Cell-Cycle Therapeutics Come of Age

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ABSTRACT

The ability to sustain unscheduled proliferation is a hallmark of cancer. The normal process of cell division occurs via the cell cycle, a series of highly regulated steps that are orchestrated at the molecular level by specific cyclins that act in association with cyclin-dependent kinases (CDKs). Cyclin D and CDK4/6 play a key role in cell-cycle progression by phosphorylating and inactivating the retinoblastoma protein, a tumor suppressor that restrains G1- to S-phase progression. The first-generation CDK inhibitors demonstrated broad activity upon several CDKs, which likely explains their considerable toxicities and limited efficacy. Palbociclib, ribociclib, and abemaciclib represent a new class of highly specific ATP-competitive CDK4/6 inhibitors that induce reversible G1-phase cell-cycle arrest in retinoblastoma-positive tumor models. Both palbociclib and ribociclib have been approved in combination with hormone-based therapy for the treatment of naïve hormone receptor–positive advanced breast cancer on the basis of an improvement in progression-free survival. In general, CDK4/6 inhibitors are cytostatic as monotherapy but demonstrate favorable tolerability, which has prompted interest in combination approaches. Combinations with phosphatidylinositol 3-kinase and mammalian target of rapamycin inhibitors in breast cancer, and inhibitors of the RAS/RAF/mitogen-activated protein kinase pathway in *RAS*-mutant cancers are particularly promising approaches that are currently being evaluated. Although the subject of intense preclinical study, predictive biomarkers for response and resistance to these drugs remain largely undefined. CDK4/6 inhibitors have emerged as the most promising of the cell-cycle therapeutics and intense efforts are now underway to expand the reach of this paradigm.

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INTRODUCTION

In cancer, the complexity of the cell-cycle regulatory machinery and the frequency with which components are deranged reflect the importance of unscheduled division to the malignant phenotype.¹ The therapeutic potential of targeting the cell cycle has long been appreciated but translation of this approach to the bedside was initially limited by the low specificity of early cell-cycle inhibitors.² The advent of highly specific inhibitors of critical cell-cycle components, most notably cyclin-dependent kinases (CDKs) 4 and 6, and an expanding appreciation of how cancer subverts the cell-cycle apparatus has ushered in a new generation of therapeutic agents to the clinic. Efforts are needed to identify effective mechanism-based combinations, establish clinically relevant biomarkers, and uncover vulnerabilities in other cell-cycle components.

THE CELL CYCLE

The classic view of the cell cycle was established by pioneering experiments in yeast and sea urchins

by Hartwell, Nurse, Hunt, and others.³ In principle, the cell cycle represents the molecular machinery by which a decision regarding the appropriateness of cell division is made and includes four phases: G₁ (cells determine whether to grow and divide or enter quiescence, G₀), S (DNA replication); G₂ (preparation for mitosis); and M (division of genetic material and cytokinesis).⁴

CDKs—in physical association with their catalytic subunits, the cyclins—are serine/threonine kinases that are responsible for phosphorylating the intracellular proteins that orchestrate the molecular events of orderly cell-cycle progression (Fig 1). Distinct CDKs and specific cyclin partners operate during different phases. For example, in G₁, CDK4 and CDK6 interact with one of three D-type cyclins (D1, D2, D3), depending on tissue context.⁵⁻⁷ Later in G₁, CDK2 and E-type cyclins orchestrate entry into S. Other CDK–cyclin pairs operate during later phases.

D-type cyclins, unlike cyclins that act at later time points, are highly responsive to extracellular mitogens.⁸ Cyclin D1, for example, increases upon signaling via estrogen and human epidermal growth factor receptors and RAS/mitogen-activated

ASSOCIATED CONTENT

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Fig 1. In the classic view of cell-cycle progression, mitogenic signaling pathways induce levels of D-type cyclins. The D-type cyclins form complexes with cyclin-dependent kinases (CDKs) 4 and 6, which then hypophosphorylate the retinoblastoma protein (Rb) during G₁. Once hypophosphorylated, Rb is primed for hyperphosphorylation by cyclin E-CDK2 complexes, which results in the release of the E2F transcription factors that are critical for entry into S phase. The later stages of the cell cycle (S, G₂, and M) are under the control of various other cyclin-CDK complexes but no longer responsive to extracellular influence. The INK4 proteins, including p15 and p16, inhibit cyclin D-CDK4/6 activity, whereas the CIP/KIP family, including p21 and p27, inhibit the remaining cyclin-CDK complexes at later stages of the cell cycle. ER, estrogen receptor; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; P, phosphate; PI3K, phosphatidylinositol 3-kinase.

protein kinase.⁹ As D-type cyclins accumulate, they associate with CDK4/6, and these complexes phosphorylate the retinoblastoma (Rb) protein, the key regulator of G₁ to S progression.¹⁰ The function of Rb depends upon the state of phosphorylation of the protein. While unphosphorylated, Rb prevents advance from G₁ to S by repressing the E2F family of transcription factors via blockade of their transactivation domains and recruitment of histone deacetylase.¹¹ Cyclin D-CDK4/6 initiates phosphorylation of Rb, which induces a conformational change that inhibits histone deacetylase binding and permits subsequent hyperphosphorylation by cyclin E-CDK2, whereupon E2Fs are released and implement a transcriptional program that allows S-phase entry.¹¹ In this model, the decision regarding G₁ to S progression is mediated by the balance of mitogenic and inhibitory signaling in G₀/G₁, which is reflected in cyclin D levels and cyclin D-CDK4/6 activity. Once Rb is hyperphosphorylated, cells commit to completing the cell cycle mostly unresponsive to external influence.

