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## CDK inhibitors as anticancer agents: Current status and future prospective

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### Abstract

The cell-cycle is tremendously complex process and strictly controlled by cellular proteins called 'Cyclin-dependent kinases' (CDKs). Because of their critical contribution in cell division and their dysregulation in many cancers, CDKs have become an intense area of research for >20 years and several CDK-inhibitors (CDKIs) were developed. Initial results with broadly acting, first generation, pan-CDK inhibitors including flavopiridol, olomoucine, UCN-01 were almost disappointing due to limited efficacy and high toxicities observed in-vivo. To overcome these failures, Second-generation CDK inhibitors with high selectivity and specificity were enormously researched and developed. Based on impressive results in clinical investigations, FDA granted approval to three highly-specific CDK4/6 inhibitors including: palbociclib (PD0332991), abemaciclib (LY2835219) and ribociclib (LEE011) for treatment of patients with 'HR-positive', 'HER2-negative' advanced or metastatic breast cancer. In this Review, we'll focus on CDKs, their contribution in cell-cycle and cancer, development and failure of pan-CDK inhibitors and currently approved CDK4/6 inhibitors with their preclinical and clinical study data that manifested their benefit in breast cancer treatment. In future, translational studies for coordinated assessment of the important biomarkers of clinical sensitivity and complete understanding of intersection of pharmacology and biology of CDKIs is required for more clinical success in the cancer treatment.

**Keywords:** Abemaciclib, Cancer, Cyclin-dependent kinases, CDKIs, Cell-cycle, Palbociclib, Ribociclib

### Introduction

Cancer is a complex, multistage process which includes both the hereditary and epigenetic changes of genes that transforms normal cells into very dangerous malignant clones [1]. It is 2<sup>nd</sup> major cause of death worldwide after heart diseases, considering for 8.8 million deaths in 2015. Globally, almost 1 out of 6 deaths is because of cancer [2]. By 2020, it is estimated to have expanded to 7.5 billion and around 15 million new cancer-cases will be diagnosed and 12 million patients of cancer will die [3, 4]. Cancers of Lung, Liver, Colorectal, Stomach and Breast are prevalent causes of cancer-deaths [5].

Intravenous cytotoxic chemotherapy is a hallmark of cancer treatment for decades. These chemotherapeutic drugs target rapidly proliferating (cancer) cells also the normal tissues. Subsequently leads to classic toxicities including gastrointestinal symptoms, alopecia, fertility problems, nerve problems etc [6, 7]. Many novel molecular targets that are cancer specific have been uncovered with the hope to exclude toxic-side effects associated with conventional cancer chemotherapy. Thus far, several novel agents yielded promising results in preclinical and also in clinical levels. These novel targets include: the vascular endothelial growth factor receptor and the basic fibroblast growth factor receptor (angiogenesis); the growth factor receptor tyrosine-kinase including epidermal growth factor receptor and HER-P/neu (proliferation); protein kinase-C (proliferation and drug resistance); cyclin-dependent kinase (proliferation); the oncogenic GTP-binding protein Ras (proliferation); matrix metalloproteinase and, angiogenin (angiogenesis and metastasis) [8].

In this review, we'll discuss about the CDKs, their critical role in cell-cycle and dysregulation in cancer, pre-clinical and clinical evidences of pan-CDK inhibitors and highly specific approved CDKIs and then trace some clinical data of CDKIs under clinical trials. At last, we'll discuss the future perspective of these inhibitors.

### Cell-cycle and CDKs

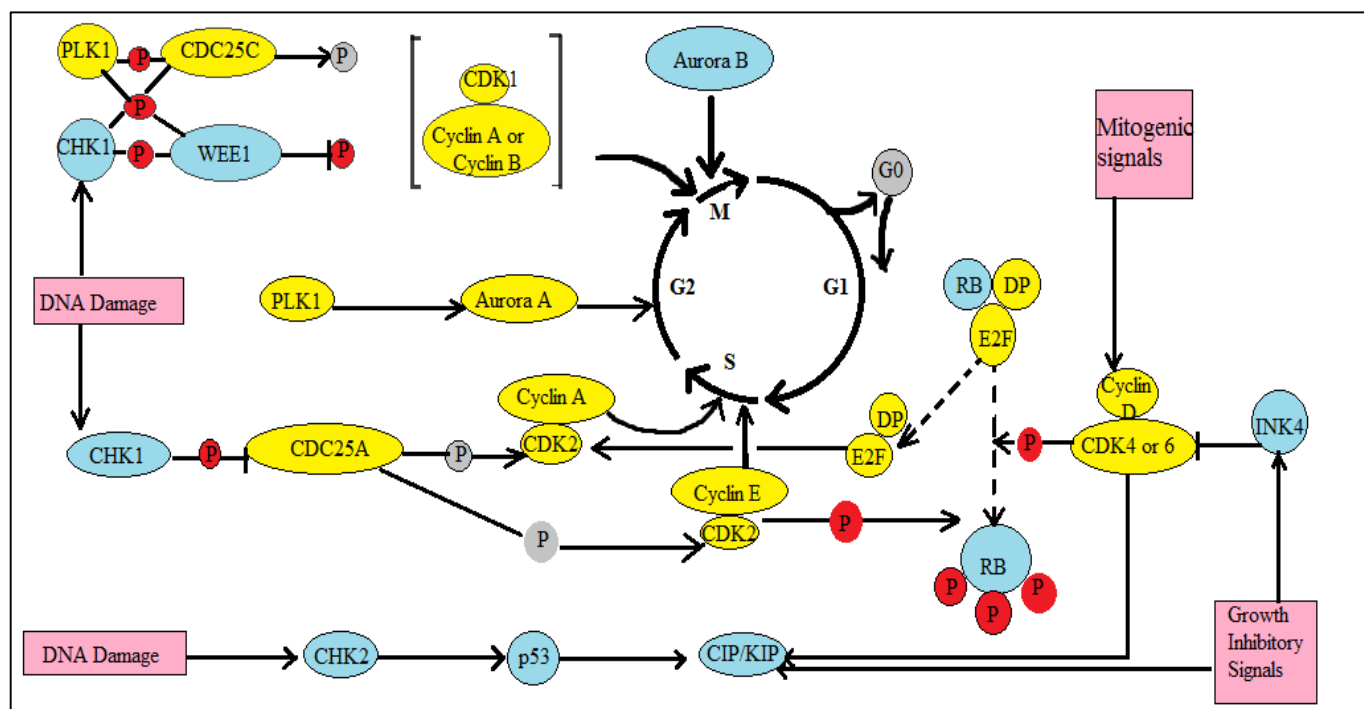
Leland H. Hartwell, R. Timothy (Tim) Hunt and Paul M. Nurse discovered the key regulators of cell-cycle. After >10 years of their discovery of CDK identification, they received the Nobel Prize and then the promise of their influential study was finally begun to be

