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Review

Cyclin-Dependent Kinase 4/6 Inhibitors: A Potential Breakthrough Therapy for Malignancies of Gastrointestinal Tract

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Abstract. *Cancer is the leading cause of death worldwide for which effective treatments remain limited. This article aimed to critically review and discuss the potential of targeting cell cycle machineries as a vital tool for cancer treatment. Cyclin dependent kinase (CDK) 4/6 inhibitors were originally approved by the United State Food and Drug Administration (US FDA) for advanced-stage breast cancer treatment. The nearly double-prolonged survival time in patients who received CDK4/6 inhibitors are superior to the conventional chemotherapy or endocrine therapy alone and, thus, these medications have been designated a breakthrough therapy by the US FDA. The requirement of CDK4/6 in the progression of cancer cells, but probably dispensable in normal cells, makes CDK4/6 a popular target for cancer treatment. The effects of CDK4/6 inhibitors in cancer may also involve the tumor microenvironment in which the therapeutic effects are synergistically pronounced. These emerging roles, hence, prompt investigations regarding their therapeutic potential in other cancers, including gastrointestinal cancer. Many preclinical and clinical studies of CDK4/6 inhibitors in gastrointestinal cancers are underway and, as a result, several new potentials are gradually reported. Contrariwise, the primary effect of this drug group is arresting the cell cycle rather than inducing cell death. The efficacy of using CDK4/6 inhibitors as a single regimen in clinical practice is then limited. In this article, the effects of CDK4/6 inhibitors on the progression of gastrointestinal cancers, at both preclinical and clinical levels are reviewed. The future directions for research and the possibility of CDK4/6 inhibitors being "breakthrough therapy" for gastrointestinal cancers are also discussed.*

Cancer is recognized as a leading cause of death worldwide, whereas effective treatments are underway and yet to be developed for malignancies in certain organs (1). Cancer cells possess malignant hallmarks that differentiate them from their normal counterparts in the same tissues (2). The abnormality at the genetic or epigenetic levels results in cellular transformation and gain the ability to compete and invade their surrounding tissues. Limitless replication is one of the cancer hallmarks (2). Cancer cells gain this ability through various molecular mechanisms, *e.g.*, amplification of proliferation-associated genes, autoactivation of growth signaling pathways, and the mutation of cell cycle inhibitor genes (3). Although these mechanisms benefit cancer cells to survive, it could be a double-sided sword for their survival. The requirement of unnatural overexpression of genes or proteins makes cancer cells dependent on those oncogenes and this dependency becomes their Achilles' heel.

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Targeting overexpressed and requisite proteins for cancer cell proliferation then turns into a hotspot of research to figure out a new therapeutic target to cure cancer (3, 4).

The cell cycle is a vital process and is highly regulated to maintain homeostasis. The main regulatory proteins contributing to cell cycle progression are cyclins and cyclin-dependent kinases (CDKs) (4). Different cyclins are responsible in each phase of the cell cycle so that the expression and degradation of cyclins are the dynamic processes throughout the cell cycle and are controlled like a cascade of regulation. To function as a cell cycle regulator, cyclins need to couple with CDKs; a serine/threonine kinase capable of phosphorylating other proteins that are involved with driving of the cycle. The early step in cell cycles starts when D-type cyclins (cyclin D1, cyclin D2, and cyclin D3) are expressed after cells are activated by growth signaling and those D-type cyclins in turn activate CDK4/6 which further phosphorylates the retinoblastoma protein (RB) (Figure 1). After RB is phosphorylated, the constraint E2F transcription factor releases and activates the transcription of genes that are needed for further phases, namely cyclin E and other CDKs (5). As D-type cyclins are gate keepers for the cell cycle, many cancers are frequently found with the aberrant D-type cyclin expression (3). An example is breast cancer in which *CCND1* genes encoding for cyclin D1 protein are largely amplified (6). This makes a group of breast cancer cells become more dependent on cyclin D1 for their proliferation while cyclin D1 is dispensable for normal mammary epithelial cells (7, 8). As aforementioned, the function of CDK4/6 is dependent on the availability of D-type cyclins for which it is required for breast cancer cells. Therefore, the development of an anticancer drug is focused on targeting the kinases which are considered druggable (9). The development of CDK4/6 inhibitors has been successful for prolonging the survival time of hormone receptor-positive breast cancer patients and pave the way for the study in other solid tumors including gastrointestinal cancers.

