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

Debu Tripathy1, Aditya Bardia2, and William R. Sellers3

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.

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 G1 (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 G1–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.

Advant 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

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

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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; 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...
Table 1. Key reported clinical trials with palbociclib and abemaciclib

<table>
<thead>
<tr>
<th>Study name/ID</th>
<th>Combination drug</th>
<th>Phase</th>
<th>Population</th>
<th>Common grade 3/4 AEs (&gt;10% of patients)</th>
<th>Clinical activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Palbociclib</strong></td>
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<tr>
<td>Breast cancer</td>
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<tr>
<td>PALOMA-1/ NCT00721409 (74)</td>
<td>Letrozole</td>
<td>II</td>
<td>ER+ ABC with no prior treatment for ABC (N = 165)</td>
<td>Neutropenia (54%), leukopenia (19%)</td>
<td>Median PFS 20.2 months vs. 5.7 months for letrozole monotherapy (hazard ratio = 0.488; P = 0.0004)</td>
</tr>
<tr>
<td>PALOMA-2/ NCT01740427 (18)</td>
<td>Letrozole</td>
<td>III</td>
<td>ER+ ABC with no prior treatment for ABC (N = 666)</td>
<td>Neutropenia (66%), leukopenia (25%)</td>
<td>Median PFS 24.8 months vs. 14.5 months for letrozole monotherapy (hazard ratio = 0.58; P &lt; 0.000001)</td>
</tr>
<tr>
<td>PALOMA-3/ NCT01942135 (19)</td>
<td>Fulvestrant</td>
<td>III</td>
<td>HR+ ABC after progression on one line of endocrine therapy for ABC (N = 521)</td>
<td>Neutropenia (62%), leukopenia (25%)</td>
<td>Median PFS 9.2 months vs. 3.8 months for fulvestrant monotherapy (hazard ratio = 0.42; P &lt; 0.001)</td>
</tr>
<tr>
<td>NCT01037790 (75)</td>
<td>None</td>
<td>II</td>
<td>Metastatic Rb+ breast cancer (N = 37, n = 33 HR+)</td>
<td>Neutropenia (54%), leukopenia (31%), lymphopenia (30%), thrombocytopenia (19%)</td>
<td>Total (N = 37): 5% PR, 38% SD &lt;6 months, 14% SD ≥6 months, CBR 19% HR+ (n = 33): 6% PR, 39% SD &lt;6 months, 16% SD ≥6 months, CBR 21%</td>
</tr>
<tr>
<td><strong>Solid tumors</strong></td>
<td></td>
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<tr>
<td>NCT00141297 (71)</td>
<td>None</td>
<td>I</td>
<td>Rb+ advanced solid tumors (N = 41)</td>
<td>Neutropenia (20%), leukopenia (10%) in cycle 1</td>
<td>27% SD for ≥4 cycles, 16% SD for ≥10 cycles (n = 37 evaluable)</td>
</tr>
<tr>
<td>NCT00420056 (76)</td>
<td>None</td>
<td>Ib</td>
<td>CD19+ /CD20+, CD5+, CD22+ MCL, with cyclin D1 positivity, t(11;14) translocation, or bcl-1/ IGH rearrangement (N = 17)</td>
<td>Neutropenia (35%), thrombocytopenia (24%), hypophosphatemia (12%)</td>
<td>6% CR, 12% PR, 41% SD, median PFS 4 months (90% CI, 2.0–14.7 months; n = 16 evaluable)</td>
</tr>
<tr>
<td><strong>Liposarcoma</strong></td>
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<tr>
<td>NCT01209598 (77)</td>
<td>None</td>
<td>II</td>
<td>Advanced CDK4-amplified, Rb+ well-differentiated liposarcoma (N = 30)</td>
<td>Neutropenia (50%), leukopenia (47%), thrombocytopenia (30%), lymphopenia (27%), anemia (17%)</td>
<td>3% PR, 12-week PFS 66% (90% CI, 51–100), median PFS 18 weeks (n = 29 evaluable)</td>
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<tr>
<td><strong>NSCLC</strong></td>
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<tr>
<td>NCT01291017 (78)</td>
<td>None</td>
<td>II</td>
<td>Previously treated, advanced Rb+, CDKN2A inactivated NSCLC (N = 19)</td>
<td>Neutropenia (16%)</td>
<td>50% SD, median PFS 12.5 weeks</td>
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<tr>
<td><strong>Abemaciclib</strong></td>
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<td><strong>Solid tumors</strong></td>
<td></td>
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<tr>
<td>NCT01394016 (79)</td>
<td>None</td>
<td>I</td>
<td>Advanced solid tumors (whole study N = 225; escalation phase n = 33)</td>
<td>Leukopenia (10%), neutropenia (10%; n = 173)</td>
<td>Breast cancer (n = 47): 23% PR, 47% SD, 25% 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 = 13): 0% PR, 13% SD, 0% ORR, 13% DCR</td>
</tr>
<tr>
<td>NCT02014129 (80)</td>
<td>None</td>
<td>I</td>
<td>Japanese patients with advanced cancer (N = 12)</td>
<td>200 mg BID (n = 6): leukopenia (33%), neutropenia (17%)</td>
<td>Maximal percent change in tumor size from baseline: –35 to –25% &gt;30% tumor shrinkage in 2 patients (HR+, HER2+ breast cancer and small intestine neuroendocrine carcinoma)</td>
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<tr>
<td><strong>Breast cancer</strong></td>
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<tr>
<td>MONARCH-1/ NCT02102490 (22)</td>
<td>None</td>
<td>II</td>
<td>HR+, HER2+. ABC that has progressed on prior endocrine therapy and chemotherapy (N = 152)</td>
<td>Diarrhea (20%) and fatigue (13%), white blood cell decreased (28%), neutrophil count decrease (22%)</td>
<td>Confirmed PR 20%, confirmed ORR 20%, CBR 42%, median PFS 6 months</td>
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</tbody>
</table>

