Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Review

Clinical Cancer Research

Ribociclib (LEE011): Mechanism of Action and Clinical Impact of This Selective Cyclin-Dependent Kinase 4/6 Inhibitor in Various Solid Tumors

Debu Tripathy[1], Aditya Bardia[2], and William R. Sellers[3]

Abstract

The cyclin D–cyclin-dependent kinase (CDK) 4/6–p16–retinoblastoma (Rb) pathway is commonly disrupted in cancer, leading to abnormal cell proliferation. Therapeutics targeting this pathway have demonstrated antitumor effects in preclinical and clinical studies. Ribociclib is a selective, orally bioavailable inhibitor of CDK4 and CDK6, which received FDA approval in March 2017 and is set to enter the treatment landscape alongside other CDK4/6 inhibitors, including palbociclib and abemaciclib. Here, we describe the mechanism of action of ribociclib and review preclinical and clinical data from phase I, II, and III trials of ribociclib across different tumor types, within the context of other selective CDK4/6 inhibitors. The pharmacokinetics, pharmacodynamics, safety, tolerability, and clinical responses with ribociclib as a single agent or in combination with other therapies are discussed, and an overview of the broad portfolio of ongoing clinical trials with ribociclib across a wide range of indications is presented. On the basis of the available data, ribociclib has a manageable tolerability profile and therapeutic potential for a variety of cancer types. Its high selectivity makes it an important partner drug for other targeted therapies, and it has been shown to enhance the clinical activity of existing anticancer therapies and delay the development of treatment resistance, without markedly increasing toxicity. Ongoing trials of doublet and triplet targeted therapies containing ribociclib seek to identify optimal CDK4/6–based targeted combination regimens for various tumor types and advance the field of precision therapeutics in oncology. *Clin Cancer Res; 23(13); 3251–62. ©2017 AACR.*

Introduction

Cancer development is often characterized by abnormal cellular proliferation and dysregulation of cell-cycle control (1). The cell cycle is regulated at different stages by various cyclin–cyclin-dependent kinase (CDK) complexes (Fig. 1). The G_1 (pre-DNA synthesis) to S (DNA synthesis) cell-cycle checkpoint is regulated by the cyclin D–CDK4/6–p16–retinoblastoma (Rb) pathway, which ensures conditions are appropriate for cell growth and division before the cell is irreversibly committed to division (2–4). In this pathway, cyclin D is the key entry point at which various mitogenic and growth arrest signaling pathways converge to regulate the cell cycle (3). In response to mitogenic signaling, levels of D-type cyclins rise and associate with CDK4 or CDK6. The resulting active cyclin D–CDK4/6 complexes phosphorylate Rb, relieving its repression of E2 transcription factors (E2F). The released E2F consequently activates the transcription of genes required for the G_1–S transition and cell-cycle progression (5, 6). The tumor suppressor and negative regulator of the cyclin D–CDK4/6 complex, p16, is a critical mediator of cellular senescence, limiting the replicative life span of cells (7).

In cancer, the cyclin D–CDK4/6–p16–Rb pathway is commonly disrupted in favor of cell-cycle progression and continued growth, rendering this pathway a key target for cancer therapeutics (5, 8). In hormone receptor–positive (HR^+) breast cancer, CDK4/6 pathway activation has also been associated with resistance to endocrine therapy (9, 10). p16 is frequently inactivated by gene deletion, point mutation, or transcriptional silencing by methylation (5). Amplification of *CDK4* and *CCND1* (encoding cyclin D1) and overexpression of cyclin D protein (e.g., through chromosome 11 translocations or inversions) are also frequent in human cancers (5, 11). The abnormalities of the cyclin D–CDK4/6–p16–Rb pathway vary across cancer types (Supplementary Table S1). The pathway can also be upregulated as a result of upstream oncogenic mutations (3, 12, 13). This provides a therapeutic potential for targeting CDK4 and CDK6 in the treatment of cancer.

Advent of CDK4/6 Inhibitors

Preclinical evidence showing a role for the cyclin D–CDK4/6–p16–Rb pathway in cancer led to the development of a first generation of broad-acting pan-CDK inhibitors (14). The first such inhibitor to enter clinical trials was flavopiridol; however, clinical studies demonstrated poor efficacy, complex pharmacokinetics, and dose-limiting toxicities due to off-target effects (14, 15). The late 1990s/early 2000s saw the development of

[1]Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas. [2]Department of Hematology/Oncology, Massachusetts General Hospital Cancer Center/Harvard Medical School, Boston, Massachusetts. [3]Novartis Institutes for BioMedical Research, Cambridge, Massachusetts.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Current address for W.R. Sellers: The Broad Institute and Dana-Farber Cancer Institute, Cambridge, Massachusetts.

Corresponding Author: Debu Tripathy, Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 1354, Houston, TX 77030. Phone: 713-792-2817; Fax: 713-563-0903; E-mail: dtripathy@mdanderson.org

doi: 10.1158/1078-0432.CCR-16-3157

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

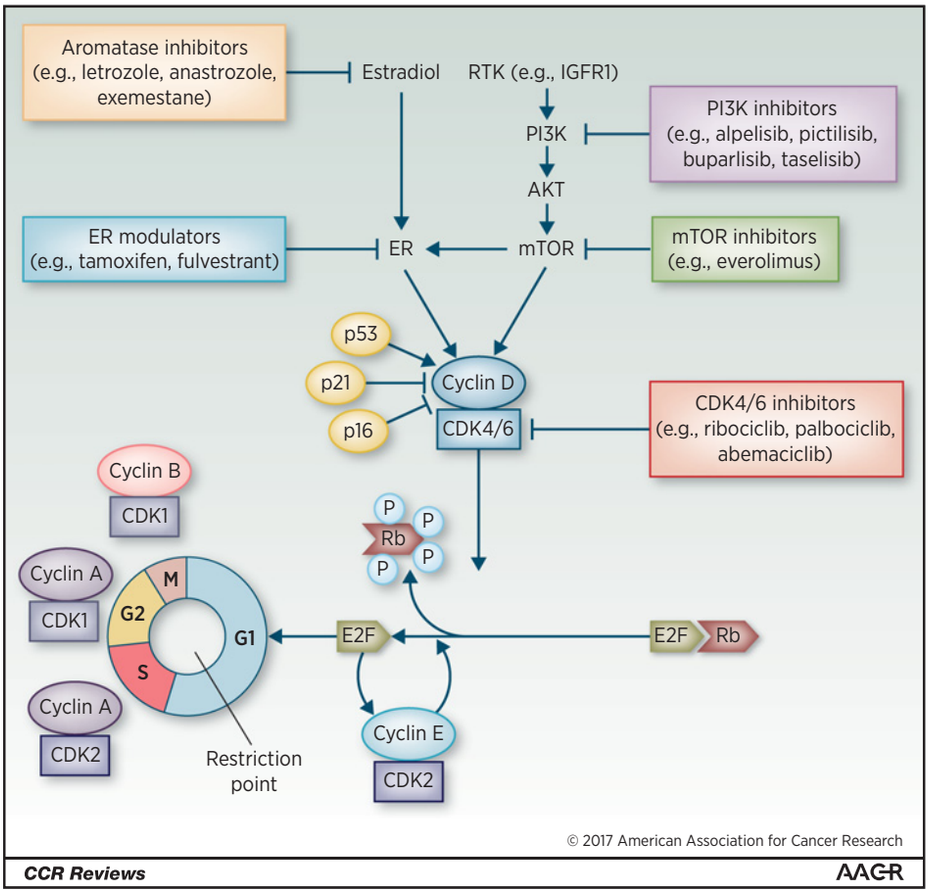

Figure 1.

The role of cyclin–CDK complexes and the cyclin D–CDK4/6–p16–Rb pathway in the cell cycle. The cell cycle is regulated by cyclins and checkpoints, with different cyclins (and cyclin–CDK combinations) and checkpoints acting at phase transition of the cell cycle. At the G_1–S cell-cycle checkpoint, mutiple mitogenic pathways, including ER and PI3K/AKT/mTOR, promote the synthesis of cyclin D, which associates with and activates CDK4/6. Activated CDK4/6 phosphorylates the Rb protein to disrupt its sequestering interaction with E2F, releasing its transcription effect and allowing the expression of genes necessary for cell-cycle progression, including cyclin E. Cyclin E associates with CDK2, which further phosphorylates Rb, resulting in progression of the cell cycle past the restriction point and irreversible S-phase entry. Additional cyclin–CDK complexes act at further cell-cycle checkpoints; cyclin A–CDK2 enables the S–G_2 transition, and cyclin A–CDK1 and cyclin B–CDK1 facilitate the onset and progression of mitosis, respectively (82). Selective inhibitors of CDK4/6, such as ribociclib, act directly on the cyclin D–CDK4/6–p16–Rb pathway to block cell-cycle progression. The pathway may also be impacted by inhibitors of other pathways acting upstream of CDK4/6, providing a rationale for dual inhibition. ER, estrogen receptor; IGFR1, insulin-like growth factor 1 receptor; RTK, receptor tyrosine kinase.

second-generation CDK inhibitors. These inhibitors showed preferential inhibition of CDK1 and CDK2 and/or increasing overall potency. Of these, dinaciclib, an inhibitor of CDK1, CDK2, CDK5, and CDK9, was most extensively studied in the clinic; however, phase II trials showed limited efficacy (14, 16, 17).