CDK-cyclin activity is regulated by two families of inhibitors.¹² The inhibitor of CDK4 (INK4) group (p15, p16, p18, and p19) specifically interferes with the association between CDK4/6 and cyclin D, with no activity upon other CDK-cyclins. Oncogenic MYC and RAS, for example, induce p16, which leads to cell-cycle arrest and senescence.^{13,14} The CIP/KIP CDK inhibitors (p21, p27, and p57), which were initially described as inhibitors of cyclin A/E-CDK2 and cyclin B-CDK1, are induced by various mechanisms. For example, p27 increases upon inhibitory signaling (eg, by transforming growth factor beta) and functions to maintain quiescence, whereas p21, a transcriptional target of p53, is upregulated by DNA damage and inhibits cyclin-CDK complexes to halt progress until repair occurs.¹⁵ During G₁ phase, p27 binds cyclin E-CDK2 to prevent Rb hyperphosphorylation and restrain G₁ to S progression. As mitogenic signaling increases cyclin D

levels, p27 shifts to complex with cyclin D-CDK4/6 and assumes a more nuanced role, with both tumor suppressive and oncogenic properties. Here, p27 functions as a molecular switch that is capable of activating or inactivating the Rb phosphorylating function of cyclin D-CDK4/6 depending on p27's own phosphorylation status at a particular tyrosine residue (Y88).^{16,17} The kinase responsible for phosphorylating p27 has recently been identified in breast cancer.¹⁷ These findings may be of clinical relevance as overexpression of p27 Y88 or the phosphorylating kinase could impart resistance to CDK4/6 inhibitors. As a corollary, the tumor suppressor activity of p16 is related to p27, as increased levels of p16 bind cyclin D-CDK4/6, which redistributes p27 to cyclin E-CDK2 and reinforces cell-cycle arrest.¹⁸

This model is oversimplified in several respects. In the traditional view, cyclin D-CDK4/6 progressively phosphorylate Rb at multiple sites, priming Rb for further phosphorylation and inactivation by cyclin E-CDK2. Recent evidence suggests that cyclin D-CDK4/6 only monophosphorylate Rb at one of 14 sites.¹⁹ These various monophosphorylated forms show different binding specificities for E2Fs and other substrates, which suggests unrecognized complexity in Rb's function during G₁ phase. Moreover, several non-E2F-dependent mechanisms of Rb control over the cell cycle exist. For example, Rb binds the cognate binding protein, S-phase kinase-associated protein 2 (SKP2), which prevents SKP2-mediated degradation of p27 and promotes cell-cycle arrest.^{20,21} Rb also colocalizes the anaphase-promoting complex with SKP2 to target SKP2 for degradation.²² Lastly, although CDK4/6 functions largely upon Rb, 71 other substrates have been identified, including the transcription factor FOXM1, which restrains senescence.²³ Cyclin D3-CDK6 and cyclin D1-CDK4 show divergent substrate specificities, which suggests unappreciated complexity in their function as well.²³

Several observations emerge from this cursory review. Because a commitment to cell division is made in late G₁ phase, inhibitors of CDK4/6–cyclin D may be of greatest therapeutic relevance.⁴ Next, alterations in various cyclins, CDKs, and their inhibitors may render specific tumors more or less sensitive to CDK4/6 inhibition. Lastly, because levels of D-type cyclins are regulated by mitogens, an appreciation of signaling pathways that are important in various cancers will help identify tumor-specific mechanisms of cell-cycle activation.

ALTERATIONS IN CELL-CYCLE COMPONENTS IN CANCER AND EARLY CDK INHIBITORS

The importance of the cyclin D–CDK4/6–Rb pathway in cancer is highlighted by the observation that nearly all tumors harbor abnormalities in a component, that alterations in upstream tumor suppressors and oncoproteins may ultimately function by influencing cell-cycle activity, and several viral oncoproteins function by inactivating Rb. Alterations in cell-cycle components, however, are variable by tumor type, which reflects the differential importance of various cyclins, CDKs, and inhibitors in normal tissue development and homeostasis.²⁴ Breast cancer illustrates this heterogeneity even within a given tumor type. Gene expression profiling has identified four distinct subtypes of breast cancer: luminal A and B (commonly hormone receptor [HR]–positive), human epidermal growth factor receptor 2 (HER2)–enriched, and basal-like (frequently HR-negative).²⁵ Cyclin D1 amplification and CDK4 copy gain are common among luminal and HER2-enriched subtypes but are rare in basal-like tumors, which harbor Rb loss or mutation and amplification of cyclin E1. In glioblastoma, melanoma, and pancreatic cancer, p16 loss is common and allows tumors to escape oncogene-induced senescence.^{26–28} In well-differentiated/dedifferentiated liposarcoma, amplification of 12q, which contains CDK4, is highly prevalent.²⁹ Mutation of Rb itself,

however, is infrequent possibly because intact Rb is helpful during the early stages of cancer progression.³⁰

The first generation of cell-cycle therapeutics demonstrated limited efficacy and considerable toxicity. This likely relates to their broad, and thus toxic, activity on CDKs that are important for mitosis and DNA transcription.² Recently, three highly selective ATP-competitive CDK4/6 inhibitors (CDK4/6i) have entered clinical development: palbociclib, ribociclib, and abemaciclib. Although mechanistically similar, differences are emerging (Table 1). A summary of completed and ongoing phase III trials is listed in Table 2.

CDK4/6 INHIBITORS AS MONOTHERAPY

Palbociclib

Palbociclib inhibits CDK4–cyclin D1, CDK4–cyclin D3, and CDK6–cyclin D2 with IC₅₀s of 11 nmol/L, 9 nmol/L, and 15 nmol/L, respectively, with no activity against 36 other kinases tested.³¹ Palbociclib abrogated phosphorylated Rb (pRb) and induced G₁ arrest in Rb-positive, but not Rb-negative, cancer cell lines. Although cytostatic effects were observed in cell lines, regressions occurred in colon, breast, and glioblastoma xenografts.³¹ In contrast, in NOTCH-driven models of T-cell acute lymphoblastic leukemia, palbociclib induced prominent apoptosis in both cell lines and animal models.⁴⁰

