ADVANCES IN DRUG DEVELOPMENT

Current Developments in Oncology Drug Research

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The Evolving Role of Cyclin-Dependent Kinase Inhibitors in Cancer Management

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H&O What is the role of cyclin-dependent kinases, and why are they a target in cancer treatment?

GS The cyclin-dependent kinases (CDKs) are a large family of enzymes divided into 2 groups: those that control cell-cycle progression and those that control transcriptional events. CDKs 4, 6, 2, and 1 typically control progression through the various phases of the cell cycle, such as the transitions from G1 to S, S to G2, and G2 to M phase, as well as mitotic progression. Inhibition of these CDKs shuts down the cell cycle and prevents cells from growing. The transcriptional CDKs control the initiation and elongation of mRNA transcripts by phosphorylating the C-terminal domain of RNA polymerase II, which makes messenger RNAs. In cancer cells, cell-cycle CDKs are frequently dysregulated, and control of the transcriptional CDKs may also be altered.

As the name implies, CDKs are activated by their association with cyclins. A cyclin forms a complex with a CDK, known as the active holoenzyme. There are also other ways in which the CDKs can be regulated, such as by endogenous inhibitors. All cells contain endogenous inhibitors of CDKs so that these kinases are activated only when they should be; the process is tightly regulated. Among cells of the body that are actively growing, such as hair cells, those in the lining of the lungs and the gut, bone marrow cells, and skin cells, growth is tightly regulated by inhibitors. In cancer cells, these inhibitors may be missing.

Cancer cells frequently demonstrate overactivity of the cell-cycle CDKs. Examples are CDKs 4 and 6, which contribute to control of progression through the G1 phase of the cell cycle, and which pair with D-cyclins to form holoenzymes. There may be overexpression of D-cyclins, or amplification of the genes encoding these cyclins. There may also be amplification of the genes that encode CDK4 or CDK6, leading to their overexpression. Most commonly, mutation, deletion, or promoter methylation of the genes encoding endogenous CDK4/6 inhibitors occurs; absence of these inhibitors allows CDKs 4 and 6 to continually drive the cell-cycle progression that gives cancer cells a growth advantage.

The transcriptional CDKs add another layer of complexity. Why would targeting transcription initiation or elongation be selective for a cancer cell? The genes that control the oncogenic state are controlled by structures known as super-enhancers, which are prone to disruption. Several of the transcriptional CDKs are associated with super-enhancers. Even slight inhibition of an enzyme can disrupt the super-enhancers and affect the transcription of genes that define the oncogenic state, disrupting expression of proteins that are critical to the cancer cell. The transcriptional CDKs appear to affect the genes that are most important to cancer cells.

In summary, this biology explains why it might be possible to target the CDKs—whether they control the cell cycle or transcriptional events—and produce an effect that is selective for tumor cells.

H&O Are there certain cancers that appear to be more responsive to CDK inhibition?

GS Current CDK inhibitor drugs currently fall into 2 classes: those that are CDK4/6-selective and those that target the family more broadly, inhibiting both cell-cycle and transcriptional CDKs. Data are rapidly emerging with the CDK4/6-selective agents. In clinical trials of

patients with breast cancer positive for the estrogen receptor (ER), the addition of a CDK4/6 inhibitor, such as palbociclib (Ibrance, Pfizer) or ribociclib (Novartis) to hormonal treatment dramatically increased progression-free survival. For example, among ER-positive patients who had not received previous treatment for advanced disease, the addition of palbociclib to letrozole (Femara, Novartis) doubled progression-free survival. Similarly, the addition of palbociclib to fulvestrant (Faslodex, AstraZeneca) increased progression-free survival in patients with metastatic breast cancer who had already received hormonal treatment. ER-positive breast cancers may be particularly dependent on cyclin D1-CDK4, accounting for these results. Among breast cancers, ER positivity may be the most predictive biomarker of clinical benefit, and results were similar irrespective of amplification of *CCND1*, the gene encoding cyclin D1, or loss of $p16^{INK4A}$, an endogenous CDK4 inhibitor.

Another disease of great interest is *KRAS*-mutant lung cancer. *KRAS* drives cell growth through CDK4. A clinical trial of abemaciclib (Lilly) has shown prolonged stabilization of disease, and even instances of tumor regression or response, in patients with *KRAS*-mutant lung cancer. Other types of cancer that may be more sensitive to CDK inhibition are those with amplification of *CDK4* or a translocation involving *CCND1*, as in liposarcoma and mantle cell lymphoma, respectively.

The story is less clear with transcriptional CDK inhibitors. Some leukemias are highly sensitive to transcriptional CDK inhibitors. For example, the novel agent dinaciclib (Merck) has shown robust activity in chronic lymphocytic leukemia. Preclinical data with a novel, selective inhibitor of CDK7 have shown that some tumor types, including small cell lung cancer and triple-negative breast cancer, are "transcriptionally addicted," and sensitive to CDK7 inhibition. Transcriptional CDK inhibitors may disrupt expression of the c-MYC oncoprotein, on which some tumors depend, as well as expression of the anti-apoptotic protein Mcl-1.