More recently, inhibitors specific for CDK4 and CDK6 have entered clinical trials. Such selective inhibitors spare CDK2 activity, avoiding inhibition of general S-phase activity. Three selective CDK4/6 inhibitors are in late-stage clinical development: ribociclib (LEE011; Novartis), palbociclib (Ibrance; PD-0332991; Pfizer), and abemaciclib (LY-2835219; Eli Lilly). Palbociclib combined with letrozole or fulvestrant has demonstrated efficacy in patients with HR^+ advanced breast cancer (ABC; Table 1; refs. 18, 19) and is approved in the United States and Europe for use in these combinations (20, 21). Abemaciclib has shown efficacy as a single agent and in combination with endocrine therapies in patients with HR^+ ABC and has entered phase III development, with FDA Breakthrough Therapy Designation granted in 2015 for patients with refractory HR^+ ABC (Table 1; refs. 22–24).

Ribociclib is a selective CDK4/6 inhibitor, which received FDA approval in March 2017 in combination with letrozole for the first-line treatment of HR^+, human epidermal growth factor receptor 2–negative ($HER2^-$) ABC (25). This review will outline the development of ribociclib and provide an overview of the preclinical and clinical data for ribociclib across various tumor types.

Characteristics of Ribociclib

Ribociclib, palbociclib, and abemaciclib are all orally bioavailable, small-molecule inhibitors that bind to the ATP cleft of CDK4 and CDK6. However, abemaciclib appears to bind more readily to

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Table 1. Key reported clinical trials with palbociclib and abemaciclib

Study name/ID	Combination drug	Phase	Population	Common grade 3/4 AEs (≥10% of patients)	Clinical activity
Palbociclib					
Breast cancer					
PALOMA-1/ NCT00721409 (74)	Letrozole	II	ER^+ ABC with no prior treatment for ABC ($N = 165$)	Neutropenia (54%), leukopenia (19%)	Median PFS 20.2 months vs. 5.7 months for letrozole monotherapy (hazard ratio = 0.488; $P = 0.0004$)
PALOMA-2/ NCT01740427 (18)	Letrozole	III	ER^+ ABC with no prior treatment for ABC ($N = 666$)	Neutropenia (66%), leukopenia (25%)	Median PFS 24.8 months vs. 14.5 months for letrozole monotherapy (hazard ratio = 0.58; $P < 0.000001$)
PALOMA-3/ NCT01942135 (19)	Fulvestrant	III	HR^+ ABC after progression on one line of endocrine therapy for ABC ($N = 521$)	Neutropenia (62%), leukopenia (25%)	Median PFS 9.2 months vs. 3.8 months for fulvestrant monotherapy (hazard ratio = 0.42; $P < 0.001$)
NCT01037790 (75)	None	II	Metastatic Rb^+ breast cancer ($N = 37$; $n = 33$ HR^+)	Neutropenia (54%), leukopenia (51%), lymphopenia (30%), thrombocytopenia (19%)	Total ($N = 37$): 5% PR, 38% SD <6 months, 14% SD ≥6 months, CBR 19% HR^+ ($n = 33$): 6% PR, 39% SD <6 months, 16% SD ≥6 months, CBR 21%
Solid tumors					
NCT00141297 (71)	None	I	Rb^+ advanced solid tumors ($N = 41$)	Neutropenia (20%), leukopenia (10%) in cycle 1	27% SD for ≥4 cycles, 16% SD for ≥10 cycles ($n = 37$ evaluable)
MCL					
NCT00420056 (76)	None	Ib	$CD19^+/CD20^+$, $CD5^+$, $CD23^-$ MCL, with cyclin D1 positivity, *t(11;14)* translocation, or *bcl-1/IgH* rearrangement ($N = 17$)	Neutropenia (35%), thrombocytopenia (24%), hypophosphatemia (12%)	6% CR, 12% PR, 41% SD, median PFS 4 months (90% CI, 2.0–14.7 months; $n = 16$ evaluable)
Liposarcoma					
NCT01209598 (77)	None	II	Advanced CDK4-amplified, Rb^+ well-differentiated or dedifferentiated liposarcoma ($N = 30$)	Neutropenia (50%), leukopenia (47%), thrombocytopenia (30%), lymphopenia (27%), anemia (17%)	3% PR, 12-week PFS 66% (90% CI, 51–100), median PFS 18 weeks ($n = 29$ evaluable)
NSCLC					
NCT01291017 (78)	None	II	Previously treated, advanced Rb^+, *CDKN2A* inactivated NSCLC ($N = 19$)	Neutropenia (16%)	50% SD, median PFS 12.5 weeks
Abemaciclib					
Solid tumors					
NCT01394016 (79)	None	I	Advanced solid tumors (whole study $N = 225$; escalation phase $n = 33$)	Leukopenia (10%), neutropenia (10%; $n = 173$)	Breast cancer ($n = 47$): 23% PR, 47% SD, 23% ORR, 49% CBR, 70% DCR, median PFS 5.8 months NSCLC ($n = 68$): 3% PR, 46% SD, 3% ORR, 49% DCR, median PFS 2 months; Melanoma ($n = 26$): 4% PR, 23% SD, 4% ORR, 27% DCR; Glioblastoma ($n = 17$): 0% PR, 18% SD, 0% ORR, 18% DCR; CRC ($n = 15$): 0% PR, 13% SD, 0% ORR, 13% DCR
NCT02014129 (80)	None	I	Japanese patients with advanced cancer ($N = 12$)	200 mg BID ($n = 6$): leukopenia (33%), neutropenia (17%)	Maximal percent change in tumor size from baseline: −35 to +25% >30% tumor shrinkage in 2 patients (HR^-, $HER2^+$ breast cancer and small intestine neuroendocrine carcinoma)
Breast cancer					
MONARCH-1/ NCT02102490 (22)	None	II	HR^+, $HER2^-$ ABC that has progressed on prior endocrine therapy and chemotherapy ($N = 132$)	Diarrhea (20%) and fatigue (13%), white blood cell decreased (28%), neutrophil count decrease (22%)	Confirmed PR 20%, confirmed ORR 20%, CBR 42%, median PFS 6 months

(Continued on the following page)

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Table 1. Key reported clinical trials with palbociclib and abemaciclib (Cont'd)

Study name/ID	Combination drug	Phase	Population	Common grade 3/4 AEs (≥10% of patients)	Clinical activity
NCT02057133 (81)	Endocrine and HER2-targeted therapies	Ib	HR^+ metastatic breast cancer ($N = 110$)	Combination with letrozole ($n = 20$): diarrhea (45%), fatigue (20%), neutropenia (20%), nausea (15%), vomiting (10%)	Combination with letrozole ($n = 20$): 6-month PFS 76%
				Combination with anastrozole ($n = 16$): diarrhea (31%), leukocyte count reduced (31%), neutrophil count reduced (25%), lymphocyte count reduced (25%), fatigue (19%)	Combination with anastrozole ($n = 16$): 6-month PFS 87%
				Combination with tamoxifen ($n = 16$): diarrhea (31%), fatigue (31%), lymphocyte count reduced (13%), leukocyte count reduced (13%)	Combination with tamoxifen ($n = 16$): 6-month PFS 73%
				Combination with exemestane ($n = 15$): diarrhea (27%), abdominal pain (20%), neutrophil count reduced (20%), lymphocyte count reduced (20%), fatigue (13%)	Combination with exemestane ($n = 15$): 6-month PFS 75%
				Combination with exemestane + everolimus (150 mg BID dose; $n = 15$): neutrophil count reduced (33%), leukocyte count reduced (33%), lymphocyte count reduced (33%), diarrhea (27%), anemia (20%), fatigue (13%), stomatitis (13%), hypokalemia (13%)	Combination with exemestane + everolimus (150 mg BID dose; $n = 15$): 6-month PFS 89%
				Combination with exemestane + everolimus (200 mg BID dose; $n = 4$): neutrophil count reduced (75%), diarrhea (25%), leukocyte count reduced (25%), platelet count reduced (25%)	Combination with exemestane + everolimus (200 mg BID dose; $n = 4$): 6-month PFS 100%
				Combination with trastuzumab (150 mg BID dose; $n = 18$): diarrhea (17%), fatigue (11%), hypokalemia (11%), lymphocyte count reduced (11%)	Combination with trastuzumab (150 mg BID dose; $n = 18$): 6-month PFS 29% (HR^+ breast cancer; $n = 11$); 38% (HR^- breast cancer; $n = 7$)
				Combination with trastuzumab (200 mg BID dose; $n = 6$): diarrhea (83%), anemia (33%), neutrophil count reduced (33%), leukocyte count reduced (33%), lymphocyte count reduced (33%), platelet count reduced (17%), fatigue (17%), abdominal pain (17%)	Combination with trastuzumab (200 mg BID dose; $n = 6$): 6-month PFS 50% (HR^+ breast cancer; $n = 5$); 0 (HR^- breast cancer; $n = 1$)
NCT01394016 (79)	None	I	Metastatic breast cancer expansion cohort ($N = 47$)	—	All ($N = 47$): 23% PR, 47% SD, DCR 70%, median PFS 5.8 months HR^+ ($n = 36$): 31% PR, 50% SD, DCR 81%, median PFS 8.8 months
NCT01394016 (79)	Fulvestrant	I	HR^+ ABC ($N = 19$)	Neutropenia (32%), leukopenia (26%), abdominal pain (11%), anemia (11%)	21% PR, ORR 21%, DCR 79%, CBR 63%
NSCLC					
NCT01394016 (79)	None	I	NSCLC expansion cohort ($N = 68$)	—	All NSCLC ($N = 68$): 3% PR, 46% SD, 49% DCR, median PFS 2 months *KRAS*-mutant NSCLC ($n = 29$): 3% PR, 31% SD ≥24 weeks, 55% DCR, median PFS 2.8 months *KRAS* wild-type NSCLC ($n = 33$): 3% PR, 12% SD ≥24 weeks, 39% DCR, median PFS 1.9 months

