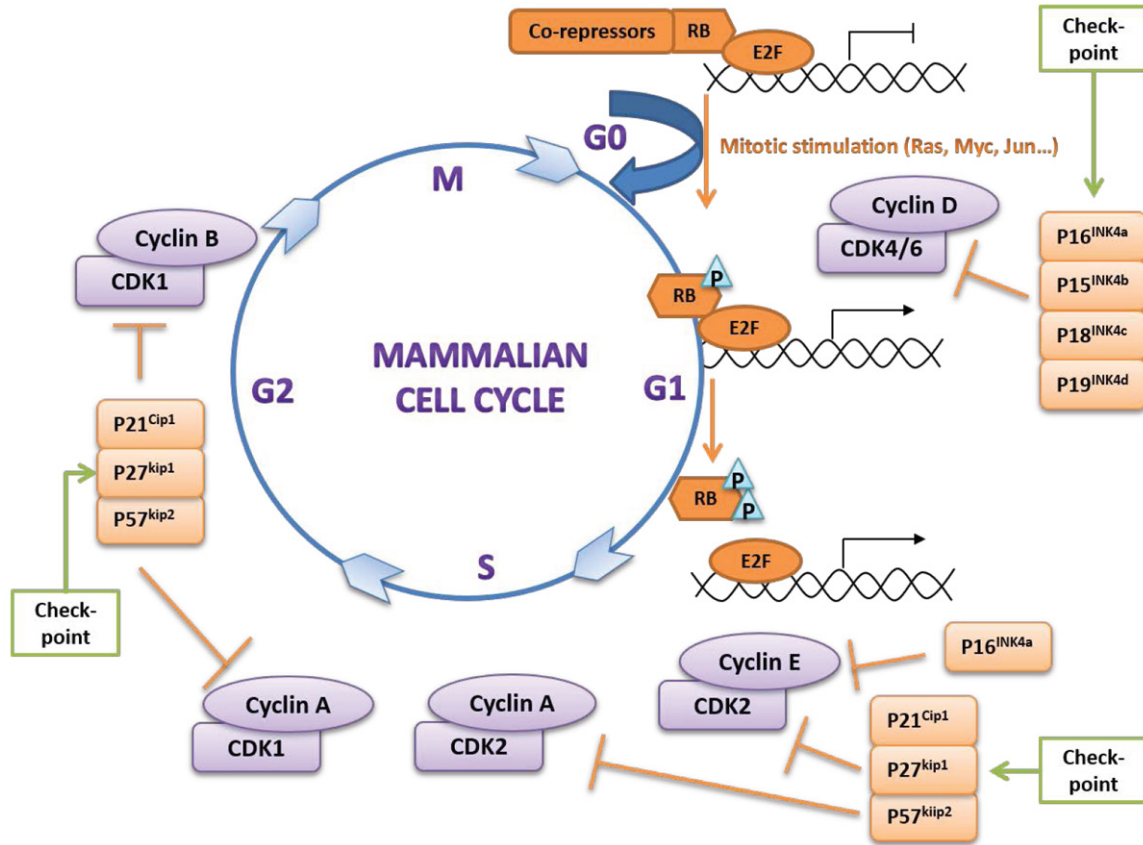


Figure 1. Major Functions of CDKs in cell cycle: Eukaryotic cell cycle is a precise process with order, which is regulated by CDKs, Cyclins and CDKIs. CDK-Cyclin complex can phosphorylate RB protein and regulate cell cycle positively, whereas CDKIs will inhibit part of the cell cycle process and play a negative regulatory role. As shown in this figure, after cell has been stimulated by mitotic signals, it then enters the G1 phase. Before it further enters the next phase, RB protein would be first phosphorylated by CDK4/6-CyclinD and CDK2-CyclinE complexes, thereby releasing E2F protein, promoting downstream cell cycle factors and transcription-related gene expression. P16^{INK4a}, P21^{Cip1}, P27^{Kip1} and P57^{Kip2} all belong to CDKIs, among which P16^{INK4a} can inhibit the activity of CDK4, prevent the phosphorylation of RB protein and the release of E2F, thereby inhibit the cell life cycle in G1 phase. P21^{Cip1}, P27^{Kip1} and P57^{Kip2}, on the other hand, can inhibit the activity of CDKs and Cyclins more extensively and block cells at different stages.

mote DNA synthesis and chromosome segregation by phosphorylation of their substrate [1, 2].

CDKs are involved not only in the cell cycle but also in the other critical cellular processes, such as gene transcription, insulin secretion, glycogen synthesis and neuronal functions [3]. So far, 21 CDKs and 5 CDK-like genes have been identified in human genome based on their homologous sequences [4]. CDK1 emerges as a key determinant of mitotic progression, whereas CDK2 is more associated with DNA replication in higher eukaryotes. In metazoans, cell cycle entry is mostly elicited by CDK4 and

CDK6, which are responsive to numerous growth-regulatory signals [5, 6]. Besides cell cycle controlling, some other CDKs including CDK7, CDK8, CDK9 and CDK11, have been shown to participate in transcriptional regulation [4]. CDK7 can phosphorylate RNA polymerase II and contribute to the initiation of transcription. CDK8 is a part of the mediator complex which regulates a plethora of genes. CDK9 can phosphorylate RNA polymerase II and thereby promote elongation of transcription. CDK11 mainly acts on the splicing machinery. Accumulating evidence suggests that these transcription-regulating CDKs have potential to be efficient therapeutic targets for cancer

treatment [7-9]. There is another type of CDKs, called atypical CDKs, which include CDK5, CDK14, CDK15, CDK16, CDK17, and CDK18 [4]. CDK5 is involved in postmitotic functions in specialized tissue settings [10, 11]. CDK14 is reported to be involved in Wnt/ β -catenin signaling pathway by combination with Cyclin Y [12, 13].

