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Review Article

Selective Cyclin-Dependent Kinase 4/6 Inhibitors as Anticancer Drugs: Moving beyond Hormone Receptor-Positive Breast Cancer

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Abstract

The cyclin D-cyclin-dependent kinase (CDK) 4/6 pathway controls the cell cycle machinery by regulating the G1-to-S-phase transition. Dysregulation of this pathway, resulting in increased cellular proliferation, is frequently observed in a variety of human cancers. Activation of cyclin D-CDK 4/6 pathway can occur through different mechanisms, including gene amplification/rearrangement, loss of negative regulatory factors, epigenetic modifications, and point mutations of different components of this pathway. Quite conspicuously, CDK 4/6 inhibitors have emerged as promising anticancer agents in various tumors in which CDK 4/6 has a pivotal role in the G1-to-S-phase cell cycle transition. The clinical use of first-generation, nonselective pan-CDK inhibitors was not progressed beyond early phase trials, due to unacceptable toxicity and lack of efficacy noted with these agents. The emergence of selective CDK 4/6 inhibitors, including ribociclib, abemaciclib, and palbociclib, has enabled us to effectively target cyclin D-CDK 4/6 pathway, at the cost of acceptable toxicity. The results of landmark Phase III trials investigating palbociclib and ribociclib in advanced hormone receptor (HR)-positive breast cancer have demonstrated a substantial clinical benefit with a well-tolerated toxicity profile. Mechanisms of acquired resistance to selective CDK 4/6 inhibitors are beginning to emerge. Clearly, a detailed understanding of these resistance mechanisms is very much essential for the rational development of post-CDK 4/6 inhibitor therapeutic strategies. Extending the use of selective CDK 4/6 inhibitors beyond HR-positive breast cancer is a challenging task and will likely require identification of clinically meaningful biomarkers to predict response and the use of combination approaches to optimize CDK 4/6 targeting.

Keywords: *Abemaciclib, palbociclib, ribociclib, selective cyclin-dependent kinase 4/6 inhibitors*

Introduction

Uncontrolled cellular proliferation, as a result of dysregulated cell division, is one of the key hallmarks of cancer, and identifying appropriate therapeutic targets to block cell division is a widely used strategy of anticancer therapy. Cyclin-dependent kinases (CDKs) control the transition from one stage of the cell cycle to the next, and they are activated upon interaction with their partner cyclins.[1] Therefore, quite conspicuously, CDKs have long been regarded as attractive therapeutic targets for cancer treatment. Unfortunately, many of the early first-generation CDK inhibitors failed in the clinical development because of nonselective pan-CDK inhibition, which was found to be toxic-to-nonmalignant cells.[2] These issues of effectiveness and toxicity of nonselective CDK inhibitors seem to have been overcome in the last decade by the development of selective CDK-targeting agents – which selectively target CDK 4/6.

Dysregulation of cyclin D-CDK 4/6 pathway is frequently observed in human cancers and results in uncontrolled cell cycle progression.[3] CDK 4/6 mediates the transition from G1 to S phase by associating with cyclin-D and regulating the phosphorylation of retinoblastoma (Rb) protein. Increased cyclin D-CDK 4/6 pathway activity can occur through several mechanisms, including overexpression of D-type cyclins, mutation or amplification of CDK 4/6, epigenetic alterations, or loss of negative regulators.[2,3] Thus, the development of selective CDK 4/6 inhibitors offers a novel therapeutic approach in the field of oncology. Following the encouraging results of early phase clinical trials, three of the selective CDK 4/6 inhibitors (e.g., abemaciclib,

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palbociclib, and ribociclib) have emerged as agents with promising anticancer activity and acceptable toxicity profile,[4-10] and among them, palbociclib and ribociclib have already received FDA approval, with landmark Phase III data available, in the setting of hormone receptor (HR)-positive, human epidermal growth factor receptor-2 (HER-2)-negative advanced breast cancer.[11-14]

In this review, we discuss the rationale of selectively targeting CDK 4/6 pathway and the challenges with regard to optimizing their use. We also provide an overview of the currently available clinical data for selective CDK4/6 inhibitors in different human cancers, other than HR-positive, HER-2 negative breast cancer.