Abbreviations: AEs, adverse events; BID, twice daily; CBR, clinical benefit rate; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; CI, confidence interval; CRC, colorectal cancer; DCR, disease control rate; ER, estrogen receptor; HER2, human epithelial receptor-2; *KRAS*, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MCL, mantle cell lymphoma; MRT, malignant rhabdoid tumor; NSCLC, non–small cell lung cancer; ORR, overall response rate; PFS, progression-free survival; PR, partial response; SD, stable disease.

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Table 2. Key characteristics of CDK4/6 inhibitors in clinical development for solid tumors

	Ribociclib (LEE011; refs. 26, 27, 31, 32)	Palbociclib (Ibrance; PD-0332991; refs. 20, 26, 28)	Abemaciclib (LY2835219; refs. 26, 29, 70)
IC_{50} (nmol/L)—on-target CDKs			
CDK4–cyclin D1	10	11	2
CDK6–cyclin D1/2/3	39	16	10
IC_{50} (nM)—on other CDKs			
CDK1–cyclin B	113,000	>10,000	1627
CDK2–cyclin A/E	76,000	>10,000	504
CDK5–p25	43,900	>10,000	355
CDK9–cyclin T	NR	NR	57
Kinase partition index	0.99	0.96	0.88
Lipophilicity (cLogP)	2.3	2.7	5.5
IC_{50} against bone marrow mononuclear cells (nmol/L)	1,700 ± 231	240 ± 43	230 ± 27
Half-life	33–42 hours	26–27 hours	17–38 hours
T_{max}	1–5 hours	6–12 hours	4–6 hours

Abbreviations: cLogP, calculated Log of the partition coefficient; NR, not reported.

the ATP cleft and forms a hydrogen bond with a catalytic residue (Lys43) that is conserved among kinases, suggesting it binds with less selectivity than ribociclib and palbociclib (26). In contrast, ribociclib and palbociclib appear to have greater lipophilicity (Table 2) and larger binding-site side chains than abemaciclib, which might reduce the number of off-target kinase ATP-binding pockets with which they interact (26). Indeed, ribociclib, palbociclib, and abemaciclib exhibit varying IC_{50} values against different CDK– and non-CDK–cyclin complexes in biochemical assays (Table 2; refs. 26–29). Interestingly, in a chemoproteomics study of CDK4/6 inhibitor activity in lung carcinoma cell lines and primary tumor samples, ribociclib was found to be significantly more selective toward CDK4 and CDK6 than palbociclib, which interacted with more than twice as many kinases than ribociclib (30).

Early Clinical Experience with Ribociclib

The first phase I clinical studies evaluated single-agent ribociclib across a range of Rb-positive advanced solid tumors and lymphomas in U.S./European ($n = 132$) and Japanese patients ($n = 17$; refs. 31, 32). The recommended phase II dose (RP2D) of single-agent ribociclib was declared as 600 mg/day on a 3-weeks-on/1-week-off schedule (31, 32). Pharmacokinetic analyses determined that ribociclib is rapidly absorbed, with a time to maximum concentration (T_{max}) of 1 to 5 hours and a half-life ($t_{1/2}$) of 33 to 42 hours (Table 2; refs. 31, 32). In Japanese patients, ribociclib exposure appeared higher on average than in non-Japanese patients, although considerable interpatient variability was observed (31, 32). In a separate, healthy volunteer study, overall exposure of a single oral dose of 600 mg ribociclib was unaffected in fed versus fasted states, indicating that ribociclib may be taken with or without food (33).

Safety analyses from the phase I studies indicated that single-agent ribociclib is associated with a manageable safety profile. The most common treatment-related adverse events (AE) were hematologic, particularly neutropenia, consistent with on-target CDK4/6 inhibitor toxicity (31, 32). Nonhematologic AEs included nausea and fatigue (31, 32). Grade 1/2 QTc prolongation was reported in patients receiving the RP2D, and subsequent trials included additional cardiac monitoring (31). AEs were generally mild to moderate in severity and were reversible upon ribociclib interruption (31, 32).

Preliminary antitumor activity was observed in the phase I studies (31, 32). Among U.S./European patients, the large majority of whom were heavily pretreated, three had partial responses (PR) and 41 had stable disease (SD); eight patients had SD for >6 months (31). The PRs occurred in a patient with head and neck acinar carcinoma and *CDKN2A* loss; another with *PIK3CA*-mutant, *CCND1*-amplified, estrogen receptor–positive (ER^+) breast cancer; and another with *BRAF*/*NRAS* wild-type, *CCND1*-amplified melanoma (31). In the Japanese population, who were also heavily pretreated, SD was observed in four patients treated at the RP2D: two with peritoneal cancer, one with esophageal cancer, and one with breast cancer (32). These data suggest that ribociclib, through its specific mechanism of action, has activity in humans.

Preclinical and Clinical Experience with Ribociclib in Individual Indications

Ribociclib has demonstrated antitumor activity in preclinical and clinical studies of a wide variety of tumor types, including breast cancer, melanoma, and neuroblastoma. Although single-agent activity has been demonstrated for ribociclib, it has also been shown to enhance the activity of combination partners and delay the development of treatment resistance in preclinical and clinical studies.

Breast cancer

Preclinical. Breast tumors frequently harbor aberrations of the cyclin D–CDK4/6–p16–Rb pathway (Supplementary Table S1; refs. 34–36), and this pathway is also implicated in resistance to endocrine therapy (3). Therefore, targeting both the cyclin D–CDK4/6–p16–Rb and ER pathways may delay the development of resistance. In a preclinical study of 50 breast cancer cell lines, ribociclib demonstrated inhibitory activity predominantly against ER^+ cell lines (37), suggesting that ER^+ breast cancer cells might be particularly susceptible to CDK4/6 inhibition. *In vivo*, ribociclib showed significant tumor growth inhibition in xenograft mouse models of ER^+ breast cancer (37). A preclinical study in CDK4/6 inhibitor–resistant breast cancer cell lines suggests that mechanisms of resistance differ between ribociclib and palbociclib. Ribociclib-resistant clones demonstrated increased E2F1 compared with increased cycle E protein levels in palbociclib-resistant clones (38). Combinations of ribociclib with endocrine therapies was also associated with potent antitumor effects in ER^+

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

breast cancer models compared with the respective single agents (38, 39).

The PI3K/AKT/mTOR pathway has also been widely implicated in breast cancer tumorigenesis and treatment resistance (8). As cyclin D1 is frequently regulated in a PI3K/AKT/mTOR–dependent manner, combinations of upstream and downstream inhibitors might act cooperatively. Indeed, combination of ribociclib with the PI3K inhibitor alpelisib (BYL719) resulted in enhanced tumor regression (−57%) versus single-agent treatments with ribociclib (−9%) and alpelisib (−15%) (39). More recently, large-scale, patient-derived breast cancer xenografts were used to evaluate such combinations in mouse preclinical trials. Treatment of 38 patient-derived xenograft (PDX) mouse models with ribociclib plus alpelisib demonstrated increased response rates and progression-free survival (PFS) with this combination compared with the single agents (40). Interestingly, the ribociclib plus alpelisib combination appeared more active in PDX models than in cell lines, suggesting a possible disconnect between *in vitro* cell-based assays and *in vivo* PDX-based results (40). Furthermore, the addition of PI3K inhibitors to ribociclib and either letrozole or fulvestrant demonstrated increased antitumor activity, including some complete regressions, without significant toxicity in mouse models of ER^+ breast cancer (37). These regressions were sustained for approximately 4 weeks posttreatment and occurred in both *PI3K/PTEN* wild-type and altered backgrounds. Taken together, the synergistic activity of ribociclib in combination with various anticancer therapies suggests that ribociclib may complement existing breast cancer treatments in the clinic.