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accomplished [9]. Originally, Cell-division is an intricate process which requires high energy [10]. It involves two successive processes, characterized by DNA replication (S-phase) and separation of replicated chromosomes into two cells (mitosis or M-phase). The cell-cycle also contains Gap phases i.e. G<sub>1</sub>, G<sub>0</sub> and G<sub>2</sub>. During G<sub>1</sub>-phase, the cell gets ready for DNA synthesis, replication of DNA happens in S-phase and is trailed by G<sub>2</sub> during which the cell gets ready for mitosis. Mitosis (M) is the process of nuclear division. The phases of mitosis include prophase, metaphase, anaphase and telophase. Dependent on the developmental and environmental signals, cells in G<sub>1</sub> may permanently or reversibly quit the cell-cycle and then enter an arrested-phase or resting-state known as G<sub>0</sub> [11, 12]. The frequency of the cells with which they get into the cell-cycle is very tightly regulated by check points, the key factor that controls the progression of cell-cycle is a group of proteins (serine-threonine kinases) called the “Cyclin Dependent Kinases (CDKs)”, As their name suggests, activation of the CDKs requires interaction with another group of proteins called the “cyclins” [13]. The timely event of each-phase of cellular

transition is necessary for maintenance of genetic unity through-out the generations and for that different complexes of CDKs and their cyclin co-partners are responsible [14]. During evolution the number of CDKs were increased and marked by a substantial extension of groups related to the cell-cycle. Human cells contain ‘20 CDKs’ and ‘29 cyclins’ [15]. CDK1 is an essential mitotic kinase and regulates G<sub>2</sub>-M transition. CDK2, 4 and 6 control the progression of cell-cycle through interphase. CDK5 doesn’t appear in cell-cycle regulation, but instead included in neuronal development and migration processes [16]. CDK7, 8 and 9 appear to regulate RNA polymerase II – dependent transcriptional initiation, elongation and further processing. CDK11 seems to involve in mRNA splicing. The function of CDK3, 10 and CDK12 to 20 are not effectively recognized yet, but secondary functions of these CDKs includes neural differentiation [17], gene transcription [18], cell death [19], DNA-damage and repair [20], cell differentiation [21], metabolism [22], development [23] and immune response [24]. Some studies showed that CDK1 with its copartners cyclin ‘A2’ and ‘B1’, alone can operate the human cell cycle [25, 26].



**Fig 1:** Major regulatory proteins involved in progression of Cell-cycle.

Cyclin-CDK complexes are activated by mitogenic signals. These complexes then phosphorylate several targets of cell-cycle, including retinoblastoma (RB) protein and promote G<sub>1</sub>–S progression. The E2F-family of transcription factors is also activated by RB hyper-phosphorylation. Growth-inhibitory signals are produced by up-regulation of CDKIs of the ‘CIP/KIP’ (CDK-interacting protein/Kinase-inhibitory protein) and ‘INK4’ families. These inhibitory signals antagonize G<sub>1</sub>–S progression. Complexes of Cyclin–CDK,

together with several other proteins including Aurora kinase (Aurora B and Aurora A) and PLK1 (Polo-like kinase 1) controls progression through S-phase and G<sub>2</sub>–M phase. Cells can also quit the cell-cycle and enter in temporary or permanent cell-cycle arrest (G<sub>0</sub> phase). Additionally, some specialized proteins can sense DNA-damage and triggers cell-cycle arrest through checkpoint kinase 1 (CHK1) in S or G<sub>2</sub> phase and via p53 and CHK2 in G<sub>1</sub>-phase [27].

**Table 1:** Known CDKs, their cyclin partners, and their functions in the human. RB: retinoblastoma protein; FOXM1: forkhead-box protein M1. [28, 29]

| CDK   | Cyclin Partener    | Function  |
|-------|--------------------|---|
| CDK1  | Cyclin A, Cyclin B | G2–M progression (mitotic entry): Nuclear envelope breakdown, Mitotic condensation, Spindle assembly  |
| CDK2  | Cyclin E, Cyclin A | G1–S progression (DNA replication): phosphorylation of RB and other replication factors, Induction of histone-synthesis, Centrosome duplication |
| CDK3  | Cyclin C           | G0 phase  |
| CDK4  | Cyclin D           | G1–S progression: RB Phosphorylation stimulates E2F, FOXM1 accumulation   |
| CDK5  | p35                | Transcription, Neuronal viability (G1–S control)  |
| CDK6  | Cyclin D           | G1–S progression: RB Phosphorylation stimulates E2F, FOXM1 accumulation   |
| CDK7  | Cyclin H           | Basal transcriptional processes: Initiation, Elongation, RNA processing   |
| CDK8  | Cyclin C           | Basal transcriptional processes: Initiation, Elongation, RNA processing   |
| CDK9  | Cyclin T           | Basal transcriptional processes: Initiation, Elongation, RNA processing   |
| CDK11 | Cyclin L           | Basal transcriptional processes: Initiation, Elongation, RNA processing   |

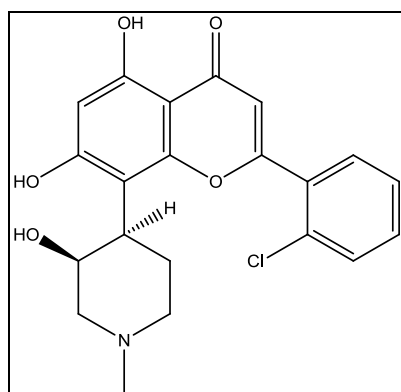
### Targeting CDKs in cancer-therapy

The cell-cycle machinery is usually dysregulated in cancer. Variety of mechanisms can be responsible for this. In some cases, certain tumor-harbor escalation of genes encoding specific ‘cyclins’ and ‘CDKs’, and hence elevating their levels in tumor cells and in other cases, genes encoding endogenous CDKs are deleted, that facilitates incontinent CDK activity resulting uncontrolled proliferation of cells. Consequently, CDKs have been considered as appealing targets for cancer-treatment and serious zone of research for >20 years. Several CDK-inhibitors have been evolved and some are under clinical investigations [30, 31]. CDK inhibitors can be ATP-competitive (interacting with CDKs in their catalytic ATP-site) or non-competitive (interacting with

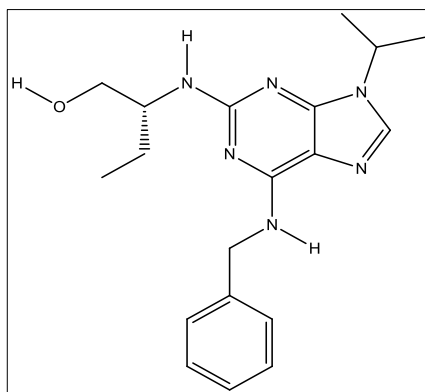
CDKs on allosteric sites) and inhibits the phosphorylation of substrates, appropriate for simultaneous blockade of cell-cycle progression and transcription, facilitating the induction of apoptosis [32].

### First generation or pan-CDK inhibitors

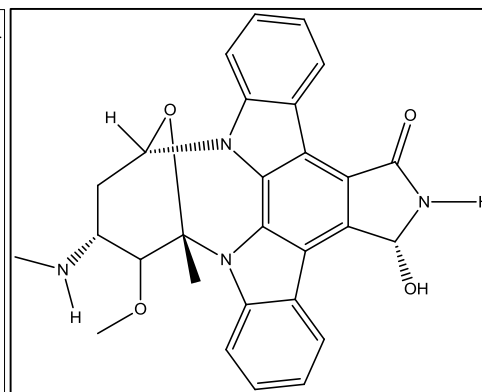
Several ATP-competitive small molecule CDKs have been emerged over the past twenty years and studied in numerous trials and in some tumor types. Majority of early compounds were non-specific towards individual CDKs which might result in limited efficacy and high toxicity and may therefore be called as pan-CDK inhibitors. These first-generation CDKs include flavopiridol, UCN-01, olomucine and roscovitine [33].