Given the important function of CDKs in regulation of cell division, gene transcription and other critical biological processes, CDK inhibitors have been developed for the treatment of various diseases caused by CDK abnormalities. Over the past 20 years, numerous compounds targeting CDK enzyme activity have emerged and have been evaluated in the clinical trial. Here, we will perform a whole mount review of the history of research and progress of CDK inhibitors, particularly their involvement in the treatment of cancer.

Pan-CDK inhibitors

CDK inhibitors have been studied since the 1990s. The first generation of CDK inhibitors are pan-CDK inhibitors, including Flavopiridol and Roscovitine, etc. The main function of these inhibitors is to block cell cycle and inhibit cell proliferation by inhibiting the CDK enzyme activity. However, the first-generation of pan-CDK inhibitors have poor selectivity and high toxicity, leading to inevitable harmful effects on normal cells. As a result, most of the pan-CDK inhibitors failed in their clinical trials [14-16]. Subsequently, second-generation CDK inhibitors, including Dinaciclib, P276-00, AT7519, TG02, Roniciclib, RGB-286638 and so on, have been developed with better selectivity and less side effects. Most of the second generation CDK inhibitors have presented efficient anti-tumor activity in preclinical trials, although the safety and efficacy of these inhibitors need to be further verified in clinical studies. At present, there are approximately 40 pan-CDK inhibitors that are in various stages of their research and development. For instance, Dinaciclib, developed by Merck company, is in phase III clinical study and has already presented a significant anti-tumor effect in the treatment of melanoma, breast cancer and leukemia. Besides, several pan-CDK inhibitors entered into phase I or phase II studies, while many other pan-CDK inhibitors have shown significant anti-

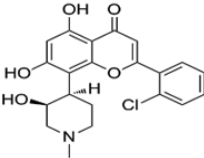
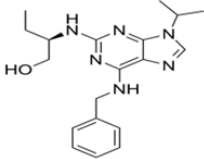
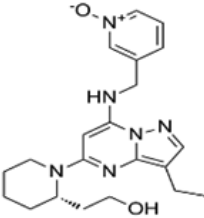
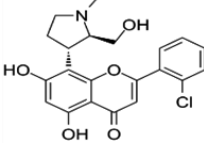
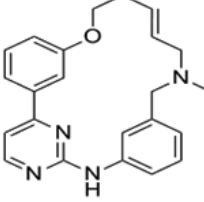
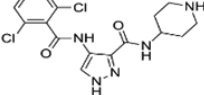
tumor activity in preclinical studies. In order to minimize the side effects of pan-CDK inhibitors, numerous studies have been conducted on drug delivery strategies, especially in the area of combination therapy. Overall, pan-CDK inhibitors have shown promising clinical efficacy despite serious side effects and safety concerns. Here, we listed pan-CDK inhibitors currently under research and development, and summarized their structures, CDK targets, developmental stages and indications of target diseases or cancers (**Table 1**). Representative pan-CDK inhibitors are described in details as follows.

Flavopiridol

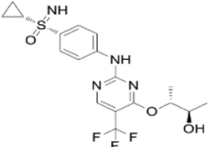
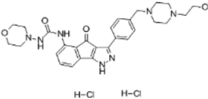
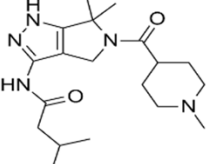
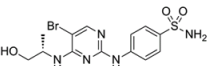
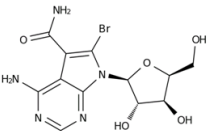
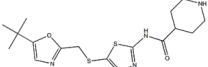
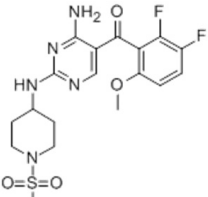
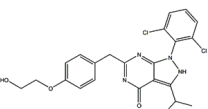
Flavopiridol (Alvocidib) belongs to the first generation of pan-CDK inhibitors. It is the first pan-CDK inhibitor that was used in clinical trials and is also one of the most widely studied pan-CDK inhibitors. Flavopiridol mainly inhibits the activities of CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 with IC_{50} values at 30, 170, 100, 60, 300 and 10 nM, respectively [17]. Since 1997, 63 clinical trials have been carried out on Flavopiridol, which is mainly administrated for the treatment of ALL, AML, CLL, lymphoma, solid tumor, gastric cancer, mantle cell lymphoma, myeloid leukemia and so on. Results from a preclinical study indicated that Flavopiridol presented significant anti-tumor activity against prostate cancer, reducing tumor volume by 85% and extending survival by 30 days. In addition, Flavopiridol can induce apoptosis of primary and recurrent/refractory AML cells by 4.3 times in vitro [18]. It can also induce apoptosis in a large number of other hematopoietic cell lines [19]. In spite of these promising progress of preclinical study, Flavopiridol presented poor efficacy in clinical trials of solid tumors. Phase I clinical studies of AML showed that after three days treatment of Flavopiridol, the number of peripheral blood cells decreased by more than 50% in 44% of patients, suggesting that Flavopiridol can induce anti-leukemia cytotoxicity. Subsequently, 45 AML patients were studied in phase II clinical study, and 16% of the patients have cardiac dysfunction during treatment process. Phase I [20] and Phase II [21] clinical trials of the CLL patients have shown that Flavopiridol can alleviate the symptoms. Due to these side effects, clinical trials with Flavopiridol only made limited progress. In order to reduce

