REVIEW

Targeting CDK4 and CDK6: From Discovery to Therapy

Charles J. Sherr[1,2], David Beach[3], and Geoffrey I. Shapiro[4]

ABSTRACT Biochemical and genetic characterization of D-type cyclins, their cyclin D–dependent kinases (CDK4 and CDK6), and the polypeptide CDK4/6 inhibitor $p16^{INK4}$ over two decades ago revealed how mammalian cells regulate entry into the DNA synthetic (S) phase of the cell-division cycle in a retinoblastoma protein–dependent manner. These investigations provided proof-of-principle that CDK4/6 inhibitors, particularly when combined with coinhibition of allied mitogen-dependent signal transduction pathways, might prove valuable in cancer therapy. FDA approval of the CDK4/6 inhibitor palbociclib used with the aromatase inhibitor letrozole for breast cancer treatment highlights long-sought success. The newest findings herald clinical trials targeting other cancers.

Significance: Rapidly emerging data with selective inhibitors of CDK4/6 have validated these cell-cycle kinases as anticancer drug targets, corroborating longstanding preclinical predictions. This review addresses the discovery of these CDKs and their regulators, as well as translation of CDK4/6 biology to positive clinical outcomes and development of rational combinatorial therapies. *Cancer Discov; 6(4); 353–67. ©2015 AACR.*

INTRODUCTION

Cyclin-dependent kinase 4 (CDK4) and closely related CDK6 play key roles in mammalian cell proliferation, where they help to drive the progression of cells into the DNA synthetic (S) phase of the cell-division cycle. Unlike CDKs 1 and 2, which act later in the cell cycle in response to periodic oscillations of cyclins E, A, and B to coordinate DNA replication with mitosis (Fig. 1), the enzymatic activities of CDK4 and CDK6 in the first gap phase (G_1) of the cycle are governed by D-type cyclins expressed in response to various extracellular signals, including stimulatory mitogens, inhibitory cytokines, differentiation inducers, cell–cell contacts, and other spatial cues. The three D-type cyclins (D1, D2, and D3) are differentially expressed, alone or in combination, in distinct cell lineages, where they assemble with CDK4 and CDK6 to form enzymatically active holoenzyme complexes. An understanding of how the three different D-type cyclins act as environmental sensors in responding dynamically to extracellular cues in various cell types helps to explain how CDK4/6 activities are differentially regulated and predicts the basis of functional interactions between mitogen signaling pathways and CDK4/6 activity in both normal and cancer cells. More than two decades after discovery of CDK4 and CDK6, drugs inhibiting their activities are now demonstrating significant efficacy in cancer treatment (for other recent reviews, see refs. 1–3). The elucidation of how signal transduction pathways activate CDK4/6 in different tumor types should pave the way for combinatorial therapies that target both cyclin D and CDK4/6 simultaneously to improve therapeutic responses.

DISCOVERY OF D-TYPE CYCLINS

The D-type cyclins were identified in 1991 by three groups of investigators under widely different experimental circumstances. At the time, no "G_1 cyclins" had yet been found in mammalian cells, whereas budding yeast (*Saccharomyces cerevisiae*) was recognized to synthesize three such cyclins (CLNs 1, 2, and 3). In yeast, the induced CLN proteins, like S phase and mitotic cyclins, associate with a single CDK (CDC28/CDK1), whose rise in activity in late G_1 is associated with the commitment of cells (START in yeast) to enter S phase (4). Using a conditionally *CLN*-deficient yeast strain, a human cDNA that complemented the *CLN* genetic deficiency was designated cyclin D1 (*CCND1*; ref. 5). In independent studies, others recovered a differentially expressed cyclin-like cDNA (originally designated *Cyl1*) that was induced during G_1 phase in murine macrophages synchronously entering the cell cycle in response to mitogen stimulation by colony-stimulating factor-1 (6). Two closely related genes, *Cyl2* and *Cyl3*, were

[1]Howard Hughes Medical Institute, Chevy Chase, MD. [2]Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee. [3]The Blizard Institute, Barts and the London School of Medicine and Dentistry, London, United Kingdom. [4]Early Drug Development Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts.

Corresponding Authors: Geoffrey I. Shapiro, Dana-Farber Cancer Institute, Mayer 446, 450 Brookline Avenue, Boston, MA 02215. Phone: 617-632-4942; Fax: 617-632-1977; E-mail: geoffrey_shapiro@dfci.harvard.edu; and Charles J. Sherr, sherr@stjude.org

doi: 10.1158/2159-8290.CD-15-0894

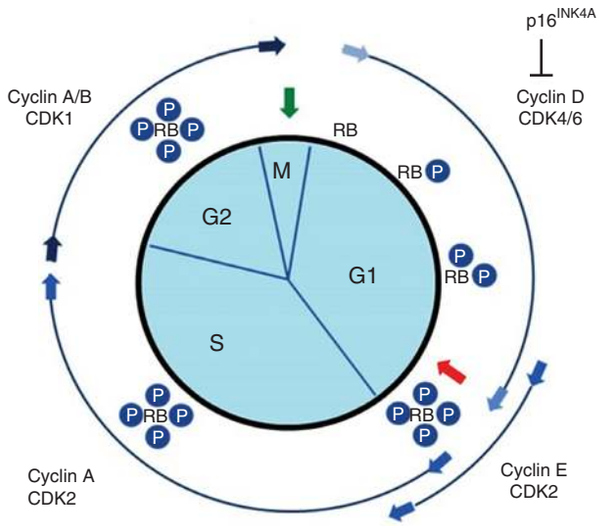

Figure 1. The cell cycle. The four phases of the mitotic cell division cycle are indicated in the inner circle, including mitosis (M phase), the cellular DNA synthesis (S) phase, and their separation by two gap (G) phases, the first (G_1) between M and S phases and the second (G_2) between S and M phases. The levels of total CDK activity are lowest in early G_1 phase and progressively increase under the agency of different cyclin–CDK complexes, reaching maximal net CDK activity as cells enter mitosis. States of RB phosphorylation (P) throughout the cell cycle are schematized. RB is dephosphorylated in M phase (green arrow) and progressively rephosphorylated in G_1, first by cyclin D–dependent CDK4/6 and later by cyclin E–dependent CDK2. RB becomes fully phosphorylated in late G_1 (red arrow), resulting in inactivation of its proliferation-suppressive function and triggering the cell's subsequent entry into S phase. The point in the cell cycle (sometimes called "the restriction point"; red arrow) at which RB becomes fully phosphorylated temporally corresponds to a late G_1 phase transition when cells lose their marked dependency on extracellular mitogens and commit to enter S phase and complete the cycle. During the S and G_2 phases, RB phosphorylation is maintained by the progressive activation of other CDKs, including cyclin A–CDK2 and cyclins A/B–CDK1. Degradation of cyclins A and B in mitosis results in the collapse of CDK activity and restores the G_1 state. INK4 proteins (the prototype $p16^{INK4A}$ is shown) specifically inhibit the cyclin D–dependent kinases to inhibit RB phosphorylation and arrest cells in G_1 phase. Arrested cells can return to a noncycling but reversible quiescent state (G_0) after mitogen withdrawal in which D-type cyclins are usually degraded or, in response to particular stress conditions, can undergo durable cell-cycle arrest (senescence). Quiescent cells restimulated with mitogens restore cyclin D synthesis and re-enter the cell cycle in early G_1, whereas senescent cells are refractory to mitogen restimulation and resist oncogenic challenge. Asynchronously dividing cells maintain mitogen-dependent cyclin D synthesis and have a contracted G_1 phase when compared with quiescent cells re-entering the division cycle (for pertinent detailed reviews, see refs. 4, 16, 41, 65).

found to be expressed in IL2-responsive lymphocytes that did not express *Cyl1* (6, 7). A fortuitous first meeting of two of the authors and subsequent prepublication exchange of the predicted amino acid sequences of *Cyl1* and human cyclin D1, as well as precipitation of human cyclin D1 with antibodies directed to the mouse CYL1 protein, revealed that the mouse and human genes were orthologs (5, 6). Concomitantly, a gene called *PRAD1* was identified at the breakpoint of a chromosomal inversion [inv(11)(p15;q13)] in parathyroid adenoma (8). Comparison of the *PRAD1* nucleotide sequence with that of human *CCND1* revealed that the two were identical, providing a key prediction that cyclin D1 has proto-oncogenic properties. As expected, *Cyl2* and *Cyl3* turned out to be equivalent to the subsequently identified human cyclin D2 (*CCND2*) and D3 (*CCND3*) genes, respectively (9, 10).