Gastrointestinal cancer is referred to as a malignancy of the gastrointestinal tract and the accessory organs, including the esophagus, stomach, pancreas, hepatobiliary tract, small intestine, large intestine, and anus (10, 11). Gastrointestinal cancer accounts for approximately a quarter of global cancer incidence and causes over one-third of cancer related mortality (10). The major gastrointestinal organs with high incidences of cancers are colorectum, stomach, liver, pancreas, and esophagus (10). The curative treatment in the early stage of each cancer is surgical resection complemented with other modalities such as targeted therapy, chemotherapy, and radiotherapy. The successful rate, however, remains low in some cancers, *e.g.*, pancreas, and in most cancers at the advanced stage. Since CDK4/6 inhibitors are appraised as breakthrough therapy in advanced-stage breast cancer which is associated with the dependency on D-type cyclins for their proliferation, the requirement of D-type cyclins for proliferation of other cancers, thereby, also holds a promise to be a potential target for therapeutic improvement. In this article, the progression of CDK4/6 investigations both at the preclinical and clinical levels in each gastrointestinal cancer are reviewed and discussed.

Esophageal Cancer

The global incidence of esophageal cancer ranks seventh and this cancer is the sixth leading cause of death from cancer (1). Two major histological subtypes of esophageal cancer are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Globally, 87% of all esophageal cancers are ESCC while 11% constitute EAC. The prevalence of ESCC is particularly high in East Asia, Eastern and Southern Africa, and Southern Europe, where it is associated with nutritional deficiencies, nitrosamines, spicy diets, hot beverages, heavy drinking, and smoking. On the other hand, EAC is much more common in North America and other parts of Europe, in which Barrett's esophagus, excess body weight, gastroesophageal reflux disease, are considered key risk factors (12). Although multiple approaches are implemented in the treatment of esophageal cancer; including esophagectomy, chemotherapy, radiotherapy, and immunotherapy, the overall survival is still limited. The overall 5-year survival rate is only 20% (13). Thus, novel effective strategies are urgently needed to improve treatment outcomes.

CDK4/6 inhibitors showed anti-tumor effects in a few preclinical studies. Overexpression of CDK4 and CDK6 have been reported in esophageal cancer (14). One study demonstrated that *in vitro* abemaciclib treatment led to decreased proliferation and increased apoptosis in 3 EAC cell lines, OE19, OE33, and FLO1. The induction of EAC in rats by esophagojejunostomy showed that 78.9% of abemaciclib-treated animals had more than 20% decreased tumor volume (15). The study of Li *et al.* determined the expression of CDK4/6 in 7 ESCC and 6 EAC cell lines and found that most of the tested cell lines have the overexpressed CDK6 (16). After knocking-down CDK6, the colony formation ability decreased dramatically. In addition, another CDK4/6 inhibitor, palbociclib, was confirmed for targeting the kinase activity, delaying cell cycle progression at the G1-S boundary, and suppressing proliferation and anchorage independence of EAC cells through activation of the RB pathway (14).

A phase II trial of palbociclib in advanced esophageal cancer was also conducted (17). Thirteen patients with RB-intact esophageal cancer; 8 patients with EAC, and 5 patients with ESCC, were treated with 125-mg daily palbociclib for days 1-21 of 28-day cycles. The results showed no objective