Palbociclib was evaluated in two phase I trials that involved Rb-positive solid tumors and lymphomas. By using a 2 week on/1 week off (21 day) schedule, the maximum tolerated dose (MTD) was 200 mg per day.⁴¹ One patient with testicular cancer achieved a partial response (PR) and 29% showed stable disease (SD) for at least two cycles. By using a 3 week on/1 week off (28 day) schedule, the MTD was 125 mg per day.⁴² Although no patients achieved a response, 13 exhibited SD for two cycles or more. Dose-limiting toxicities (DLTs) were related to myelosuppression with grade 3 to

Table 1. Profiles of the CDK4/6 Inhibitors in Advanced Clinical Development

Variable	Palbociclib (Ibrance; Pfizer, New York, NY)	Ribociclib (Kisqali, Novartis, Basel, Switzerland)	Abemaciclib (Lilly, Indianapolis, IN)
IC ₅₀	CDK4, 9–11 nM ³¹ CDK6, 15 nM	CDK4, 10 nM ³² CDK6, 39 nM	CDK4, 2 nM ³³ CDK6, 10 nM
RP2D	125 mg orally per day (3 weeks on, 1 week off)	600 mg orally per day (3 weeks on, 1 week off)	200 mg orally twice per day (continuously)
Regulatory approvals	HR-positive, HER2-negative ABC in combination with letrozole as initial endocrine-based therapy (2015); and in combination with fulvestrant after progression on first-line endocrine therapy (2016)	HR-positive, HER2-negative ABC in combination with letrozole as initial endocrine-based therapy (2017)	None
DLTs (phase I studies)	Neutropenia, thrombocytopenia	Mucositis, pulmonary embolism, neutropenia, febrile neutropenia, thrombocytopenia, QTc prolongation	Fatigue
Most common grade 3 and 4 toxicities: single agent at RP2D ^{34–36}	Neutropenia, 54% Thrombocytopenia, 19% Anemia, 5% Sepsis, 3%	Neutropenia, 28% Thrombocytopenia, 9% Anemia, 3% Fatigue, 3% Diarrhea, 3%	Neutropenia, 10% Thrombocytopenia, 7% Diarrhea, 5% Anemia, 4% Fatigue, 3%
ORR as single agent in HR + ABC ^{34–36}	6% (n = 33)	—	17%–31% (n = 132; n = 36)

Abbreviations: ABC, advanced breast cancer; CDK, cyclin-dependent kinase; DLT, dose-limiting toxicity; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IC₅₀, concentration needed to inhibit CDK activity by half; ORR, RP2D, recommended phase II dose.

Table 2. Completed and Ongoing Phase III Trials With Selective CDK4/6 Inhibitors, Palbociclib, Ribociclib, and Abemaciclib

Patient Population	Line of Therapy	Arm	Primary End Point	Secondary End Points	Grade 3 and 4 Toxicities	Comment
Palbociclib ER-positive, HER2-negative ABC; premenopausal (n = 666) PALOMA-2 ^{37a}	First line: no prior systemic therapy (either endocrine or chemotherapy) for ABC*	Palbociclib plus letrozole (n = 444) Placebo plus letrozole (n = 222)	PFS: 24.8 months v 14.5 months (hazard ratio, 0.58; 95% CI, 0.46 to 0.72 months; <i>P</i> < .001)	ORR†: 55.3% v 44.4% (OR, 1.55; <i>P</i> = .03) OS: Not mature	Neutropenia, 66.4%; leukopenia, 24.8%; anemia, 5.4%; fatigue, 1.8% Neutropenia, 1.4%; leukopenia, 0%; anemia, 1.8%; fatigue, 0.5%	Dose reduction required in 36% of patients on palbociclib plus letrozole v placebo plus letrozole Grade 3 and 4 febrile neutropenia occurred in eight patients (1.4%) with palbociclib plus letrozole v placebo plus letrozole Benefit was independent of age, performance status, disease site, prior chemotherapy, prior endocrine therapy, and disease-free interval after adjuvant treatment
HR-positive, HER2-negative ABC; pre- or postmenopausal (n = 521) PALOMA-3 ³⁸	Second line: progression after prior endocrine therapy for ABCs; one prior line of chemotherapy allowed	Palbociclib plus fulvestrant (n = 347)	PFS: 9.5 months v 4.6 months (hazard ratio, 0.46; 95% CI, 0.36 to 0.59; <i>P</i> < .001)	ORR†: 24.6% v 10.9% (OR=2.69; <i>P</i> = 0.0012)	Neutropenia, 65%; leukopenia, 28%; anemia, 3%; increased AST, 3%	A <i>PIK3CA</i> mutation was detected in plasma in 33% of patients; however, neither <i>PIK3CA</i> mutational status nor hormone-receptor expression level affected response to palbociclib.
HR-positive HER2-negative breast cancer; pre- or postmenopausal (n = 1,100) PENLOPE-B	Adjuvant: patients with high risk of relapse after completion of neoadjuvant chemotherapy and surgery	Placebo plus fulvestrant (n = 174) Palbociclib (13 cycles); placebo (13 cycles)	Invasive disease-free survival	OS: Not mature Distant disease-free survival, OS, patient-reported outcomes, quality-adjusted life years, safety	Neutropenia, 1%; leukopenia, 1%; anemia, 2%; increased AST, 3% —	Accrual ongoing: this study will enroll a population of patients with breast cancer who are at high risk of relapse according to the clinical-pathologic stage-estrogen/grade 1 score Accrual ongoing
HR-positive, HER2-negative breast cancer; pre- or postmenopausal (n = 4,600) PALLAS	Adjuvant: open to all patients with stage II or III breast cancer after surgery	Palbociclib (2 years) plus standard adjuvant endocrine therapy (at least 5 years) Standard adjuvant endocrine therapy (at least 5 years)	Invasive disease-free survival	Distant recurrence-free survival, locoregional recurrence-free survival, and OS	—	Accrual ongoing: Exploratory outcomes—correlation of cell cycle (CDKN2A, pRb, cyclin D) and breast cancer (<i>PTEN</i> , <i>ERBB2</i> , <i>BRCA1/2</i>) biomarkers with outcomes
HR-positive, HER2-negative ABC; postmenopausal (n = 600) PEARL	Second-line: resistant to prior NSAI (letrozole or anastrozole)§ One prior line of chemotherapy allowed	Palbociclib plus endocrine therapy (exemestane or fulvestrant) Capecitabine 1,250 mg/m ² twice per day for 2 of every 3 weeks	PFS (overall and stratified by <i>ESR1</i> mutational status)	ORR, CBR, OS	—	Accrual ongoing: Exploratory outcomes—correlation of cell cycle (CDKN2A, pRb, cyclin D) and breast cancer (<i>PTEN</i> , <i>ERBB2</i> , <i>BRCA1/2</i>) biomarkers with outcomes