Taken together, these earliest reports defined a distinct family of three D-type cyclins (i) that acted as mitogen sensors during the G_1 phase of the cell cycle; (ii) that were expressed in various combinations in a cell lineage–specific manner; (iii) whose functions were evolutionarily conserved but with no one of them being essential for cell-cycle progression; (iv) that were predicted to activate then-novel CDKs, and (v) that had latent proto-oncogenic capabilities.

DISCOVERY OF CDK4 AND CDK6

The presumption that D-type cyclins might allosterically regulate a novel CDK was validated by the discovery of CDK4, which was revealed to physically bind to, and be enzymatically activated by, any of the three D-type cyclins (11). A related cyclin D–dependent kinase, CDK6, with similar properties was identified 2 years later (12). Although CDKs 1 and 2, in complexes with cyclins E, A, and B, drive cell-cycle progression through S phase and M phase (mitosis), the cyclin D–dependent CDKs act during G_1 phase to propel quiescent cells that have entered the cell cycle, or proliferating cells that have completed mitosis, toward S phase (Fig. 1). Unlike CDKs 1 and 2 that phosphorylate many hundreds of cellular protein substrates (13), CDK4 is a surprisingly fastidious enzyme that has a restricted propensity to phosphorylate the retinoblastoma protein (RB1, hereafter RB) and two other RB-family proteins [RB2 (p130), RBL1 (p107); refs. 11, 14–16], but very few other substrates (17).

RB is a canonical tumor-suppressor gene in retinoblastoma and in many other cancers as well (18, 19). The RB protein undergoes periodic phosphorylation as cells traverse the division cycle. RB is dephosphorylated as cells exit mitosis, and the hypophosphorylated form detected in G_1 phase becomes hyperphosphorylated (inactivated) in late G_1 and remains so throughout progression through S phase to mitosis (refs. 20–23; Fig. 1). The role of hypophosphorylated (active) RB to restrict proliferation and act as a potent tumor-suppressor gene was highlighted by studies indicating that RB's growth-suppressive function could be inactivated by its binding to DNA tumor virus oncoproteins (human papillomavirus E7, adenovirus E1A, and SV40 T antigen; refs. 24–27). In mammalian cells stimulated by mitogens to enter the division cycle from a quiescent state (G_o), CDK4/6-mediated RB phosphorylation was first detected in mid-G_1 phase after induction of cyclin D but prior to activation of cyclin E– and A–dependent CDK2 (12, 28). Together, these results implied that the role of CDK4/6 was to phosphorylate RB, priming it for inactivation by other CDKs later in G_1, and releasing E2F transcription factors from RB constraint to allow their coordinate induction of a suite of genes whose activities are jointly required for initiation of S phase (reviewed in detail in refs. 16, 29–31).

ENTER $p16^{INK4a}$

Early controversies quickly arose around the issue of how, and even whether, the D-type cyclins regulated the cell cycle, the respective roles that CDK4 and other CDKs might play as RB kinases, and what the putative G_1 signaling pathways

might be. The discovery of a highly specific 16-kDa polypeptide inhibitor of CDK4 encoded by the *INK4a* (formally *CDKN2A*) gene (32) provided compelling data that CDK4 acts upstream of RB. Key features were that $p16^{INK4a}$ bound to and potently inhibited cyclin D–CDK4 kinase activity but spared holoenzyme complexes containing other CDKs, and that expression of $p16^{INK4a}$ inhibited RB phosphorylation and arrested cells in the G_1 phase of the cell cycle. Importantly, cells lacking functional RB were resistant to $p16^{INK4a}$-mediated cell-cycle arrest, implying that the ability of CDK4 (and later, CDK6) to drive G_1 phase progression required RB (33–35) and predicting that chemical CDK4/6 inhibitors would show efficacy only if RB were functionally intact.

Given the role of RB as a tumor suppressor, it was intuited that $p16^{INK4a}$ would play a similar role in inhibiting tumor formation. Within months of its discovery, the *INK4a* (*CDKN2A*) and the genetically linked *INK4b* gene (*CDKN2B*; ref. 36) were implicated in a reverse genetic screen to play tumor-suppressive roles in familial melanoma (37). A flurry of subsequent papers soon identified $p16^{INK4a}$ as a frequent target of inactivating mutations and deletions in many human cancers and revealed that loss of function of $p16^{INK4a}$ and RB generally occur as mutually exclusive events in tumor cells. In turn, the realization that *CCND1* in particular was a target of translocation in certain tumors [for example, in mantle cell lymphoma (MCL)] or was amplified (for example, in breast cancer) reinforced the view that cyclin D1 (and, by presumption, CDK4) were oncoproteins. After compiling data from numerous independent reports, mutations in the "RB pathway" were soon proposed to be a hallmark of cancer (19, 38).

REGULATION OF CDK4 AND CDK6 BY D-TYPE CYCLINS: IMPLICATIONS FOR CANCER TREATMENT

In many cell types, transcription of *CCND1* and cyclin D1 assembly with CDK4 each depend on activation of a RAS-dependent kinase cascade that relies on the sequential activities of RAF1, MEK1 and MEK2, and ERKs (39–42). In serum-deprived fibroblasts lacking endogenous cyclin D expression, ectopically expressed cyclin D1 does not associate with CDK4 (28), but assembly of cyclin D–CDK complexes occurs in response to enforced expression of constitutively active MEK (43). HSC70 associates with newly synthesized cyclin D1 and is a component of the mature catalytically active cyclin D1–CDK4 complex (44). CDK4, like several other kinases, similarly requires molecular chaperones to be properly folded and to assemble into productive complexes. In the cytoplasm, newly synthesized CDK4 is detected within high–molecular weight complexes that also contain HSP90 and CDC37 (45, 46). Release from the chaperone complex enables CDK4 to interact with D-type cyclins or, alternatively, to dimerize with $p16^{INK4a}$, yielding inactive CDK4. Under normal physiologic circumstances in young animals, $p16^{INK4a}$ is not expressed; however, it is induced by a variety of hyperproliferative stress signals whose oncogenic effects are countered by $p16^{INKa}$-induced cell-cycle arrest (47). Competition between mitogen-activated D-type cyclins and stress-activated $p16^{INK4a}$ for CDK4 binding determines whether cells undergo G_1 arrest or enter S phase. Presumably, HSP90 inhibition might also complement CDK4 inhibitors in preventing RB phosphorylation and enforcing cell-cycle arrest.

Naturally occurring pan-CDK inhibitors, including $p21^{CIP1}$ and $p27^{KIP1}$, facilitate cyclin D–CDK assembly and the nuclear import of the resulting complexes without inhibiting CDK4/6 kinase activity (41, 48–51). Posttranslational modification of these CIP/KIP proteins by mitogen-triggered tyrosine phosphorylation may explain their loss of CDK-inhibitory activity when acting as "assembly factors" in binding cooperatively to D cyclins and CDK4/6 (52, 53). The subsequent importation of assembled cyclin D–CDK complexes into the nucleus allows them to access and phosphorylate RB. Under normal circumstances, asynchronously dividing cells periodically express peak nuclear cyclin D1 levels at G_1–S, after which cyclin D1 export into the cytoplasm and its increased turnover is triggered during S phase (54).