H&O What are the toxicities associated with CDK inhibitors?

GS For the CDK4/6-selective inhibitors, the main toxicity is low blood cell counts. In general, this is mild and reversible, although in some patients, myelosuppression may be more severe. In early studies of breast cancer and lung cancer, the novel agent abemaciclib has been associated with fatigue and diarrhea, the latter manageable with an antidiarrheal regimen. Abemaciclib is associated with less neutropenia than the other CDK4/6 inhibitors, and can be administered continuously; in contrast, palbociclib and ribociclib are typically administered 3 weeks of every 4.

H&O Could you please describe your research of CDK4/6 inhibitors?

GS We have conducted clinical work with several CDK4/6-selective inhibitors, including palbociclib, abemaciclib, and ribociclib. When these drugs shut down the cancer cells during the G1 phase, there are several possible biological outcomes. In some cases, G1 arrest is easily overcome and reversible. In other instances, cells go into a deep, prolonged resting state known as senescence. Finally, in other cases, CDK4/6 inhibition can kill cancer cells. Understanding what dictates these disparate outcomes is of great interest in the CDK4/6 inhibitor field.

Some of our pivotal clinical work demonstrated that palbociclib hits its target in cancer cells. The initial in-human testing of palbociclib showed stable disease among a variety of tumor types, but only occasional instances of tumor shrinkage. It was therefore critical to demonstrate that palbociclib engaged the CDK4/6 target before launching into large phase 2 and phase 3 programs. We conducted a pilot study in mantle cell lymphoma, a lymphoma defined by an oncogenic translocation of *CCND1*, leading to overactivity of CDK4. This pilot study, published in 2012, enrolled 17 patients. Biopsies taken before and after treatment demonstrated that CDK4 activity was reduced in tumor cells because phosphorylation of the primary CDK4 target, the retinoblastoma protein, was reduced. Additionally, we used an experimental positron emission tomography (PET) isotope, fluorothymidine, and demonstrated that tumors had marked reduction of fluorothymidine uptake after exposure to palbociclib, consistent with G1 arrest of the cancer cells.

The study provided compelling data that palbociclib hits the CDK4 target. An exciting finding was that 5 of the patients, all very heavily pretreated, were able to stay on the study for more than a year. Among these 5 patients, 3 experienced a partial response or a complete response that lasted upwards of 900 days. The demonstration of target engagement provided confidence in the mechanism of palbociclib's action during the development of the drug in diseases such as breast cancer, liposarcoma, gastrointestinal cancers, and lung cancer.

We have also participated in early phase 1 studies of abemaciclib and ribociclib. In the abemaciclib study, we were able to apply some of our assays for target engagement to ultimately determine that abemaciclib should be administered twice a day. The twice-daily schedule has shown activity in ER-positive breast cancer and lung cancer. Notably, among patients with ER-positive breast cancer, abemaciclib has shown activity when given as monotherapy, without hormonal treatment.

Our research, and that of many other groups, is now

focused on understanding the mechanisms of resistance to CDK 4 and 6 inhibitors and on strategies to overcome resistance. Some of the mechanisms of resistance described to date include amplification of the genes encoding the target kinases or of the genes encoding other cell-cycle proteins, such as cyclin E; increased activity of CDK2; and loss of the retinoblastoma protein (a critical CDK4/6 target).

H&O What is known about inhibition of CDK2 and CDK1?

GS Research has shown that in some cell types, selective inhibition of CDK2 is compensated by CDK1, and vice versa. Therefore, this finding has driven the selection of compounds that are capable of inhibiting both CDKs. Many of the drugs that hit CDK 1 and 2 also hit the transcriptional CDKs, such as CDKs 9 and 7. Inhibitors of CDK 1 and 2 block the cell cycle, and inhibitors of CDK9 affect transcription of some of the genes that are important for maintaining the oncogenic state. In preclinical models, hitting all 3 targets together has a strong effect against cancer cells.

The development of inhibitors of CDKs 1, 2, and 9 has been delayed because these agents have been associated with more toxicity than the CDK4/6 selective inhibitors. For example, CDK9 inhibition depletes the anti-apoptotic protein Mcl-1; although this effect may be beneficial against cancer cells, Mcl-1 depletion can affect the viability of neutrophils. Therefore, development of drugs with CDK9 inhibitory activity has been complicated by neutropenia.

H&O How do CDKs affect the cellular response to DNA damage?

GS CDKs 1 and 2 do not just phosphorylate proteins involved in the cell cycle, but also phosphorylate many proteins in the DNA damage response and repair pathways. For example, we showed several years ago that CDK1 phosphorylates BRCA1, an event that is needed for efficient recruitment of the BRCA1 protein to sites of damaged DNA.