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Table 2. Completed and Ongoing Phase III Trials With Selective CDK4/6 Inhibitors, Palbociclib, Ribociclib, and Abemaciclib (continued)

Patient Population	Line of Therapy	Arm	Primary End Point	Secondary End Points	Grade 3 and 4 Toxicities	Comment
Ribociclib HR-positive, HER2-negative ABC; postmenopausal (n = 668) MONALEESA-2 ^{39,63}	First line: no prior systemic therapy (either endocrine therapy or chemotherapy) for ABC*	Ribociclib plus letrozole (n = 334)	PFS: NR v 14.7 months (hazard ratio, 0.56; 95% CI, 0.43 to 0.72; <i>P</i> < .001)	ORR†: 52.7% v 37.1% (<i>P</i> < .001) OS: Not mature	Neutropenia, 59.3%; leukopenia, 21.0%; hypertension, 9.9%; increased ALT, 9.3%	Dose reductions occurred in 54% of patients in the ribociclib plus letrozole group compared with 7% in the placebo plus letrozole group
		Placebo plus letrozole (n = 334)			Neutropenia, 0.9%; leukopenia, 0.6%; hypertension, 10.9%; increased ALT, 1.2%	Prolongation of the QTc interval > 480 ms occurred in 3.3% of ribociclib-treated patients
					—	Accrual complete
HR-positive, HER2-negative ABC; pre- and perimenopausal (n = 660) MONALEESA-7	First line: no prior endocrine therapy for ABC*; one prior line of chemotherapy for ABC allowed	Ribociclib plus either tamoxifen or NSAI (letrozole or anastrozole) plus goserelin; placebo plus either tamoxifen or NSAI (letrozole or anastrozole) plus goserelin	PFS	ORR, OS, QOL	—	Accrual complete
	First or second line: no prior endocrine therapy for ABC or after progression on first-line endocrine therapy for ABC; no prior chemotherapy for ABC allowed	Ribociclib plus fulvestrant; placebo plus fulvestrant	PFS	ORR, OS, QOL	—	Accrual complete
HR-positive, HER2-negative ABC; postmenopausal (n = 725) MONALEESA-3						
Abemaciclib HR-positive, HER2-negative ABC; postmenopausal (n = 450) MONARCH-3	First-line: no prior systemic therapy (either endocrine/chemotherapy) for ABC*	Abemaciclib plus NSAI (letrozole or anastrozole); placebo plus NSAI (letrozole or anastrozole)	PFS	ORR, OS, PK, QOL	—	Accrual complete
	First or second line: no prior endocrine therapy for ABC or after progression on first-line endocrine therapy for ABC; no prior chemotherapy for ABC allowed	Abemaciclib plus fulvestrant; placebo plus fulvestrant	PFS	ORR, OS, PK, QOL	—	Accrual complete
Advanced KRAS mutant NSCLC (n = 550) JUNIPER	Third line: progression after platinum-based chemotherapy and one other systemic therapy (or deemed ineligible for second-line chemotherapy)	Abemaciclib plus best supportive care; erlotinib plus best supportive care	OS	PFS, ORR, PK	—	Accrual ongoing

NOTE: Drug dosing was as follows unless otherwise noted: Palbociclib: 125 mg daily for 3 weeks on and 1 week off (28 day cycles); ribociclib: 600 mg once daily for 3 weeks on and 1 week off (28 day cycles); abemaciclib: 200 mg twice daily continuously; letrozole: 2.5 mg once daily continuously; anastrozole: 1 mg daily continuously; tamoxifen: 20 mg daily continuously; fulvestrant: 500 mg intramuscularly days 1 and 15 of cycle 1, then 500 mg intramuscularly day 1 of all subsequent cycles; exemestane: 25 mg daily continuously; and goserelin 3.6 mg subcutaneously monthly.

Abbreviations: ABC, advanced (metastatic or locally advanced) breast cancer; CBR, clinical benefit rate; CDK, cyclin-dependent kinase; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MONALEESA, Mammary Oncology Assessment of LEE011's Efficacy and Safety; NR, not reached; NSAI, nonsteroidal aromatase inhibitor; NSCLC, non-small-cell lung cancer; OR, odds ratio; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PK, pharmacokinetics; pRb, phosphorylated retinoblastoma protein; QOL, quality of life.

†Overall response rate among patients with measurable disease by RECIST v1.1.

‡Pre- or perimenopausal women must receive concomitant goserelin.

§Progression ≤ 12 months from prior adjuvant endocrine therapy or ≤ 1 month from prior advanced/metastatic endocrine breast cancer therapy.

4 neutropenia observed in 20% to 25%, which likely reflects the requirement for CDK4/6 in hematopoietic cell-cycle entry. The 28-day schedule was associated with rapid recovery from neutropenia and selected for further development.

