Cyclin-Dependent Kinases as valid targets for cancer treatment.

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ABSTRACT

Inefficacy of conventional chemotherapeutic drugs and appearance of several side effects such as hair loss and anemia, nausea, vomiting, diarrhea, infections, fatigue and destruction of the immune system, due to inability to discriminate normal cells and cancerous cells, have led to development of new anti proliferative drugs with less side effect and more efficacy. Cell cycle and cell regulation play significant role in drug discovery as they provide new opportunities for discovery of new drug target for treatment of cancers. CDKs are introduced as significant target for anticancer drugs as they are directly and indirectly involved in cell cycle events such as progression, transcription and DNA repair, so development of Cdk inhibitors become goal of many drug discovery companies and researchers, for treatment of cancers. In this paper we focused on Cdk's, their regulatory role in cell cycle and their prominent characteristics as valid target for drug discovery. Finally, we have reviewed 14 upcoming anticancer candidates whose target is CDKs. These anticancer drugs are such as Flavopiridol, Roscovitine, Dinaciclib, SNS032, AT7519, PD0332991, RGB-286638, P276-00, BAY-1000394, TG02/SiG1317, EM-1421, PHA-848125, LEE-011 and LY2835219 which are currently under investigation for treatment of cancer. We hope this paper gives informative background for understanding importance of Cdk's as effective targets for cancer therapy with high effective results and minimum side effect.

KEYWORDS: Cdk's, Kinases, Inhibitors, Anticancer drugs, Drug discovery,

INTRODUCTION:

Conventional chemotherapeutic drugs are non specific target chemical substances, used for treatment of cancer( Gore L et al., 2013). Due to their inability to distinguish accurately normal cells and cancerous cells because of their non specific target interaction , they are susceptible to normally rapidly dividing cells too, as a result, they produce several side effects due to damage to normal cells. These side effects are mostly including, diarrhea, anemia, hair loss vomiting, infections, nausea, fatigue and destruction of the immune system. To overcome such disadvantages, new anti cancer drug candidates are developed, which show specific target interaction with high affinity towards specific target. New drugs for cancer treatment are designed based on their specific target involved in cell cycle regulation to provide exact cancer therapy(Gore L et al., 2013) The Cell cycle is a harmonious combination process at molecular level which leads in division of cell and its proliferation, and its connection with cancers has assumed greater significance. The cell cycle is important source for target identification, helps in understanding underlying pathways of cancers to facilitate new opportunities to discover new target for cancer therapy (Matthews et al., 2010; Moen et al., 2010). Regulation of cell cycle is done by several regulatory factors, among them, Cdk's show significance, and they are serine/threonine kinases. They are activated and inactivated by cyclin and cdk inhibitor respectively (Matthews et al., 2010; Moen et al., 2010). Protein kinases is regulated by binding to cyclin hence named cyclin dependent kinases, the complex cyclin-cdk's phosphorylate their substrates on serines and theonines for cell cycle progression. Several cellular activities and functions such as growth and development processes and homeostasis of eukaryotic cells are controlled by signaling pathway of CDKs. Since 1980s evidences have shown clearly the role of protein kinases in carcinogenesis and tumor growth (Matthews et al., 2010; Meijer, 2003). Unrevealing the regulation mechanism of Cdk's led to the Nobel Prize award to Krebs and Fischer in 1992 (Matthews et al., 2010). The set of protein kinases in organism’s genome is called Kinome , the set of human’s kinome consist of 518 known proteins. Included in seven families . Cdk's are classified in 13 subfamilies (CDK1 to CDK13) but new subfamilies are investigated.
Based on their role in cell activities they can be divided in two groups. First are involved in progression of cell (CDK1, CDK2, CDK3, CDK4, CDK6), and second group involved in regulation of transcription (CDK7-CDK9 and CDK11-CDK13). CDKs regulation is controlled by members of cyclin proteins. Evidences has demonstrated that the CDKs expression remains quite constant, while cyclin concentration undergoes variation during cell cycle. Cyclin concentration abnormality directly contributed in cell deregulation which involved in tumor formation and development (Schwob, 2001). Cancer treatment involved in CDKs inhibition has assumed great importance as it showed better therapy as compared to conventional chemotherapy (Mariale G and Belmont P, 2014).

**Regulation of cell cycle by Cyclin-Cdks dependent kinases:**

In eukaryotic cells, including human cells, DNA replication occurs at specified time in S phase that is transiently separated by G1 and G2, (pre synthetic and post synthetic phases respectively), the factors that govern and regulate separation of these phases are Cyclins and Cyclin dependent kinases. (Morgan, David O. 2007.)