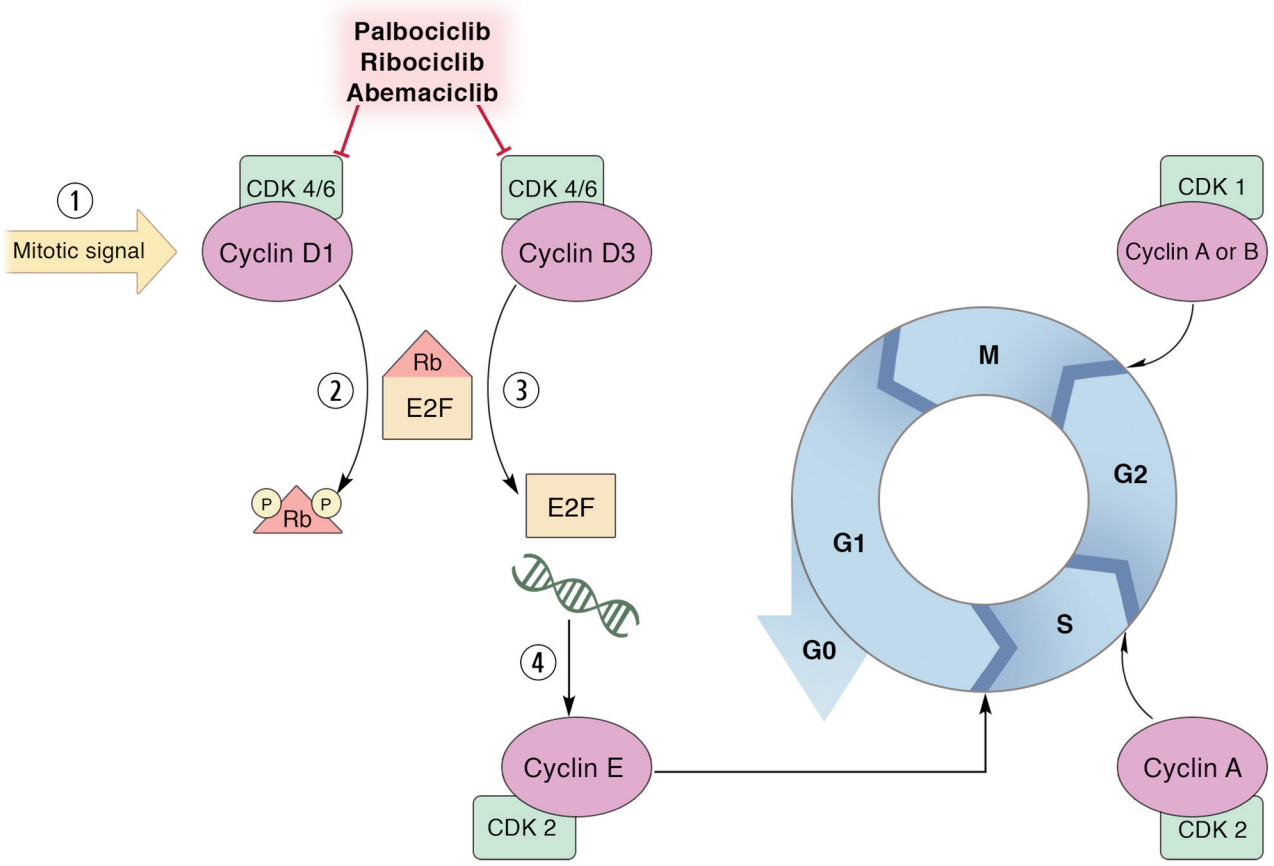

Figure 1. *The effect of cyclin dependent kinase (CDK) 4/6 inhibitors. CDK 4/6 inhibitors, i.e., palbociclib, ribociclib, abemaciclib, inhibit the kinase function of the CDK4/6 when they are bound with their cyclin partners (D-type cyclins) and then as they prevent the phosphorylation of retinoblastoma protein (RB). Hypo-phosphorylated RB thus restrains E2F transcription factor and prevents the transcriptional activities that resulted in arresting the cell cycle at the* G_1 *phase.*

responses. In another phase I study of palbociclib in the Japanese patients, one esophageal cancer patient with palbociclib monotherapy at the 125-mg dose experienced stable disease >24 weeks (18). In a recently completed phase II trial of palbociclib in patients with advanced or metastatic esophageal and/or gastric cancer, however, neither complete nor partial responses were observed (NCT01037790) (17). Only 6 of 19 (31.6%) patients showed stable disease. In the meantime, among other CDK4/6 inhibitors, ribociclib, was also studied in another phase I clinical trial as a single agent in Japanese patients with advanced solid tumors. Among those, 9 patients with esophageal cancer were treated with 400/600 mg ribociclib on 3-weeks-on/1-week-off dosing schedules. Nonetheless, no patient in the study achieved complete or partial response, with only one patient experienced stable disease for ≥5 cycles (19). Given the limited efficacy demonstrated in these studies, CDK4/6 inhibitor monotherapy is not warranted. An ongoing trial of abemaciclib with the PD-1 inhibitor pembrolizumab in gastroesophageal cancers, however, may provide more details about the efficacy of combination strategies (NCT03997448) (20).

Gastric Cancer

Gastric cancer (GC) remains an important problem worldwide and its incidence ranks fifth and mortality ranks fourth (1). The reported incidence rates are highest in Eastern Asia and Eastern Europe. Although multiple treatments have been made in GC, the 5-year survival rate of patients with advanced GC is still less than 15%. Thus, novel drugs and new therapeutic targets are needed for GC patients. Previous studies suggested aberrant expression of Cyclin D-CDK4/6 in GC (21) as possible targets. The importance of cyclin D1 for GC cell proliferation and invasiveness was recently reported (22). Thus, the administration of CDK4/6 inhibitors might be another alternative approach that could improve the therapeutic outcome for GC patients.