CDK inhibitors in cancer therapy

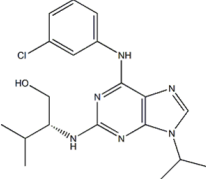
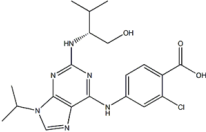
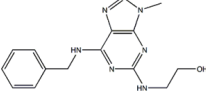
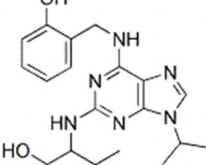
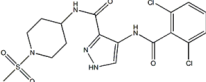
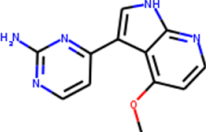
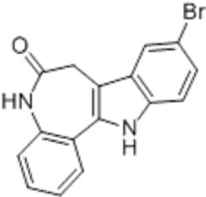
Table 1. Pan-CDK inhibitors under development (updated to April, 2021)

No.	Name	Alternative Name	CAS No.	Formula	Published CDK preference	Other Main Target	Highest Clinical Phase	Indications/targeting cancers	Chemical structure
1	Flavopiridol	Alvocidib, L868275, HMR-1275	146426-40-6	$C_{21}H_{20}ClNO_5$	1, 2, 4, 6, 7, 9		Phase II	ALL/AML/CLL/MM/lymphoma/MCL	
2	R-roscovitine	CYC202, Seliciclib, Roscovitine	186692-46-6	$C_{19}H_{26}N_6O$	1, 2, 5, 7, 9		Phase II	NSCLC/CD/NPC/Metastatic breast cancer/Advanced solid tumor	
3	Dinaciclib	SCH 727965, SCH-727965	779353-01-4	$C_{21}H_{28}N_6O_2$	1, 2, 5, 9		Phase III	CLL/MCL/NSCLC/Melanoma/Breast tumor	
4	P276-00	Rivaciclib hydrochloride, P276	920113-03-7	$C_{21}H_{21}Cl_2NO_5$	1, 4, 9		Phase II	BC/Head and neck cancer	
5	TG02	SB1317, TG-02; SB-1317	937270-47-8	$C_{23}H_{24}N_4O$	1, 2, 5, 7, 9	JAK2, FLT3	Phase II	Glioblastoma/anaplastic astrocytoma/CLL/Hematological neoplasm	
6	AT7519	AT 7519, AT-7519	844442-38-2	$C_{16}H_{17}Cl_2N_5O_2$	1, 2, 4, 5, 6, 9		Phase II	MM/CLL/MCL/Non-Hodgkin lymphoma/Solid tumor	

CDK inhibitors in cancer therapy

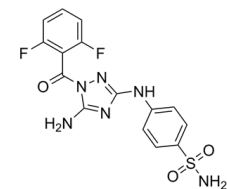
7	Roniciclib	BAY1000394	1223498-69-8	$C_{18}H_{21}F_3N_4O_3S$	1, 2, 3, 4, 7, 9	Phase II	SCLC	
8	RGB-286638	RGB286638	784210-87-3	$C_{29}H_{37}Cl_2N_7O_4$	1, 2, 3, 4, 5, 9	Phase I	Hematological Malignancies	
9	PHA-793887	PHA 793887, PHA793887	718630-59-2	$C_{19}H_{31}N_5O_2$	1, 2, 4, 5, 7, 9	Phase I	Solid tumor	
10	ZK304709 [96]	ZK-304709, ZK 304709	477588-78-6	$C_{13}H_{16}BrN_5O_3S$	1, 2, 4, 7, 9			
11	Xylocydine [97, 98]		685901-63-7	$C_{12}H_{14}BrN_5O_5$	7, 9			
12	SNS032 [99-102]	BMS-387032, SNS-032	345627-80-7	$C_{17}H_{24}N_4O_2S_2$	1, 2, 4, 7, 9	Phase I	B-lymphoid Malignancies, CLL, Tumors	
13	R547 [103-105]	Ro 4584820	741713-40-6	$C_{18}H_{21}F_2N_5O_4S$	1, 2, 3, 4, 7, 9	Phase I	Neoplasms	
14	RGB286147 [106]	CDK/CRK inhibitor, CDK7 inhibitor IV	784211-09-2	$C_{23}H_{22}Cl_2N_4O_3$	1, 2, 3, 4, 7			

CDK inhibitors in cancer therapy

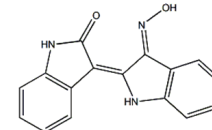
16	Purvalanol A [107-110]	NG60	212844-53-6	$C_{19}H_{25}ClN_6O$	1, 2, 4, 5, 7		
17	Purvalanol B [107]	NG95, NG-95	212844-54-7	$C_{20}H_{25}ClN_6O_3$	1, 2, 5		
18	Olomoucine		101622-51-9	$C_{15}H_{18}N_6O$	1, 2, 5	ERK1/MAP	
19	Olomoucine II	IN1181	500735-47-7	$C_{19}H_{26}N_6O_2$	1, 2, 7, 9		
20	NVP-LCQ195 [111]	AT9311, AT-9311, LCQ195, LCQ-195	902156-99-4	$C_{17}H_{19}Cl_2N_5O_4S$	1, 2, 3, 5, 7, 9	CLK3, CHK2	
21	Meriolin 3 [112, 113]		954143-48-7	$C_{12}H_{11}N_5O$	1, 2, 5, 9, 3, 4, 6, 7		
22	Kenpauillone [114, 115]	NSC664704, 9-Bromopauillone	142273-20-9	$C_{16}H_{11}BrN_2O$	1, 2, 5	GSK-3β	