Cyclins as regulatory factors are unable to perform their function lonely during cell cycle regulation so for this reason they come in contact with Cdk's to activate them for their function. The ascent and descent in the concentration of cyclins is properly timed, depending upon Cdk's, which perform phosphorylation of substrates for the cell cycle progression. Several cyclins have been identified that activate different Cdk's during phases of cell cycles of mammalian cells. They are named as follow:

- Cyclin D activates Cdk4 and Cdk6 in late G1 phase which result in inactivation of Retinoblastoma protein for repression of transcription of certain genes and transition of G1 to S phase.
- Cyclin E activates Cdk2 in late G1 phase that leads in initiation of DNA synthesis.
- Cyclin A activate Cdk2 in S phase which result in completion of DNA synthesis.
- Cyclin B activate Cdk1 late G2/M phase that result in transition of the cell cycle from G2 to M phase. (Rastogi .S. C., et al 2008)

**Activation of Cdk's by cyclins:**

At a proper time, the cyclins stimulate different Cdk's and phosphorylate substrates to form a complex which ride the cell cycle generator, including integration of signals for confirmation of progression of cell cycle and targets in check points pathways.

For an example, Cdk4 and Cdk6 complex with cyclin D in late G1 and perform phosphorylation of substrate Retinoblastoma (Rb) protein which in turn bind to factor E2F for it’s inactivation so that the cell cycle transfers from G1 to S phase. In this step, Rb serves as a regulatory protein and E2F is a necessary factor for certain gene transcription. When Cdk4 and Cdk6 phosphorylate Rb then Rb releases E2F factor to allow cell cycle progression.

- Similarly Cyclin E and Cdk2 combines transiently and form a complex in late G1, after which degradation of cyclin E quickly occurs to release Cdk2, which then form a complex with cyclin A for completion of DNA synthesis in S phase.
- In addition a complex forms between cyclin B and Cdk1 in late G2/M phase for transition of cell cycle from G2 to M phase.
- It is important to know that imbalance in production of cyclins or production at improper time leads to abnormality and mutation in the cell cycle, resulting in aberrant growth and unlimited proliferation, as seen in cancerous cells. (Morgan, David O. 2007., Rastogi .S. C., et al 2008)

**Proper destruction of Cdk's in cell cycle regulation**

When the level of G1 Cyclin accelerates, they complex with their Cdk's and signal the cell to assemble the chromosomes for duplication. S phase promoting factor (SPF) which comprising cyclin A bound to Cdk2, moves in the nucleus and prepare the cell to replicate it’s DNA and Centrosomes. While DNA synthesis is going on, cyclin E is eliminated and the level of mitotic cycling begins to accelerates in G2 phase. An M phase promoting factor including mitotic cyclins and M phase Cdk's begins spindle assembly, condensation of chromosome and breakdown of nuclear envelope. At this point M phase promoting factor activates APC (anaphase promoting complex), bringing about following actions:

Separation of sister chromatids at the metaphase and movement of them to poles for completion of mitosis. Destruction of mitotic cyclins by conjugation with the protein ubiquitin(Ub), which targets them for destruction by proteasomes. Turning on the synthesis of G1 cyclins for the next round of the cycle. Keeping the newly synthesized DNA under protection in S phase by the protein geminin to prevent from rereplication before mitosis destruction.

Proper destruction of cell cycle regulators via the ubiquitin-mediated proteolytic route is essential. Three enzymes, Ub-activating, Ub-conjugating and Ub-ligase continuously function to form a
Fourteen anticancer drugs with Cdk inhibitory property along with their trial report:

Fourteen anticancer drugs are reviewed here are Flavopiridol, Roscovitine, Dinaciclib, SNS032, AT7519, PD0332991, RGB-286638, P276-00, BAY-1000394, TG02/SIG1317, EM-1421, PHA-848125, LEE-011 and LY2835219 (Mariaule G and Belmont P, 2014).

1.1. Flavopiridol (also known as alvocidib) is a flavonoid derived from a native plant from India called rohitukine. This plant derivative showed ATP-competitive property and was together produced by Sanofi-Aventis and the US National Cancer Institute (NCD).[1]. This is a potent inhibitor for several CDKs involved in cell cycle regulation: CDK1 (IC50: 30 nM), CDK2 (IC50: 100 nM), CDK4 (IC50: 20 nM), CDK6 (IC50: 60 nM) and CDK7 (IC50: 10 nM) (Mariaule G and Belmont P, 2014). It also has shown ability to inhibit CDK9 (IC50: 10 nM), which has crucial role in RNA transcription regulation via activation of RNA polymerase II by phosphorylation process (Schwob, 2001; Kaur et al., 1992). Inhibition ability of this compound is seen at two levels: one during phase G1 and second during the shift from G2 phase to M phase. It is currently under clinical development in phase II for investigation for anti-cancer property for the treatment of chronic lymphoid leukemia and administration mode of the drug is intravenous (Schwob, 2001; Kaur et al., 1992).