The preclinical results of the anti-tumor efficacy of CDK4/6 inhibitors in GC are promising. A recent study demonstrated that palbociclib could significantly inhibit cell proliferation and induce cell senescence, cell cycle arrest, and apoptosis in human GC cell lines; AGS and HGC-27, by regulating the Notch pathway (23). The study by Wang *et al.* showed that human GC cell lines, namely, AGS, KATO-III, NCI-N87, and HS746T, treated with palbociclib exhibited cell cycle arrest in G_1 phase and decreased number of cells in the G_2/M. Similar to other studies, palbociclib inhibited cell proliferation *via* modulation of the cell cycle, and numerous signaling pathways, including p53, PI3K/AKT, Ras-ERK, JNK/MAPK, Wnt/β-catenin, and Smad (24). Palbociclib-induced autophagy and senescence also play crucial roles in decreasing cell proliferation in GC (25).

The clinical efficacy of palbociclib in GC, however, was very limited. A phase II clinical trial of palbociclib in 5 patients with advanced GC failed to demonstrate relevant anti-tumor effect (17). Although the efficacy of palbociclib monotherapy was not encouraging, the combination of CDK4/6 inhibitors with other agents becomes more interesting. Another phase II non-randomized, single arm, open label study of abemaciclib in combination with pembrolizumab in patients with unresectable or metastatic GC who have received at least two lines of prior therapy is ongoing (NCT03997448). The results of this trial may allow the further clinical investigation and the elucidation of relevant mechanisms of the action (20).

Liver Cancer

The most prevalent primary liver cancer is hepatocellular carcinoma (HCC) which accounted for 75-80% of cases (1). The second most prevalent type of liver cancer is the malignancy of intrahepatic bile duct epithelial cells, so called cholangiocarcinoma. HCC is the sixth most common malignancy and has been in the top five leading causes of cancer-related deaths for decades (26, 27). Several studies showed that D-type cyclins and CDK4/6 are essential for the proliferation of HCC cells and associated with poor prognosis of patients (28). The aberrant regulation of the cyclin D-CDK4-RB pathway was reported in up to 73% in HCC cases (29). Thus, targeting CDK4/6 is a promising approach for the treatment of HCC and many studies showed the consistent effectiveness of CDK4/6 inhibitors.

CDK4/6 inhibitors, namely palbociclib, show a very potent effect on HCC cell cycle arrest as similar as to that seen in estrogen receptor positive breast cancer cells. The major effect involves the cyclin D-CDK4/6-RB pathway in which a positivity of RB expression is a crucial factor for drug response (30). Given that RB loss or impairment is found in less than 30% in HCC, the possibility of using palbociclib for HCC treatment is sound. Palbociclib also exerted a synergistic effect on HCC cell inhibition when combined with sorafenib, a pan-kinase inhibitor approved for targeted therapy in HCC. The effect of palbociclib, however, was reversible after pausing the drug. Thus, the 3-week on and 1-week off regimen used in patients with breast cancer might not be effective for HCC and patients might have high risk to develop adverse effects of palbociclib. The other studies also supported that combination of palbociclib with sorafenib exerted a very potent effect probably *via* modulation of multiple pathways. The combination of other pathway inhibitors such as BAY-117082, an inhibitor of the nuclear factor-kB (NF-kB) pathway, showed a satisfactory effect (31). The inhibitory effects of palbociclib may also be involved in more than the cell cycle regulations *via* CDK4/6 inhibition (32). The study of Hsieh *et al.* found that palbociclib could activate AMP-activated protein kinase and inhibited protein-phosphatase 5 which resulted in the induction of autophagy and apoptosis of HCC cells *in vitro* and *in vivo* (33). These effects remained in palbociclib-treated HCC cells even if CDK4/6 were knocked down, suggesting some CDK4/6 independent effects of palbociclib. The inhibitory effects of ribociclib and abemaciclib, another two CDK4/6 inhibitors, however, were diminished in CDK4/6 depleted cells which, in contrast, suggested palbociclib specific effects. All these studies suggested that combined palbociclib with an appropriate kinase inhibitor should synergize the effect of palbociclib and was better than using either drug alone.

Apart from palbociclib, another two CDK4/6 inhibitors also exerted an effective inhibition on the growth of HCC cells. Abemaciclib showed a potent effect on cell cycle arrest similar to palbociclib (34). Inhibitory effects of ribociclib on HCC cell proliferation are also promising; nonetheless, this drug is probably highly effective in cells with a high expression of RB and low p16 proteins (28). Ribociclib also synergized the effect of infigratinib, a fibroblast growth factor receptor (FGFR) inhibitor, and reversed the resistant phenotype of HCC cells to infigratinib (26). As infigratinib is highly selective to FGFR in HCC cells, the combination of this drug with ribociclib is then anticipated as an alternative treatment for HCC with overexpression of FGFR.