CDK inhibitors in cancer therapy

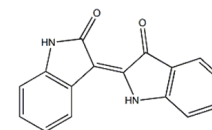
23 JNJ-7706621 [116-121] Aurora kinase/CDK inhibitor, JNJ7706621 443797-96-4 $C_{15}H_{12}F_2N_6O_3S$ 1, 2, 3, 4, 6, 9 Aurora A/B



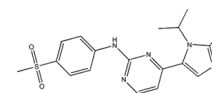
24 Indirubin-3'-Monoxime [122, 123] 160807-49-8 $C_{16}H_{11}N_3O_2$ 1, 2, 5 GSK-3 β



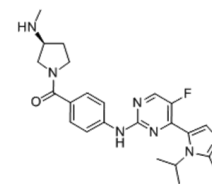
25 Indirubin [122, 124] Isoindigotin; Indigopurpurin 479-41-4 $C_{16}H_{10}N_2O_2$ 1, 2, 4, 5 GSK-3 β Phase IV Childhood Acute Promyelocytic Leukemia



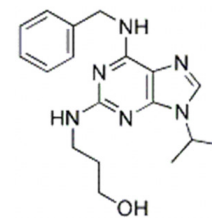
26 AZD-5438 [125] AZD5438; AZD 5438 602306-29-6 $C_{18}H_{21}N_5O_2S$ 1, 2, 4, 5, 7, 9 Phase I Neoplasms



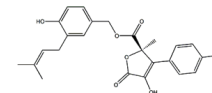
27 AZD5597 [126] compd (S)-8b; AZD 5597 924641-59-8 $C_{23}H_{28}FN_7O$ 1, 2 CYP, hERG



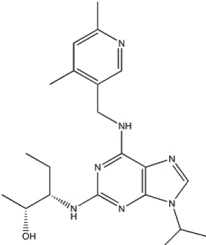
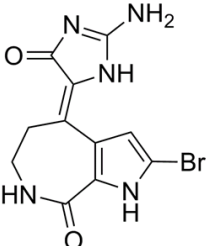
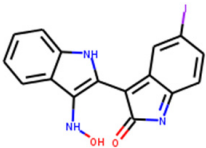
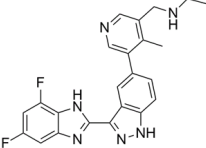
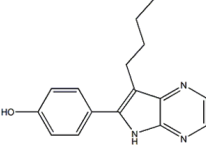
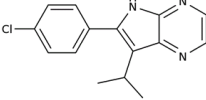
28 Bohemine [110, 127] 189232-42-6 $C_{18}H_{24}N_6O$ 1, 2, 9



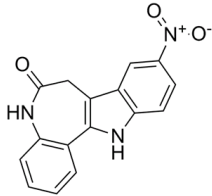
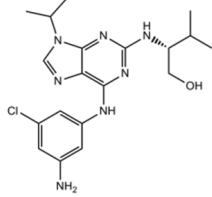
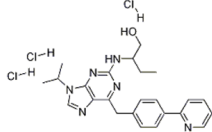
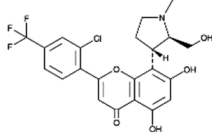
29 Butyrolactone I [128] 87414-49-1 $C_{24}H_{24}O_7$ 1, 2, 5



CDK inhibitors in cancer therapy

30	CYC065	CYC-065, CYC 065	1070790-89-4	$C_{21}H_{31}N_7O$	2, 9		Phase I	AML, MDS, Advanced cancer, Relapsed/Refractory CLL	
31	10Z-Hymenialdisine [129]	(Z)-Hymenialdisine, HD; Hymenialdesine;	82005-12-7	$C_{11}H_{10}BrN_5O_2$	1, 2, 3, 4, 5, 6	MEK1, GSK-3 β			
32	5-Iodo-indirubin-3'-monoxime [124]		331467-03-9	$C_{16}H_{10}IN_3O_2$	1, 5	GSK-3 β			
33	AG024322 [130, 131]	AG-024322	837364-57-5	$C_{23}H_{20}F_2N_6$	1, 2, 4, 6, 7, 9		Phase I	Neoplasms, Non-Hodgkin lymphoma	
34	Aloisine A [132]	RP107	496864-16-5	$C_{16}H_{17}N_3O$	1, 2, 5	GSK-3 α , GSK-3 β			
35	Aloisine B [132]	RP90	496864-14-3	$C_{15}H_{16}N_3Cl$	1, 5	GSK-3 β			

CDK inhibitors in cancer therapy

36	Alsterpaullone [133, 134]	9-Nitropaullone, NSC705701	237430-03-4	$C_{16}H_{11}N_3O_3$	1, 2, 5	GSK-3 α , GSK-3 β	
37	Aminopurvalanol [135]	NG97, NG-97, Aminopurvalanol A	220792-57-4	$C_{19}H_{26}ClN_7O$	1, 2, 5	ERK1, ERK2	
38	R-CR8 [136, 137]	(R)-CR8, CR8, (R)-Isomer	294646-77-8	$C_{24}H_{29}N_7O$	1, 2, 5, 7, 9	CK1	
39	Voruciclib [138-141]	P1446A-05	1000023-04-0	$C_{22}H_{19}ClF_3NO_5$	4, 6, 9	Phase I CLL/Melanoma	

side effects, researchers are trying to combine Flavopiridol with other drugs to treat cancer so as to improve the clinical efficacy of Flavopiridol [22, 23].