Clinical. The combination of ribociclib and endocrine therapies in HR^+, $HER2^-$ breast cancer is being investigated in phase Ib to III studies (Table 3; Supplementary Table S2). These studies have demonstrated that ribociclib exposure is unaffected by combinations with exemestane, letrozole, or fulvestrant in patients with HR^+ ABC (41, 42). Similarly, letrozole exposure when combined with ribociclib was within the range of values observed from single-agent letrozole (41, 43). The addition of ribociclib to exemestane, letrozole, or fulvestrant was also associated with manageable tolerability profiles in all studies (Table 3). The most common AEs were hematologic, and grade 3/4 neutropenia was frequent but uncomplicated (42, 44–46). QT prolongation has been observed following ribociclib treatment in combination with endocrine therapy; cases were predominantly grade 1 to 2 in severity, with prolongation limited by proactive dose modifications (31, 46). AEs were manageable and reversible upon treatment interruption (46, 47). In the phase III MONALEESA-2 study of ribociclib plus letrozole in the first-line treatment of postmenopausal women with HR^+, $HER2^-$ ABC, ribociclib dose intensity was 88% despite dose interruptions, and only 7.5% of patients discontinued ribociclib plus letrozole treatment due to AEs (46).

Clinical activity of ribociclib was observed in studies of HR^+ ABC (Table 3; refs. 42, 44–46). In MONALEESA-2, where 668 patients with HR^+ ABC were randomized to receive ribociclib plus letrozole or placebo plus letrozole, ribociclib plus letrozole significantly increased PFS relative to placebo plus letrozole in the first-line setting (median PFS: not reached vs. 14.7 months; hazard ratio = 0.56; $P = 3.29 \times 10^{-6}$; ref. 46). The PFS rate at 12 months was 72.8% versus 60.9% in the ribociclib and placebo groups, respectively (46). A significant hazard ratio benefit for ribociclib plus letrozole was also observed across all prespecified patient subgroups, including older patients (≥65 years) and those with visceral metastases, bone-only disease, or *de novo* ABC (46, 48, 49). In the ribociclib plus letrozole arm, decreased tumor size at the initial evaluation (∼week 8) was observed in 76% of evaluable patients with measurable disease (50). In newly diagnosed grade II/III HR^+, $HER2^-$ invasive breast cancer, a randomized presurgical study demonstrated an enhanced reduction in expression of the Ki67 marker for cell proliferation upon combination of ribociclib and letrozole (≥92%) versus letrozole alone (69%), further supporting a role for ribociclib in enhancing the antitumor effects of its combination partner (43). An ongoing trial (NCT02712723) is investigating ribociclib plus letrozole in the neoadjuvant setting (Supplementary Table S2). Preliminary clinical activity has also been established with ribociclib plus fulvestrant in pretreated HR^+, $HER2^-$ ABC, and PRs were observed in patients who received prior fulvestrant (42). When evaluating potential biomarkers of response, preliminary clinical activity with ribociclib plus letrozole or fulvestrant was reported in patients with ER^+ breast cancer tumors carrying alterations in PI3K/AKT/mTOR or cyclin D–CDK4/6–p16–Rb pathways, suggesting a possible benefit in patients whose tumors carry these alterations (42, 45).

Encouraging preliminary clinical activity has also been demonstrated with triplet therapy of ribociclib, exemestane, and everolimus (mTOR inhibitor), as well as ribociclib, letrozole, and alpelisib (PI3Kα-selective inhibitor) in pretreated patients with HR^+ ABC (Table 3; Supplementary Table S2; refs. 47, 51, 52). Although ribociclib exposure remained unaltered by combination with everolimus, exposure to everolimus, which is metabolized by cytochrome P450 3A4 (CYP3A4), increased 1.5- to 3-fold when combined with ribociclib (41). However, use of lower doses of everolimus (e.g., 2.5 mg/day) resulted in exposures within the ranges achieved with single-agent everolimus dosing (e.g., 5–10 mg/day), with potentially lower toxicity (41, 52). Triplet therapy with ribociclib, everolimus, and exemestane in pretreated patients with ER^+ ABC was associated with manageable safety (Table 3; ref. 52). During triplet therapy with ribociclib, alpelisib, and letrozole, pharmacokinetic indices for ribociclib and alpelisib were generally consistent with historic single-agent data (51). The combination of ribociclib, alpelisib, and letrozole also demonstrated an acceptable safety profile (Table 3; ref. 51). Both triplet regimens have demonstrated antitumor activity in patients whose ER^+ breast cancer tumors harbor PI3K/AKT/mTOR and/or cyclin D–CDK4/6–p16–Rb pathway modifications (51, 52). Further evaluation and validation of biomarkers of response are ongoing.

Melanoma

Preclinical. The now-refined genetic landscape of melanoma has highlighted the centrality of RAS signaling in this disease. Indeed, activating mutations occur in critical components of this pathway, including $BRAF^{V600}$(35%–50%), *NRAS* (10%–25%), and *NF1* (∼15%; refs. 53, 54). The cyclin D–CDK4/6–p16–Rb pathway is also commonly dysregulated in melanomas. Mutations, deletions, or hypermethylation of *CDKN2A* are key driver alterations in melanomas, and *CCND1* (cyclin D1) and *CDK4* are frequently amplified (Supplementary Table S1; refs. 53–57). Germline mutations in *CDKN2A* and *CDK4* are also linked to familial melanoma (58). High *CCND1* and low *CDKN2A* copy numbers have been associated with reduced PFS with BRAF inhibitors (59).

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Table 3. Clinical experience with ribociclib combinations

Study name/ID	Combination drug	Phase	Population	MTD/RP2D/dose	Reported grade 3/4 AEs	Clinical activity
HR$^+$, HER2$^-$ breast cancer						
CLEE011X2106/ NCT01857193 (44)	Exemestane	Ib	Postmenopausal women with ER$^+$, HER2$^-$ ABC previously treated with letrozole or anastrozole ($N = 14$ treated with ribociclib + exemestane)	**Evaluated dose:** **Ribociclib:** 600 mg/day (3 weeks on/1 week off) **Exemestane:** 25 mg/day	Neutropenia (71%), leukopenia (36%), lymphopenia (14%), ALT elevation (14%), AST elevation (14%), anemia (14%)	2 unconfirmed PR, 4 SD
CLEE011X2107/ NCT01872260 (45, 47)	Letrozole	Ib	Pretreated and treatment-naïve postmenopausal women with ER$^+$, HER2$^-$ ABC ($N = 47$ treated with ribociclib + letrozole)	**RP2D:** **Ribociclib:** 600 mg/day (3 weeks on/1 week off) **Letrozole:** 2.5 mg/day	**All patients:** Neutropenia and neutrophil count reduced (60%), ALT elevation (4%), AST elevation (4%), asthenia (2%), constipation (2%), nausea (2%), UTI (2%), fatigue (1%) **First line ($n = 28$):** Neutropenia and neutrophil count reduced (64%), ALT elevation (4%), AST elevation (4%), asthenia (4%), nausea (4%) **Previously treated ($n = 19$):** Neutropenia and neutrophil count reduced (53%), ALT elevation (5%), AST elevation (5%), constipation (5%), fatigue (5%), UTI (5%)	**First line ($n = 28$):** 2 CR, 11 PR, 3 unconfirmed PR, 5 SD, 4 NCRNPD, ORR 46%, DCR 89%, CBR 79%, Median PFS 25.3 months **Previously treated ($n = 19$):** Median PFS 5.5 months
CLEE011X2108/ NCT02088684 (42)	Fulvestrant	Ib	Postmenopausal women with HR$^+$, HER2$^-$ ABC ($N = 28$).	**Ribociclib:** 600 mg/day (3 weeks on/1 week off) or 400 mg/day (continuous) **Fulvestrant:** 500 mg on days 1 and 15 of cycle 1 and day 1 of subsequent cycles	**Ribociclib 600 mg/day 3 weeks on/1 week off; $n = 13$):** Neutropenia (62%), fatigue (15%), leukocyte count reduced (15%), ALT elevation (8%), AST elevation (8%) **Ribociclib 400 mg/day (continuous; $n = 15$):** Neutropenia (33%), leukocyte count reduced (7%), ALT elevation (7%), AST elevation (7%)	**Ribociclib 600 mg/day 3 weeks on/1 week off; ($n = 13$):** 3 PR, 9 SD, 1 NCRNPD **Ribociclib 400 mg/day (continuous; $n = 15$):** 2 PR, 7 SD, 5 NCRNPD
CLEE011X2106/ NCT01857193 (52)	Everolimus + exemestane	Ib	Postmenopausal women with ER$^+$, HER2$^-$ ABC previously treated with letrozole or anastrozole ($N = 77$ treated with ribociclib + everolimus + exemestane)	**RP2D:** **Ribociclib:** 300 mg/day (3 weeks on/1 week off) **Everolimus:** 2.5 mg/day **Exemestane:** 25 mg/day	Neutropenia (31%), neutrophil count reduced (18%), leukocyte count reduced (12%), anemia (7%), thrombocytopenia (7%), lymphopenia (7%), ALT elevation (5%), AST elevation (4%), lymphocyte count reduced (4%)	ORR 9%, DCR 73%, CBR 26%
CLEE011X2107/ NCT01872260 (51)	Alpelisib + letrozole	Ib	Pretreated and treatment-naïve postmenopausal women with ER$^+$, HER2$^-$ ABC ($N = 46$ treated with ribociclib + alpelisib + letrozole)	**RP2D:** **Ribociclib:** 300 mg/day (3 weeks on/1 week off) **Alpelisib:** 200 mg/day **Letrozole:** 2.5 mg/day	Increased ALT (30%), increased AST (26%), hyperglycemia (17%), neutropenia (17%), fatigue (13%), reduced neutrophil count (4%), anemia (4%), thrombocytopenia (2%), vomiting (2%), nausea (2%)	ORR 16%, DCR 70%, CBR 26%