1.2. (R) Roscovitine (also known as seliciclib) is a cyclin-dependent kinase inhibitor developed by Cyclacel Pharmaceuticals, Inc (Mariaule G and Belmont P, 2014). This anticancer drug candidate shows inhibitory activity towards several CDKs such as CDK1 (2.7 µM), CDK2 (0.1 µM), CDK7 (0.5 µM) and CDK9 (0.8 µM). Weak inhibitory activity of the candidate is reported towards CDK4 and CDK6 (IC50 > 100 µM). The inhibitory performance of the drug candidate is in the form of phosphorylation of retinoblastoma protein (pRb protein) and RNA polymerase II as well. (R)-Roscovitine has been investigated for treatment of lung cancer (non-small cell lung cancer, NSCLC) and undergone in phase II trial and administration mode of the drug is orally (McClue et al., 2002).

1.3. Dinaciclib (also known as SCH-727965), is a potent cyclin-dependent kinase inhibitor under evaluation at Merck. It shows inhibitory activity towards CDK1 (IC50: 3 nM), CDK2 and CDK5 (IC50: 1 nM), and also of CDK9 (IC50: 4 nM) (Mariaule G and Belmont P, 2014). The evidences have shown this novel anticancer candidate shown more efficiency than flavopiridol in its ability to inhibit CDks by high selectivity power towards different CDks. The anticancer drug has undergone phase...
III clinical trial for treatment of lymphocytic leukemia in 2012 and administration mode of the drug is intravenous (Mariaule G and Belmont P., 2014)

1.4. SNS032,(previously known as BMS-387032) being developed by Sunesis. The compound has ability to selectivity inhibit CDK2 (IC50: 38 nM), CDK7 (IC50: 62 nM) and CDK9 (IC50: 4 nM due to its specific thiazole unit (Diaz et al., 2009).The inhibitory performance of the compound is done is cell cycle as well as transcription process. The anticancer candidate is in phase I investigational trial for the treatment of of chronic lymphoid leukemia along with multiple myeloma and administration mode of the drug is intravenous (Tong et al., 2010).

1.5. AT7519 is a potent cyclein-dependent inhibitor developed by Astex Pharmaceuticals. It has inhibitory activity towards several CDKs and shows its performance is both cell cycle regulation and RNA polymeraseII-dependent transcription (Mahadevan et al., 2011) The higher inhibitory activity is towards CDK1, 2, 4, 6 and 9 with IC50 of 10-210 nM. less potent inhibitory activity is towards CDK3 and little activity towards CDK7. AT7519 is in phase I of clinical trial for treatment of refractory solid tumors and administration mode of the drug is intravenous (Mariaule G and Belmont P., 2014).

1.6. PD0332991(also known as palbociclib),developed by Pfizer. This anticancer drug has strong inhibitory activity towards CDK4 (IC50: 11 nM) and CDK6 (IC50: 16 nM)(Mariaule G and Belmont P., 2014). PD033299 prevents pRb protein from phosphorylation which led in cell cycle arrest at the G1 phase. PD033299 is in phase III investigational trial for breast cancer treatment and administration mode of the drug is orally (Guha.M., 2012)

1.7. RGB-286638 is product of Agennix. It has ability to inhibit several CDKs such as CDK1 (IC50: 2 nM), CDK2 (IC50: 3 nM), CDK3 (IC50: 5 nM), CDK4 (IC50: 4 nM) and CDK9 (IC50: 1 nM) (Cirstea et al., 2013).This anticancer candidate is in the end of phase I clinical trial for treatment of solid tumors and multiple myeloma (Mariaule G and Belmont P., 2014).

1.8. P276-00 ( flavopridol’ s analog) produced by Nicholas Piramal company has shown efficient inhibitory activity towards CDK9 (IC50: 20 nM) and also towards CDK1 (IC50: 79 nM), CDK2 (IC50: 224 nM) and CDK4 (IC50: 63 nM)(Kim, K.S. et al., 2000 ; De Azevedo et al., 1996). It completed phase II clinical trial for treatment of locally advanced head and neck cancer, relapsed and/or refractory mantle cell lymphoma but still no result obtained in 2012/2013 and administration mode of the drug is intravenous (Kim, K.S. et al., 2000 ; Murthi, K.K et al., 2000).