A separate RAS signaling pathway commanded by PI3K and AKT (protein kinase B) negatively regulates glycogen synthase kinase 3β (GSK3β) to prevent its phosphorylation of cyclin D1 on a single threonine reside (Thr286; refs. 55, 56). PI3K/AKT-mediated inhibition of cyclin D1 Thr286 phosphorylation prevents the nuclear export of cyclin D1–CDK complexes and blocks cyclin D1 recognition by the FBX4-containing ubiquitin ligase that triggers cyclin D degradation (55–58). Moreover, certain cyclin D mutants or C-terminally truncated variants encoded by an alternatively spliced mRNA (D1b) that lacks the Thr286 codon are retained in the nucleus during S phase and are proposed to confer a neo-oncogenic function that triggers DNA re-replication, aneuploidy, and tumorigenesis (59–61). Thus, altered cyclin D turnover in tumor cells may be reflected by persistent and intense nuclear cyclin D1 expression.

RAS signaling, highlighted in the above discussion, is by no means the only arbiter of the life history of D-type cyclins in cells, as many receptor-mediated signals—for example, the T-cell receptor, cytokine and hormone receptors (HR), and the machinery that monitors cell adhesion—all converge to positively or negatively regulate the levels of individual D-type cyclins in different cell types. In short, cyclin D transcription, assembly with CDK4/6, nuclear transport, and stability are each mitogen-dependent steps governed by distinct signaling pathways. The central conclusion is that D-type cyclins act as mitogen sensors to govern G_1 phase progression. Cancer-specific mutations, such as those affecting receptor tyrosine kinases (RTK), RAS, RAF, PI3K, or PTEN, or genetic alterations leading to aberrant hormone and cytokine receptor signaling can enhance cyclin D–dependent CDK4/6 activity. Conversely, cell type–specific RTK, RAF/MEK/ERK, and PI3K/AKT inhibitors, particular hormone or interleukin antagonists, or antiproliferative cytokines, such as TGFβ, can increase the threshold for CDK4/6 activation and synergize with CDK4/6 inhibitors to induce G_1 phase cell-cycle arrest (19).

Despite the high frequency of mutations epistatically targeting the RB signaling pathway in cancer cells, inactivation of the individual *Ccnd1*, *Cdk4/6*, and *Cdkn2a* genes in mice, while leading to specific developmental defects when disrupted alone or in combination, established that their functions were nonessential for the cell cycle *per se* (62–64). In contrast, the demonstration that inactivation of these genes can prevent oncogene-induced tumor development in mouse models reinforced the view that these enzymes might be suitable cancer-specific drug targets (65).

EARLY ATTEMPTS TO DEVELOP A CHEMICAL CDK4/6 INHIBITOR

Based on founding discoveries in the early 1990s that provided proof-of-principle that CDK4 inhibition might retard cancer cell development, David Beach and Giulio Draetta founded Mitotix, Inc. Although inhibitors, such as flavopiridol and roscovitine, that broadly targeted CDKs were then available (1), the Mitotix scientific advisory board (Beach D, Draetta G, Sherr CJ, Bishop JM, Kirschner M, Rothstein R, Nicolau KC, and Folkman J) decided at its first meeting in late 1993 to try to develop a drug that would specifically inhibit CDK4. At this early stage, the significance of RB phosphorylation by CDK4 still remained a subject of intense debate, particularly given that cyclin E/A–dependent CDK2 robustly catalyzes RB phosphorylation at the G_1–S transition (Fig. 1). Moreover, CDK6 had not yet been discovered (12), and the demonstration that the ability of $p16^{INK4a}$ to arrest the cell cycle depended upon functional RB had not thus far been firmly established (33–35). Assays for cyclin D–CDK4 RB kinase activity were optimized at Mitotix for high-throughput screening of chemical libraries, and several lead compounds were identified. These preliminary efforts fueled a partnership with DuPont Merck chemists who tried to synthesize derivative compounds that inhibited CDK4, but not other CDKs, and whose pharmacologic and medicinal properties would facilitate further drug development. However, no suitable drugs were identified, and Mitotix was sold during the dot-com bust in 2000–2001.

Soon thereafter, chemists at Parke-Davis developed a specific CDK inhibitor (PD0332991) that would eventually become Pfizer's palbociclib (IBrance; refs. 66, 67). Palbociclib is an orally bioavailable, low nanomolar reversible inhibitor of CDK4/6 (Table 1), which exhibited no significant activity against a wide panel of other kinases; these included cyclin E– and A–driven CDK2 and cyclin B–CDK1 that are more than 1,000-fold less sensitive to the drug (Table 1). Palbociclib arrested the proliferation of tumor cell lines that retain functional RB, blocking its phosphorylation on CDK4/6-specific sites. Treated breast, colon, and lung cancer cell lines, which are primarily driven by cyclin D1–CDK4, as well as myeloid and lymphoid leukemia cells that primarily depend on cyclins D2/D3 and CDK6, accumulated in G_1 phase, exhibited loss of the proliferation marker Ki67, and downregulated canonical E2F target genes (IC_{50} values for cultured cells: 40 to 170 nmol/L). In several xenograft models, the drug was efficacious in inducing tumor stasis or regression and was tolerated without significant toxicities at daily doses up to 150 mg/kg for up to 50 days of treatment (66, 67). Although withdrawal of palbociclib after several weeks was accompanied by tumor regrowth, the re-emerging cancers remained drug-sensitive, suggesting that recurring tumors did not acquire therapeutic resistance. Tumor xenografts lacking *CDKN2A* were sensitive to the drug, whereas those lacking functional RB were refractory to palbociclib treatment. Although several lines of evidence argue for CDK4/6-independent roles of D-type cyclins as transcriptional cofactors for HRs (68) and

Table 1. Key characteristics of CDK inhibitors

Drug	Palbociclib (Pfizer) (PD0332991, Ibrance)	Ribociclib (Novartis) (LEE011)	Abemaciclib (Eli Lilly) (LY2835219)
IC_{50} (*in vitro* kinase assay, recombinant proteins)	CDK4 (D1): 11 nmol/L CDK4 (D3): 9 nmol/L CDK6 (D2): 15 nmol/L CDK1: >10 μmol/L CDK2: >10 μmol/L (66, 67)	CDK4: 10 nmol/L CDK6: 39 nmol/L CDK1: >100 μmol/L CDK2: >50 μmol/L (1, 89)	CDK4 (D1): 0.6–2 nmol/L CDK6 (D1): 2.4–5 nmol/L CDK 9: 57 nmol/L CDK1: >1 μmol/L CDK2: >500 nmol/L (1, 88)
PK	T_{max} 4.2–5.5 hr $t_{1/2}$ 25.9–26.7 hr (69, 70)	T_{max} 4 hr $t_{1/2}$ 24–36 hr (90, 91)	T_{max} 4–6 h $t_{1/2}$ 17–38 h (crosses blood:brain barrier; refs. 92, 93)
PD	Reduced RB phosphorylation in paired tumor biopsies, along with reduced fluorothymidine-PET uptake (75)	Reduced RB phosphorylation and Ki67 expression in paired tumor biopsies (90)	Reduced RB phosphorylation and topoisomerase IIα expression in paired tumor and skin biopsies (92)
Dosing	125 mg daily (3 weeks, 1-week drug holiday) or 200 mg daily (2 weeks, 1-week drug holiday; refs. 69, 70)	600 mg daily (3 weeks, 1-week drug holiday; ref. 90)	200 mg twice daily (continuous dosing; ref. 92)
Major dose-limiting toxicities	Neutropenia, thrombocytopenia	Neutropenia, thrombocytopenia	Fatigue
Other reported adverse events	Anemia, nausea, anorexia, fatigue, diarrhea (69, 70)	Mucositis Prolonged EKG QTc interval Elevated creatinine Nausea (90)	Diarrhea Neutropenia (92)

for phosphoproteins, such as FOXM1, as alternative CDK4/6 substrates (17), the observed preclinical effects of palbociclib were consistent with the notion that inhibition of CDK4/6 is a key mechanism underlying antitumor activity (66). Clinical development of CDK4/6 inhibitors, briefly summarized in Table 2, is discussed below.