Dinaciclib

Dinaciclib (SCH727965), developed by Merck & Co. Ltd., has entered phase III clinical trials, and showed impressive anti-tumor activity in lung cancer, breast cancer, and chronic lymphocytic carcinoma. Dinaciclib mainly inhibits the activity of CDK9, thus preventing the phosphorylation of the carboxyl terminus of RNA polymerase II, which plays a transcriptional inhibitory role and induces cell apoptosis. Strikingly, it has been proved that Dinaciclib has the best therapeutic efficacy for leukemia. In acute lymphoblastic leukemia, Dinaciclib inhibited the growth of T-ALL cells and prolonged the survival time of mouse tumor xenograft models. In preclinical experiments, Dinaciclib combined with Panobinostat can induce MLL-AF9 tumor cell apoptosis. The number of leukocytes was significantly reduced in the mouse tumor model, showing a stronger survival advantage, with median survival increased from 33 days to 52 days [24]. Further studies demonstrated that Dinaciclib can eliminate many cytokines in the microenvironment, such as CD40L, BAFF, IL-4, etc., which are essential for the growth of CLL cells [25]. These studies indicated that Dinaciclib has great potential as a clinical treatment agent for CLL. Clinical trial results also showed that Dinaciclib was superior to Flavopiridol in the treatment of CLL. Recent studies have further demonstrated that Dinaciclib has a more remarkable anti-tumor effect when combined with PD1 monoclonal antibodies [26], making Dinaciclib a potential promising therapeutic target in clinical setting.

P276-00

P276-00 can effectively inhibit CDK1, CDK4 and CDK9 with IC_{50} values at 79 nM, 63 nM and 20 nM, respectively. P276-00 showed significant cytotoxicity against mantle cell lymphoma (MCL) cells in vitro [27]. In the Phase II clinical trial of MCL, 13 patients with relapsed and refractory MCL were treated with p276-00. Overall, both drug resistance and anti-tumor effects were significant. At present, the molecular mechanism of p276-00 in the treatment of MCL remains unclear [28]. Other studies have

shown that p276-00 can arrest the cell cycle in the G1 phase, thereby inducing apoptosis of head and neck cancer cells [29]. The anti-tumor activity and safety of p276-00 was evaluated in a phase II clinical study in patients with recurrent and locally advanced head and neck cancer. The results suggested that P276-00 had good anti-tumor activity, while its safety needs to be further evaluated.

TG02

TG02 is a novel oral poly-kinase inhibitor that mainly inhibits CDK1, CDK2, CDK7 and CDK9 activities with IC_{50} values at 9 nM, 5 nM, 37 nM and 3 nM, respectively. Preclinical studies have shown that TG02 alone or in combination with TMZ can inhibit the proliferation of glioblastoma cells [30]. Phase I clinical studies have been conducted in China to determine the clinical dose and efficacy of TG02. The results showed that TG02 is effective in the treatment of hematological malignancies, and TG02 therapy has been found to promote tumor deposition and prolong survival in a variety of mouse models of leukemia. TG02 has broad-spectrum of anti-CDKs and anti-JAK2/Kit activity, which provides a theoretical basis for clinical treatment of patients with hematologic diseases [31]. Further studies have shown that Carfilzomib (the second-generation proteasome inhibitor) combined with TG02 improve the efficacy of relapsed/refractory multiple myeloma (MM) [32]. In conclusion, TG02 has shown promising therapeutic potentials in clinical trials, although further investigation is still needed in the future.

AT7519

AT7519 is a potent pan-CDK inhibitor that mainly inhibits CDK1, 2, 4, 6 and 9. Studies have shown that AT7519 not only has inhibitory activity against a variety of solid tumors, but also can inhibit hematologic malignancies. Preclinical trials have proved that AT7519 can induce apoptosis in various neuroblastoma cell lines [33]. In addition, AT7519 also induces neutrophils apoptosis and reduces inflammatory response in a pneumonia model. So, AT7519 has been evaluated as a potential agent for ARDS (acute respiratory distress syndrome with neutrophil dominant) in many studies [34]. The efficacy of AT7519 in patients with advanced refractory solid tumors or non-Hodg-

kin's lymphoma has been evaluated in phase I clinical trials. Phase I clinical trials also provided guidance for dosages of AT7519 to be used in Phase II clinical trials, with a recommended dose of 27.0 mg/kg. AT7519 is in phase II clinical trials for the treatment of relapsed mantle cell lymphoma and recurrent refractory chronic lymphocytic leukemia. Furthermore, AT7519 in combination with Onalespib (HSP90 inhibitor) for the treatment of metastatic or unresectable solid tumors and AT7519 in combination with Bortezomib for the treatment of multiple myeloma are also in clinical trials [35]. Together, AT7519 exhibited great potential for clinical application.

Roniciclib

Roniciclib is an oral pan-CDK inhibitor. A study at the National University of Singapore Cancer Institute indicated that Roniciclib combined with cisplatin has a significant synergistic anti-tumor effect [36]. Another preclinical study showed that Roniciclib induced apoptosis of medullary thyroid cancer cells. The combination of Roniciclib and Soafenib further inhibited tumor growth in xenograft models compared to Roniciclib alone [37]. To date, the safety and tolerated dose of Roniciclib in patients with advanced malignancy have been evaluated in phase I clinical trials, and Roniciclib in combination with conventional chemotherapy agents for the treatment of extensive non-small cell lung cancer (ED-SCLC) has entered phase II clinical trials [38]. Unfortunately, the results showed that the combination treatment produced significant side effects and cytotoxicity, so the phase II clinical trial was terminated [39]. In addition to ED-SCLC, phase II trials in non-small cell lung cancer (NSCLC) and advanced breast cancer have also failed to meet expectations. Therefore, the future development of Roniciclib might need to be re-optimized in terms of the dosage and administration strategy.