(Continued on the following page)
<table>
<thead>
<tr>
<th>Study name/ID</th>
<th>Combination drug</th>
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<th>Population</th>
<th>Common grade 3/4 AEs (≥10% of patients)</th>
<th>Clinical activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02057133 (81)</td>
<td>Endocrine and HER2-targeted therapies</td>
<td>Ib</td>
<td>HR+ metastatic breast cancer (N = 110)</td>
<td>Combination with letrozole (n = 20): diarrhea (45%), fatigue (20%), neutropenia (20%), nausea (15%), vomiting (10%)</td>
<td>Combination with letrozole (n = 20): 6-month PFS 76%</td>
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<tr>
<td>NCT02057133 (81)</td>
<td></td>
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<td>Combination with anastrozole (n = 16): diarrhea (31%), leukocyte count reduced (31%), neutrophil count reduced (25%), lymphocyte count reduced (25%), fatigue (19%)</td>
<td>Combination with anastrozole (n = 16): 6-month PFS 87%</td>
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<tr>
<td>NCT02057133 (81)</td>
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<td>Combination with tamoxifen (n = 16): neutrophil count reduced (20%), lymphocyte count reduced (13%), fatigue (19%)</td>
<td>Combination with tamoxifen (n = 16): 6-month PFS 73%</td>
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<tr>
<td>NCT02057133 (81)</td>
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<td>Combination with exemestane (n = 15): diarrhea (27%), abdominal pain (20%), neutrophil count reduced (20%), lymphocyte count reduced (20%), fatigue (13%)</td>
<td>Combination with exemestane (n = 15): 6-month PFS 75%</td>
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<tr>
<td>NCT02057133 (81)</td>
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<td>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%)</td>
<td>Combination with exemestane + everolimus (150 mg BID dose; n = 15): 6-month PFS 89%</td>
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<tr>
<td>NCT02057133 (81)</td>
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<td>Combination with exemestane + everolimus (200 mg BID dose; n = 4): neutrophil count reduced (75%), diarrhea (25%), leukocyte count reduced (25%), platelet count reduced (25%)</td>
<td>Combination with exemestane + everolimus (200 mg BID dose; n = 4): 6-month PFS 100%</td>
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<tr>
<td>NCT02057133 (81)</td>
<td></td>
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<td>Combination with trastuzumab (150 mg BID dose; n = 18): diarrhea (17%), fatigue (11%), hypokalemia (11%), lymphocyte count reduced (11%)</td>
<td>Combination with trastuzumab (150 mg BID dose; n = 18): 6-month PFS 29% (HR+ breast cancer; n = 11); 38% (HR- breast cancer; n = 7)</td>
</tr>
<tr>
<td>NCT02057133 (81)</td>
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<td>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%), fatig (17%), abdominal pain (17%)</td>
<td>Combination with trastuzumab (200 mg BID dose; n = 6): 6-month PFS 50% (HR+ breast cancer; n = 5); 0 (HR- breast cancer; n = 1)</td>
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</table>