PALBOCICLIB CLINICAL DEVELOPMENT

Phase I studies of palbociclib were conducted in patients with advanced cancers that express RB, establishing recommended phase II doses of 125 mg daily for 3 weeks on/1 week off (3/1 schedule) or 200 mg daily for 2 weeks on/1 week off (2/1 schedule; refs. 69, 70). Dose-limiting toxicities were neutropenia and thrombocytopenia that precluded continuous dosing. Changes in absolute neutrophil count and platelets as related to plasma palbociclib exposure were established using an E_{max} model. Given the reliance of myeloid development in the mouse on cyclin D2– and D3–driven CDK6 (62, 71, 72), these results might have been anticipated. Other side effects were mild, including fatigue, diarrhea, anemia, and nausea, with overall good tolerability on both dosing schedules. Palbociclib exhibited linear pharmacokinetics, with a median time to maximal concentration (T_{max}) of ~5 hours, and was eliminated with a mean plasma half-life of ~26 hours (Table 1). In the phase I experience, a partial response

Table 2. Highlights of representative completed and ongoing clinical trials with CDK4/6 inhibitors

Cancer type	Drug(s) (trial phase)	Description and outcome	References
Monotherapy trials			
Advanced solid tumors (various) NCT00141297	Palbociclib (phase I, completed)	Drug dosage, PK/PD, and dose-limiting toxicities were established. Stable disease realized in 19 of 74 patients.	(69, 70)
Advanced solid tumors (various) NCT01237236	Ribociclib (phase I)	Drug dosage, PK/PD, and dose-limiting toxicities established in 85 patients. Reductions in Ki67 and phosphorylated RB documented in paired pre- and posttreatment tumor biopsies. Stable disease for >6 treatment cycles in 14% of patients.	(90, 91)
Advanced solid tumors (various) NCT01394016	Abemaciclib (phase I)	Drug dosage, PK/PD, and dose-limiting toxicities determined. Drug efficiently crosses the blood:brain barrier to equal plasma concentrations.	(92)
Advanced solid tumors or hematologic malignancies (various) NCT02187783	Ribociclib (phase II)	SIGNATURE: to determine efficacy of treatment in previously treated patients preidentified as having CDK4/6 pathway–activated tumors (including p16 loss or CDK4/6 or cyclin D1/D3 amplification).	(133) Outcome not reported
MCL NCT00420056	Palbociclib (PD study, completed)	Of 17 patients receiving drug on the 3/1 schedule, reductions in RB phosphorylation and tumor proliferation (Ki67 and fluorothymidine PET) occurred during the first cycle in most patients. Five heavily pretreated patients achieved PFS of >1 year.	(75)
MCL NCT01739309	Abemaciclib (phase II)	Of 22 patients with relapsed or refractory disease who received >6 treatment cycles, there were five partial responses, and 9 patients with stable disease.	(105)
Liposarcoma NCT01209598	Palbociclib (phase II)	Of 30 patients who had progressed on prior therapy, 66% were progression-free after 12 weeks on a 2/1 schedule. Eight patients remained on study for >40 weeks with tumor regressions in 4 patients and one complete response.	(69, 77, 80)
Breast cancer NCT01037790	Palbociclib (phase II)	37 patients enrolled with 2 partial responses and 5 with stable disease for a clinical benefit rate of 19% overall and 29% in $HR^+/HER2^-$ negative disease.	(82)
Breast cancer NCT01394016	Abemaciclib (phase I)	Expansion cohort of phase I trial. Evaluation in 25 heavily pretreated patients with $ER^+/HER2^-$ disease in which 72% exhibited overall clinical benefit. Drug was also evaluated in 11 patients with $HR^+/HER2^+$ disease (with 100% control rate); 5 patients with $HR^-/HER2^+$ disease exhibited stable disease of only brief duration. Median duration of response for all treated HR^+ patients was 13.4 months with 8.8-month PFS.	(92, 95, 96)
Breast cancer NCT02102490	Abemaciclib (phase II)	MONARCH-1. Based on monotherapy responses seen in expansion cohort of the phase I trial. Evaluating monotherapy for patients whose disease has progressed despite prior chemotherapy.	Not reported

(*continued*)

Table 2. Highlights of representative completed and ongoing clinical trials with CDK4/6 inhibitors (*Continued*)

Cancer type	Drug(s) (trial phase)	Description and outcome	References
Breast cancer NCT02308020	Abemaciclib (phase I)	Designed to exploit traversal of the blood–brain barrier by abemaciclib. Assessment in breast cancer patients with brain metastases in 3 cohorts: (1) $HR^+/HER2^+$; (2) $HR^+/HER2^-$; (3) patients eligible for resection, 5–14 days prior to surgery.	Not reported
NSCLC NCT01291017	Palbociclib (phase II)	16 patients enrolled with advanced disease and evidence of *CDKN2A* loss on the 3/1 schedule. 8 patients were progression-free >4 months.	(101)
NSCLC NCT01394016	Abemaciclib (phase I)	Expansion cohort in phase I trial. In 15 of 31 patients who remained on trial for >6 months, overall disease control rate was 49% with 6-month PFS in 26%. Patients with tumors harboring *KRAS* mutation showed greater disease control.	(92, 102)
GBM NCT01394016	Abemaciclib (phase I)	Expansion cohort in phase I trial. Two of 17 patients showed decreases in tumor size and prolonged time to progression.	(92)
Melanoma NCT01394016	Abemaciclib (phase I)	Expansion cohort in phase I trial. 26 patients enrolled. Partial response observed in a patient with tumor harboring *NRAS* mutation and *CDKN2A* loss.	(92)
Germ cell tumor NCT01037790	Palbociclib (phase II)	30 patients enrolled to 3/1 schedule, based on preliminary efficacy seen in patients with growing teratoma syndrome in phase I trial. 24-week PFS rate 28%, with efficacy predominantly in patients with teratoma or teratoma with malignant transformation.	(73, 74)
Hormonal combinations in ER^+ breast cancer			
Breast cancer NCT00721409 NCT01740427	Palbociclib Letrozole (phase II/III)	PALOMA-1: 165 postmenopausal women with advanced $ER^+/HER2^-$ disease who had not received systemic treatment for advanced disease were randomized to receive letrozole vs. letrozole/palbociclib. Mean PFS was 10.2 months with letrozole alone and 20.2 months for the combination. *CCND1* amplification and *CDKN2A* loss did not predict benefit. Provisional FDA approval for this indication was obtained in early 2015, and data are awaited from phase III evaluation (PALOMA-2).	(83)
Breast cancer NCT01942135	Palbociclib Fulvestrant (phase III)	PALOMA-3: Interim analysis of ongoing phase III study of pre- and postmenopausal women with advanced $ER^+/HER2^-$ disease reported PFS of 9.2 months with combination vs. 3.8 months with fulvestrant alone.	(84)
Breast cancer NCT02107703	Abemaciclib Fulvestrant (phase III)	MONARCH-2: Fulvestrant with or without abemaciclib.	Not reported
Breast cancer NCT02246621	Abemaciclib Aromatase inhibitors (phase III)	MONARCH-3: Anastrozole or letrozole with placebo or abemaciclib.	Not reported
Breast cancer NCT01919229	Ribociclib Letrozole (phase II, completed)	MONALEESA 1: Presurgical study of letrozole vs. letrozole/ribociclib in early breast cancer patients.	Results pending
Breast cancer NCT01958021	Ribociclib Letrozole (phase III)	MONALEESA 2: First-line metastatic trial in postmenopausal patients randomizing letrozole to letrozole/ribociclib.	Not reported
Breast cancer NCT02422615 (phase III)	Ribociclib Fulvestrant (phase III)	MONALEESA 3: Randomized double-blind, placebo-controlled study in postmenopausal women with $HR^+/HER2^-$ advanced disease who have received no or 1 line of endocrine treatment.	Not reported
Breast cancer NCT02278120	Ribociclib Aromatase inhibitors Tamoxifen Goserelin (phase III)	MONALEESA 7: Randomized double-blind, placebo-controlled study of tamoxifen or an aromatase inhibitor with goserelin along with ribociclib or placebo in pre- or perimenopausal women with $HR^+/HER2^-$ breast cancer.	Not reported
Breast cancer NCT01872260	Ribociclib Letrozole BYL719 (phase I/II)	Example of triplet therapy combining CDK4/6 inhibition, hormonal therapy, and an α isoform-selective PI3K inhibitor.	(116)