Mantle cell lymphoma is characterized by the t(11;14) translocation, which results in constitutive expression of cyclin D1 and was among the first malignancies in which palbociclib was evaluated.⁴³ In a study that enrolled 17 relapsed patients, one complete response and two PRs were observed. Five patients who continued palbociclib for at least 1 year demonstrated a reduction in summed 3-deoxy-3-[¹⁸F]-fluorothymidine positron emission tomography standard uptake value of at least 70% at week 3, which was consistent with palbociclib-induced growth arrest. A $\geq 90\%$ reduction in pRb was necessary but not sufficient to predict clinical benefit. This study provided evidence of palbociclib's on-target activity, but why some patients with suppression of pRb failed to derive benefit remains unclear.⁴⁴

Studies in well-differentiated/dedifferentiated liposarcoma,^{45,46} advanced germ-cell tumors,^{47,48} ovarian cancer,⁴⁹ non-small-cell lung cancer (NSCLC),⁵⁰ and KRAS-mutant colon cancer⁵¹ showed stable disease in a subset of patients. Palbociclib monotherapy was evaluated in 37 patients with advanced breast cancer (ABC), of whom 84% were HR positive, HER2 negative.³⁴ Enrollment in the triple-negative arm was closed after rapid progression. Median progression-free survival (mPFS) was significantly longer for HR-positive (4.5 months) compared with HR-negative (1.5 months) patients.

Ribociclib

Ribociclib inhibits CDK4 and CDK6, with IC_{50} s of 10 nmol/L and 39 nmol/L, respectively⁵² (Table 1). Ribociclib was active in Rb-positive models of breast cancer, melanoma, neuroblastoma, and liposarcoma, with suppression of pRb and induction of G₁ arrest.^{32,53} In liposarcoma cell lines, chronic exposure led to recovery of pRb with release from G₁ arrest, which implied potential benefits from intermittent dosing.⁵⁴ In neuroblastoma models, MYC amplification, which typically signifies poor prognosis, imparted sensitivity to ribociclib.⁵³ Although the mechanism was not elucidated, MYC is known to directly antagonize p21 and p27 and to induce G₁ cyclins and may serve an analogous role to HR positivity in breast cancer.⁵⁵

Ribociclib was evaluated in a phase I dose-escalation study in Rb-positive solid tumors and lymphomas using two schedules: 3 weeks on/1 week off (28-day cycle) and continuous dosing, but myelosuppression precluded the development of continuous administration.³⁵ On the 28-day schedule, DLTs included febrile neutropenia, thrombocytopenia, and QTc prolongation. The recommended phase II dose (RP2D) was 600 mg per day, below the MTD of 900 mg per day, owing to a lower incidence of QTc prolongation. Among 132 evaluable patients, there were three PRs and eight patients with SD disease for > 6 months. At the RP2D, the most common adverse events were grade 3 and 4 neutropenia and thrombocytopenia, which occurred in 28% and 9%, respectively.

Abemaciclib

Abemaciclib demonstrates IC_{50} s of 2 nmol/L and 10 nmol/L for CDK4 and CDK6, respectively³³ (Table 1). In preclinical

studies, activity was also noted on CDK9–cyclin T1 (IC_{50} , 57 nmol/L), though effects were of uncertain significance. Antitumor activity mediated by reversible G₁ arrest was demonstrated in several models, and, in colon xenografts, no acquired resistance developed after prolonged dosing.³³ Abemaciclib was evaluated in a phase I study in advanced solid tumors using once per day and twice per day regimens, with the latter selected as a result of the sustained inhibition of pRb in pharmacodynamic correlates.³⁶ DLTs were related to fatigue and the MTD/RP2D was 200 mg twice per day.

One hundred ninety-two patients were treated in tumor-specific cohorts. All-grade toxicities, mostly grade 1 and 2 in severity, included diarrhea (63%), nausea (45%), and fatigue (41%). Myelosuppression was less common, with only 10% experiencing grade 3 and 4 neutropenia. In breast cancer, the clinical benefit rate (complete response + PR + SD ≥ 24 weeks) for HR-positive and HR-negative patients was 61% versus 11%, respectively, and responses occurred exclusively in HR-positive patients (31% v 0%), including some with HER2-positive disease. Among 68 heavily pretreated patients with NSCLC, SD for > 24 weeks occurred in 31% of KRAS-mutant versus 12% of KRAS wild-type patients. Evidence of activity was noted in smaller cohorts with ovarian cancer, melanoma, and glioblastoma, the latter reflecting abemaciclib's penetration of the blood-brain barrier. In MCL, abemaciclib monotherapy resulted in a response rate of 23%.⁵⁶

Despite mechanistic similarities, early-phase studies have demonstrated some differences between CDK4/6i. DLTs for abemaciclib were related to fatigue, whereas myelosuppression, particularly neutropenia, was dose limiting for palbociclib and ribociclib. Lower rates of myelosuppression presumably permit the continuous administration of abemaciclib.

CDK4/6 INHIBITORS IN COMBINATION THERAPIES

As the cell cycle operates downstream from oncogenic signaling pathways and CDK4/6i exhibits low response rates but favorable tolerability, further clinical development is focusing on mechanism-based combinations, and ongoing approaches are listed in Table 3.

Combinations With Hormonal Agents in Breast Cancer

In a pivotal preclinical study, gene expression profiling identified genes that correlate with palbociclib sensitivity in breast cancer.⁵⁷ Genes that were upregulated in palbociclib-sensitive cell lines were exclusively luminal, whereas nonluminal markers were over-represented in resistant lines. Thus, HR-positive and HER2-enriched tumors seem to be dependent on cyclin D–CDK4/6 regulation of Rb for G₁ to S progression. Mechanistically, activation of the estrogen receptor (ER) pathway induces cyclin D1 levels and combining hormone blockade with CDK4/6i cooperatively reduces cyclin D–CDK4/6 activity.⁵⁸ Furthermore, breast cancers that are resistant to hormonal agents remain dependent on cyclin D1.^{59,60} In preclinical studies, palbociclib and tamoxifen were synergistic and palbociclib resensitized tamoxifen-resistant cell lines to endocrine-based therapy.³¹

In view of these findings, the development of CDK4/6i has focused on HR-positive ABC in combination with endocrine-based

Table 3. Active Clinical Trials Studying Cyclin-Dependent Kinase 4/6 Inhibitors in Combination With Other Therapies (excluding combinations in breast cancer limited to hormonal/HER2 agents alone)