Flavopiridol



Roscovitine



UCN-01

### Flavopiridol

Flavopiridol (also known as alvocidib) is a ‘semi-synthetic flavonoid’ and derived from ‘rohitukine’, a chromone alkaloid. This ATP-competitive CDKI was jointly developed by ‘Sanofi-Aventis’ and the ‘US National Cancer Institute’ (NCI). It is the foremost highly investigated CDKI thus far, with >60 clinical-trials performed between 1998 and 2016 [34, 35]. Flavopiridol potentially inhibits CDK1 (IC50: 30nM), CDK2 (IC50: 100nM), CDK4 (IC50: 20nM), CDK6 (IC50: 60nM), CDK7 (IC50: 10nM) and CDK9 (IC50: 10nM) and causes cell-cycle arrest in G1 and G2-phases [36]. Although, flavopiridol resulted in remarkable in-vitro activity due to its broad-spectrum, comprehensive nature substantially in-vivo study results were quite disappointing [37]. Afterwards another phase-I study with flavopiridol demonstrated significant clinical efficacy using novel dosing schedule of drug administration in patients with refractory CLL. A comparatively short-infusion time of 4 hours resulted in ‘45%’ PR (19 out of 42 patients) with a median-response

duration >12 months. TLS (Tumor-lysis syndrome) was the most serious toxicity observed in the phase-I study, but it was dose limiting and observed in 44-55% of patients [38, 39]. In phase II studies, significant clinical responses were observed with flavopiridol in haematological malignancies, like mantle-cell lymphoma (MCL) and chronic-lymphocytic leukaemia (CLL) [40, 41, 42]. Although, exclusion of patients with WBC <200 × 10<sup>9</sup>/L, implementation of aggressive TLS-prophylaxis and reduction in flavopiridol dosing resulted in great improvement in the tolerability of drug but TLS still occurred in ‘44%’ pts [38, 39]. Durable responses were achieved by patients having high-disease burden and high-risk genetic features [43]. In 2014, Tolero Pharmaceuticals reported positive results of phase-II trial in patients with AML (acute myeloid leukemia) in annual meeting of ‘American Society of Clinical Oncology’. It was revealed that flavopiridol additionally possesses a synergistic effect with other anticancer agents like cisplatin, irinotecan or docetaxel. Thus, co-administration of flavopiridol with other cytotoxic-agents

has been proved as useful tactic to reduce its amount, so limiting the side-effects [34, 44]. A randomized phase-II trial was performed to compare 'flavopiridol, cytarabine and mitoxantrone' (FLAM) combination with 'cytarabine and daunorubicin' (7+3) in patients with AML (newly diagnosed). In this study, better CR-rate was observed with FLAM in comparison with 7+3 [45]. In another Phase-II trial, combination treatment of 'flavopiridol and cisplatin' in patients with primary peritoneal and ovarian cancer elicited a clinical response i.e. SD in 25% and CR in 17.5% of pts. Though the results were preliminary and far from definitive, the authors concluded that combination treatments using these agents merit further studies in platin-resistant and platin-sensitive tumors. Presently, flavopiridol is under phase-III trial [46].

### Olomoucine and Roscovitine

Olomoucine was another pan-CDK inhibitor to be developed. It selectively inhibit CDK1, 2, 5 and probably CDK7 (but not CDK4). It was shown to inhibit 50% growth in 'National Cancer Institute' (NCI) panel of sixty tumor cell-lines (IC<sub>50</sub> ¼ 60:3 mM). It typically arrests cells in 'G1-S' and 'G2-M' transitions [47]. Roscovitine (also known as seliciclib) is derived from olomoucine and exerts similar selectivity for CDKs. Its inhibitory-activity for CDKs (particularly for CDK1) was 5-10 folds greater than olomoucine. Roscovitine (CYC202, developed by Cyclacel) is a purine-based, small molecule, orally bioavailable, highly selective ATP competitive inhibitor of several CDKs [48]. It is highly active against human CDK1, 2, 7, 9 and to less extent CDK4/6. The mechanism for R-roscovitine includes down control of RNA polymerase-II dependent transcription and improved expression of E2F1. It also decreases RB phosphorylation [33, 49]. Roscovitine was gone into clinical-trial in 2001 by 'Cyclacel pharmaceuticals, Inc'. In Phase-I setting, PR was observed in 1 out of 56 pts [50]. A subsequent 'randomized, blinded, Phase-II trial' (APPRAISE) was performed in 187 patients with advanced NSCLC (non-small cell lung cancer) however, this investigation was ended as a result of poor PFS rates, and results were not published [51]. Roscovitine was also undergone several other phase-I and phase-II clinical trials in some human cancers as monotherapy and also as combination therapy. This orally administered compound showed appropriate bioavailability in phase-I studies. Subsequently, Phase-II clinical study of 'roscovitine' along with 'gemcitabine and cisplatin' as 'first-line treatment' and with 'docetaxel' as 'second-line treatment' was conducted in 52 pts with NSCLC. In this study, PR was observed in 9 pts, SD in 21 pts treated with 'roscovitine/gemcitabine/cisplatin' and PR in 2, SD in 1 patient treated with 'roscovitine/docetaxel' [52]. In another phase-II study in patients with nasopharyngeal cancer, roscovitine was evaluated as single-agent and resulted in notable tumor shrinkage [53]. Because of the impressive results of R-roscovitine as a powerful cytotoxic agent, certain bio-isoester were made by some changes in the purine hetero atom to achieve similar action as of roscovitine and also to make them less-toxic, high specific and to increase the short half-life of roscovitine in human. In 2015, it was licenced to 'ManRos Therapeutics' by 'Cyclacel' for treatment of cystic-fibrosis [54]. R-roscovitine is still under clinical-investigation along with chemotherapy for solid tumors [55].

### UCN-01

UCN-01 is a staurosporine analog, initially developed as a specific 'protein kinase C inhibitor'. It was later subsequently

demonstrated to inhibit CDK 1 and 2 in in-vitro models at higher concentrations (IC<sub>50</sub> ¼ 300– 600 nM) and thus can be characterized as a 'CDK inhibitor' [52]. In preclinical models, the drug arrests cell-cycle in G1/S phase, hypophosphorylation of pRb and induction of p21. The dose limiting toxicities (DLTs) observed in phase-I studies were nausea, vomiting, hyperglycemia, pulmonary dysfunction and hypotension. The recommended phase-II dose of UCN-01 was 42.5 mg/m<sup>2</sup> per day administered on a 72-hour continuous-infusion schedule [53]. A Phase-II study of 'UCN-01' along with irinotecan was performed in patients with metastatic TNBC (triple negative breast cancer) but results were unimpressive [54]. Another Phase-II study of 'UCN-01' in patients having metastatic melanoma showed insufficient clinical efficacy of this drug as monotherapy [55]. Several combination trials of UCN-01 along with chemotherapeutic-agents is under investigation.