(*continued*)

Table 2. Highlights of representative completed and ongoing clinical trials with CDK4/6 inhibitors (*Continued*)

Cancer type	Drug(s) (trial phase)	Description and outcome	References
MAP kinase pathway combinations			
BRAF-mutant melanoma NCT01777776	Ribociclib LGX818 (phase I/II)	Phase I study followed by the randomized phase II of LGX818 vs. LGX818/ribociclib in BRAF inhibitor–naïve population and assessment of LGX818/ribociclib in BRAF inhibitor–resistant population.	Not reported
NRAS-mutant melanoma NCT01781572	Ribociclib Binimetinib (phase Ib/II)	Preliminary phase I results among 14 patients demonstrated 6 with partial response and 6 with stable disease (4 of whom had >20% regression).	(114)
RAS-mutant cancers NCT02022982	Palbociclib PD0325901 (phase I/II)	Phase I trial with expansion in *KRAS*-mutant NSCLC.	Not reported
RAS-mutant cancers NCT02065063	Palbociclib Trametinib (phase I)	Phase I trial with expansion in *NRAS*-mutant melanoma.	(137)
Other combinations			
MCL NCT02159755	Palbociclib Ibrutinib (phase I)	Trial of palbociclib and ibrutinib in previously treated MCL.	Not reported
Small cell lung cancer NCT02499770	G1T28 (ref. 130) Etoposide Carboplatin (phase I)	Phase I followed by randomization of etoposide/carboplatin ± G1T28; first trial utilizing CDK4/6 inhibitor to protect bone marrow function from effects of chemotherapy in an RB-negative tumor type.	Not reported

Abbreviation: GBM, glioblastoma multiforme.

was achieved in a patient with growing teratoma syndrome (73), with clinical benefit later confirmed in a larger group of patients with teratoma (74): Stable disease was noted in 19 of 74 patients with advanced solid tumors, with 9 patients receiving 10 or more cycles on either the 3/1 or 2/1 schedule.

Mantle Cell Lymphoma

In order to document pharmacodynamic effects of palbociclib-mediated CDK4/6 inhibition, a pilot study was conducted in patients with MCL (75), a subtype associated with a [t(11:14(q13;q32)] translocation that drives ectopic cyclin D1 expression in B cells that normally express cyclins D2 and D3 (76). Seventeen previously treated patients received palbociclib on the 3/1 schedule. All patients underwent 2-deoxy-2-[(18)F] fluoro-D-glucose (FDG) and 3-deoxy-3[(18)F]fluorothymidine (FLT) PET to study tumor metabolism and proliferation, respectively, in concert with pretreatment and on-treatment lymph node biopsies to assess RB phosphorylation and markers of proliferation and apoptosis. Substantial reductions in the summed FLT-PET maximal standard uptake value (SUV_{max}), as well as in RB phosphorylation and Ki67 expression, occurred after 3 weeks in most patients, with significant correlations among these end points. These results definitively demonstrated that palbociclib mediated CDK4/6 inhibition with consequent G_1 arrest in patients' MCL cells. Five of 17 heavily pretreated patients [including those who had received prior chemotherapy (bortezomib) and stem cell transplant] achieved progression-free survival (PFS) of >1 year (range, 15–30 months), with one complete and two partial responses. Of note, responses were not immediate but occurred after 4 to 8 cycles. Although those patients with prolonged clinical benefit demonstrated marked first-cycle reductions in summed FLT SUV_{max} and in expression of phosphorylated RB and Ki67, such decreases also occurred in patients who did not go on to achieve prolonged benefit, suggesting population heterogeneity in responses after initial CDK4/6 inhibition.

Liposarcoma

Palbociclib has also been evaluated in patients with RB-positive, *CDK4*-amplified well-differentiated or dedifferentiated liposarcoma, who had progressed on prior therapy (77). Among 30 patients treated on the 2/1 schedule, 66% were progression-free at 12 weeks, exceeding the protocol predefined endpoint of 12-week PFS of 40%. Of note, 8 patients remained on study for more than 40 weeks, including three with well-differentiated and five with dedifferentiated disease. In addition, regressions were noted in 4 patients; in two instances, these were documented late in the treatment course, reminiscent of delayed responses observed in the MCL study. One patient with a dedifferentiated tumor achieved partial response at 74 weeks and went on to achieve a complete response; another patient demonstrated reduction of a dedifferentiated component within a well-differentiated lesion that resulted in a 30% tumor decrease over a 1-year period. These results suggest that gradual tumor regression can occur after initial CDK4/6 inhibitor–mediated tumor growth inhibition.

A fundamental question concerns how continuous administration of a seemingly cytostatic drug can sometimes lead to

tumor regression, whether in xenograft models or in patients. An issue is whether reversible G_1 arrest (quiescence), which occurs in all RB-positive cell lines exposed to CDK4/6 inhibitors, can lead to a durable state of cell-cycle exit (senescence) marked by resistance to mitogenic stimulation or oncogenic challenge. Although it is relatively easy to finger senescent cells in culture, it is considerably more problematic in a clinical setting to evaluate the significance of suboptimal senescence-associated biomarkers, whether expressed alone or in combination (78). Despite these caveats, there is considerable evidence that senescent cells secrete cytokines that attract immune cells and lead to tumor clearance, providing a potential explanation for tumor regression (79).

In an intriguing study (80), palbociclib treatment of seven RB-positive liposarcoma cell lines, each with chromosome 12q14 amplification of linked *CDK4* and *MDM2*, arrested in a G_1 state within 48 hours of drug exposure. Three of the cell lines subsequently expressed several senescence biomarkers and failed to resume proliferation when palbociclib was withdrawn. Similar results were obtained with other CDK4/6 inhibitors (Table 1, and see below). Proteolytic turnover of MDM2 was required for the induction of senescence, whereas cell lines that only underwent transient G_1 arrest did not reduce MDM2 in response to palbociclib. MDM2 turnover was found to depend on its E3 ligase activity and the expression of ATRX, and is postulated to facilitate stabilization of a senescence-activating protein(s) in a p53-independent fashion. Importantly, in an analysis of seven paired tumor biopsies among patients in the liposarcoma trial, among 3 patients without clinical benefit (time to progression < 84 days), there was no evidence of reduced MDM2 in the on-treatment biopsy. In contrast, among 4 patients who achieved demonstrable clinical benefit, remaining progression free for 168, 376, 500+, and 800+ days (including the patient with complete response), the on-treatment biopsies demonstrated marked reduction in MDM2 expression. Of note, all of the on-treatment biopsies showed reduced expression of phosphorylated RB. Therefore, similar to the experience in MCL, reduced RB phosphorylation appears necessary but not sufficient to define patients ultimately destined to achieve clinical benefit with sustained disease control. Elucidating the factors that distinguish transient CDK4/6-inhibitory drug–induced cytostasis from durable cell-cycle withdrawal in various cancer types remains a formidable challenge.

Breast Cancer

The use of palbociclib in breast cancer is relatively advanced. Preclinical data utilizing palbociclib demonstrated the substantial sensitivity of HR-positive cell lines, compared with HR-negative cell lines, in part related to a higher incidence of RB negativity in the latter breast cancer subgroup (81). In a phase II study of palbociclib on the 3/1 schedule in RB-positive advanced breast cancer, 31 patients with $HR^+/HER2^-$ disease and 4 patients with $HR^-/HER2^-$ disease were enrolled (82). Of the $HR^+/HER2^-$ group, 1 patient achieved partial response and 5 had stable disease for ≥6 months, with median PFS of 3.8 and 1.5 months for the $HR^+/HER2^-$ and $HR^-/HER2^-$ groups, respectively. Stratification related to degree of prior treatment demonstrated that patients with HR^+ tumors who had received more than 2 lines of anti-estrogen therapy had significantly longer PFS than those who had received fewer therapeutic cycles (5 vs. 2 months); the degree of prior cytotoxic regimen exposure did not affect outcome. Notably, in this trial, 24% of patients had treatment interruption and 51% had dose reduction, all for cytopenias.