Overview of Cyclin D-Cyclin-Dependent Kinase 4/6 Pathway Dysregulation

Principle mechanisms by which the cyclin D-CDK 4/6 pathway can become dysregulated in various human cancers are amplification of the genes encoding cyclin D1 (CCND1) or deletion of the locus encoding CDKN2A. According to the published data, amplification of CCND1 is frequently found in some human cancers, for example, breast cancer (35% of cases), head-and-neck cancer (26%–39%), endometrial cancer (26%), pancreatic adenocarcinoma (25%), and nonsmall cell lung cancer (NSCLC) (5%–30%).[15,16] In a recently reported landmark study, which investigated the role of routine molecular screening to identify actionable mutations in advanced refractory cancer patients, Cassier *et al*. found CCND1 amplification and homozygous deletion of CDKN2A in 17% and 21% of patients, respectively.[17]

The cyclin D-CDK 4/6 pathway can be dysregulated by multiple other mechanisms also, for example, mutations in the genes encoding various components of this pathway, epigenetic alterations, and mutations in the upstream factors. Haluska and Hodi found that about 20% of familial malignant melanoma cases harbor CDKN2A mutations.[18,19] Epigenetic modifications of the CDKN2A gene have been reported in human ovarian cancer.[20] Jackson *et al*. highlighted the importance of mutations in the upstream factors as a mechanism of cyclin D-CDK 4/6 pathway dysregulation in malignant rhabdoid tumors, where the INI1/SMARCB1 gene is frequently mutated.[21]

Biologic Rationale of Selectively Inhibiting Cyclin-Dependent Kinase 4/6 in Human Cancers

The ideal CDK-targeted agents should block CDK-mediated signaling in malignant cells and at the same time should spare the aspects of CDK activity which are critical for the survival of nonmalignant cells, thus avoiding toxicity. Inhibition of CDK1 by nonspecific inhibitors could affect all cell types and result in toxicity, as evidenced by the reported fact that mouse embryos lacking CDK1 fail to develop beyond the blastocyst stage.[22] In addition, nonspecific targeting of CDKs might also result in inhibition of CDKs 7, 8, and 9, the exact functions of which are less well established.[23] Clearly, toxicity is a major concern regarding nonselective CDK-targeted agents because CDKs play a critical role in the proliferation of both normal cells and cancer cells.

The difficulty in finding a therapeutic window wherein CDK inhibition is both safe and effective was reflected in the early clinical experience with various nonselective CDK inhibitors, for example, flavopiridol and seliciclib. To date, the most well-studied nonselective CDK inhibitor is flavopiridol, which showed limited clinical benefit, mainly because of its complex pharmacokinetics and high levels of off-target effects.[24] Seliciclib, a purine-based compound that inhibits CDKs 1, 2, 5, 7, and 9, failed to demonstrate effective clinical activity in Phase I studies.[25]

It is possible that cancers with known aberrations in the cyclin D-CDK 4/6 pathway will be more sensitive to CDK 4/6 inhibition than normal cells.[26] Furthermore, selective inhibitors spare CDK2 activity which allows normal cells to continue to function and proliferate. In addition, in contrast to the cytotoxic effects of pan-CDK inhibitors, selective CDK 4/6 inhibitors are usually found to have cytostatic effects, which might further limit the potential of these agents to cause significant clinical toxicity.[27]

Selective Cyclin-Dependent Kinase 4/6 Inhibitors in Cancer Therapy

As discussed earlier, after the encouraging results from preclinical studies, three CDK4/6 inhibitors have currently reached early phase clinical trials – abemaciclib, palbociclib, and ribociclib with published Phase III data available for palbociclib and ribociclib, in the setting of HR-positive, HER-2-negative advanced breast cancer.[11-14]

The next part of this review will focus on the currently available preclinical and clinical data of selective CDK 4/6 inhibitors in different human cancers, other than the archetypal model of ER-positive, HER-2-negative luminal breast cancer.

Preclinical Data

Abemaciclib

It has been shown to reduce the phosphorylation of Rb1 in colorectal cancer and melanoma xenografts, thus inducing G1 arrest.[28] Abemaciclib has also been demonstrated to induce growth regression in vemurafenib-resistant melanoma models, in which expression of cyclin D1 was noted to be elevated in conjunction with mitogen-activated protein kinase (MAPK) pathway reactivation *in vitro*.[29]

Ribociclib

Single-agent ribociclib has been shown to inhibit the growth of neuroblastoma and liposarcoma cell lines, by inducing

G1 arrest and reducing Rb1 phosphorylation.[30] It inhibits CDK 4/6 effectively even at nanomolar concentrations.

Palbociclib

It has been shown to be active in mantle cell lymphoma xenografts[31] and glioblastoma cell lines.[32] Moreover, activity of palbociclib in combination with bortezomib has been demonstrated in both acute myeloid leukemia and myeloma.[33,34] In ovarian cancer cell lines, a response to palbociclib was found to be most marked in Rb1-proficient cell lines with low p16INK4A expression, and amplification of cyclin E1 was associated with resistance.[35]

Data from Early Phase Clinical Trials

After the publication of promising results from preclinical research, quite conspicuously, selective CDK 4/6 inhibitors have been investigated in early phase clinical trials also.