RGB-286638

The main target of RGB-286638 is CDK9, so RGB-286638 is involved in controlling of cell cycle and regulation of gene transcription [40]. Preclinical experiments have shown that RGB-286638 can induce apoptosis of various human cancer cell lines. Intravenous injection of RGB-286638 for 5 consecutive days had the

best inhibition effect on tumor growth in solid tumor and hematoma mouse models. Based on experience in preclinical trials, a phase I clinical trial of RGB-286638 is currently being conducted to evaluate safety and drug resistance in patients with recurrent or refractory blood cancer [41]. The clinical application of RGB-286638 still needs further investigation.

PHA-793887

The low concentration of PHA-793887 inhibits the phosphorylation of Rb protein, and thus prevents the progression of cell cycle [42]. In vitro experiments showed that PHA-793887 had a certain toxic effect on leukemia cells. Subcutaneous xenograft model and primary leukemia cell dissemination model were used to evaluate the therapeutic effect of PHA-793887 in vivo, and the results sound promising [43]. However, in a phase I clinical trial, there were 19 patients who showed severe hepatotoxicity. Therefore, the clinical application of PHA-793887 is still under development [44].

Specific CDK inhibitors

The biggest challenge in the clinical application of pan-CDK inhibitors is their low specificity and significant side effects on normal somatic cells. In order to solve this problem, researchers have successfully developed a variety of specific CDK inhibitors, including CDK4/6-, CDK7-, CDK9-, CDK12/13-inhibitors etc. Each type of tumor is associated with its own CDK expression landscape, selection of appropriate specific CDK inhibitors for relevant patients is therefore expected to assure the therapeutic effect, and to avoid toxic and side effects as well. At present, a variety of specific CDK inhibitors have shown significant anti-tumor effects in preclinical and clinical studies. Here, we briefly summarized the characteristics of some specific CDK inhibitors and their anti-tumor activity.

CDK4/6 inhibitors

CDK4/6 inhibitors are the first ones that were approved by FDA for clinical treatment. These inhibitors specifically inhibit CDK4/6 and show limited toxicity to normal cells. There are three FDA-approved CDK4/6 inhibitors and they are Palbociclib produced by Pfizer, Ribociclib pro-

duced by Novartis and Abemaciclib produced by Eli Lilly. Palbociclib is the first and most popular CDK4/6 inhibitor, which reached \$2.135 billion of global sales in 2016, and is expected to reach \$7 billion in 2022. Ribociclib is very similar to Palbociclib in structure, but Abemaciclib is quite different. In vitro studies indicated that Palbociclib has almost equivalent inhibition effect on CDK4 and CDK6, while Abemaciclib and Ribociclib are more potent against CDK4 than CDK6 [45-47].

All three CDK4/6 inhibitors can effectively arrest cell cycle from G1 to S phase by blocking the phosphorylation of Rb protein, and thus inhibit the proliferation of Rb-positive tumor cells. These inhibitors are currently approved for the first-line treatment of HR⁺ advanced breast cancer, which can effectively reduce resistance to mono-endocrine therapy and significantly extend survival in HR⁺/HER2⁻ breast cancer patients. Recent studies have shown that, besides blocking of the cell cycle, CDK4/6 inhibitors also suppress tumor growth through multiple other mechanisms, including enhancing cytostasis caused by signaling pathway inhibitors, inducing senescence, regulation of cell metabolism, and even promoting anti-tumor immune responses [48]. These novel molecular mechanisms provide a theoretical basis for combination therapy with CDK4/6 inhibitors. For instance, CDK4/6 inhibitors combined with hormone receptor antagonist letrozole have been applied for breast cancer therapy. Many other combination therapies involving CDK4/6 inhibitors are currently under clinical trials for a variety of diseases including anti-cancer therapy.

CDK7 inhibitors

CDK7 has dual functions of cell cycle controlling and transcriptional regulation, which make CDK7 a potential target for cancer therapy. Several CDK7 specific inhibitors have been shown significant anti-tumor activity, including non-covalent inhibitors BS-181, ICEC0942, LDC4297, QS1189 and covalent inhibitors THZ1, THZ2, YKL-5-124. BS-181 is the first highly selective CDK7 inhibitor. Preclinical studies have shown that BS-181 inhibits cancer cell proliferation and xenograft tumor growth, but its bioavailability is poor and cell permeability is insufficient [49]. ICEC0942 (CT7001) is the first

oral CDK7 inhibitor, which is developed from BS-181 and has higher drug-like properties compared with BS-181 [50, 51]. Notably, ICEC0942 entered clinical trials in 2017 and is currently being investigated in phase I/II trials for a variety of therapies for advanced malignancies, including monotherapy or combination therapy for triple-negative breast cancer, castrate resistant prostate cancer (CRPC), and combination therapy with Fulvestrant for patients with HR⁺/HER2⁻ breast cancer. (ClinicalTrials.gov identifier: NCT03363893).

THZ1 is one of the most widely studied CDK7 covalent inhibitors. Preclinical studies have shown that THZ1 has strong anti-tumor activity in several cancer types [52-57]. Of note, THZ1 has been shown to inhibit not only CDK7 activity, but also CDK12 and CDK13 activity. In order to obtain a more specific inhibitor of CDK7, researchers developed the inhibitor YKL-5-124 by combining the covalent warhead of THZ1 with the pyrrolidinopyrazole core of PAK4 inhibitor PF-3758309 [58]. YKL-5-124 has a potent inhibition specificity against CDK7, but has no inhibitory activity against CDK12 and CDK13. Preclinical studies have shown that YKL-5-124 can potentiate genomic instability and trigger anti-tumor immune response in small cell lung cancer, which provides a theoretical basis for the combination therapy of CDK7 inhibitors and immunotherapy [59]. SY-1365, a THZ1 derived CDK7 inhibitor, entered phase I clinical trial in advanced solid tumors in May 2017 to evaluate its efficacy in the treatment of ovarian and breast cancer [60] (ClinicalTrials.gov identifier: NCT03134638). SY-5609 is another selective CDK7 inhibitor. Preclinical studies have shown that SY-5609 combined with Fulvestrant have potent anti-tumor activity against ovarian cancer, TNBC and ER⁺ breast cancer [61, 62]. SY-5609 entered phase I clinical trials in January 2020 for the treatment of advanced solid tumors and in combination with Fulvestrant in patients with HR⁺/HER2⁻ breast cancer (ClinicalTrials.gov identification: NCT04247-126).