Preclinical studies reported that CDK4/6 inhibitors are promising for HCC treatment, however, clinical studies are still lacking at the present time. More clinical investigation is needed to ascertain the efficacy for the practice. Since the window of toxicity is known from studies in other cancers, the results of clinical trials may be available in a near future and the direction of HCC treatment will be therefore clarified.

Biliary Tract Cancer

Biliary tract cancer includes the malignancy of gallbladder and bile duct epithelia in which the latter is also called

cholangiocarcinoma (CCA). CCA is considered relatively rare, and the prevalence is approximately 3% of all gastrointestinal cancer. The prevalence of CCA, however, is particularly in high in the Southeast Asian countries where the carcinogenesis is closely associated with parasitic infections. Intrahepatic CCA is sometimes classified as primary liver cancer in which the prevalence has accounted for 30% of liver cancer. Intrahepatic CCA, however, naturally differs from hepatocellular carcinoma and even possesses heterogenous biology between the intrahepatic and extrahepatic subtypes. This results in difficulty of developing therapeutic strategies and makes this disease dismal and fatal.

The study of Sitthithumcharee *et al*. (35) showed that the CCA cells overexpressed cyclin D1 and thus makes the cancer cells depend on the activities of CDK4/6 for their proliferation. The treatment of cells with CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) thus induced cell cycle arrest and cellular senescence. The inhibitory effects were also shown in patient-derived xenografts. The major affected pathways are RB-dependent, and the pharmacological efficacy is associated with the *KRAS* mutation signature. The increased therapeutic efficiency of palbociclib was also shown in the combination with the pan-mTOR inhibitor, MLN0128, in another study (36). Greater effects of CDK4/6 inhibitors were shown in the combination with the inhibitors of other pro-carcinogenic pathways, namely Wnt/β-catenin and Notch 3 (37). Since the primary effect of palbociclib is to arrest the cell cycle which limits the efficacy of cancer treatments, the combined treatment with MLN0128 which promotes CCA cell apoptosis thus provides a new hope for CCA treatment. Not only pan-mTOR inhibition, the inactivation of focal adhesion kinase (FAK) and CDK4/6 by a combination of FAK inhibitor, PND1186, and palbociclib also showed a greater effect (38). In addition to a combination of CDK4/6 inhibitors and other chemical agents, palbociclib also sensitized CCA cells to radiotherapy (39). The underlying mechanism is by inhibiting the repairing of DNA damage after radiation and, hence, promote cells to apoptosis.

The preclinical studies of CDK4/6 inhibitors, especially palbociclib, showed promising roles of this drug group for CCA treatment. The clinical study, however, is limited and no data are available to date. Up to this current search, the study of CDK4/6 inhibitors for the treatment of gallbladder cancer was not available. Altogether, the investigations on effects of CDK4/6 inhibitors for biliary tract cancers are relatively limited compared with other gastrointestinal cancers and more studies are needed to guide the direction of future practice in this cancer group.

Pancreatic Cancer

Pancreatic cancer (PC), especially pancreatic ductal adenocarcinoma (PDAC), is one of the deadliest cancers with a very poor prognosis. The 5-year survival rate of patients with PC who have standard chemotherapy is less than 10% (40). Most PC harbor mutations of *KRAS* which is a regulating pathway of cell cycle machinery expressions and of *CDKN2A*, is a gene encoding p16 protein that acts as a natural cell cycle inhibitor. It is approximated that PC harboring these mutations are comprised of more than 90% of cases (41). Therefore, the study of cell cycle inhibitors, especially CDK4/6 inhibitor which has been approved for some cancers are then of the great interest. Many studies, both preclinical and clinical trials, are attempting to determine the effectiveness of using this drug group.