(Continued on the following page)

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Table 3. Clinical experience with ribociclib combinations (Cont'd)

Study name/ID	Combination drug	Phase	Population	MTD/RP2D/dose	Reported grade 3/4 AEs	Clinical activity
CLEE011A2201/ NCT01919229 (43)	Letrozole	II	Postmenopausal women with HR^+, $HER2^-$ Grade II/III, invasive, early breast cancer who have received no prior breast cancer treatment ($N = 14$)	**Ribociclib:** 600 mg/day or 400 mg/day **Letrozole:** 2.5 mg/day	All AEs were mild/moderate with no grade 3/4 AEs	**Ribociclib 400 mg/day + letrozole:** 96% decrease in Ki67 **Ribociclib 600 mg/day + letrozole:** 92% decrease in Ki67 **Letrozole only:** 69% decrease in Ki67
MONALEESA-2/ NCT01958021 (46)	Letrozole	III	Postmenopausal women with HR^+, $HER2^-$ ABC who have received no prior treatment for advanced disease ($N = 668$)	**Ribociclib:** 600 mg/day (3 weeks on/1 week off) **Letrozole:** 2.5 mg/day	**Ribociclib + letrozole arm vs. placebo + letrozole arm:** Neutropenia (59% vs. 1%), leukopenia (21% vs. 1%), hypertension (10% vs. 11%), increased ALT (9% vs. 1%), lymphopenia (7% vs. 1%), increased AST (6% vs. 1%)	**Ribociclib + letrozole arm vs. placebo + letrozole arm:** Median PFS NR (95% CI, 19.3–NR) vs. 14.7 months (95% CI, 13.0–16.5); hazard ratio = 0.56; $P = 3.29 \times 10^{-6}$ ORR 41% vs. 28% ($P < 0.001$) CBR 80% vs. 72% ($P = 0.02$)
***NRAS*- or *BRAF*-mutant melanoma**						
CMEK162X2114/ NCT01781572 (62)	Binimetinib	Ib/II	Patients with advanced *NRAS*-mutant melanoma ($N = 22$ received ribociclib + binimetinib)	**MTD:** **Ribociclib:** 200 mg/day (3 weeks on/1 week off) **Binimetinib:** 45 mg BID **RP2D:** ongoing	CPK elevation (18%), neutropenia (9%), acneiform (4%), dermatitis (4%), rash (4%)	7 PR, 11 SD, 33% had 20%–30% tumor shrinkage, CBR 86%
CLEE011X2105/ NCT01777776 (61)	Encorafenib	Ib/II	Patients with advanced $BRAF^{V600}$-mutant melanoma ($N = 28$ received ribociclib + encorafenib)	**MTD:** ongoing **RP2D:** ongoing	Hand–foot syndrome (11%), rash (4%), myalgia (4%)	Two confirmed PRs, 3 unconfirmed PRs, 10 SD, 1 SD >9 cycles

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BID, twice daily; CBR, clinical benefit rate; CI, confidence interval; CPK, creatine phosphokinase; DCR, disease control rate; NCRNPD, not complete response nor progressive disease; NR, not reached; ORR, overall response rate; UTI, urinary tract infection.

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Approved treatments for *BRAF*-mutant melanoma include targeted therapy with BRAF and/or MEK inhibitors (60). Binimetinib (MEK162), a MEK inhibitor, and encorafenib (LGX818), a selective BRAF inhibitor, have demonstrated antitumor activity as single agents in *NRAS*- and *BRAF*-mutant melanomas, respectively, and in combination in *BRAF*-mutant melanomas (61–63). The combination of ribociclib and binimetinib demonstrated enhanced tumor regression [34% tumor/control (T/C) ratio] in xenograft models of $NRAS^{Q61K}$-mutant melanoma relative to single-agent binimetinib (12% T/C) or ribociclib (32% T/C; refs. 27, 62). In $BRAF^{V600E}$-mutant melanoma models, low-dose ribociclib exhibited synergistic activity with encorafenib (27, 61). The addition of ribociclib to encorafenib also appeared to prevent resistance to encorafenib (27, 61), suggesting that simultaneous inhibition of BRAF and cyclin D–CDK4/6–p16–Rb pathways may provide a therapeutic benefit in the clinical setting. The enhanced antitumor activity of ribociclib plus encorafenib relative to single agents was confirmed in a large-scale *in vivo* screen of PDX melanoma models, where the combination was associated with a 100% response rate, including 87% PR and complete response (CR; exceeding the 72% PR and CR for binimetinib plus encorafenib; ref. 40). The combination also resulted in a significant improvement in PFS and delayed the development of resistance in the tumor models ($P = 1.8 \times 10^{-3}$), with no drug–drug interactions (40).

Clinical. The combinations of ribociclib plus binimetinib or encorafenib have been evaluated in two dose-escalation/expansion phase Ib/II studies of advanced *NRAS*- and *BRAF*-mutant melanoma, respectively (Table 3; refs. 61, 62). In patients with advanced *NRAS*-mutant melanoma, the combination of ribociclib and binimetinib did not affect the pharmacokinetic exposures of either drug (41, 62). Common AEs experienced with ribociclib plus binimetinib included creatine phosphokinase (CPK) elevation, acneiform dermatitis, nausea, rash, edema, leukopenia, and creatinine elevation, whereas grade 3/4 AEs included CPK elevation, neutropenia, acneiform dermatitis, and rash (Table 3; ref. 62). This combination was also associated with preliminary antitumor activity, including cases of PR (33%) and SD (52%; Table 3; ref. 62). In patients with advanced $BRAF^{V600}$-mutant melanomas, the combination of ribociclib and encorafenib resulted in reduced ribociclib exposure and increased encorafenib exposure, most likely due to encorafenib-mediated CYP3A4 induction and to ribociclib-mediated CYP3A4 inhibition, respectively (41). The combination of ribociclib and encorafenib demonstrated clinical activity and an acceptable tolerability profile, with AEs consisting of hand–foot syndrome, nausea, pruritus, rash, and myalgia (Table 3; ref. 61). Cases of PR and SD were observed in patients receiving ribociclib plus encorafenib whose tumors exhibited p16 loss or *CDK4* mutation (61). There was little evidence of response in patients resistant to BRAF inhibition (61). The use of ribociclib and binimetinib and/or encorafenib combinations in melanoma is being investigated further in ongoing trials (Supplementary Table S2).

Neuroblastoma

Preclinical. Neuroblastoma is frequently driven by oncogenic activation of anaplastic lymphoma kinase (*ALK*), often resulting in cyclin D1 upregulation and cell proliferation (64). Consistent with this, extensive overexpression of cyclin D–CDK4/6 components has been observed in neuroblastomas (65). Array-based data showed that cyclin D1 expression is 3 to 8 times higher in neuroblastoma tumors than in libraries of 18 other common malignancies, including breast cancer (65). High expression of CDK4 and CDK6 and deletions of *CDKN2A* have also been associated with these tumors (Supplementary Table S1; refs. 65–67).

Ribociclib activity has been profiled against >500 cell lines in the Cancer Cell Line Encyclopedia study, whereby neuroblastoma cell lines were identified as particularly sensitive to ribociclib (27). Here, ribociclib induced cytostasis at nanomolar concentrations in 12 of 17 human neuroblastoma–derived cell lines (67). In sensitive neuroblastoma cell line–derived xenograft mouse models, treatment with ribociclib was accompanied by a reduction in phosphorylated Rb, Ki67, and cell proliferation, leading to significant tumor growth delay throughout the treatment period ($P < 0.0001$; ref. 67). CDK4/6 is therefore an attractive target for neuroblastoma treatment.