CDK9 inhibitors

CDK9 regulates cellular transcriptional elongation and mRNA maturation, and has become an attractive therapeutic target for many cancers, especially those caused by dysregulation of

transcription [63, 64]. Several CDK9 inhibitors have been developed, and their significant anti-tumor activity has been demonstrated in preclinical studies, such as Fadraciclib, AZD-4573, CDKI-73, MC180295, etc. In addition, Fadraciclib combined with temozolomide can effectively suppress MYCN-amplified neuroblastoma long-term [65]. AZD-4573 is a highly selective CDK9 inhibitor that can down-regulate the expression of oncogenic genes such as MCL-1. Preclinical studies have demonstrated that AZD-4573 has significant anti-cancer efficacy in hematologic malignancies [66, 67]. CDKI-73 combined with Olaparib has a synergistic therapeutic effect in BRCA1-proficient ovarian cancer, which facilitates the use of CDK9 as a predictive biomarker for PARP inhibitors in clinical practice [68]. MC180295 can dephosphorylate SWI/SNF protein Brg1, thereby promoting gene activation and leading to the restoration of tumor suppressor gene expression. Additionally, CDK9 inhibition sensitizes to the immune checkpoint inhibitor α -PD-1 *in vivo*, making it an excellent target for epigenetic therapy on cancer [69]. The results of these preclinical studies have promoted the development of CDK9 inhibitors for clinical application.

A recent review summarized the progress of sixteen CDK9 inhibitors in various stages of clinical development for cancer therapy. Four of them, P276-00, ZK-304709, BAY-1000394 and SNS-032, have been terminated in clinical trials due to their poor selectivity and high toxicity. Other inhibitors, including Alvocidib, TP-1287, P-1446, BAY-1143572, BAY-1251152, TG-02, (R)-Roscovitine, Fadraciclib, Dinaciclib, AT7519, BTX-A51 and AZD-4573, are currently being evaluated in several clinical trials [70]. Actually, most of these compounds are not very specific, they also inhibit other CDKs, such as Dinaciclib which also targets CDK1, 2, 5, TG-02 which also targets CDK1, 2, 3, 5, AT7519 which also targets CDK1, 2, 4, 5, 6, etc. [71-73]. Thus, they are also considered as pan-CDK inhibitors. Due to the high homology of these CDKs in the catalytic domain, the development of specific CDK9 inhibitors remains a major challenge.

CDK12 inhibitors

CDK12 is another critical transcriptional regulator besides CDK7 and CDK9 among CDK fam-

ily. It can bind to cyclin K to phosphorylate the CTD region of RNA polymerase II, thereby promote transcription elongation [74]. Several novel functions of CDK12 in cancer, especially breast cancer, have been revealed in recent studies. These novel functions are achieved by regulation of a variety of biological activities, including c-MYC expression, Wnt/ β -catenin signaling, RNA splicing, ErbB-PI3K-AKT signaling, MAPK signaling as well as noncanonical NF- κ B pathway, and DNA damage response (DDR) signaling [75-80]. In recent years, several CDK12 inhibitors have been developed. These inhibitors include SR-4835 and THZ531, which presented strong anti-tumor activity in preclinical studies. SR-4835 is a highly selective dual inhibitor of CDK12 and CDK13, which can suppress the expression of core DNA damage response proteins. This can provoke a “Brca-ness” phenotype that leads to deficiencies in DNA damage repair, thereby promote the synergistic effect of DNA damage chemotherapy and PARP inhibitors in TNBC [81, 82]. THZ531 is another covalent inhibitor of CDK12 and CDK13, which can significantly down-regulate the expression of DNA damage response genes and key super-enhancer-related transcription factors [83]. Recent studies indicated that THZ531 has a striking synergistic effect with sorafenib in the treatment of hepatocellular carcinoma [84]. To date, the inhibitors targeting CDK12 in clinical trials have all been pan-CDK inhibitors, including Dinaciclib. Therefore, development of CDK12 inhibitors with high specificity and drug properties is needed.

Combination therapy of CDK inhibitors and PD1-PDL1 antibodies

Through decades of research, cancer immunotherapy has emerged as a powerful and effective strategy for cancer treatment. In 1992, Dr. Honjo identified PD1 (programmed death receptor 1) and demonstrated PD1 expression in T cells. In 1999, Dr. Chen identified PDL1 (B7-H1) and demonstrated high PDL1 expression in immune and tumor cells. The interaction between PDL1 and PD1 induces T cell apoptosis and negatively regulates lymphocyte activation. Thus, blocking PD1-PDL1 immune checkpoints promotes T cell activation, which facilitates the cytotoxic effect of T cells on tumor cells. Although the blockade of the immune checkpoint PD1-PDL1 has achieved remark-

able success in the clinical treatment of a variety of cancers, the majority of cancer patients still failed to respond to the immunotherapy. In addition, drug resistance may occur during the targeted therapy of PD1-PDL1. Therefore, many trials have been conducted to improve the responsiveness of cancer patients to immunotherapy through combination therapy strategies. Recent studies have shown that some CDK inhibitors can enhance the anti-tumor immune response. In preclinical and clinical trials, some CDK inhibitors have demonstrated potent anti-tumor activity when used in combination with PD1-PDL1 immunotherapy.