In the initial survey of breast cancer cell lines with palbociclib, synergistic growth-inhibitory activity was noted with the estrogen antagonist tamoxifen, including activity in a model of acquired tamoxifen resistance. This work prompted extensive clinical investigation of CDK4/6 inhibitors with anti-estrogen agents in estrogen receptor (ER)–positive breast cancer. After phase I work demonstrated that full-dose palbociclib on the 3/1 schedule could be combined with the standard dose of the aromatase inhibitor letrozole (2.5 mg once daily), a randomized phase II study was conducted (PALOMA-1/TRIO-18; NCT00721409) comparing letrozole with the combination of palbociclib and letrozole (1:1) in postmenopausal women with $ER^+/HER2^-$ breast cancer who had not received systemic treatment for advanced disease (83). Two cohorts were sequentially enrolled. In the first group, 66 patients were selected based on $ER^+/HER2^-$ status alone; in the second, the 99 patients enrolled were also required to have breast cancer harboring *CCND1* amplification, loss of *CDKN2A*, or both. PFS was the primary endpoint. In cohort 1, median PFS was 5.7 months for letrozole alone, compared with 26.1 months for the combination group (hazard ratio, 0.299), whereas in cohort 2, median PFS was 11.1 months for the letrozole group compared with 18.1 months for the combination group (hazard ratio, 0.508). When the entire population of 165 patients was considered, median PFS was 10.2 months for letrozole alone and 20.2 months for the combination (hazard ratio, 0.488), indicating a significantly improved PFS when palbociclib is added to letrozole in first-line systemic treatment for advanced $ER^+/HER2^-$ breast cancer. Based on these data, a phase III, double-blind, placebo-controlled study in a similar population of 650 patients is ongoing and awaiting further analysis (PALOMA-2; NCT0170427). Nonetheless, given the significant benefit of the combined regimen in extending PFS in the phase II trial, palbociclib was provisionally approved by the FDA in early 2015 for use in patients with $ER^+/HER2^-$ breast cancer.

It is noteworthy that *CCND1* amplification and *CDKN2A* loss do not appear to contribute to the ability to select patients who are most likely to benefit from combined hormonal treatment and CDK4/6 inhibition. Instead, the study confirmed monotherapy data suggesting that ER positivity may be the most effective predictive marker for identification of patients with breast cancer for CDK4/6 inhibitor–based treatment, reflective of the importance of CDK4 activity in the proliferation of ER^+ breast cancer cells, irrespective of genomic alterations in the pathway. Possibly, *CCND1* amplification and *CDKN2A* loss may contribute to determining response to letrozole alone, again reflecting substantial biology linking cyclin D1 and the ER (68); however, larger studies will be required for definitive conclusions to be drawn. Moreover, these findings highlight an emerging theme that genetic alterations in the "RB signaling pathway," except for RB loss itself, may not serve as informative biomarkers in distinguishing patient responses to CDK4/6 inhibition.

Palbociclib combined with the ER antagonist fulvestrant has also been studied in a 2:1 randomized, placebo-controlled

phase III study enrolling 521 patients with advanced ER$^+$/HER2$^-$ breast cancer who had progressed during prior endocrine therapy (PALOMA-3; NCT01942135; ref. 84). Consistent with the results of the study with letrozole, an interim analysis reported PFS of 9.2 months with palbociclib–fulvestrant and 3.8 months with placebo–fulvestrant (hazard ratio, 0.42). This study enrolled premenopausal and perimenopausal patients, with benefit of the combination observed across groups.

Many additional questions about these trials require further clinical assessment. For example, although combination treatment was well tolerated overall, even without increased incidence of febrile neutropenia, grade 3–4 neutropenia was still significantly more common in the palbociclib-containing combinations compared with hormonal therapy alone, necessitating dose interruptions and reductions and suggesting that confirmation of pharmacodynamic effects utilizing lower doses of palbociclib may prove worthwhile. Although diabetogenic effects in long-term treated patients have not been reported, predicted side effects of targeted therapy may involve glucose intolerance, based on requirements of pancreatic β cells for CDK4 (85, 86) and effects of p16^{INK4a} on age-dependent islet cell regeneration (87). In addition, to date, the effect of palbociclib on overall survival is unknown, with follow-up ongoing in the PALOMA-2 and PALOMA-3 trials. It will also be of interest to determine whether palbociclib, used alone or in combination with other agents, has utility in women whose tumors are resistant to anti-estrogen treatment based on *ESR1* mutation. Finally, the success of the addition of palbociclib to hormonal therapy in metastatic disease has also generated interest in the use of such combinations in the adjuvant setting; a pilot study assessing the feasibility of 2 years of combined palbociclib and hormonal treatment is currently under way (NCT01864746).

DEVELOPMENT OF ADDITIONAL CDK4/6 INHIBITORS: RIBOCICLIB AND ABEMACICLIB

Two additional CDK4/6 inhibitors, ribociclib (LEE011) and abemaciclib (LY2835219; Table 1), are in active clinical development (Table 2). Like palbociclib, these orally bioavailable and highly selective CDK4/6-targeting agents exhibit IC_{50} values in the low nanomolar range, whereas other CDK family members are far less sensitive (1, 88, 89). All three inhibitors compete for binding of ATP to CDK4/6, so variations in potency and specificity reflect their somewhat different chemical structures (illustrated in ref. 1).

Ribociclib

Phase I clinical development of ribociclib in patients with tumors with documented RB positivity utilized a Bayesian logistic regression model incorporating an overdose control principle to guide dose escalation (NCT01237236; refs. 90, 91). Among 85 patients treated, dose-limiting toxicities occurred in 10, including neutropenia, thrombocytopenia, pulmonary embolism, hyponatremia, QTcF prolongation, and elevated creatinine, resulting in a recommended phase II dose of 600 mg daily on the 3/1 schedule. Plasma exposure increases were slightly higher than dose-proportional; the mean half-life at the 600 mg dose level was 36.2 hours. Among paired tumor biopsies from 40 patients, reductions of ≥50% from baseline in Ki67 and phospho-pRB were documented in 55% and 42% of samples, respectively; correlations with clinical outcome have not been reported. Partial responses were seen in a patient with *PIK3CA*-mutated, *CCND1*-amplified, ER$^+$ breast cancer and in a second patient with wild-type *BRAF*/*NRAS*, *CCND1*-amplified melanoma. Stable disease for ≥6 cycles was observed in 14% of the treated population. Despite the expanded use of ribociclib in the clinical arena alone or in concert with other drugs (Table 2), published outcome data are not yet available.

Abemaciclib

In contrast with palbociclib and ribociclib, the dose-limiting toxicity of abemaciclib is fatigue (NCT01394016; ref. 92). Diarrhea and hematologic toxicity also occur with this agent, although the former is manageable with supportive medications and/or dose reduction, and the latter is milder than with the other compounds, allowing continuous daily dosing without interruption. The reasons for reduced hematologic toxicity and other observed nonhematologic toxicities are under active investigation, but may be related to a greater selectivity for CDK4 over CDK6 with abemaciclib (88), its greater relative potency and potential to target CDK9 (Table 1), and/or its distribution into the central nervous system (93). Both once-daily and twice-daily schedules have been evaluated with a recommended phase II dose of 200 mg twice daily (92). The agent demonstrates dose-proportional pharmacokinetics, with a median time to maximal plasma concentration of 4 to 6 hours and terminal elimination half-life ranging from 17 to 38 hours. Abemaciclib is widely distributed (93) such that concentrations can be detected in cerebrospinal fluid that approximate those in plasma (92), forecasting its potential use in treating brain tumors. Pharmacodynamic assessment of target engagement in keratinocytes from skin biopsies demonstrated both reduced RB phosphorylation and topoisomerase IIα expression by 4 hours after treatment; however, despite the half-life, in patients treated once daily, there was partial reversibility in samples obtained directly prior to the next dose (92). Therefore, 200 mg twice daily was selected as the dose and schedule for further development, to ensure persistent target coverage over the dosing interval, as was shown to be important in preclinical pharmacokinetic/pharmacodynamics modeling in mice bearing human tumor xenografts (94).