Clinical. The efficacy and safety of single-agent ribociclib in patients with neuroblastoma was evaluated as part of the phase I study investigating ribociclib in 31 pediatric patients [median age (range): 5 (1–20) years] with malignant rhabdoid tumors (MRT), neuroblastoma, or CDK4/6 pathway-activated tumors (68). Consistent with single-agent data in adults, the MTD and RP2D were 470 mg/m^2/day (adult equivalent dose ≈800 mg/day) and 350 mg/m^2/day (adult equivalent dose ≈600 mg/day), respectively, on a 3-weeks-on/1-week-off dosing schedule (68). Ribociclib was rapidly absorbed, with a median T_{max} of 2 to 5 hours regardless of age (69). As in adults, ribociclib exposure in pediatric patients appeared to accumulate 2- to 3-fold in plasma, reaching steady state within 8 days (69). Clearance was 2 to 3 times slower in pediatric patients compared with adults, presumably due to their lower body weight (69). Ribociclib was well tolerated with mild-to-moderate, reversible AEs, the majority of which were hematologic (68). Three patients, two with neuroblastoma and one with MRT, received ribociclib for ≥4 cycles; SD was the best overall response (68). Ribociclib remains the only CDK4/6 inhibitor investigated in a clinical trial of neuroblastoma.

Ongoing Trials with Ribociclib

Multiple trials of ribociclib are ongoing across different tumor types, including $BRAF^{V600}$- and *NRAS*-mutant melanoma, non–small cell lung cancer, teratoma, liposarcoma, myelofibrosis, and gynecologic cancers; these are summarized in Supplementary Table S2. The most advanced trials are investigating ribociclib combinations in HR^+ breast cancer. MONALEESA-3 is evaluating the addition of ribociclib to fulvestrant in patients with HR^+ ABC who have received no or only one line of prior endocrine therapy. MONALEESA-7 is investigating the combination of ribociclib and tamoxifen or nonsteroidal aromatase inhibitors plus goserelin in pre/perimenopausal women with HR^+ ABC. MONALEESA-7 is the only trial entirely dedicated to investigating CDK4/6 inhibition in the pre/perimenopausal setting. In addition, based on preclinical rationale, a number of additional doublet and triplet combination studies are underway, including combinations of ribociclib with endocrine therapy and PI3K pathway inhibition (Supplementary Table S2).

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Ribociclib in Perspective

The clinical data for ribociclib add to the wealth of emerging information supporting use of CDK4/6 inhibitors in the treatment of cancer. Certain differences among ribociclib, palbociclib, and abemaciclib, including pharmacokinetic factors, target selectivity, and toxicities, are likely to influence their activity or utility in individual settings. Pharmacokinetic data with ribociclib demonstrate a long half-life compared with palbociclib and abemaciclib (Table 2). Although the half-lives of ribociclib and palbociclib enable once-daily, 3-weeks-on/1-week-off dosing (14), the pharmacokinetic/pharmacodynamic profile of abemaciclib favors twice-daily, continuous dosing (29, 70). The convenience of intermittent versus continuous dosing and its impact on treatment adherence and outcomes remain to be explored. Once-daily, continuous dosing of ribociclib in combination with endocrine therapies is being evaluated in ongoing breast cancer trials (NCT02088684, NCT02712723, NCT02732119; ref. 42). Pharmacokinetic data indicate that ribociclib can be taken with or without food, whereas palbociclib must be administered with food (20, 33), and that ribociclib may be absorbed more rapidly than palbociclib and abemaciclib (Table 2; refs. 70, 71). Preclinically, ribociclib appears to have less toxicity against bone marrow mononuclear cells compared with palbociclib and abemaciclib (Table 2; ref. 26), which may potentially translate into fewer hematologic toxicities. Hematologic toxicities were reported with ribociclib plus letrozole in MONALEESA-2 and with palbociclib plus letrozole in PALOMA-2 (18, 46). Differences in target selectivity also lead to variations in safety, with abemaciclib demonstrating increased frequency of gastrointestinal AEs versus ribociclib or palbociclib (Table 1; ref. 14). Ribociclib is generally well tolerated, with predictable AEs that are easily manageable by dose adjustment or treatments. Finally, preclinical data suggest that both ribociclib and abemaciclib can cross the blood–brain barrier, supporting further exploration with central nervous system tumors (72, 73).

Conclusions

Ribociclib is a promising, selective CDK4/6 inhibitor in the late stages of clinical development, demonstrating preclinical and clinical activity across a range of tumor types, including HR^+ breast cancer. The preclinical, clinical, and pharmacokinetic profiles of ribociclib in a variety of tumor types make it an important addition to the class of CDK4/6 inhibitors. Given the selectivity of ribociclib toward CDK4/6, the addition of ribociclib to existing anticancer therapies in doublet and triplet combinations has been successful, enhancing efficacy of existing therapies with minimal increases in toxicity in preclinical and clinical studies. This result is being explored extensively across a range of tumor types and in combination with a variety of anticancer agents (Supplementary Table S2). Establishing validated biomarkers of clinical response to ribociclib will help define patient populations who will benefit most from treatment, improve treatment outcomes, and identify effective drug combinations to mitigate treatment resistance. The comprehensive and robust portfolio of unique and ongoing clinical studies of doublet and triplet therapies containing ribociclib is likely to shape the future landscape of cancer therapeutics.

Disclosure of Potential Conflicts of Interest

D. Tripathy reports receiving commercial research grants and editorial support from Novartis and is a consultant/advisory board member for Nektar Therapeutics, Novartis, Pfizer, and Puma Biotechnology. A. Bardia is a consultant/advisory board member for Genentech/Roche, Novartis, and Pfizer. W.R. Sellers is the former vice president/global head of oncology for Novartis, holds ownership interest in Novartis, and is a consultant/advisory board member for Astex Pharmaceuticals, EMD Serrano, Peloton Therapeutics, and Servier Pharmaceuticals. No other potential conflicts of interest were disclosed.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NCI or the NIH.

Acknowledgments

The authors thank Abbie Saunders, PhD, for medical editorial assistance with this manuscript.

Grant Support

Financial support for medical editorial assistance was provided by Novartis Pharmaceuticals Corporation. The project described was supported in part by award number P30CA014089 from the NCI (to D. Tripathy). A. Bardia was supported by the NIH (4K12CA087723–14).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 15, 2016; revised January 12, 2017; accepted March 24, 2017; published OnlineFirst March 28, 2017.

References

1. Abukhdeir AM, Park BH. P21 and p27: roles in carcinogenesis and drug resistance. Expert Rev Mol Med 2008;10:e19.
2. Caldon CE, Daly RJ, Sutherland RL, Musgrove EA. Cell cycle control in breast cancer cells. J Cell Biochem 2006;97:261–74.
3. Lange CA, Yee D. Killing the second messenger: Targeting loss of cell cycle control in endocrine-resistant breast cancer. Endocr Relat Cancer 2011;18: C19–24.
4. Johnson A, Skotheim JM. Start and the restriction point. Curr Opin Cell Biol 2013;25:717–23.
5. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. J Clin Oncol 2006;24:1770–83.
6. Baker SJ, Reddy EP. CDK4: A key player in the cell cycle, development, and cancer. Genes Cancer 2012;3:658–69.
7. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature 2005;436: 720–4.
8. Hosford SR, Miller TW. Clinical potential of novel therapeutic targets in breast cancer: CDK4/6, src, JAK/STAT, PARP, HDAC, and PI3K/AKT/mTOR pathways. Pharmgenomics Pers Med 2014;7:203–15.
9. Thangavel C, Dean JL, Ertel A, Knudsen KE, Aldaz CM, Witkiewicz AK, et al. Therapeutically activating RB: Reestablishing cell cycle control in endocrine therapy-resistant breast cancer. Endocr Relat Cancer 2011; 18:333–45.
10. Miller TW, Balko JM, Fox EM, Ghazoui Z, Dunbier A, Anderson H, et al. ERalpha-dependent E2F transcription can mediate resistance to estrogen deprivation in human breast cancer. Cancer Discov 2011;1:338–51.
11. Beroukhim R, Mermel C, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463:899–905.
12. Kobayashi S, Shimamura T, Monti S, Steidl U, Hetherington C, Lowerll A, et al. Transcriptional profiling identifies cyclin D1 as a critical downstream effector of mutant epidermal growth factor receptor signaling. Cancer Res 2006;66:11389–98.