Palbociclib, the first CDK4/6 inhibitor approved by the US FDA, has been studied in PC for a decade. In 2012, Liu *et al*. (42) found that PD-0332991 (which was later approved in a generic name of palbociclib) exerted a potent anti-proliferative effect on PC cells. The treatment using palbociclib, however, could induce epithelial-mesenchymal transition (EMT) and probably increase metastatic ability of PC by activating the transcriptional activities of the SMAD4 pathway. This resulted in the concern for using palbociclib for PC treatment. The same group of authors then applied SB505124 which is the inhibitor of type-I transforming growth factor-β receptor to block the EMT. The better inhibitory effect on PC cells after combination with SB505124 was observed compared with using palbociclib alone. The later studies of CDK4/6 inhibitors were carried forward and they found what might provide only a modest effect if they were used as single agents (43). In addition, CDK4 expression itself could not be a good predictor for the response to CDK4/6 inhibitor (40). The anti-proliferative effects were more pronounced when CDK4/6 inhibitors were combined with a certain chemotherapy (44). The importance of selecting the right chemotherapeutic drug was reported by the study of Kumaraswamy *et al*. (43). The combination of palbociclib and taxane resulted in a satisfactory anti-tumor effect. The administration of palbociclib at the same time with gemcitabine conversely ablated the effects of both drugs compared with the single agent treatment (43). These effects were suspected to be the mechanistically result of G_1 arrest by palbociclib which protected PC cells form undergoing the S phase which is the point of gemcitabine's action. The in-depth mechanisms were then recently discovered by Savador-Babero *et al*. (45, 46) in that the sequence of applying chemotherapeutic drugs and CDK4/6 inhibitors mattered. CDK4/6 inhibitors should be given after the treatment of chemotherapy as it then allowed the cells to undergo the primary effects of each chemotherapeutic drug, *e.g*., breaking of DNA strands and inhibiting the microtubule polymerization and de-polymerization. In addition to the cell cycle arrest of chemotherapeutic-treated cells re-entering the cell cycle, CDK4/6 inhibitors also prevented the repairing of DNA that had been bombarded by the chemotherapeutic drug

resulting in cell apoptosis. The sequence of administration of CDK4/6 inhibitor for patients, hence, needs to be taken into the account, otherwise the results of combination could be worsened and distort the clinical outcome.

The results of combining palbociclib with the other agents rather than those chemotherapeutic drugs are also promising. The co-treatment of PC cells with an mTOR inhibitor demonstrated a great effect *in vitro* and *in vivo*. The administration of the mTOR inhibitor also prevented the resistance to CDK4/6 inhibitors which is likely an adaptive ability for PC cells carrying *KRAS* mutation (47). The combination of palbociclib with trametinib, a MEK inhibitor, also showed a potent inhibitory effect on PC growth *in vivo* (48). This combination also increased the infiltration of antigen presenting cells and cytotoxic T cells into the PC tumor microenvironment. Therefore, adding the immunotherapeutic agents such as anti-PDL1 to the group of the combination of palbociclib and trametinib resulted in a super-synergistic effect. The phase I clinical trial of palbociclib and trametinib combination also showed a suggestive result. Among 9 patients with solid cancers, 2 patients with PC receiving palbociclib-trametinib had a partial remission (49). As this study only recruited a limited number of patients, more investigation is needed to clarify the actual benefit. Studies of the inhibitory effects of palbociclib are ongoing as well as the discovery of new mechanisms (50) and the development of drug delivery studies are also reported (51).

Apart from palbociclib, another two CDK4/6 inhibitors; abemaciclib and ribociclib, are also reported for their therapeutic aims in PC. Abemaciclib exerted a potent effect on the inhibition of the PDAC xenograft model and the effects were greater when combined with the inhibitor of HuR (ELAVL1), a positive regulator of cyclin D1, and Yes-associated protein 1 (YAP1) (51). The combination of ribociclib with MEK162, a MEK inhibitor (53), or sorafenib, a pan-kinase inhibitor (41), also showed an effective inhibition of PC growth and increased apoptosis of PC cells. In a phase I clinical trial of an add-on of ribociclib and everolimus, an inhibitor of mTOR pathway, compared to the standard chemotherapy of gemcitabine and 5-fluorouracil, the results did not show any satisfactory effect (54). Among 21 patients receiving add-on combination of ribociclib and everolimus, only 2 of them had stable disease whereas the rest of them did not respond well. Therefore, more clinical studies are extremely needed to clarify how benefit the patients can get compared with the risk of developing the adverse effects of multiple drug administration.

Colorectal Cancer

Colorectal cancer (CRC) is one of the most problematic cancers for public health worldwide. In 2020, the incidence of CRC ranked third whereas the mortality ranked second in both males and females (1). Curative-intent surgery and a standard chemotherapy regimen provided a satisfactory result, however, the recurrence and the resistance to chemotherapy remained. One of the oncogenic driver genes frequently found mutated in colorectal cancer is *KRAS*. The frequency of *KRAS* mutation in colorectal cases was reported up to 50% (55). The development of targeted therapy, hence, aims to target Ras associated pathways and its downstreams, *e.g.*, Raf, MEK, ERK. Although, CRC with the *KRAS* mutation responds well to the inhibitors of the extracellular signal-regulated kinases (ERK) pathway, a subfamily of mitogen-activated protein kinase (MAPK), when modest effects were observed in some models of preclinical studies which may not suffice for clinical translation. Because a major group of patients with CRC are likely to benefit from targeting ERK downstream, the therapeutic agent synergizing the effects of ERK pathways inhibitors are then of interest to develop a novel treatment regimen.