Type of Advanced Cancer	Combination Agent	Phase	Trial
Palbociclib			
Solid tumors	FU plus oxaliplatin	I	NCT01522989
Solid tumors	Carboplatin or cisplatin	I	NCT02897375
Solid tumors (<i>PI3K</i> mutation)	Taselisib or pictilisib	I	NCT02389842
Solid tumors (<i>EGFR</i> , <i>HER2-4</i> alterations)	Neratinib	I	NCT03065387
NSCLC (<i>KRAS</i> mutation)	PD-0325901 (MEK 1/2 Inhibitor)	I and II	NCT02022982
Squamous lung, pancreatic, head and neck or other cancers with <i>PI3K</i> dependance	PF-05212384 (<i>PI3K</i> /mTOR inhibitor)	I	NCT03065062
Pancreatic	Nab-paclitaxel	I	NCT02501902
Head and neck	Cetuximab	II	NCT02499120
Prostate	Androgen deprivation therapy	II	NCT02059213
Breast (HR-positive, HER2-negative)	Pembroluzumab plus letrozole	I	NCT02778685
Breast (HR-positive, HER2-negative)	Everolimus plus exemestane	I and II	NCT02871791
Breast (HR-positive, HER2-negative)	PF-05212384 plus letrozole or fulvestrant	I	NCT02684032
Breast (HR-positive, HER2-negative, <i>PIK3Cα</i> mutant)	GDC-0077 (<i>PI3K</i> inhibitor) plus letrozole	I	NCT03006172
Breast	Paclitaxel	I	NCT01320592
Mantle cell lymphoma	Ibrutinib	I	NCT02159755
Mantle cell lymphoma	Bortezomib	I	NCT01111188
Ribociclib			
Melanoma (<i>NRAS</i> mutant)	Binimetinib	I and II	NCT01781572
Melanoma (<i>BRAF</i> mutant)	Binimetinib and LGX818 (RAF inhibitor)	I and II	NCT01543698
Castrate resistant prostate	Enzalutamide	I and II	NCT02555189
Castrate resistant prostate	Docetaxel and prednisone	I and II	NCT02494921
Pancreatic	Everolimus	I and II	NCT02985125
Pancreatic and colon (<i>KRAS</i> mutation)	Trametinib	I and II	NCT02703571
Soft tissue sarcomas	Doxorubicin	I	NCT03009201
Well-differentiated/dedifferentiated liposarcoma	HDM201 (HDM2 inhibitor)	I and II	NCT02343172
NSCLC (<i>ALK</i> mutation)	Ceritinib	I and II	NCT02292550
Head and neck	Cetuximab	I	NCT02429089
Neuroendocrine tumors	Everolimus	II	NCT03070301
Glioma	Radiotherapy		NCT02607124
Ovarian (platinum sensitive)	Platinum chemotherapy	I	NCT03056833
Endometrial	Everolimus plus letrozole	II	NCT03008408
Breast	Capecitabine	I	NCT02754011
Breast	Paclitaxel	I	NCT02599363
Breast (HR-positive, HER2-negative)	Everolimus plus exemestane	I and II	NCT01857193
Breast (HR-positive, HER2-negative)	Fulvestrant plus alpelisib or buparlisib	I and II	NCT02088684
Breast (HR-positive, HER2-negative)	Letrozole plus alpelisib	I	NCT01872260
Breast (HR-positive, HER2-negative)	Letrozole plus buparlisib	I	NCT02154776
Neuroblastoma (<i>ALK</i> mutation)	Ceritinib	I	NCT02780128
Myelofibrosis	Ruxolitinib plus PIM447 (PIM kinase inhibitor)	I	NCT02370706
Abemaciclib			
Solid tumors	LY3300054 (anti-PD-L1)	I	NCT02791334
Solid tumors (<i>RAS</i> /MAPK alteration)	LY3214996 (ERK1/2 inhibitor)	I	NCT02857270
Breast (<i>NOTCH</i> alteration)	LY3039478 (<i>Notch</i> inhibitor)	I	NCT02784795
Breast (HR-positive, HER2-negative)	Exemestane plus everolimus or LY3023414 (<i>PI3Kα</i> /mTOR inhibitor) plus fulvestrant	I	NCT02057133
Stage IV NSCLC, breast (HR-positive, HER2-negative)	Pembroluzimab	I	NCT02779751
Stage IV NSCLC	Necitumumab	I	NCT02411591
Stage IV NSCLC	Pemetrexed, gemcitabine, ramucirumab or pembroluzimab	I	NCT02079636
Pancreatic	LY3023414 or galunisertib	II	NCT02981342
Glioblastoma	Temozolomide	II	NCT02977780
Mantle cell lymphoma	Ramucirumab	I	NCT02745769

NOTE. Studies similar in concept may only be listed once.

Abbreviations: ERK, extracellular regulated kinase; FU, fluorouracil; HER2, human epidermal growth factor receptor; HR, hormone receptor; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NSCLC non-small-cell lung cancer; PD-L1, programmed death-ligand 1; *PI3K*, phosphatidylinositol 3-kinase; *PI3Kα*, *PI3K* catalytic subunit alpha; PIM, proviral integration site for Moloney murine leukemia virus.

therapy.³⁴ PALOMA-1 was a randomized, open-label, phase II study of palbociclib plus letrozole versus letrozole alone in treatment-naïve ER-positive, HER2-negative ABC.⁶¹ The study initially accrued a separate cohort that required cyclin D1 amplification, p16 loss, or both. An interim analysis found these biomarkers were unlikely to enrich for efficacy. Among the entire population, mPFS, the primary end point, was 20.2 months for

palbociclib plus letrozole versus 10.2 months for letrozole alone, a highly significant improvement. With the combination, neutropenia was common, but febrile neutropenia was rare. PALOMA-1 led to approval of this combination for treatment-naïve HR-positive, HER2-negative ABC. The randomized, placebo-controlled, phase III PALOMA-2 confirmed the results from PALOMA-1.⁶²

In the phase III PALOMA-3 study, patients with HR-positive, HER2-negative ABC with progression on prior endocrine treatment were randomly assigned to the selective ER degrader fulvestrant plus palbociclib versus fulvestrant plus placebo.³⁸ Thirty-four percent of patients received prior chemotherapy. With a median follow-up of 8.9 months, mPFS was significantly improved at 9.5 months versus 4.6 months, respectively. Benefit was observed regardless of prior endocrine therapy, HR expression level, or phosphatidylinositol 3-kinase (*PI3K*) mutational status. PALOMA-3 resulted in approval for palbociclib plus fulvestrant for treatment of patients with HR-positive, HER2-negative ABC after experiencing progression on first-line endocrine therapy.