13. Solit D, Garraway L, Pratilas C, Sawai A, Getz G, Basso A, et al. BRAF mutation predicts sensitivity to MEK inhibition. Nature 2006;439:358–62.
14. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov 2015;14:130–46.
15. Dickson MA. Molecular pathways: CDK4 inhibitors for cancer therapy. Clin Cancer Res 2014;20:3379–83.
16. Mita MM, Joy AA, Mita A, Sankhala K, Jou YM, Zhang D, et al. Randomized phase II trial of the cyclin-dependent kinase inhibitor dinaciclib (MK-7965) versus capecitabine in patients with advanced breast cancer. Clin Breast Cancer 2014;14:169–76.
17. Stephenson JJ, Nemunaitis J, Joy AA, Martin JC, Jou YM, Zhang D, et al. Randomized phase 2 study of the cyclin-dependent kinase inhibitor dinaciclib (MK-7965) versus erlotinib in patients with non-small cell lung cancer. Lung Cancer 2014;83:219–23.
18. Finn RS, Martin M, Rugo HS, Jones S, Im S, Gelmon K, et al. Palbociclib and letrozole in advanced breast cancer. N Engl J Med 2016;375:1925–36.
19. Turner NC, Ro J, Andre F, Loi S, Verma S, Iwata H, et al. Palbociclib in hormone-receptor-positive advanced breast cancer. N Engl J Med 2015;373:209–19.
20. Pfizer. IBRANCE® (palbociclib) prescribing information. New York, NY: Pfizer. Available from: http://Labeling.pfizer.com/ShowLabeling.aspx?id=2191.
21. Pfizer. IBRANCE® (palbociclib) receives approval in European Union for the treatment of women with HR+/HER2- metastatic breast cancer. New York, NY: Pfizer. Available from: http://www.pfizer.com/news/press-release/press-release-detail/ibrance_palbociclib_receives_approval_in_european_union_for_the_treatment_of_women_with_hr_her2_metastatic_breast_cancer.
22. Dickler M, Tolaney S, Rugo H, Cortes J, Diéras V, Patt D, et al. MONARCH 1: Results from a Phase II study of abemaciclib, a CDK4 and CDK6 inhibitor, as monotherapy, in patients with HR+/HER2- breast cancer, after chemotherapy for advanced disease. J Clin Oncol 34, 2016 (suppl; abstr 510).
23. Goetz M, Beeram M, Beck T, Conlin A, Dees E, Dickler M, et al. Abemaciclib, an inhibitor of CDK4 and CDK6, combined with endocrine and HER2-targeted therapies for women with metastatic breast cancer. In: Proceedings of the Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2015 Dec 8–12; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2016;76(4 Suppl):Abstract nr P4-13-25.
24. Lilly. Lilly receives FDA breakthrough therapy designation for abemaciclib - a CDK 4 and 6 inhibitor - in advanced breast cancer. Indianapolis, IN: Lilly. Available from: https://Investor.lilly.com/releasedetail.cfm?releaseid=935735.
25. Novartis. Novartis Kisqali (ribociclib, LEE011) receives FDA approval as first-line treatment for $HR^+/HER2^-$ metastatic breast cancer. Available from: https://www.novartis.com/news/media-releases/novartis-kisqali-ribociclib-lee011-receives-fda-approval-first-line-treatment.
26. Chen P, Lee N, Hu W, Xu M, Ferre R, Lam H, et al. Spectrum and degree of CDK drug interactions predicts clinical performance. Mol Cancer Ther 2016;15:2273–81.
27. Kim S, Loo A, Chopra R, Caponigro G, Huang A, Vora S, et al. LEE011: An orally bioavailable, selective small molecule inhibitor of CDK4/6–reactivating rb in cancer. In: Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; 2013 Oct 19–23; Boston, MA. Philadelphia (PA): AACR; Mol Cancer Ther 2013;12(11 Suppl):Abstract nr PR02.
28. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther 2004;3:1427–38.
29. Gelbert LM, Cai S, Lin X, Sanchez-Martinez C, Del Prado M, Lallena MJ, et al. Preclinical characterization of the CDK4/6 inhibitor LY2835219: *in-vivo* cell cycle-dependent/independent anti-tumor activities alone/in combination with gemcitabine. Invest New Drugs 2014;32:825–37.
30. Sumi N, Kuenzi B, Knezevic C, Rix L, Rix U. Chemoproteomics reveals novel protein and lipid kinase targets of clinical CDK4/6 inhibitors in lung cancer. ASC Chem Biol 2015;10:2680–6.
31. Infante J, Cassier P, Gerecitano J, Witteveen P, Chugh R, Ribrag V, et al. A phase I study of the cyclin-dependent kinase 4/6 inhibitor ribociclib (LEE011) in patients with advanced solid tumors and lymphomas. Clin Cancer Res 2016;22:5696–705.
32. Yamada Y, Ishikawa N, Kakizume T, Tajima T, Hewes B, Doi T. A phase I study of single-agent ribociclib in Japanese patients with advanced solid tumors. In: Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; 2015 Nov 5–9; Boston, MA. Philadelphia (PA): AACR; Mol Cancer Ther 2015;14(12 Suppl 2): Abstract nr B31.
33. Dhuria SV, Siddani R, Kosecki CM, Germa C, Mondal S. A phase I food-effect study of the ribociclib (LEE011) drug-in-capsule (DiC) formulation in healthy subjects. J Clin Oncol 33, 2015 (suppl; abstr e13577).
34. The Cancer Genome Atlas Research Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.
35. Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. Nat Rev Cancer 2011;11:558–72.
36. Geradts J, Wilson PA. High frequency of aberrant p16(INK4A) expression in human breast cancer. Am J Pathol 1996;149:15–20.
37. O'Brien N, Di Tomaso E, Ayala R, Tong L, Issakhanian S, Linnartz R, et al. *In vivo* efficacy of combined targeting of CDK4/6, ER and PI3K signaling in ER+ breast cancer. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5–9; San Diego, CA. Philadelphia (PA): AACR; Cancer Res 2014;74(19 Suppl):Abstract nr 4756.
38. Lenihan C, Bouchekioua-Bouzaghou K, Abdulghani R, Chupin J, Shia A, Schmid P. CDK4/6 inhibitor resistant ER-positive cells remain dependent on estrogen signalling and retain sensitivity to endocrine therapy. In: Proceedings of the 2016 San Antonio Breast Cancer Symposium; 2016 Dec 6–10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2017;77 (4 Suppl):Abstract nr P3-03-12.
39. Parasuraman S, Caponigro G, Loo A, Cao Z, Kim S, Issa I, et al. LEE011, a potent and selective CDK4/6 inhibitor, under preclinical and clinical investigation. In: Proceedings of the 12th International Congress on Targeted Anticancer Therapies; 2014 Mar 5–7; Washington, DC. TAT Congress; 2014. Oral presentation O4.4.
40. Gao H, Korn J, Ferretti S, Monahan J, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med 2015;21:1318–25.
41. Bhansali S, Parasuraman S, Hewes B, Einolf H, Umehara K, Matano A, et al. Application of SimCyp® and real-time pharmacokinetic assessments in defining dose in novel–novel combinations with potential drug–drug interactions in cancer patients. In: Proceedings of the 2014 Annual Meeting American College of Clinical Pharmacology; 2014 Sep 14–16: Atlanta, GA. Ashburn (VA): American College of Clinical Pharmacology; 2014. Poster 27.
42. Tolaney S, Forero-Torres A, Boni V, Bachelot T, Lu Y-S, Maur M, et al. Ribociclib + fulvestrant in postmenopausal women with HR+, HER2− advanced breast cancer (ABC). In: Proceedings of the 2016 San Antonio Breast Cancer Symposium; 2016 Dec 6–10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2017;77(4 Suppl):Abstract nr P4-22-12.
43. Curigliano G, Gómez Pardo P, Meric-Bernstam F, Conte P, Lolkema M, Beck J, et al. Ribociclib plus letrozole in early breast cancer: a presurgical, window-of-opportunity study. Breast 2016;28:191–8.
44. Bardia A, Modi S, Chavez-MacGregor M, Kittaneh M, Marino A, Matano A, et al. Phase Ib/II study of LEE011, everolimus, and exemestane in post-menopausal women with ER+/HER2− metastatic breast cancer. J Clin Oncol 32:5s, 2014 (suppl; abstract 535).
45. Munster P, Ismail-Khan R, Garcia-Estevez L, Mayer I, Becerra C, Hamilton E, et al. Phase Ib safety, efficacy, and molecular analysis of ribociclib (LEE011) plus letrozole for the treatment of ER+, HER2− advanced breast cancer. In: Proceedings of the 2016 San Antonio Breast Cancer Symposium; 2016 Dec 6–10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2017;77(4 Suppl):Abstract nr P4-22-18.
46. Hortobagyi G, Stemmer S, Burris H, Yap Y, Sonke G, Paluch-Shimon S, et al. Ribociclib as first-line therapy for HR-positive, advanced breast cancer. N Engl J Med 2016;375:1738–48.
47. Juric D, Hamilton E, Garcia Estevez L, De Boer R, Mayer I, Campone M, et al. Phase Ib/II study of LEE011 and BYL719 and letrozole in ER^+, $HER2^-$ breast cancer: safety, preliminary efficacy and molecular analysis. In: Proceedings of the Thirty-Seventh Annual CTRC-AACR San Antonio Breast Cancer Symposium; 2014 Dec 9–13; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2015;75(9 Suppl):Abstract nr P5-19-24.