In an expansion cohort in the phase I trial, abemaciclib has also been evaluated as monotherapy in advanced breast cancer among heavily pretreated patients with a median of 7 prior systemic therapies (92, 95, 96). In the initial experience, among 25 patients with HR$^+$/HER2$^-$ disease, the clinical benefit rate was 72%, including 7 partial responses (28%). Only one of the partial responders received concomitant hormonal therapy, suggesting a higher response rate for abemaciclib as a single agent than palbociclib. Although dose reductions for fatigue and diarrhea from 200 to 150 mg twice daily occurred, 150 mg still produced pharmacodynamic CDK4/6 inhibition and was administered continuously without interruption, raising the possibility of the importance of continuous target inhibition. Importantly, activity was observed irrespective of concomitant *PIK3CA* mutation. Like the palbociclib experience, among 4 patients with HR$^-$/HER2$^-$ cancers, all still had progressive disease. Abemaciclib has also been combined

successfully at full dose with fulvestrant in a small group of patients, with acceptable safety and promising efficacy (96, 97), underscoring the potential value of combinatorial regimens as seen with palbociclib (see below).

HER2-amplified breast cancer cell lines have also demonstrated sensitivity to CDK4/6 inhibition. In addition, genetically engineered mouse models clearly revealed the importance of cyclin D1–CDK4 activity for the initiation and maintenance of HER2-driven breast cancers (98–100). The initial abemaciclib trial enrolled 11 patients with $HR^+/HER2^+$ and 5 patients with $HR^-/HER2^+$ disease. The disease control rate in the $HR^+/HER2^+$ group was 100%, with 4 partial responses, whereas 3 patients with $HR^-/HER2^+$ disease had stable disease of brief duration (92, 95, 96). Although patients with $HR^+/HER2^+$ disease are likely to derive benefit from CDK4/6 inhibition, further work will be required to determine if activity is driven primarily by HR status and whether there is benefit for patients with $HR^-/HER2^+$ disease. Of note, for the entire HR^+ population treated with abemaciclib, the median duration of response was 13.4 months and median PFS was 8.8 months.

As with breast cancer, patients with non–small cell lung cancer (NSCLC) have been evaluated in a phase II trial of palbociclib (NCT01291017) and in a phase I expansion cohort of abemaciclib. In the palbociclib study, among 16 previously treated patients, 8 achieved stable disease ≥ 4 months (101). With abemaciclib (92, 102), 68 patients have been treated with a range of one to ten prior systemic therapies. Two partial responses were observed, including 1 patient with *KRAS*-mutant NSCLC and 1 patient with squamous NSCLC and copy-number loss of *CDKN2A*. Thirty-one (46%) patients had stable disease, including 15 patients who remained on trial ≥ 6 months (4 of whom for >12 months), for an overall disease control rate of 49% and 6-month PFS rate of 26%. In addition, patients with *KRAS*-mutant NSCLC fared best; the disease control rate for the *KRAS* mutation–positive population was 55% (16/29 patients), whereas that for the population with wild-type *KRAS* was 39% (13/33 patients). These data are consistent with the importance of CDK4 activity in preclinical mouse models of KRAS-driven NSCLC, where CDK4 ablation or inhibition has induced synthetic lethality (103).

The activity of abemaciclib has also been explored in glioblastoma multiforme (GBM) and melanoma (92). Among 17 GBM patients, 2 patients had a decrease in tumor size and received treatment without progression for >16 months. In preclinical models, codeletion of *CDKN2A* and *CDKN2C*, encoding $p16^{INK4A}$ and $p18^{INK4C}$, respectively, dictated increased sensitivity to CDK4/6 inhibition, and it will be of interest to determine whether responding cases met this prediction (104). Among 26 melanoma patients, 1 patient with a tumor harboring *NRAS* mutation and *CDKN2A/B* alteration achieved a confirmed partial response. In melanoma models, CDK4/6 inhibition has been shown to cause destabilization of FOXM1, an event linked to the induction of senescence (17); whether FOXM1 expression was altered by abemaciclib in this tumor is unknown.

Abemaciclib has also been evaluated in a population of patients with relapsed or refractory MCL similar to those treated with palbociclib (NCT01739309; ref. 105). Among 22 patients, grade 3–4 neutropenia and thrombocytopenia were more common than in the solid tumor treatment setting, occurring in approximately one third of patients. Overall, there were 5 partial responses and 9 patients who achieved stable disease as the best response; among these 14 patients, 8 received ≥ 6 cycles. Taken together, the palbociclib and abemaciclib trials demonstrate that CDK4/6 inhibition can achieve durable disease control in patients with relapsed or refractory MCL.

ADDITIONAL APPLICATIONS OF COMBINATORIAL APPROACHES WITH CDK4/6 INHIBITORS

MEK Inhibitor Combinations in RAS-Driven Cancers

Consistent with early findings indicating that the cellular life history of D-type cyclins is highly dependent on RAS signaling, preclinical synergistic effects of CDK4/6 inhibition and MAP kinase inhibition in melanoma and pancreatic cancer models have stimulated substantial interest in the development of these combinations (106–108). Possibly, the predominantly cytostatic effects of CDK4/6 inhibitors might be reprogrammed to induce senescence or apoptosis in response to drugs targeting RTKs and/or downstream RAS signaling pathways that are essential for cell viability. For example, in an inducible mouse model of *NRAS*-mutant melanoma, pharmacologic inhibition of MEK activates apoptosis, but not cell-cycle arrest (109). Therefore, cell death is balanced by continued proliferation, leading to tumor stasis *in vivo*. In contrast, genetic extinction of *NRAS* induces both of these effects. CDK4 was identified as the critical driver of this differential phenotype, so that combined inhibition of CDK4 and MEK led to apoptosis with blockade of continued proliferation, resulting in net tumor regression and substantial synergy in therapeutic efficacy. Consistent with these results, combined CDK4 and MEK inhibition has led to increased apoptosis and/or reduced viability in colony formation assays in human melanoma and pancreatic cancer cell lines. However, although potent inhibitors of RAS–RAF–MEK–ERK signaling efficiently silence ERK phosphorylation, interruption of feedback circuits can result in rebound of ERK activity (110). Determinants of primary resistance are not the same in different tumor types that upregulate various RTKs or their ligands in response to pathway inhibition (111–113). Drug dosing schedules or the addition of specific RTK inhibitors may hold the key to circumventing these potential problems.

This work was translated to a phase I trial combining ribociclib (3/1 schedule) with the MEK inhibitor binimetinib (twice daily continuously) in *NRAS*-mutant melanoma (NCT01781572; ref. 114). In a preliminary report of 14 patients receiving the first two dose levels, including ribociclib at 200 or 300 mg with binimetinib at 45 mg, 6 patients had achieved partial response and 6 had stable disease, including 4 with >20% tumor shrinkage. Tumor regression was often early and accompanied by major symptomatic improvement. The drug combination was not without severe adverse effects, with dose-limiting toxicities, including acute renal injury, elevated creatine phosphokinase, peripheral edema, and arrhythmia most likely due to binimetinib or to consequences of combinatorial treatment. Other common treatment-related toxicities included rash, anemia, nausea, diarrhea, and fatigue. Possibly, antitumor drug synergy between various CDK4/6 and MEK inhibitors may allow reduced dosing schedules

to temper toxicities. Despite these issues, this early experience prompted other trials in which palbociclib is being combined with other MEK inhibitors, including PD0325901 (NCT02022982) or trametinib (NCT02065063).

Combined Inhibition of CDK4/6 and PI3K Pathway Signaling

Combined CDK4/6 and PI3K or PI3K/mTOR signaling has been investigated preclinically primarily in models of breast cancer, where activation of the PI3K pathway occurs frequently, with the most promising results emerging with PI3Kα isoform-selective drugs. Recently, it has been recognized that in sensitive cells, as well as in tumors from patients who responded to PI3K inhibition, there is repression of RB phosphorylation, whereas in cell lines with reduced sensitivity or in tumors from patients who did not respond to PI3K inhibition, RB phosphorylation is sustained. A combinatorial drug screen utilizing multiple *PIK3CA*-mutant cell lines with only modest sensitivity to PI3K inhibitors revealed that combined ribociclib-mediated CDK4/6 and PI3K inhibition synergistically reduces cell viability *in vitro* and leads to tumor regressions *in vivo* (115). In addition to overcoming intrinsic resistance in these models, the combination also reverses adaptive resistance to PI3K inhibition. The addition of a PI3K inhibitor to combined CDK4/6 inhibition and hormonal therapy is particularly attractive for *PIK3CA*-mutant ER^+ breast cancers (116).