Results from the phase III MONALEESA-2 study, which randomly assigned patients with treatment-naïve HR-positive, HER2-negative ABC to ribociclib plus letrozole versus placebo plus letrozole were recently reported and showed a significant improvement in PFS for that combination, which resulted in regulatory approval.^{52,63} At 18 months, PFS was significantly improved with the combination at 63% versus 42%, respectively. A phase Ib study evaluated the combination of abemaciclib with various hormone-based therapies, showing safety and preliminary efficacy with diarrhea the chief toxicity.⁶⁴ Results of several randomized, phase III studies with abemaciclib are awaited. CDK4/6i is being extensively studied in adjuvant and neoadjuvant settings as well.

Other Combination Approaches in Breast Cancer

Although 45% of luminal breast cancers harbor mutations in *PIK3CA*, *PI3K* inhibitor (*PI3Ki*) monotherapy has proven disappointing.²⁵ *PI3Ki*-sensitive cell lines demonstrate suppression of pRb, whereas resistant lines show persistent pRb mediated by mammalian target of rapamycin. Unfortunately, combining *PI3K* with mammalian target of rapamycin inhibitors appears toxic.⁶⁵ In a combinatorial drug screen, ribociclib was the strongest resensitizing agent for *PI3Ki* in *PI3Ki*-resistant models, which resulted in suppression of pRb and synergistic effects. In *PIK3CA*-mutant breast cancer xenografts, the combination seemed to be effective as initial therapy or after acquired *PI3Ki* resistance.⁶⁵ A phase Ib/II study established a tolerable combination of ribociclib, alpelisib (*PI3K* α -specific inhibitor), and letrozole in women with ER-positive, HER2-negative ABC.⁶⁶

Combinations With Targeted Therapies in Other Cancers

Mutations in the *RAS* proto-oncogene are common in cancer, but oncogenic *RAS* has proven to be resistant to pharmacologic inhibition, and targeting downstream signaling components RAF, mitogen-activated protein kinase kinase (MEK), and extracellular regulated kinase to approximate *RAS* inhibition results in limited success. In an inducible *NRAS*-mutant, genetically engineered melanoma mouse model, pRb persisted despite MEK inhibitor (MEKi) monotherapy but not with *NRAS* extinction, and a systems biology approach identified CDK4 as the driver of these divergent phenotypes.⁶⁷ Combining MEKi with palbociclib was synergistic and induced regressions in vivo that were not observed with either monotherapy.⁶⁷ A phase Ib/II study is evaluating ribociclib with the MEKi, binimetinib, in *NRAS*-mutant melanoma.⁶⁸ Among 22 patients in phase I, many who received prior immunotherapy, the overall response rate was 41% and mPFS was 6.7 months. These

results compare favorably with a phase II study of binimetinib monotherapy.⁶⁹

In *KRAS*-mutant colon cancer, monotherapy with either MEKi or CDK4/6i has been similarly disappointing^{51,70}; however, combination of palbociclib and MEKi was synergistic in *KRAS*-mutant colon cancer cell lines with tumor regressions in xenografts.^{71,72} *KRAS* is also mutated in a subset of NSCLC, and a synthetic lethal interaction was reported for MEKi and genetic inactivation of CDK4 in mouse models—findings that were not recapitulated with ablation of other CDKs.⁷³ These studies suggest a role for CDK4/6i in combination with inhibitors of the *RAS*/mitogen-activated protein kinase pathway, but the mechanistic underpinnings of these combinations likely differ by tumor. For example, in *BRAF*-mutant melanoma, palbociclib antagonized vemurafenib-mediated cytotoxicity.⁷⁴ Clinical studies with MEK, extracellular regulated kinase, and RAF inhibitors are ongoing in several tumor types (Table 3).

PI3K signaling is implicated in B-cell survival, and expression of the *PI3K* δ subunit is restricted to hematopoietic cells and, thus, is a potential therapeutic target. In advanced MCL, the *PI3K* δ -specific inhibitor, GS101, transiently inhibited pAKT with little effect on cell-cycle arrest or apoptosis⁷⁶; however, induction of G₁ arrest with palbociclib followed by treatment with GS101 sensitized cell lines to *PI3K* δ inhibition. The mechanism for G₁ sensitization involved induction of *PIK3IP1*, an endogenous inhibitor of *PI3K*, which is expressed during G₁. Thus, palbociclib seemed to favorably reprogram gene expression, which allowed the combination to work. Similarly, in multiple myeloma, changes in gene expression correlate with the length of G₁ arrest, with some genes remaining either suppressed or activated after release from G₁.⁷⁷ Treatment with palbociclib resulted in the favorable expression of proapoptotic and antiapoptotic genes as well as IRF4, which normally protects myeloma cells from bortezomib killing. The combination of palbociclib, bortezomib, and dexamethasone was evaluated in a phase I and II trial in myeloma for which, among 25 evaluable patients, overall response rate was 20%.⁷⁸ Although the challenges that exist in translating schedule-based mechanisms to the clinic may limit these approaches, vulnerabilities that are created by alterations in gene expression via G₁ arrest warrant further examination.