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.
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 IC50 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 (Tmax) of 1 to 5 hours and a half-life (t1/2) 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 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+...
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 increased 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 × 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 preclinical 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 PRRs 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 unchanged 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). During triplet therapy with ribociclib, everolimus, and fulvestrant 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 BRAFV600E (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).
Table 3. Clinical experience with ribociclib combinations

<table>
<thead>
<tr>
<th>Study name/ID</th>
<th>Combination drug</th>
<th>Phase</th>
<th>Population</th>
<th>MTD/RP2D/dose</th>
<th>Reported grade 3/4 AEs</th>
<th>Clinical activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLEE01X2106/ NCT0851793 (44)</td>
<td>Exemestane</td>
<td>Ib</td>
<td>Postmenopausal women with ER+, HER2- ABC previously treated with letrozole or anastrozole (N = 14 treated with ribociclib + exemestane)</td>
<td>Evaluated dose: Ribociclib: 600 mg/day (3 weeks on/1 week off) Exemestane: 25 mg/day</td>
<td>Neutropenia (71%), leukopenia (36%), lymphopenia (14%), ALT elevation (14%), AST elevation (14%), anemia (14%)</td>
<td>2 unconfirm PR, 4 SD</td>
</tr>
<tr>
<td>CLEE01X2107/ NCT08712260 (45, 47)</td>
<td>Letrozole</td>
<td>Ib</td>
<td>Pretreated and treatment-naïve postmenopausal women with ER+, HER2- ABC (N = 47 treated with ribociclib + letrozole)</td>
<td>RP2D: Ribociclib: 600 mg/day (3 weeks on/1 week off) Letrozole: 2.5 mg/day</td>
<td>All patients: Neutropenia and neutrophil count reduced (60%), ALT elevation (4%), AST elevation (4%), anemia (4%), constipation (2%), nausea (2%), UTI (2%), fatigue (1%)</td>
<td>First line (n = 28): Neutropenia and neutrophil count reduced (64%), ALT elevation (4%), AST elevation (4%), anemia (4%), constipation (2%), nausea (2%)</td>
</tr>
<tr>
<td>CLEE01X2108/ NCT08888884 (42)</td>
<td>Fulvestrant</td>
<td>Ib</td>
<td>Postmenopausal women with HR+, HER2- ABC (N = 28)</td>
<td>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</td>
<td>Ribociclib 600 mg/day 3 weeks on/1 week off; n = 13: Neutropenia (62%), fatigue (15%), leucocyte count reduced (15%), ALT elevation (8%), AST elevation (8%) Ribociclib 400 mg/day (continuous; n = 15): Neutropenia (33%), leucocyte count reduced (7%), ALT elevation (7%), AST elevation (7%)</td>
<td>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</td>
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<tr>
<td>CLEE01X2106/ NCT0851793 (52)</td>
<td>Everolimus + exemestane</td>
<td>Ib</td>
<td>Postmenopausal women with ER+, HER2- ABC previously treated with letrozole or anastrozole (N = 77 treated with ribociclib + everolimus + exemestane)</td>
<td>RP2D: Ribociclib: 300 mg/day (3 weeks on/1 week off) Everolimus: 2.5 mg/day Exemestane: 25 mg/day</td>
<td>Neutropenia (31%), leucocyte count reduced (18%), anemia (7%), thrombocytopenia (7%), lymphopenia (7%), ALT elevation (7%), AST elevation (7%) Ribociclib 600 mg/day 3 weeks on/1 week off; n = 13: Neutropenia (31%), leucocyte count reduced (18%), anemia (7%), thrombocytopenia (7%), lymphopenia (7%), ALT elevation (7%), AST elevation (7%)</td>
<td>ORR 9%, DCR 73%, CBR 26%</td>
</tr>
<tr>
<td>CLEE01X2107/ NCT08712260 (51)</td>
<td>Alpelisib + letrozole</td>
<td>Ib</td>
<td>Pretreated and treatment-naïve postmenopausal women with ER+, HER2- ABC (N = 46 treated with ribociclib + alpelisib + letrozole)</td>
<td>RP2D: Ribociclib: 300 mg/day (3 weeks on/1 week off) Alpelisib: 200 mg/day Letrozole: 2.5 mg/day</td>
<td>Alpelisib 300 mg/day (continuous; n = 60): Neutropenia (7%), fatigue (31%), reduced neutrophil count (4%), anemia (4%), thrombocytopenia (2%), vomiting (2%), nausea (2%)</td>
<td>ORR 16%, DCR 70%, CBR 26%</td>