### Reasons for failure of first generation or pan-CDK inhibitors

To some extent, the reasons for failure of non-specific CDKs in clinical trials can be clarified by 3 key ideas:

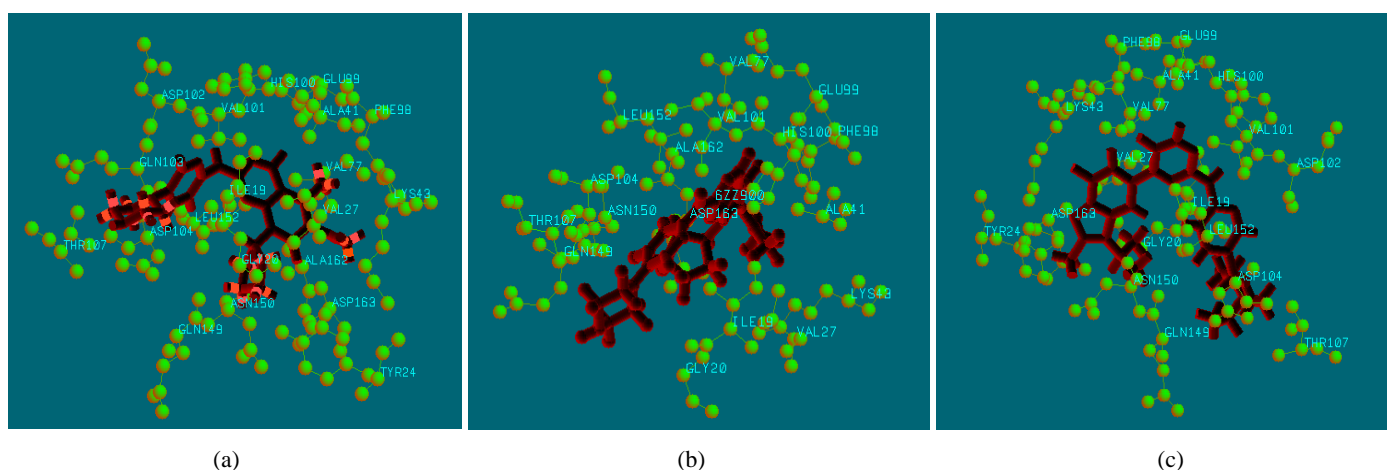
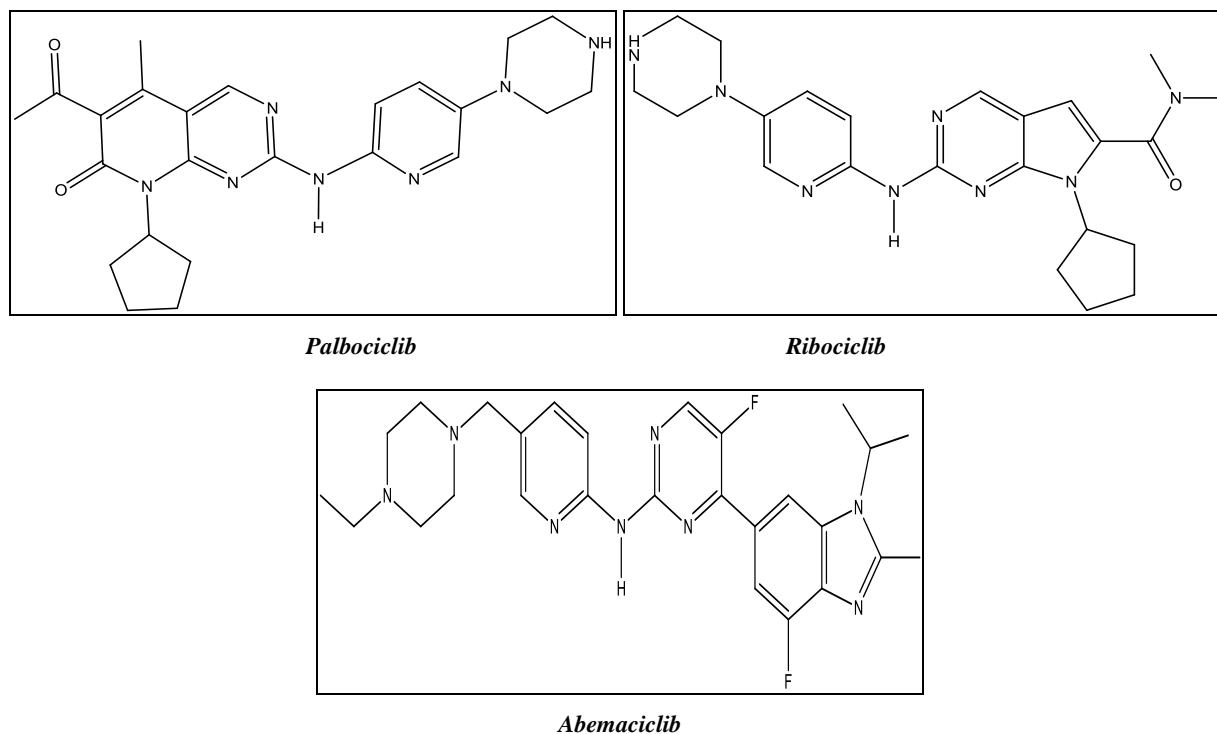
- 1) Inadequate understanding of mode of action: For several CDKs with low specificity or selectivity, there's absence of clearness with the point what CDKs are truly being repressed and hence the corresponding mechanism that could result in therapeutic effectiveness of drug molecule [56].
- 2) An absence of excellent biomarker to estimate the response of tumors to CDKs is additionally thought to be a cause of their failures so far.
- 3) Lack of therapeutic window: Most of the CDKs target several other proteins which are crucial for proliferation and survival of normal cells (e.g. 'CDK1' and 'CDK9'). The intrinsic inability of these drugs to discriminate between 'healthy' and 'cancerous' tissues, limits their power to attain therapeutic levels. Consistently, non-selective CDKs lead to the toxicities including myelosuppression, diarrhoea, nausea and anaemia [28].

### Second-generation or specific CDK 4/6 Inhibitors

Generally, first-generation pan-CDKs were associated with low-therapeutic index and high toxicities at concentrations required to inhibit their targets. To overcome these limitations, highly specific or selective, second-generation CDK inhibitors including palbociclib (PD-0332991), abemaciclib (LY-2835219) and ribociclib (LEE011) were developed [57]. These CDK 4/6 inhibitors have been widely investigated pre-clinically in *in vitro* and *in vivo* models of different tumor entities including breast cancer, mBC, leukemia, glioma, melanoma, hepatocellular carcinoma, sarcoma, lung adenocarcinoma, ovarian cancer, renal cancer, pancreatic cancer, and prostate cancer. In most of the studies, palbociclib, abemaciclib and ribociclib were found to be highly-selective against 'CDK4' and 'CDK6' with IC<sub>50</sub> values of <40 nM. All three agents inhibit cell-proliferation in Rb-positive cells, expression of protein and transcription of E2F target genes, which ultimately results in G0/G1 arrest. These agents also demonstrated dose-dependent inhibition of growth in tumor-xenograft models [58]. In addition, all the study reports revealed that RB-expression is essential for sensitivity to these CDK inhibitors. CDK 4/6 inhibitors are presently under investigation in >80 clinical-trials. The clinical-trial results of CDK4/6 inhibitors along with

hormone therapy have demonstrated a remarkable improvement in PFS rates in patients with ‘advanced HR+, HER2-negative breast cancer’ as compared to hormone

therapy alone [56]. Based on the encouraging clinical trial results, palbociclib, abemaciclib and ribociclib have` been approved by the USFDA [59-61].