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

48. Burris H, Chan A, Campone M, Blackwell K, Winer E, Janni W, et al. First-line ribociclib + letrozole in patients with HR+, HER2− advanced breast cancer (ABC) presenting with visceral metastases or bone-only disease: a subgroup analysis of the MONALEESA-2 trial. In: Proceedings of the 2016 San Antonio Breast Cancer Symposium; 2016 Dec 6–10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2017;77(4 Suppl): Abstract nr P4-22-16.
49. O'Shaughnessy J, Petrakova K, Sonke G, André F, Conte P, Arteaga C, et al. First-line ribociclib plus letrozole in patients with de novo HR+, HER2− ABC: A subgroup analysis of the MONALEESA-2 trial. In: Proceedings of the 2016 San Antonio Breast Cancer Symposium; 2016 Dec 6–10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2017;77(4 Suppl): Abstract nr P4-22-05.
50. Andre F, Stemmer S, Hortobagyi G, Burris H, Shimon S, Campone M, et al. Ribociclib + letrozole for firstline treatment of HR+, HER2− ABC: efficacy, safety, and pharmacokinetics. Eur J Cancer 2016;69:S7.
51. Juric D, Ismail-Khan R, Campone M, García-Estévez L, Becerra C, De Boer R, et al. Phase Ib study of ribociclib and alpelisib and letrozole in ER+, HER2− advanced breast cancer: Safety, preliminary efficacy, and molecular analysis. In: Proceedings of the Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2015 Dec 8–12; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2016;76(4 Suppl): Abstract nr P3-14-01.
52. Bardia A, Modi S, Oliveira M, Campone M, Ma B, Dirix L, et al. Triplet therapy with ribociclib, everolimus, and exemestane in postmenopausal women with HR+/HER2− advanced breast cancer. In: Proceedings of the Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2015 Dec 8–12; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2016;76(4 Suppl): Abstract nr P6-13-01.
53. The Cancer Genome Atlas Research Network. Genomic classification of cutaneous melanoma. Cell 2015;161:1681–96.
54. Rajkumar S, Watson IR. Molecular characterisation of cutaneous melanoma: Creating a framework for targeted and immune therapies. Br J Cancer 2016;115:145–55.
55. Maelandsmo GM, Flørenes VA, Hovig E, Øyjord T, Engebraaten O, Holm R, et al. Involvement of the pRb/p16/cdk4/cyclin D1 pathway in the tumorigenesis of sporadic malignant melanomas. Br J Cancer 1996;73:909–16.
56. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. Cell 2012;150:251–63.
57. Smalley K, McArthur G. The current state of targeted therapy in melanoma: this time it's personal. Semin Oncol 2012;39:204–14.
58. Potrony M, Badenas C, Aguilera P, Puig-Butille JA, Carrera C, Malvehy J, et al. Update in genetic susceptibility in melanoma. Ann Transl Med 2015;3:210.
59. Nathanson K, Martin A, Wubbenhorst B, Grescock J, Letrero R, D'Andrea K, et al. Tumor genetic analyses of patients with metastatic melanoma treated with the BRAF inhibitor dabrafenib (GSK2118436). Clin Cancer Res 2013;19:4868–78.
60. National Comprehensive Cancer Network. National Comprehensive Cancer Network guidelines. Melanoma version 1.2017. Fort Washington (PA): National Comprehensive Cancer Network. Available from: https://www.nccn.org/professionals/physician_gls/pdf/melanoma.pdf.
61. Taylor M, Sosman J, Gonzalez R, Carlino M, Kittaneh M, Lolkema M, et al. Phase Ib/II study of LEE011 (CDK4/6 inhibitor) and LGX818 (BRAF inhibitor) in *BRAF*-mutant melanoma. Ann Oncol 2014;25 Suppl 4: iv374–5.
62. Sosman J, Kittaneh M, Lolkema M, Postow M, Schwartz G, Franklin C, et al. A phase 1b/2 study of LEE011 in combination with binimetinib (MEK162) in patients with NRAS-mutant melanoma: early encouraging clinical activity. J Clin Oncol 32:5s, 2014 (suppl; abstr 9009).
63. PR Newswire. Array BioPharma and pierre fabre announce COLUMBUS phase 3 study of encorafenib plus binimetinib for BRAF-mutant melanoma met primary endpoint. Available from: http://www.prnewswire.com/news-releases/array-biopharma-and-pierre-fabre-announce-columbus-phase-3-study-of-encorafenib-plus-binimetinib-for-braf-mutant-melanoma-met-primary-endpoint-300333794.html.
64. Wellmann A, Doseeva V, Butscher W, Raffeld M, Fukushima P, Stetler-Stevenson M, et al. The activated anaplastic lymphoma kinase increases cellular proliferation and oncogene up-regulation in rat 1a fibroblasts. FASEB J 1997;11:965–72.
65. Molenaar JJ, Ebus ME, Koster J, van Sluis P, van Noesel CJ, Versteeg R, et al. Cyclin D1 and CDK4 activity contribute to the undifferentiated phenotype in neuroblastoma. Cancer Res 2008;68:2599–609.
66. Molenaar JJ, Koster J, Ebus ME, van Sluis P, Westerhout EM, de Preter K, et al. Copy number defects of G1-cell cycle genes in neuroblastoma are frequent and correlate with high expression of E2F target genes and a poor prognosis. Genes Chromosomes Cancer 2012;51:10–9.
67. Rader J, Russell MR, Hart LS, Nakazawa MS, Belcastro LT, Martinez D, et al. Dual CDK4/CDK6 inhibition induces cell-cycle arrest and senescence in neuroblastoma. Clin Cancer Res 2013;19:6173–82.
68. Geoerger B, Bourdeaut F, Dubois SG, Dewire MD, Marabelle A, Pearson AD, et al. Phase I study of LEE011 (CDK4/6 inhibitor) in patients with malignant rhabdoid tumors, neuroblastoma, and cyclin D–CDK4/6 pathway-activated tumors. Ann Oncol 2014;25(Suppl 4):iv151–2.
69. Bhansali S, Parasuraman S, Matano A, Davis C, Chakraborty A. Pharmacokinetics of LEE011, a CDK4/6 inhibitor, in adult and pediatric patients. Presented at the 2014 American College of Clinical Pharmacology; 2014 Sep 14–16; Atlanta, GA. Ashburn (VA): ACCP; 2014:Poster 1-10-2000545.
70. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: From discovery to therapy. Cancer Discov 2016;6:353–67.
71. Flaherty KT, Lorusso PM, Demichele A, Abramson VG, Courtney R, Randolph SS, et al. Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. Clin Cancer Res 2012;18:568–76.
72. Raub TJ, Wishart GN, Kulanthaivel P, Staton BA, Ajamie RT, Sawada GA, et al. Brain exposure of two selective dual CDK4 and CDK6 inhibitors and the antitumor activity of CDK4 and CDK6 inhibition in combination with temozolomide in an intracranial glioblastoma xenograft. Drug Metab Dispos 2015;43:1360–71.
73. Patel Y. PDTB-12.CNS penetration of the CDK4/6 inhibitor ribociclib (LEE011) in non-tumor bearing mice and mice bearing orthotopic pediatric brain tumors. Neuro Oncol 2016;18 Suppl 6:vi152.
74. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015;16:25–35.
75. DeMichele A, Clark AS, Tan KS, Heitjan DF, Gramlich K, Gallagher M, et al. CDK4/6 inhibitor palbociclib (PD0332991) in rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. Clin Cancer Res 2015;21:995–1001.
76. Leonard JP, LaCasce AS, Smith MR, Noy A, Chirieac LR, Rodig SJ, et al. Selective CDK4/6 inhibition with tumor responses by PD0332991 in patients with mantle cell lymphoma. Blood 2012;119:4597–607.
77. Dickson MA, Tap WD, Keohan ML, D'Angelo SP, Gounder MM, Antonescu CR, et al. Phase II trial of the CDK4 inhibitor PD0332991 in patients with advanced CDK4-amplified well-differentiated or dedifferentiated liposarcoma. J Clin Oncol 2013;31:2024–8.
78. Gopalan P, Pinder M, Chiappori A, Ivey A, Villegas A, Kaye F. A phase II clinical trial of the CDK 4/6 inhibitor palbociclib (PD 0332991) in previously treated, advanced non-small cell lung cancer (NSCLC) patients with inactivated CDKN2A. J Clin Oncol 32:5s, 2014 (suppl; abstr 8077).
79. Patnaik A, Rosen L, Tolaney S, Tolcher A, Goldman J, Gandhi L, et al. Efficacy and safety of abemaciclib, an inhibitor of CDK4 and CDK6, for patients with breast cancer, non-small cell lung cancer, and other solid tumors. Cancer Discov 2016;6:740–53.
80. Fujiwara Y, Tamura K, Kondo S, Tanabe Y, Iwasa S, Simomura A, et al. Phase I study of abemaciclib, an inhibitor of CDk4 and 6, as a single agent for Japanese patients with advanced cancer. Cancer Chemother Pharmacol 2016;78:281–8.
81. Beeram M, Tolaney S, Beck J, Dickler M, Conlin A, Dees E, et al. A phase 1b study of abemaciclib in combination with endocrine and HER2-targeted therapies for patients with metastatic breast cancer. Ann Oncol 2016;27:vi552–87.
82. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: A changing paradigm. Nat Rev Cancer 2009;9:153–66.

Published OnlineFirst March 28, 2017; DOI: 10.1158/1078-0432.CCR-16-3157

Clinical Cancer Research

Ribociclib (LEE011): Mechanism of Action and Clinical Impact of This Selective Cyclin-Dependent Kinase 4/6 Inhibitor in Various Solid Tumors

Debu Tripathy, Aditya Bardia and William R. Sellers

Clin Cancer Res 2017;23:3251-3262. Published OnlineFirst March 28, 2017.