Abemaciclib

The first-in-human Phase I trial of abemaciclib enrolled 75 patients with advanced solid tumors.[4] The dose-limiting toxicity was Grade 3 fatigue. The most common treatment-related adverse events (AEs) included diarrhea (52%), nausea (32%), fatigue (21%), vomiting (21%), and neutropenia (19%). Pharmacodynamic evidence of targeted CDK4/6 inhibition was observed, as shown by a decrease in Rb phosphorylation in the skin. In an expansion cohort of this trial in patients with NSCLC, 51% achieved at least stable disease (SD), with 41% of patients receiving at least 4 cycles of treatment.[5]

Ribociclib

The initial Phase I dose escalation study of single-agent ribociclib enrolled 128 patients with Rb+ advanced solid tumors and lymphomas.[6] The most common AEs were neutropenia (45%), leukopenia (44%), nausea (43%), and fatigue (42%).[6] Among 110 evaluable patients, three had confirmed partial response (PR). Prolonged SD for at least 4 and 6 cycles was seen in 24% and 15% of patients, respectively.[6] In a trial of 14 patients with NRAS-mutated melanoma who received ribociclib in combination with the MEK inhibitor binimetinib, six patients had a PR.[7] Another Phase I study investigated ribociclib in pediatric patients with malignant rhabdoid tumors, neuroblastoma, or other cyclin D-CDK 4/6-INK4-Rb pathway-activated tumors.[36] Ribociclib was well tolerated in the pediatric population, with a similar safety profile to that seen in adults.[36]

Palbociclib

Two of Phase I studies investigating palbociclib in patients with Rb1-expressing (Rb+) cancers have shown signs of efficacy manifesting predominantly as SD.[8,9] Flaherty *et al*. reported the first-in-human Phase I dose escalation study of palbociclib, including 41 patients with advanced solid tumors.[8] The most common all-grade nonhematologic AEs after Cycle 1 included fatigue (n = 10; 24%), diarrhea (n = 6; 15%), and nausea, dyspnea, and arthralgia (n = 5; 12% each). Pharmacodynamic decreases in neutrophil and platelet counts correlated with increasing palbociclib exposure. During the 7-day rest period in Cycle 1, both cell types recovered, indicating that this effect was fully reversible. Preliminary signs of clinical activity were observed, with 10 patients (27%) achieving SD for at least 4 cycles and 6 patients (16%) having SD for at least 10 cycles.[8] In a third single-arm study comprising 17 patients with relapsed mantle cell lymphoma, five patients had a PFS duration of >12 months, with one complete response and two PRs.[10]

Vaughn *et al*. reported a Phase I trial of three patients with growing teratoma syndrome.[37] The efficacy of palbociclib has been investigated further in a Phase II study of thirty patients with relapsed, Rb1-proficient germ-cell tumors, in which eight patients had a PFS duration of >24 weeks.[38]

In a Phase II trial of thirty patients with Rb+ advanced well-differentiated or dedifferentiated liposarcoma, palbociclib treatment resulted in a 12-week PFS rate of 66%, with one patient having a PR.[39] Finally, in a Phase II trial of palbociclib in 19 patients with previously treated, advanced NSCLC exhibiting Rb expression and CDKN2A inactivation, the median PFS was 12.5 weeks, and five patients remained on study for at least 24 weeks.[40]

Data from Phase III Randomized Trials

Phase III randomized studies to investigate the therapeutic efficacy of selective CDK4/6 inhibitors are currently ongoing in a variety of cancers, but till now, the only published data are available for patients with HR-positive advanced/metastatic breast cancer.[11-14] The results of two currently ongoing Phase III RCTs in lung cancer patients are eagerly awaited. One of them (NCT02152631/JUNIPER) is comparing abemaciclib with erlotinib in Stage IV NSCLC patients with a detectable KRAS mutation who have progressed after platinum-based chemotherapy, taking PFS and OS as primary endpoints. The second study (NCT02154490/Lung-MAP) is intended to compare palbociclib with docetaxel in recurrent stage IIIB–IV squamous cell lung cancer, positive for CDK4/6, CCND1, CCND2, and CCND3 expression.