**Fig 2:** Active-site residues of CDK6 bound to (a) Palbociclib, (b) Ribociclib and (c) Abemaciclib

Palbociclib (6-acetyl-8-cyclopentyl-5-methyl-2-[[5-(piperazin-1-yl) pyridin-2-yl] amino]-7H, 8H-pyrido [2, 3-d] pyrimidin-7-one)

**Description:** Palbociclib (also called PD0332991 and IBRANCE, from Pfizer), a pyridopyrimidin derivative was the first of the novel selective-CDK4/6 inhibitor to gain FDA-approval for treatment of ‘HR+MBC’ [54, 62]. It was originally developed by David Fry and Peter Toogood in 2001, though many years have been taken to prove its potential therapeutic value, finally phase-II clinical trials started in 2009 [63, 64]. Its anti-tumor potential was evaluated in some tumor types including; hepatocellular carcinoma, T-cell acute lymphoblastic leukemia (T-ALL), renal cell carcinoma, neuroblastoma, mantle cell lymphoma, myeloma, pancreatic ductal adenocarcinoma, medulloblastoma, NSCLC, melanoma, esophageal adenocarcinoma and broadly in breast cancer [65].

Based on ‘PALOMA-1’ study results, in February 2015, the USFDA granted ‘accelerated approval’ to Palbociclib. In ‘PALOMA-3’ study, higher PFS rates were observed with Palbociclib + fulvestrant and based on this; on February 19, 2016 the USFDA approved ‘palbociclib’ along with ‘fulvestrant’ for treatment of women with ‘HR+, HER2-negative ABC or MBC’ [73]. Palbociclib received ‘regular approval’ on March 31, 2017 for HER2-negative breast cancer, alongside an aromatase-inhibitor [74]. Palbociclib is presently under-investigation in >50 clinical studies including extensive variety of cancer types.

### Ribociclib

**Molecular formula:** C<sub>23</sub>H<sub>30</sub>N<sub>8</sub>O

**IUPAC Name:** 7-cyclopentyl-N, N-dimethyl-2-[[5-(piperazin-1-yl) pyridin-2-yl] amino]-7H-pyrrolo [2, 3-d] pyrimidine-6-carboxamide

**Discription:** Ribociclib (also known as LEE011 and Kisqali, from Novartis), a pyrolo-pyrimidine derivative is another orally administered selective-inhibitor of CDK4/6 to gain US FDA approval to treat some 'metastatic breast cancers' along with an aromatase inhibitor. Similarly to palbociclib; it blocks RB-phosphorylation and ultimately arrests the cell-cycle in various tumors [54, 75]. Furthermore, ribociclib showed anti-tumor activity in xenografts of neuroblastoma (including senescence induction) [76], liposarcoma [77], Ewing sarcoma [78] and rhabdomyosarcoma [79].

The Monaleesa-3 clinical trial of ribociclib (LEE011) along with fulvestrant for treatment of men and postmenopausal women suffering from 'HR+, HER2- ABC' who had received no or only one line of prior endocrine treatment is currently ongoing [84]. Encouraging preliminary clinical-efficacy was also found with triplet-therapy including 'ribociclib, everolimus, and exemestane'. The combination was also found feasible and permits lower dosing of EVE (leads to better tolerability. Further investigations on this study are currently ongoing [85]. Ribociclib received 'Breakthrough Therapy' and 'Priority Review' designations from USFDA in August 2016 and November 2016, respectively. On March 13, 2017, the USFDA granted 'regular approval' to ribociclib along with an 'aromatase inhibitor' as initial endocrine-based therapy for treatment of postmenopausal women with 'HR+, HER2-ABC or MBC' [86]. Ribociclib is presently investigating in >30 clinical trials involving several tumour types.

#### Abemaciclib

**Molecular formula:** C<sub>27</sub>H<sub>32</sub>F<sub>2</sub>N<sub>8</sub>

**IUPAC Name:** N-[5-[(4-ethylpiperazin-1-yl) methyl]pyridin-2-yl]-5-fluoro-4-(7-fluoro-2-methyl-3-propan-2-ylbenzimidazol-5-yl) pyrimidin-2-amine

**Description:** Abemaciclib (also known as LY2835219, from Eli Lilly), a pyrimidine-benzimidazole derivative, structurally different from other inhibitors (such as palbociclib and ribociclib) is third parallel USFDA approved CDK4/6 inhibitor [54]. Abemaciclib inhibit not only CDK4/6 but also CDK9 and PIM1 and eminently displays more noteworthy selectivity for CDK4 compared with CDK6. Similarly to palbociclib and ribociclib, it inhibits RB-phosphorylation and arrest the cell-cycle at G1-phase [87]. Abemaciclib demonstrated anti-tumour activity in xenograft models of colorectal cancer, acute-myeloid leukemia (AML), lung cancer, glioblastoma, mantle-cell lymphoma [88], NSCLC, melanoma [89] and bladder cancer [90]. Abemaciclib also crosses the 'blood-brain barrier' (BBB) and shows prolong survival rate in an intracranial glioblastoma xenograft model [91, 92]. It suggests the potential efficacy of abemaciclib against primary as well as metastatic tumors, involving the CNS. On September 28, 2017, FDA granted approval of abemaciclib treatment under the market name Verzenio for the treatment of HR-positive and HER2-negative advanced or metastatic breast cancer [93].

#### CDK inhibitors in ongoing-clinical trials

Till date, many CDKs other than Palbociclib, Abemaciclib and Ribociclib have been developed and some of them have been patented because of their high-inhibition profiles against CDKs and are investigating thoroughly under clinical trials.

#### Future perspective and conclusion

The translational path to impressively target the cell-cycle has been a long journey from basic science studies to eventual preclinical and then clinical testing. Based on the, frequent dysregulation of cell-cycle pathways in cancer by 'CDK hyper-activation', the CDKs and their regulators have become an attractive set of target for cancer-treatment and several ATP-competitive CDKs are being developed. First-generation pan CDKs, targeting multiple CDKs were developed but not progressed for further phase-II trials because of high toxicity and low therapeutic index. After unsatisfactory outcomes in clinical-trials of non-selective pan CDKs, the significance of specificity and selectivity of drug molecule for particular target now has been broadly accepted and gave a strong impetus for the development and success of second generation CDKs as anticancer agents. Till now, 3 highly-selective CDK 4/6 inhibitors (palbociclib, abemaciclib and ribociclib) have been approved by the USFDA for breast-cancer treatments and have an established safety profile. All these approved inhibitors are being further investigating in ongoing-clinical trials involving an extensive variety of cancer types. Palbociclib, the 'first FDA-approved agent' for treatment of 'HR-positive ABC', demonstrated clinical efficacy in endocrine sensitive, refractory settings and chemotherapy exposed disease. Moreover, its drug combination also confirmed the activity in premenopausal women without compromise in efficacy. Besides the clinical success of Palbociclib, abemaciclib and ribociclib in clinic, it's quite a daunting task to optimize CDKs in clinical practice. Currently, there's an emergence of integrated evaluation of biomarkers of clinical sensitivity and selection of appropriate patient populations is needed to provide the basis for rational-drug combinations. Despite many encouraging preclinical-studies, effectively-designed clinical trials will eventually be needed to characterize the ideal use of CDK4/6 inhibitors for a given tumor. The future of targeted therapy along with established agents is highly encouraging and promises more clinical successes in the complicated field of anticancer drug development through more clear understanding of regulatory mechanisms of the cell-cycle, comprehensive understanding of the intersection of pharmacology and biology of CDKs, incorporation of more specific targeted-agents and ultimately from the results of ongoing trials.