1.9. BAY-1000394, produced by Bayer, not only it shows inhibitory activity towards CDKs are functional in the cell cycle such as CDK1, CDK2, CDK3 and CDK4) but also implicated in the transcription regulation (CDK7, CDK9 (Siemeister, G. et al., 2012). This anticancer candidate is in phase clinical trial for several types of tumors and administration mode of the drug is orally (Lücking, U. et al., 2013).

1.10. TG02/SG1317, developed by S*BIO and owner legally by Tragara pharmaceuticals in 2008. TG02 inhibits CDK9, CDK5, CDK2, CDK3 and CDK1 (Mariaule G and Belmont P., 2014). It is in phase I clinical trial for treatment of chronic lymphocytic leukemia and small lymphocytic lymphoma and administration mode of the drug is orally (Goh, K.C et al 2012 and Poulsen, A. et al., 2013).

1.11. EM-1421, known as terameprocol developed by Erimos company of pharmaceutics. It shows inhibitory property towards CDK1. It is in phase I/II clinical trials. It is evaluated for treatment of refractory solid tumors and administration mode of the drug is intravenous (Galons .H et al., 2013).

1.12. PHA-848125 also known as milciclib is product of Nerviano. This anticancer candidate has selective inhibitory activity towards several CDKs such as CDK1 (IC50: 2 nM), CDK2 (IC50: 3 nM), CDK4 (IC50: 5 nM), CDK5 (IC50: 4 nM) . It is currently in phase II clinical trial for treatment of patients suffering from thymic carcinoma. According to result of the drug candidate in phase II reported at ASCO (American Society of clinical oncology 2014), 43 patients have been treated and out of 30 patients whose data are accessible ,14 patients showed successful response to the drug with a progression free survival rate at 3 months of 46.7%. Recently treatment of mesothlioma is patented and administration mode of the drug is orally (Mariaule G and Belmont P., 2014).

1.13. LEE-011 is another CDK inhibitor candidate developed by Astex Pharmaceuticals™ and Novartis. It shows high selectivity for CDK4 and CDK6. It is in phase II Clinical trial and used for treating patients suffering from breast cancer and administration mode of the drug is orally (Mariaule G and Belmont P., 2014).

1.14. LY2835219, also named abemaciclib developed by Eli Lilly. It show selective inhibitory activity for CDK4/CDK6, and also inhibits the phosphorylation of pRb protein by blocking cells at G1 phase. It is in phase I clinical trial investigated for treatment of non-small-cell
lung carcinoma. This anticancer drug has shown positive results for breast cancer in combination with hormone therapies in phase I clinical trial. A study in phase III is planned for LY2835219 in 2014 which includes combination of the drug with fulvestrant for patients with HER2-negative metastatic breast cancer and administration mode of the drug is orally (Mariaule G and Belmont P., 2014).

CONCLUSION:
Cyclin dependent kinases are the promise target for anticancer drugs, as they are involved in cell cycle events like progression, control, transcription and DNA repair, understanding the role and pathways involved Cdks give informative knowledge of them for being effective target for cancer therapy. It is notable that study of Cyclins and their activation function on Cdks, time of their activation, and their concentration regarding Cdks activation are essential in understanding cell cycle and designing Cdks as promising target with this background that imbalance in production of cyclins, production at improper time and their concentration disproportion leads to aberrant growth and unlimited proliferation, as seen in cancerous cells. Also understanding the role of each Cdks in cell cycle and their connection with other regulatory factors and complex formation with other factors are supportive for more detailed information about their selection for target. After conformation of validation of Cdks as drug target CDKs inhibitors are developing to take a place in cancer therapy in order to minimize side effect of the chemotherapy and radiation and increase the effectiveness of cancer treatment. However the selectivity done by the candidates between CDKs and even against other kinases are very poor which result in emerging difficulties for drug dosing in clinical dosing status. Administration in both cases, high dose and low dose lead in toxicity and inefficiency respectively. Therefore we agree to provide such negative outcome is combination of these drug candidates with classical cytotoxic agents. This combination is feasible efficiently in some inhibitors such as flavopiridol or BAY-1000394. In addition the poor selectivity also can be resulted from high homology’s degree between CDKs family, mostly within ATP-binding site. Although these anticancer drugs with Cdk inhibitory property are evaluating but still the exact and specific Cdks which are involved in tumor development are not specifically identified. Moreover there are still no anticancer drugs in market with Cdk inhibitor as their target so these drugs are going to be the first drugs with cdk inhibitory property will be launched in market soon. Therefore this research is very sensitive and interesting.

Conflicts of Interest
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Ethics
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REFERENCES
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