Combination of Selective Cyclin-Dependent Kinase 4/6 Inhibitors with Other Therapies

Till date, most of the published data of combining selective CDK 4/6 inhibitors with other therapeutic modalities are in the setting of HR-positive advanced breast cancer. However, at the same time, there are few encouraging published clinical data of different combination approaches in other human cancers also.

A number of combination strategies with selective CDK 4/6 inhibitors are being tried as treatment options

in hematological malignancies, including combination with bortezomib in patients with multiple myeloma.[41] Moreover, preclinical evidence supports the combination of CDK4 inhibition with ibrutinib or PI3K inhibitors in the treatment of mantle cell lymphoma.[42]

Preclinical evidence of effectiveness also exists for CDK 4/6 inhibition in combination with MAPK-pathway inhibition with MEK or BRAF inhibitors in melanoma and colorectal cancer. Combination of CDK 4/6 inhibitors with RAS/RAF/MEK/ERK pathway inhibitors is a promising therapeutic approach in melanoma. Selective CDK4/6 inhibition with abemaciclib can also resensitize melanoma cell lines with BRAF V600E mutation to vemurafenib after the development of acquired resistance.[29] There is an ongoing Phase Ib/II (NCT01781572) study, investigating the combination of ribociclib with the MEK inhibitor binimetinib (MEK162) in patients with NRAS-mutant melanoma. Common AEs experienced with this combination included acneiform dermatitis, nausea, rash, edema, and leukopenia.[7] This combination was also associated with significant antitumor activity, including cases of PR (33%) and SD (52%).[7] In BRAF V600E-mutant melanoma models, low-dose ribociclib exhibited synergistic activity with encorafenib (LGX818) – a selective BRAF inhibitor.[43] The addition of ribociclib to encorafenib also appeared to prevent resistance to encorafenib.[43] In a Phase Ib/II study, the combination of ribociclib and encorafenib demonstrated clinical activity and an acceptable toxicity profile.[44] Triplet combination of ribociclib with binimetinib and encorafenib is also being explored in a currently ongoing Phase II study (NCT02159066/LOGIC-2).

Challenges of Extending the use of Selective Cyclin-Dependent Kinase 4/6 Inhibitors beyond Hormone Receptor-Positive Breast Cancer

Although a number of potential biologic biomarkers of sensitivity of selective CDK 4/6 inhibitors are available (e.g., cyclin D, CDKN2A, and Rb1 status), ER-positive status in breast cancer is the only biomarker currently confirmed for clinical use. Some human cancers, such as mantle cell lymphoma, probably have subtype-specific sensitivity to selective CDK 4/6 inhibitors, thus ameliorating the need for selection markers. However, for most of the other human cancer subtypes, biomarkers are essential in identifying selective dependence on cyclin D1-CDK 4/6 pathway. In an ongoing Phase II/III study (NCT02154490/Lung-MAP), there is a treatment arm in which patients with recurrent squamous cell lung cancer are being allocated to receive palbociclib on the basis of aberrations in CDK4 and CCND1-3. In another ongoing trial (NCT02187783/SIGNATURE), patients are being allocated to ribociclib therapy on the basis of CCND/CDKN2A/CDK4 aberrations.

Further research work is required to identify biomarkers of resistance to selective CDK 4/6 inhibitors in various human cancers. Loss of Rb1 function is an established mechanism of primary resistance to CDK4/6 inhibitors *in vitro*, but this and other biomarkers of resistance are yet to be validated in clinical setting. Loss of Rb1 function is rarely found in ER-positive breast cancer although data are limited regarding the changing frequency of Rb1 loss with the development of resistance to prior therapies. Amplification of E2F or loss of CDKN1A, which are both commonly observed in a variety of human cancers and are linked to tamoxifen resistance, has been proposed as other potential biomarkers of resistance.[45]

Conclusions

The clinical use of selective CDK 4/6 inhibitors, either alone or as combination approach, now has proven efficacy in patients with advanced stage ER-positive, HER-2-negative breast cancer. Extending the clinical use of selective CDK 4/6 inhibition outside HR-positive advanced breast cancer will require identification of human cancer subtypes, and those are dependent on the cyclin D-CDK 4/6 pathway for their growth. Moreover, it will also require identification of clinically useful biomarkers to expand indications and effective drug combinations to overcome resistance. Although some of the published preclinical and early phase clinical data seem to be very much encouraging regarding the useful implementation of selective CDK 4/6 inhibitors in various other human cancers, these results must be confirmed in Phase III trials before any firm conclusions can be made. There is also an urgent need for prospective biomarker-driven clinical trials to identify appropriate target population, for whom selective CDK 4/6 inhibition will be cost-effective.

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Conflicts of interest

There are no conflicts of interest.

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