Several studies have shown that *KRAS* mutation signatures of CRC respond well to palbociclib treatment as the ERK pathway influenced by Ras mutation directly involves the expression of cell cycle regulatory proteins. The expression of D-type cyclins and the transcription factors that regulate cell mitosis such as forkhead box M1 (FOXM1) are under the regulation of ERK (56). Reciprocally, CDK4/6 is also reported to control FOXM1 stability by their kinase functions (57). The combination of MEK inhibitors; namely trametinib (58), PD0325901 (56), and palbociclib, thereby, exerted the synergistic effects on growth inhibition of CRC *in vitro* and *in vivo*. The super-synergistic effects on inhibiting cell proliferation were observed when MEK inhibitor and palbociclib were given in combination with cetuximab, a monoclonal antibody targeting epidermal growth factor receptor (EGFR) (59). In this particular circumstance, palbociclib can also induce apoptosis of CRC cells. For instance, in hypoxic conditions, CRC cells developed hypoxia-resistance by upregulation of the transcription factor hypoxia-inducible factor 1α (HIF1α) (60). The treatment of palbociclib downregulated HIF1α expression and also affected other pro-proliferative and pro-survival pathways such as ERK and glycogen synthase kinase 3β (GSK3β). Moreover, palbociclib also synergized the effect of the chemotherapeutic drug, irinotecan, and increased apoptotic cell death of CRC. The synergistic effects are not only reported with chemotherapeutic or targeted therapeutic drugs. Palbociclib also exerted synergistic effects with radiotherapy in CRC cells with wild type p53 (61). A few studies, however, showed that not all *KRAS* mutated CRC responded well to palbociclib. A specific mutation of *KRAS* at p.G12D made CRC cells less sensitive to palbociclib (62). The other factors, such as high p27 expression, could compromise the inhibitory activity of palbociclib in CRC (63). Although

Table I. *Published clinical trials of cyclin-dependent kinase 4/6 inhibitors in gastrointestinal cancer.*

Study (Author, year of publication, Ref.)	Region of study	Cancer type	Therapeutic regimen	Key outcomes
Tamura *et al*., 2016 (18)	Japan	3 Colorectal cancers 1 Esophageal cancer 1 Pancreatic adenocarcinoma	Palbociclib monotherapy (Phase I)	1/3 with colorectal cancer had stable disease 1/1 with esophageal cancer had partial response 1/1 pancreatic cancer had progressive disease
Doi *et al*., 2017 (19)	Japan	9 Esophageal squamous cell carcinomas	Ribociclib monotherapy (Phase I)	1/9 esophageal had stable disease
Karasic *et al*., 2020 (17)	United States	5 Gastric adenocarcinomas 3 Gastroesophageal junction adenocarcinomas 8 Esophageal adenocarcinomas 5 Esophageal squamous cell carcinomas	Palbociclib monotherapy (Phase II)	No objective response
Weinberg *et al*., 2020 (53)	United States	12 Metastatic pancreatic adenocarcinomas	Ribociclib plus everolimus (mTOR inhibitor)	2 patients had stable disease
Kato *et al*., 2021 (49)	United States	6 Pancreatic adenocarcinomas 1 Colorectal cancer 1 Gastrointestinal stromal tumor	Palbociclib plus trametinib (MEK inhibitor) (Phase I)	2/6 pancreatic adenocarcinoma had partial remission 2/6 pancreatic adenocarcinoma had stable disease 1/1 colorectal cancer had stable disease 1/1 gastrointestinal stromal tumor had stable disease

MEK: Mitogen-activated protein kinase kinase; mTOR: mammalian target of rapamycin.

promising data suggests palbociclib as a good candidate for CRC treatment, more investigation is needed to clarify who will benefit from the treatment. The discovery of the molecular signature of palbociclib response may assist in clinical judgement to determine candidates who would have maximal benefit over the risk of adverse drug reaction.

Apart from palbociclib, another CDK4/6 inhibitor, abemaciclib, is also effective for CRC treatment both in KRAS and BRAF mutations. The synergistic effect was pronounced when abemaciclib was combined with LY3009120, a pan-raf inhibitor (64). Using machine learning to identify the prognostic signature of colon adenocarcinoma, Linares-Blanco *et al*. found that several genes are potential candidates for therapeutic targeting including fatty acid binding protein 6 (FABP6) (65). The *in silico* molecular docking revealed that FABP6 strongly interacted with abemaciclib. This suggested the novel potential strategies to improve the efficacy of abemaciclib. Biological validation is still lacking and needs further study. Altogether, the CDK4/6 inhibitors are promising for therapeutic aims in a large proportion of patients with *KRAS* mutated CRC but findings of biomarkers for drug responses are needed with more clinical studies and are required to make appropriate decisions.