Dinaciclib enhances anti-PD1 mediated tumor suppression

Dinaciclib, a potent CDK inhibitor of CDK1, 2, 5, 9 and 12, can induce apoptosis in various tumor cells as described above. Hossain et al. reported that combination therapy with Dinaciclib and anti-PD1 antibody presented considerable anti-tumor activity. This combination therapy can induce T cell infiltration and DC activation, suggesting that combination therapy can improve anti-tumor immune response and lead to tumor regression. In addition, in combination with anti-PD1 antibodies, Dinaciclib can induce immunogenic cell death (ICD) to convert tumor cells into endogenous vaccines [26]. Together, this study has paved a new path in solving the toxic and side-effect issues of pan-CDK inhibitors, thus increases the application possibility of pan-CDK inhibitors.

CDK4/6 inhibitors augment the anti-tumor efficacy of PD1-PDL1 immune checkpoint blockade

CDK4 and CDK6 are fundamental drivers of the cell cycle and are required for the initiation and progression of various malignancies. Pharmacological inhibitors of CDK4/6 have displayed significant activity against several solid tumors. In a mouse tumor model study, Goel et al. found that CDK4/6 inhibitors not only induce tumor cell cycle arrest, but also promote anti-tumor immunity [85]. On one hand, CDK4/6 inhibitors activate expression of endogenous retroviral elements in tumor cells, thereby stimulates the production of type III interferons and simultaneously enhances tumor antigen presentation. On the other hand, CDK4/6 inhibi-

tors markedly suppress the proliferation of regulatory T cells. Based on these two functions, clearance of tumor cells mediated by cytotoxic T cell is significantly promoted by treatment with CDK4/6 inhibitors. This study provided a theoretical basis for combination therapy using CDK4/6 inhibitors and PD1-PDL1 antibodies.

Abemaciclib is another CDK4/6 inhibitor, which has been clinically approved in the treatment of HR⁺ breast cancer. In a recent study, Schaer et al. reported that treatment with Abemaciclib can promote human T cell activation and can up-regulate expression of antigen presentation genes in breast cancer cells [86]. Further study indicated that Abemaciclib monotherapy can increase T cell inflammatory and delay tumor growth. Combination therapy with Abemaciclib and anti-PDL1 antibody can induce immunological memory and tumor elimination. These results suggested that combination therapy with Abemaciclib and anti-PDL1 antibody effectively stimulated both innate and adaptive immune response. Taken together, combination therapy with Abemaciclib and anti-PDL1 antibody have presented a great potential in clinical application.

Since the efficacy of PDL1 antibody therapy depends on the protein abundance of PDL1, Zhang et al. investigated the regulatory mechanism of PDL1 expression and stability [87]. They found that CDK4 is involved in the regulation of PDL1. Another study further proved that combination therapy with CDK4/6 inhibitors and anti-PDL1 antibody presented a remarkable anti-tumor activity [88]. Together, these findings reinforce the potential of combination therapy with CDK4/6 inhibitors and PD1-PDL1 antibody in clinical application. To date, in addition to the combination of CDK4/6 inhibitors and PDL1 antibody, other combination therapies are also being tried [89-92]. Combination therapies are expected to play an important role in future cancer therapy.

Other combination therapies

CDK12 mutant cases are known to be associated with elevated neoantigen burden and increased tumor T cell infiltration and clonal expansion [93]. CDK12 inactivation defines a distinct class of metastatic castration-resistant prostate cancer (mCRPC) that may benefit from immune checkpoint immunotherapy [93, 94].

Indeed, a phase II clinical trial was conducted in patients with metastatic prostate cancer harboring CDK12 deficiency (ClinicalTrials.gov Identifier: NCT03570619). These patients were administered with immune-checkpoint inhibitor in combination with nivolumab and ipilimumab followed by nivolumab monotherapy. A recent study also reported that SR-4835, a CDK12 and CDK13 specific inhibitor, induced immunogenic cell death, thereby enhanced the anti-tumor activity of PD1-PD-L1 immune checkpoint therapy in breast cancer [95]. Moreover, YKL-5-124 (CDK7 inhibitor) and MC18029 (CDK9 inhibitor) are also being studied in combination therapy with PD1/PD-L1 as mentioned above [59, 69]. These findings suggested that the combination of CDK12 inhibitors and PD1-PDL1 immunotherapy will be a promising strategy for cancer treatment.

Future perspective

CDK inhibitors developed in the early stage lack efficacy and selectivity in clinical practice, and the therapeutic effect is limited. Pan-CDK inhibitors have displayed remarkable anti-tumor efficacy. However, due to their low specificity and severe side effects, pan-CDK inhibitors have not been approved for clinical (cancer) treatment. Optimization of pan-CDK inhibitors together with combination therapy might hold some promise, and more relevant clinical trials are actually underway.

In contrast to pan-CDK inhibitors, great progress has been achieved in terms of selective Cdk4/6 inhibitors. Palbociclib has been licensed since 2015 for the treatment of hormone responsive, Rb positive breast cancer. Subsequently, Abemaciclib developed by Eli Lilly and Ribociclib developed by Novartis were also approved by the FDA. The biggest challenge in research and development of CDK inhibitors might be dealing with the adverse effects and potential drug tolerance. Further understanding of the behind mechanism and exploring ideal combination therapy might help overcome the selectivity and drug tolerance of CDK inhibitors.

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Disclosure of conflict of interest

None.

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