**Table 2:** Some Selected Successful Clinical Trials of Palbociclib.

| Clinical Trial Phase  | Aim of the study   | Condition   | Dose  | Clinical responses   | References |
|---|--|---|---|--|------------|
| Phase I   | To identify the maximum-tolerated dose` (MTD) and dose-limiting toxicities` (DLTs) | Rb-positive advanced malignancies (n=41)  | once daily(QD) for 21 of 28 days  | a) Acceptable-safety profile as monotherapy<br>b) SD in 1 out of 5 pts (patients), that was maintained $\geq 10$ cycles<br>c) DLT was neutropenia observed in 5 patients, other manageable toxicities were nausea, diarrhea and fatigue<br>d) The MTD` and recommended phase-II dose was found` to be 125 mg QD (oral).  | 66,67      |
| phase II  | Assessment of tumor response and tolerability of palbociclib as` monotherapy       | Rb- positive MBC (n=37)   | 125 mg` daily for 21 of 28 days   | a) SD in 5pts for >6 months, PR in 2pts, CBR=19%<br>b) Overall PFS=3.7 months and significant benefit observed in pts with less prior chemotherapy<br>c) Out of 2 patients with HR-positive, HER2-positive disease, 1 had PR and another had stable disease` (SD) for 5 months<br>d) DLT was cytopenia, all grade 3 /4 toxicities were neutropenia`, thrombocytopenia and anaemia  | 68         |
| PALOMA 1 (an open label randomized, phase-II` clinical trial)                     | To analyze the effectiveness of `palbociclib+ letrozole` with `letrozole alone`    | postmenopausal pts with advanced` ER+ and HER2- MBC, no-prior systemic treatment, (n=165) | Letrozole alone (n=81)<br>Vs.<br>letrozole (2.5 mg daily + palbociclib 125 mg. (n=84) | a) PFS=10.2 months for `letrozole group` and 20.2 months for `Palbociclib+letrozole group`<br>b) hazard ratio [HR], 0.488; 95% confidence interval [CI], 0.319–0.748; $P < .001$ )<br>c) Overall survival` (OS) =37.5 months (palbociclib + letrozole group) and 33.3 months (letrozole alone group)<br>d) Neutropenia, fatigue and leukopenia were the most-common adverse events` (AEs) in the Palbociclib+letrozole group | 69         |
| PALOMA 2 Phase-III Clinical trial   | To analyze the effectiveness of `palbociclib+ letrozole` with `letrozole alone`    | postmenopausal pts with no-prior systemic therapy` for ABC (n=666)                        | `P (125mg/d; for 21 of 28 days) + L (2.5 mg/d)` or `Letrozole alone every 28 days`    | a) higher PFS=24.8 months in Patients receiving `letrozole+Palbociclib` as compare to the `letrozole alone` (14.5 months)<br>b) ORR was also higher with Palbociclib (55.3%), CBR` was 84.9% vs. 70.3%<br>c) AEs: Neutropenia (79.5 vs. 6.3%), nausea (35.1% vs. 26.1%) and fatigue (37.4 vs. 27.5%)<br>d) Remarkable clinical benefit and safety of P+L in ER+/HER2–ABC pts was confirmed                                   | 70         |
| PALOMA 3 An international, randomized, multicenter, double blind, phase III study | To compare Fulvestrant + palbociclib Vs. Fulvestrant + placebo                     | Patients with HR+/HER2 negative MBC` who had progressed disease on previous ET, (n= 521)  | Palbociclib+ fulvestrant (n=347)<br>Vs. fulvestrant + placebo (n=174)                 | a) Significantly better median PFS= 9.5 months for `Palbociclib + fulvestrant` vs. 4.6 months for `fulvestrant + placebo`<br>b) Most-common grade3/4 AEs include neutropenia (65% in the fulvestrant+palbociclib group and 1% in the fulvestrant+placebo group), anaemia ([3% vs. 2%), leucopenia (28% vs. 1%)   | 71, 72     |

**Table 3:** Some Selected Successful Clinical Trials of Ribociclib

| Clinical Trial Phase  | Aim of the study   | Condition  | Dose   | Clinical responses  | References |
|---|--|--|--|---|------------|
| Phase I   | To establish the 'MTD' and/or 'RP2D' (recommended dose for expansion) of ribociclib (LEE011) and to evaluate its safety-profile and toxicities | Rb+ advanced solid tumors` and lymphomas, (n=132)  | escalating doses of LEE011 on a 21 of 28 days or continuous schedule   | a) Acceptable safety-profile as monotherapy<br>b) PR in 3 Out of 110 evaluable pts, SD in 24% and 15% of pts (for $\geq 4$ and $\geq 6$ cycles respectively)<br>c) The 'MTD and RP2D' declared as 900 and 600 mg./d on 21 of 28 d schedules, respectively.<br>d) DLTs include: mucositis, pulmonary embolism, neutropenia, asymptomatic thrombocytopenia, QTcF-prolongation` (>500 ms), hyponatremia and increased creatinine.<br>e) All grades` drug-related AEs include neutropenia (40%), leukopenia (36%), nausea (35%), and fatigue (27%). | 80         |
| Phase Ib  | To evaluate the 'tolerability' and 'safety' of ribociclib + letrozole  | Post-menopausal women with ER+, HER2- ABC, (n=47)  | LEE (600mg; QD; 3wks on/1wk off) + LET (2.5 mg QD)   | a) Acceptable safety-profile<br>b) Demonstrated clinical activity, particularly in Treatment Naive group (CR in 1 patient, ORR: 39%, CBR: 73%)<br>c) Drug-related Grade`3/4 AEs were neutropenia` (43%), lymphopenia` (4%), and leucopenia` (2%).   | 81         |
| Phase Ib/II (3-arm study)   | To investigate the combination of ribociclib (LEE), alpelisib (BYL) and letrozole (LET)  | Post-menopausal women with` ER+, HER2- ABC, (n=98)   | LET: 2.5 mg(QD) (continuous) along with escalating-doses of` either 'LEE (QD) (3-wks-on/1-wk-off)' or 'BYL QD (continuous)', or 'both' in 28-day cycles, A1: 'LEE+LET' (41 pts), A2: 'BYL+LET' (21 pts) A3:'LEE+BYL+LET'(36 pts) | a) Acceptable safety-profile and` significant clinical-activity<br>b) In 'A1', 6 pts exhibit known responses: 1 PR, 2 SD, 2 PD and 1 NCRNPD (non-CR, non-PD).<br>c) In 'A2', 5 pts exhibit known responses: SD in 2 and NCRNPD in 3pts.<br>d) In A3, evaluable pts: 27; PR: 7%, unconfirmed PR: 15%, SD: 22%, NCRNPD: 22% and PD: 19%<br>e) The most-common (all grade >35%) study drug-related AEs were: hyperglycemia, nausea, neutropenia and fatigue  | 82,83      |
| Phase III (MONALEESA-2, a 'randomized', 'double-blind', 'placebo-controlled', international clinical trial) | To evaluate the clinical efficacy of ribociclib + letrozole  | post-menopausal women with 'HR+, HER2-negative ABC or MBC' who received no-prior therapy (n=668) | Ribociclib;600mg + letrozole;2.5mg (n=334) or placebo+ letrozole;2.5mg (n=334) once daily, 3-wks-on/1-wk-off)  | a) Significantly Improved PFS rates.<br>b) The estimated median PFS was '14.7 month' in the 'placebo-containing arm'. ORR was 52.7% in the 'ribociclib + letrozole' arm and 37.1% in the 'placebo + letrozole' arm.<br>c) All grade3/4 AEs (reported in >2%) were leukopenia, neutropenia, abnormality in liver functions, lymphopenia, nausea, fatigue and vomiting.   | 75         |