Prospective and Future Direction of Research

Although CDK4/6 inhibitors are approved for use in some cancers and hold a very high promise for use in the GI cancers, information at present is still limited. Studies at the preclinical level showed great inhibitory effects on GI cancer cell proliferation, but in clinical studies, only a modest effect was observed. The key clinical trials that may suggest the future direction of the study of CDK4/6 inhibitors on GI cancers are summarized in Table I. Some CDK4/6 inhibitors are likely affecting more than inhibiting CDK4/6 which could increase their efficacy. On the other hand, these off-target effects can cause unexpected adverse reactions in patients. The inhibition of the G_1 phase of the cell cycle may also disturb the effects of other drugs targeting the downstream process in cell proliferation, such as DNA

Table II. *Potential combination of cyclin-dependent kinase 4/6 inhibitors and other agents for gastrointestinal cancer treatment.*

CDK4/6 inhibitor	Combined agent	Cancer	Study level	Ref.
Palbociclib	Sorafinib (pan-kinase inhibitor)	Hepatocellular carcinoma	Preclinical	30
	BAY-117082 (Nuclear factor-kB inhibitor)	Hepatocellular carcinoma	Preclinical	31
	MLN0128 (pan-mTOR inhibitor)	Cholangiocarcinoma	Preclinical	36
	PND1186 (focal adhesion kinase inhibitor)	Cholangiocarcinoma	Preclinical	38
	Radiotherapy	Cholangiocarcinoma	Preclinical	39
	SB505124 (type-I transforming growth factor-β receptor inhibitor)	Pancreatic cancer	Preclinical	46
	Paclitaxel	Pancreatic cancer	Preclinical	43
	Gemcitabine (With a certain sequence)	Pancreatic cancer	Preclinical	45
	Trametinib (MEK inhibitor)	Pancreatic cancer	Preclinical	48
	Trametinib (MEK inhibitor)	Pancreatic cancer	Clinical trial	49
	Hydroxychloroquine	Pancreatic cancer	Preclinical	51
	Trametinib (MEK inhibitor)	Colorectal cancer	Preclinical	56
	PD0325901 (MEK inhibitor)	Colorectal cancer	Preclinical	58
	Trametinib (MEK inhibitor) and cetuximab (epidermal growth factor receptor inhibitor)	Colorectal cancer	Preclinical	59
	Irinotecan	Colorectal cancer	Preclinical	61
Ribociclib	Infigratinib (fibroblast growth factor receptor inhibitor)	Hepatocellular carcinoma	Preclinical	26
	MEK162 (MEK inhibitor)	Pancreatic cancer	Preclinical	53
	Sorafinib	Pancreatic cancer	Preclinical	41
	Everolimus (mTOR inhibitor)	Pancreatic cancer	Clinical trial	54
Abemaciclib	Pembrolizumab (anti-PD1)	Esophageal cancer Gastric cancer	Clinical trial	20
	ELAVL1 (HuR inhibitor)	Pancreatic cancer	Preclinical	52
	LY3009120 (pan-raf inhibitor)	Colorectal cancer	Preclinical	64

CDK: Cyclin-dependent kinase; HuR: Hu antigen R; MEK: mitogen-activated protein kinase kinase; mTOR: mammalian target of rapamycin; PD-1: programmed cell death protein 1; Raf: RAF proto-oncogene serine/threonine-protein kinase.

replication or mitosis. This could antagonize the effect of other therapeutic aims. The potential combinations that may result in synergistic effects for each drug are summarized in Table II. In summary, studies to fully understand the comprehensive mechanisms of these inhibitors are needed for an appropriate selection and the safety of administration to the patients. More clinical trials, especially a randomized controlled trial, are urgently needed to guide clinical practice of these potentially breakthrough-therapy medications.

Conflicts of Interest

The Authors declare that they do not have conflicts of interest. We did not receive any specific funding for this work. CS received research grants from the National Research Council of Thailand (N41A640108), The Medical Council of Thailand, Faculty of Medicine (IN65126), and Fundamental Fund of Khon Kaen University.

Authors' Contributions

FZ, YZ, CS conceptualized the paper, reviewed literature, and wrote first draft of manuscript. CS supervised and revised the manuscript. TK summarized the literature and created the table and figure. All Authors critically reviewed and approved the final manuscript.

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