CDK12 is a recently recognized CDK that controls the transcription of genes involved in DNA repair pathways. It is thought that blocking CDK1 and disrupting BRCA1 function, or blocking CDK12 and disrupting the expression of the DNA repair genes and proteins, will sensitize cells to DNA damage. We are studying the use of CDK1/2 and CDK12 inhibitors in combination with DNA-damaging agents, including chemotherapy and radiation. Because CDK1 and CDK12 inhibition can impact the proficiency of the homologous recombination repair pathway, inhibitors of these CDKs can also sensitize cells to poly(ADP-ribose) polymerase (PARP) inhibitors. Therefore, we have been trying to convert cancer cells that are homologous recombination repair–proficient to a homologous recombination repair–deficient state by blocking CDK12 or CDK1, and then sensitizing them to PARP inhibition. Such a strategy may greatly extend the use of PARP inhibitors beyond patients with tumors that are already homologous recombination repair–deficient, such as those that are *BRCA*-associated. In the clinic, we are using dinaciclib, a drug that blocks CDKs 1 and 12, together with the PARP inhibitor veliparib (AbbVie). This combination has shown preliminary clinical benefit in patients with breast cancer, prostate cancer, ovarian cancer, and other gynecologic malignancies.

H&O Are there differences in the ways CDK inhibitors impact cancer cells vs noncancerous cells?

GS This question raises the issue of how drugs that block CDKs could be selective for cancer cells. Inhibitors against cell-cycle CDKs can block the cycles of normal cells, causing toxicity. For example, a CDK4/6 inhibitor can block cycling of a marrow stem cell, leading to neutropenia. The activity of the CDKs is much greater in cancer cells than nontransformed cells, and therefore CDK inhibitors may have greater effects in cancer cells.

Recently, we demonstrated that when blocking CDKs 1 and 2, the arrest in normal cells is greater than that in cancer cells. When the cells are arrested, they are not sensitive to DNA damage. With CDK1/2 inhibition, it is possible to obtain better arrest in a normal cell and protect it from DNA damage, whereas in a cancer cell, the arrest is less complete and the CDK inhibitor-DNA damaging agent combinations remain sensitive to DNA damage. Therefore, the CDK DNA-damaging agent combinations may have selectivity for transformed cells compared with those that are not transformed.

Some of the transcriptional CDKs have a much greater effect in cancer cells than in normal cells. As previously discussed, in cancer cells, these CDKs control the expression of the genes necessary for maintenance of the oncogenic state. Inhibitors of transcriptional CDKs reduce expression of these genes, with greater effects in cancer cells than in normal cells.

H&O Are there any other types of CDK inhibitors in development?

GS Recent studies are focused on the development of highly selective transcriptional CDK inhibitors that also bind to the CDK covalently, rather than reversibly. THZ1 is a selective inhibitor of CDK7 that has shown striking activity in preclinical models of triple-negative breast

cancer and small cell lung cancer. A THZ1 derivative will be entering a first-in-human clinical trial this year.

H&O Are CDK inhibitors being studied in combination with other drug classes?

GS We already discussed the combination of CDK4/6 inhibitors with hormonal therapy in ER-positive breast cancer. CDK4/6 inhibitors are also being combined with signal transduction inhibitors, such as inhibitors of the mitogen-activated protein (MAP) kinase pathway (eg, inhibitors of MEK and ERK) and the phosphoinositide 3 (PI3) kinase pathway (eg, inhibitors of PI3-kinase). The rationale is that combining signal transduction inhibition and cell-cycle inhibition will produce even stronger effects on cancer cell proliferation. CDK4/6 inhibitors are also being combined with chemotherapy agents in sequenced combinations.

As previously mentioned, inhibitors of other CDK family members are being combined with chemotherapy agents and PARP inhibitors.

Although there are some concerns that both classes of CDK inhibitors could affect T-cell proliferation, there is also evidence that CDK inhibition may beneficially affect the immune microenvironment. For this reason, combinations with immune checkpoint blockade are also being explored.

H&O Are there any characteristics or biomarkers that can predict response to CDK inhibition?

GS So far, for the CDK4/6-selective compounds, there has not been a good biomarker, other than ER positivity in breast cancer. Additional work will be required to determine whether amplification of the genes encoding cyclin D1 or CDK4/6 can predict the response to a CDK inhibitor in other disease types. However, current clinical data have not shown that these markers are predictive. Even patients without these markers can achieve prolonged clinical benefit from a CDK4/6 inhibitor.

Work is also ongoing to define biomarkers that may predict response to transcriptional CDK inhibitors, including *MYC* amplification, *CCNE1* (cyclin E) amplification, or *MCL1* amplification.

H&O Has the success of CDK inhibitors provided insight into how cancer develops or progresses?

GS The success of CDK4/6-selective inhibitors suggests how critical these kinases are for tumor development and maintenance. Alterations in their expression often represent early steps in tumor development. CDKs may be downstream recipients of the activation of many signaling pathways. Additionally, the success of CDK inhibitors has prompted researchers to revisit older data evaluating how CDKs control the growth of cancer cells and how their inhibition may lead to reduced tumor proliferation, senescence, or tumor cell death.

Disclosure

Dr Shapiro has received research funding from Pfizer for investigator-initiated studies, and has served on advisory boards for Pfizer, Eli Lilly, G1 Therapeutics, and Vertex Pharmaceuticals. He has participated as an investigator in clinical trials of palbociclib, abemaciclib, and ribociclib.

Suggested Readings

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