**Table 4:** Clinical Data of abemaciclib

| Clinical Trial Phase  | Aim of the study  | Condition   | Dose  | Clinical responses   | References |
|---|---|---|---|--|------------|
| Phase I<br>'dose escalation study'  | To evaluate the pharmaco-kinetic profile, efficacy and safety of abemaciclib  | Advanced cancer,<br>(n=225)   | Dose escalation: n=33,<br>tumor-specific cohorts for breast cancer: n=92,<br>single-agent therapy (abemaciclib): n=47,<br>NSCLC: n=68, glioblastoma: n=17, melanoma:<br>n=26, colorectal cancer: n=15, and HR-positive<br>breast cancer: n=19, combination treatment with<br>abemaciclib + fulvestrant. | a) The maximum tolerable dose of abemaciclib was found as 200mg. every 12 hrs<br>b) DCR (Disease control rate): [CR+PR+SD] was higher for HR+ tumors (81%) Vs. HR-tumors (33%),<br>c) CBR (CR+PR+SD for $\geq 24$ wks): 61%, Median duration of response (DR): 13.4 mo. (95% CI, 3.7–13.4) and median PFS: 8.8 mo. (95% CI, 4.2–16.0).<br>d) The most-common TEAEs involved leukopenia, neutropenia, anemia, thrombocytopenia, anorexia, nausea, vomiting, diarrhea, fatigue, weight loss, increased creatinine.   | 87         |
| Phase Ib  | Assessment of the safety, efficacy and pharmacokinetics (PK) of abemaciclib along with endocrine or HER2-negative` targeted therapies | Metastatic breast cancer,<br>(n=65)   | Part A: abemaciclib (150-200mg. every 12 hrs) with letrozole (2.5mg/d )<br>Part B: anastrozole (1mg/d),<br>Part C: tamoxifen (20mg/d),<br>Part D: exemestane (25mg/d),<br>Part E: exemestane (25 mg/d) + everolimus (5mg/d),<br>Part F: trastuzumab (6-8mg/kg every 21 days)                            | a) Abemaciclib along with endocrine-therapies demonstrated controllable safety and efficacy.<br>b) DCR` (CR+PR+SD): 67% for PartA+B [`nonsteroidal aromatase inhibitors` (36 pts)] and 75% for Part C [tamoxifen (16 pts)].<br>c) TEAEs ( $\geq 20\%$ overall in `Parts A-D`) were diarrhea, vomiting, nausea, fatigue, abdominal pain, neutropenia, decreased appetite, and anemia.   | 94         |
| MONARCH 1, a phase-II single-arm study  | Assessment of` clinical safety and efficacy of` abemaciclib as `monotherapy`  | HR+/HER2-MBC who had progressed disease on or after endocrine-therapy and chemotherapy<br>(n=132) | 200mg every 12 hrs  | a) The primary endpoint of ORR by investigator assessment at 12 mo was 19.7% (95% CI: 13.3, 27.5) and didn`t change at 18 mo<br>b) At the 18 mo update, 7 pts (5.3%) remained on treatment (6 PR and 1 SD). At 18 mo, median OS` was 22.3 mo (95% CI: 17.7, NR) and the survival rate was 58.7%.<br>c) Grade 3/4 TEAEs include neutropenia (25.0%), diarrhea (19.7%), fatigue (13.6%), leukopenia (6.8%), anemia (4.5%) and nausea (4.5%).<br>d) The results of` 18 month analysis demonstrated more favorable benefit-risk profile of` abemaciclib as single-agent than expected from available chemotherapies. | 95         |
| neoMONARCH a `randomized`, `multicenter`, `open-label phase-II neoadjuvant study` | To compare the effectiveness of abemaciclib + anastrozole Vs. abemaciclib monotherapy Vs. anastrozole monotherapy                     | Early-stage HR+, HER2-BC<br>(n=223)   | Abemaciclib; 150mg, PO; every 12hrs + anastrozole; 1mg/d, PO, [QD] for 2 wks; followed by pts receiving abemaciclib 150mg [PO; Q12H] and anastrozole 1mg [QD] for subsequent 14 wks.  | a) An interim analysis of 9 months results; abemaciclib, given either as `monotherapy` or in `combination with anastrozole` demonstrated significantly ( $p<0.001$ , $n=64$ )` greater suppression of Ki67 as compare to anastrozole alone.  | 96         |
| MONARCH 2, a double-blind Phase 3 trial   | To analyze the safety and effectiveness of `abemaciclib + fulvestrant` with `fulvestrant alone`                                       | HR+/HER2- ABC<br>(n=669)  | abemaciclib or placebo (150 mg; BD) + fulvestrant (500 mg, per label).  | a) Significantly Prolonged PFS with `Abemaciclib+fulvestrant` as compare to `fulvestrant alone` (16.4 v 9.3 months)<br>b) ORR of `abemaciclib+fulvestrant`: 48.1% compared with 21.3% in the control arm.<br>c) Most common AEs include diarrhea (86.4% Vs 24.7%), neutropenia (46.0% Vs 4.0%), nausea (45.1% Vs 22.9%) and fatigue (39.9% Vs 26.9%) in the abemaciclib Vs. placebo arms.  | 97         |