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(Continued on the following page)
<table>
<thead>
<tr>
<th>Study name/ID</th>
<th>Combination drug</th>
<th>Phase</th>
<th>Population</th>
<th>MTD/RP2D/dose</th>
<th>Reported grade 3/4 AEs</th>
<th>Clinical activity</th>
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<tbody>
<tr>
<td>CLEE01A2201/</td>
<td>Letrozole</td>
<td>II</td>
<td>Postmenopausal women with HR⁺, HER2⁻ Grade II/III invasive, early breast</td>
<td>Ribociclib: 600 mg/day or 400 mg/day Letrozole: 2.5 mg/day</td>
<td>All AEs were mild/moderate with no grade 3/4 AEs</td>
<td>Ribociclib 400 mg/day + letrozole: 96% decrease in Ki67</td>
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<td>NCT01919229</td>
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<td>cancer who have received no prior breast cancer treatment (N = 14)</td>
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<td></td>
<td>Ribociclib 600 mg/day + letrozole: 92% decrease in Ki67</td>
</tr>
<tr>
<td>MONALEESA-2/</td>
<td>Letrozole</td>
<td>III</td>
<td>Postmenopausal women with HR⁺, HER2⁻ ABC who have received no prior</td>
<td>Ribociclib: 600 mg/day (3 weeks on/1 week off) Letrozole: 2.5 mg/day</td>
<td></td>
<td>Letrozole only: 69% decrease in Ki67</td>
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<tr>
<td>NCT01958021</td>
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<td>treatment for advanced disease (N = 668)</td>
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<tr>
<td><strong>NRAS- or BRAF-mutant melanoma</strong></td>
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<tr>
<td>CMEK162X2114/</td>
<td>Binimetinib</td>
<td>Ib/Ii</td>
<td>Patients with advanced NRAS- mutant melanoma (N = 22 received ribociclib + binimetinib)</td>
<td>MTD: Ribociclib: 200 mg/day (3 weeks on/1 week off) Binimetinib: 45 mg BID RP2D: ongoing</td>
<td>CPK elevation (18%), neutropenia (9%), acneiform (4%), dermatitis (4%), rash (4%)</td>
<td>7 PR, 11 SD, 33% had 20–30% tumor shrinkage, CBR 86%</td>
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<tr>
<td>NCT01781572</td>
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<td><strong>Encorafenib</strong></td>
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<td>Patients with advanced BRAFV600-mutant melanoma (N = 28 received ribociclib + encorafenib)</td>
<td>MTD: ongoing RP2D: ongoing</td>
<td>Hand-foot syndrome (11%), rash (4%), myalgia (4%)</td>
<td>Two confirmed PRs, 3 unconfirmed PRs, 10 SD, 1 SD &gt;9 cycles</td>
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<td>CLEE01X2105/</td>
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**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.
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<sup>K61Q</sup>-mutant melanoma relative to single-agent binimetinib (12% T/C) or ribociclib (32% T/C; refs. 27, 62). In BRAF<sup>V600E</sup>-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 × 10<sup>−7</sup>), 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, acneform 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<sup>V600E</sup>-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<sup>2</sup>/day (adult equivalent dose ≈800 mg/day) and 350 mg/m<sup>2</sup>/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<sub>max</sub> 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<sup>V600E</sup> 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<sup>+</sup> breast cancer. MONALEESA-3 is evaluating the addition of ribociclib to fulvestrant in patients with HR<sup>+</sup> 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/peri-menopausal women with HR<sup>+</sup> ABC. MONALEESA-7 is the only trial entirely dedicated to investigating CDK4/6 inhibition in the pre/peri-menopausal 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).
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 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 Serono, Peloton Therapeutics, and Servier Pharmaceuticals. No other potential conflicts of interest were disclosed.

Disclaimer

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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


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