Interactions between immune checkpoint blockade and the cell cycle are poorly understood. Of some concern, at least in T lymphocytes, programmed death-1 signaling inhibits cell-cycle progression by stabilizing p27.⁷⁹ In the neoadjuvant breast cancer setting, however, abemaciclib was shown to increase tumor infiltration by cytotoxic, but not regulatory T cells.⁸⁰ Combination studies with immunotherapy are ongoing in breast cancer and NSCLC. Studies that have evaluated chemotherapy in combination with CDK4/6i have reported mixed results. In preclinical studies of breast cancer, palbociclib inhibited the cytotoxic effects of doxorubicin.⁸¹ When pancreatic cancer cell lines were treated with palbociclib followed by chemotherapy, antagonism was observed with agents that acted in S and M phase, including gemcitabine and taxanes⁴⁴; however, in a clinical trial of advanced HR-positive breast cancer, palbociclib and paclitaxel were successfully combined on a unique alternating schedule on the basis of preclinical optimization.⁸² Similarly, fluorouracil and palbociclib were also safely combined in patients with advanced solid tumors without antagonism.⁸³ Clinical trials are ongoing with platinum therapies in ovarian cancer, docetaxel in prostate cancer, and doxorubicin in

sarcoma. The effectiveness of radiotherapy in combination with CDK4/6i also depended on proper sequencing in preclinical studies of atypical rhabdoid tumor and brainstem glioma and is under clinical evaluation for treatment of CNS malignancies.^{84,85}

BIOMARKERS AND MECHANISMS OF RESISTANCE

Although considerable preclinical literature has evaluated biomarkers for CDK4/6i, validation in prospective clinical trials has lagged. Most studies find that Rb-negative models are unaffected by CDK 4/6i, with some notable exceptions.^{86,87} HR-positive status in breast cancer remains the only biomarker that is used clinically.

A large number of preclinical studies, particularly in melanoma and glioblastoma, have implicated the loss of p16 in palbociclib sensitivity.⁸⁸⁻⁹⁰ Gene expression profiling of ovarian cancer cell lines revealed that low p16, along with higher levels of Rb and lower cyclin E, implied sensitivity.⁹¹ In pancreatic cell lines, resistance to palbociclib was associated with increased cyclin E1.⁴⁴ In renal cell carcinoma models, loss of p16 again correlated with sensitivity along with low expression of E2F1 mRNA.⁹² In sarcoma cell lines, sensitivity correlated with higher CDK4 expression.⁹³

These findings could be explained by mechanistic considerations. Low p16 reflects the loss of a critical endogenous inhibitor of CDK 4/6 activity, which could be restored by pharmacologic CDK4/6i.²⁴ Similarly, higher levels of cyclin D and CDK4 suggest dependence on this complex for G₁- to S-phase progression. In contrast, higher levels of cyclin E and E2Fs, both downstream from Rb, reflect the bypass of cyclin D-CDK4/6 regulation of Rb and predict resistance; however, none of these biomarkers has been clinically validated. In PALOMA-1, p16 and cyclin D1 added no predictive significance to HR positivity for patients who received palbociclib plus letrozole, though an analysis from PALOMA-2 showed a trend toward increased benefit for p16-negative patients.^{37,61} In the phase I study of ribociclib, none of 29 patients with deletion of p16 was among those who remained on drug for ≥ 8 weeks.³⁵ An integrated, comprehensive analysis of Rb pathway activity may be needed to identify sensitive tumors rather than the analysis of just one component in isolation.

Response to CDK4/6i may depend on whether neoplastic cells enter quiescence, a reversible nonproliferative state, or senescence, in which cells permanently exit the cell cycle unresponsive to mitogens. Indeed, some liposarcoma cell lines express senescence markers after palbociclib treatment, whereas others do not, and the senescence response seems to require reduced levels of MDM2.⁹⁴ This mechanism was recapitulated in glioma, breast, and lung cancer and was p53 independent. The protein ATRX, a member of the SWI/SNF chromatin remodeling complex, seems to be necessary for regulating MDM2 levels in responsive cells. Among seven paired pre- and post-treatment biopsies from patients with liposarcoma in a clinical study of palbociclib, those with favorable clinical outcomes showed reduced MDM2, whereas those with unfavorable outcomes had stable or increased levels. The precise mechanism requires additional investigation, though interconnections between MDM2 and Rb clearly exist.¹⁴

An understanding of acquired resistance will speed the development of combination strategies. Several mechanisms have been proposed. When HR-positive breast cancer cell lines received prolonged CDK4/6i, the remaining viable cells acquired CDK6 amplification with persistent expression of pRb.⁹⁵ Knockdown of CDK6 rendered resistant cells sensitive to CDK4/6i. In another breast cancer study, palbociclib-treated cell lines recovered pRb and returned to cycling after 72 hours of treatment.⁹⁶ This process was mediated by the activation of the PI3K pathway, which resulted in increased levels of G₁- to S-phase cyclins, including cyclin D1, which could complex with CDK2 to phosphorylate Rb. Addition of PI3Ki to palbociclib had synergistic antiproliferative effects by suppressing cyclins D1 and E2.⁴⁴ HR-positive breast cancer cell lines that received palbociclib for 4 months showed amplification of cyclin E1 and Rb mutation. This acquired resistance could not be overcome with the addition of PI3Ki; however, the combination in treatment-naïve tumors forestalled the development of resistance.⁹⁶

CONCLUSIONS

The success of next-generation CDK inhibitors reflects a detailed understanding of the cell cycle's molecular mechanisms and insightful preclinical investigations that have identified tumors that are most likely to benefit. Much work remains to ensure that CDK4/6i achieve maximal impact. Development of effective combinations requires a detailed mechanistic understanding of interactions between the cell cycle and other tissue-specific oncogenic alterations in cancer. Combinations with chemotherapy and immunotherapy may still prove effective, and well-designed clinical studies are needed. Clinical trials should incorporate correlatives to evaluate putative biomarkers and identify mechanisms of de novo and acquired resistance that are relevant to CDK4/6i combination approaches. Our appreciation of cancer-related vulnerabilities at other points in the cell cycle is expanding and holds additional therapeutic promise.^{97,98} The era of cell-cycle therapeutics—much delayed but now with newfound promise—is likely just beginning.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Cell-Cycle Therapeutics Come of Age

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