**Table 5:** Some selected ongoing-clinical trials of CDKIs

| <b>Drug Candidate</b>    | <b>Phase</b> | <b>Combination</b>                                     | <b>Condition</b>  | <b>Study Sponsor</b>  | <b>Clinical Trials.gov Identifier</b> |
|--------------------------|--------------|--|---|---|---------------------------------------|
| CYC065                   | Phase I      | -  | Advanced Cancers  | Cyclacel Pharmaceuticals, Inc.  | NCT02552953                           |
| Dinaciclib               | Phase I & II | Pembrolizumab  | ABC or MBC, TNBC  | Jo-Chien University of California, San Francisco                      | NCT01676753                           |
| PD-0332991               | Phase II     | -  | Breast cancer, Ovarian Cancer, Rectal cancer, Adult-solid tumor, Colon cancer, Melanoma, Teratoma                                     | Abramson Cancer Center of the University of Pennsylvania              | NCT01037790                           |
| Palbociclib (PD-0332991) | Phase II     | -  | Metastatic Urothelial Carcinoma (UC)  | UNC-Lineberger Comprehensive Cancer Center, Collaborator: Pfizer      | NCT02334527                           |
| AT7519                   | Phase I      | -  | Advanced Malignant Solid Neoplasm, Metastatic Malignant Solid Neoplasm  | National Cancer Institute (NCI)                                       | NCT02503709                           |
| Palbociclib              | Phase II     | -  | UC  | UNC-Lineberger Comprehensive Cancer Center Collaborator: Pfizer       | NCT02334527                           |
| Dinaciclib               | Phase I      | Epirubicin Hydrochloride                               | ER-Negative, HER2/Neu Negative Male Breast Carcinoma, PR- Negative Recurrent Breast Carcinoma, Stage IV Breast Cancer, TNBC           | NCI   | NCT01624441                           |
| Dinaciclib               | Phase I & II | Ofatumumab   | Chronic Lymphocytic Leukemia, Pro-lymphocytic Leukemia, Recurrent Small Lymphocytic Lymphoma, Refractory Chronic Lymphocytic Leukemia | NCI   | NCT01515176                           |
| Dinaciclib               | Phase I      | MK2206   | Pancreatic Cancer   | NCI   | NCT01783171                           |
| Dinaciclib               | Phase I      | Bortezomib & Dexamethasone                             | Recurrent Plasma Cell Myeloma   | NCI   | NCT01711528                           |
| Dinaciclib SCH 727965    | Phase II     | -  | Stage IV Melanoma   | NCI   | NCT00937937                           |
| Trilaciclib              | Phase II     | Gemcitabine & Carboplatin                              | Triple-Negative Breast Neoplasms, Breast Neoplasm, Breast Cancer, TNBC  | G1 Therapeutics, Inc.   | NCT02978716                           |
| Ribociclib               | Phase I & II | -  | High-Grade Glioma, Diffuse Intrinsic-Pontine Glioma, Bithalamic High Grade Glioma   | Children's Hospital Medical Center, Cincinnati Collaborator: Novartis | NCT02607124                           |
| Palbociclib              | Phase I & II | Binimetinib  | Lung Cancer   | Dana-Farber Cancer Institute, Collaborators: Pfizer, Array BioPharma  | NCT03170206                           |
| PD-0332991               | Phase I      | 5-FU & Oxaliplatin                                     | Advanced Solid Tumor Malignancies   | Georgetown University, Collaborator: Pfizer                           | NCT01522989                           |
| PD0332991                | Phase II     | -  | Ovarian Epithelial Carcinoma  | Jonsson Comprehensive Cancer Center                                   | NCT01536743                           |
| PD0332991                | Phase II     | Anastrozole & Goserelin                                | Breast Neoplasms  | Washington University School of Medicine                              | NCT01723774                           |
| Trilaciclib              | Phase I & II | Carboplatin, Placebo & Etoposide                       | SCLC  | G1 Therapeutics, Inc.   | NCT02499770                           |
| LY2835219                | Phase I      | Fulvestrant  | Advanced cancer   | Eli Lilly and Company   | NCT01394016                           |
| Abemaciclib (LY2835219)  | Phase III    | Fulvestrant & Placebo                                  | Breast Neoplasm   | Eli Lilly and Company   | NCT02107703                           |
| LEE011                   | Phase I      | Letrozole & BYL719                                     | ER+ ABC   | Novartis Pharmaceuticals  | NCT01872260                           |
| LEE011                   | Phase I & II | BYL719 & fulvestrant & BKM120                          | ABC   | Novartis Pharmaceuticals  | NCT02088684                           |
| LEE011                   | Phase III    | Tamoxifen, Letrozole, Anastrozole, Goserelin & Placebo | ABC or MBC  | Novartis Pharmaceuticals  | NCT02278120                           |
| PD 0332991               | Phase I      | bortezomib   | MCL   | Weill Medical College of Cornell                                      | NCT01111188                           |

|                          |                |                                      |  |   |             |
|--------------------------|----------------|--------------------------------------|--|---|-------------|
|                          |                |                                      |  | University, Collaborators: Millennium Pharmaceuticals, Inc., Pfizer   |             |
| Ribociclib               | Phase I        | Tamoxifen & Goserelin                | Breast Cancer  | H. Lee Moffitt Cancer Center and Research Institute, Collaborator: Novartis                                   | NCT02586675 |
| Abemaciclib              | Phase I        | -                                    | Diffuse Intrinsic Pontine Glioma, Brain Tumor, Solid Tumor, Ewing-Sarcoma, Neuroblastoma, Rhabdomyosarcoma, Rhabdoid Tumor Osteosarcoma, | Emory University  | NCT02644460 |
| Palbociclib              | Phase II       | -                                    | Metastatic Malignant Neoplasm to Brain   | Massachusetts General Hospital<br>Collaborator: Pfizer  | NCT02896335 |
| Palbociclib (PD-0332991) | Phase III      | Placebo                              | Breast Cancer, HR-Positive, Her2-normal Postneoadjuvant Treatment With CDK 4/6 Inhibitor CPS-EG Score                                    | German Breast Group, Collaborators: Pfizer, AGO Study Group, NSABP Foundation Inc, Breast International Group | NCT01864746 |
| Ribociclib               | Phase III      | Placebo & Adjuvant endocrine therapy | Breast Cancer  | Novartis Pharmaceuticals  | NCT03078751 |
| Palbociclib              | Phase II & III | -                                    | Recurrent Squamous Cell Lung Carcinoma, Stage IV Squamous Cell Lung Carcinoma  | Southwest Oncology Group in Collaborators: National Cancer Institute (NCI)                                    | NCT02785939 |

\*Clinical trial data obtained from ClinicalTrials.gov. <sup>[98]</sup>

## Abbreviations

**CDK:** Cyclin Dependent Kinase  
**CDKIs:** Cyclin Dependent Kinase Inhibitors  
**RB:** Retinoblastoma  
**CIP:** CDK Interacting Protein  
**KIP:** Kinase Inhibitory Protein  
**PLK:** Polo-Like Kinase  
**FOXM1:** Forkhead Box Protein M1  
**CHK:** Checkpoint  
**AML:** Acute Myeloid Leukemia  
**BC:** Breast Cancer  
**TNBC:** Triple Negative Breast Cancer  
**HR+:** Hormone Receptor Positive  
**ER+:** Estrogen Receptor Positive  
**HER:** Human Epidermal Growth Factor Receptor  
**ALL:** Acute Lymphocytic Leukemia  
**MCL:** Mantle Cell Lymphoma  
**NSCLC:** Non-Small Cell Lung Cancer  
**SCLC:** Small Cell Lung Cancer  
**ABC:** Advanced Breast Cancer  
**CLL:** Chronic Lymphocytic Leukemia  
**UC:** Metastatic Urothelial Carcinoma  
**MBC:** Metastatic Breast Cancer  
**AEs:** Adverse Events  
**TLS:** Tumor lysis syndrome  
**TEAE:** Treatment-Emergent Adverse Events  
**MTD:** Maximum Tolerated Dose  
**OS:** Overall Survival  
**PFS:** Progression Free Survival  
**CR:** Complete Response  
**PR:** Partial Response  
**SD:** Stable Disease  
**PD:** Progressive Disease  
**DCR:** Disease Control Rate  
**CBR:** Clinical Benefit Rate  
**DR:** Duration of Response  
**HR:** Hazard Ratio  
**ORR:** Objective Response Rate  
**USFDA:** United States Food & Drug Administration

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