Combined PI3K and CDK4/6 inhibition may also be of substantial interest in squamous cell NSCLC and head and neck cancers, where amplification of both *CCND1* and *PIK3CA* are common events (117, 118), as well as in pancreatic cancer, where genetically engineered mouse models have suggested the importance of the PI3K pathway downstream from activated KRAS (119). $p16^{INK4A}$-deficient pancreatic cancer cell lines may be inherently resistant to CDK4/6 inhibitors; synergistic compromise of cell proliferation and viability have been demonstrated with PI3K inhibitors (108), as well as with inhibitors of IGF1R both *in vitro* and *in vivo* (120). Of note, sensitivity to combined CDK4/6 and IGF1R inhibition correlated with reduced activity of mTORC1, and combined inhibition of CDK4/6 with temsirolimus recapitulated the effects of the IGF1R combination (120). Although these data are provocative, recent results in pancreatic patient-derived xenografts suggest that CDK4/6 inhibition alone may be highly effective in suppressing proliferation, raising the question of whether established cell lines are adequate for assessing therapeutic sensitivities (121). A combinatorial drug screen in dedifferentiated liposarcoma cell lines also identified CDK4 and IGF1R as synergistic drug targets. In this work, the phosphorylation of multiple proteins and cell viability in response to systematic drug combinations were measured in order to derive computational models of the signaling network in dedifferentiated liposarcoma. The models predicted that the observed synergy of CDK4 and IGF1R inhibitors depends on activated AKT; consistent with this prediction, combined inhibition of CDK4 and IGF1R cooperatively suppressed activation of proteins within the AKT pathway (122).

Combined inhibition of CDK4/6 and PI3Kδ has been examined in models of MCL, where idelalisib (Zydelig) monotherapy achieves transient inhibition of AKT phosphorylation but only modest effects on cell proliferation. MCLs express low levels of the negative PI3K regulator PIK31P1 compared with normal peripheral B cells; however, prolonged CDK4/6 inhibition has been shown to induce expression of PIK3IP1 in these cells and thereby cooperates with PI3Kδ inhibition to lead to robust apoptosis (123). Similar mechanistic considerations may underlie synergism of CDK4/6 inhibition with ibrutinib-mediated BTK inhibition; in addition, combined CDK4/6–PI3Kδ inhibition may also be an effective strategy following the development of acquired ibrutinib resistance (124).

ADDITIONAL THERAPEUTIC OPTIONS

Enthusiasm for the use CDK4/6 inhibitors in cancer treatment raises obvious questions about how they might be leveraged in combination with other therapeutic modalities, including cytotoxic chemotherapy, irradiation, immune checkpoint blockade, and angiogenesis inhibition. Because CDK4/6 inhibitors induce G_1 phase arrest in tumors expressing functional RB, they may well blunt the effects of cytotoxic drugs or ionizing irradiation (IR), which kill cancer cells in S- or M-phase (2). Preclinical studies revealed that CDK4/6 inhibitors protect both cancer cells and normal hematopoietic cells from cytotoxicity induced by IR and DNA-damaging agents (125); in turn, immune checkpoint inhibitors rely on the ability of antibodies to PD-1/PD-L1, CTLA-4, and other analogous negative regulatory molecules to restore proliferative T-cell expansion and differentiation, both of which depend on cyclin D2/D3 and CDK4/6 (126, 127). Perhaps clever combinatorial drug sequencing schedules might circumvent these potential problems (128), but relevant data are largely unavailable.

Conversely, CDK4/6 inhibition might be used to transiently arrest normal hematopoietic stem and progenitor cells during chemotherapy or radiation exposure. For example, in a mouse RB-competent MMTV–HER2-driven breast cancer model, the antitumor activity of carboplatin was compromised by palbociclib administration, whereas in an RB-deficient model, palbociclib coadministration protected against carboplatin-induced hematologic toxicity (129). Given that multilineage myelosuppression is a major dose-limiting toxicity of chemotherapy, transient administration of a selective CDK4/6 inhibitor may render CDK4/6-dependent stem and progenitor cells resistant to chemotherapy to preserve hematopoietic function (130). Such trials have recently been initiated in small cell lung cancer, a routinely RB-negative tumor type (NCT02499770).

CONCLUSIONS

It has taken well over two decades to exploit the scientific insights that provided early proof-of-principle that CDK4/6 inhibitors might prove useful for cancer therapy. The founding discoveries, although firmly supported by biochemical and genetic data, predated the available chemistry technologies required to exploit them. Not for failure of trying, it took a decade before chemists at Parke-Davis developed palbociclib, and the merger of the company with Warner-Lambert and

then Pfizer, coupled with a lack of enthusiasm for palbociclib monotherapy based on early phase I trials, challenged the company to champion this drug candidate over many others in their burgeoning pipeline (131). In retrospect, one might have predicted that combinatorial therapies with drugs targeting mitogen-dependent signaling pathways regulating D-type cyclins would synergize with CDK4/6 inhibitors to prevent tumor cell proliferation. Whether chosen purposefully or empirically, the combined use of palbociclib and letrozole, a "cyclin D1 inhibitor," in patients with ER^+ breast cancer revealed potent antitumor activity and ultimately led to FDA approval of palbociclib in early 2015.

Many tumors lacking $p16^{INK4a}$ or overexpressing D-type cyclins have shown exquisite sensitivity to CDK4/6 inhibitors, whereas many normal cells are relatively resistant, implying that cancer cells become "addicted" to RB pathway mutations (132). As indicated by trials with breast cancer (83) and under study in a SIGNATURE trial (NCT02187783), amplification of D-type cyclins or CDK4/6 and p16 inactivation may not predict objective responses to CDK4/6-inhibitory therapy (133). Although profound resistance to CDK4/6 inhibitors is conferred by RB inactivation *per se*, it may well prove that mutations affecting other cell-cycle regulators, such as amplification of cyclin E, loss of $p27^{KIP1}$ or $p21^{CIP1}$, and activation of CDK2, would bypass the requirement of tumor cells for CDK4/6 activity, thereby also conferring *de novo* resistance (62–64). In addition, alterations in expression of such cell-cycle regulators, including cyclin E and $p27^{KIP1}$, as well as D-type cyclins themselves, have been demonstrated in adaptation to CDK4/6 inhibition, potentially contributing to acquired resistance (108, 134–136). Of note, some of these adaptations are reversible after drug removal, suggesting, perhaps counterintuitively, the potential value of intermittent dosing schedules for maintenance of cell-cycle arrest.

At present, it remains unclear whether the cytostatic effects alone of CDK4/6 inhibition can efficaciously control tumor progression or whether definitive tumor cell elimination under the duress of continued target engagement is a prerequisite for durable clinical responses. Additional drugs targeting mitogenic signaling pathways in RB-positive tumors may be able to convert cytostatic responses to durable cell-cycle arrest (senescence) or cell death (apoptosis). In the next few years, we are likely to see combinations of targeted therapies—some obvious combinations being RAF/MEK/ERK and PI3K inhibitors used in conjunction with those targeting CDK4/6—for which trials are now under way. Despite the decades required for drug discovery and clinical applications, much remains to be learned. Future work will indicate whether the promise of CDK4/6 inhibitors, most advanced for breast cancer, can be validated and extended to other cancers.

Disclosure of Potential Conflicts of Interest

C.J. Sherr has consulted at a 2-day workshop for Eli Lilly. G.I. Shapiro reports receiving a commercial research grant from Pfizer and is a consultant/advisory board member for Eli Lilly, EMD Serono, G1 Therapeutics, and Vertex Pharmaceuticals. No potential conflicts of interest were disclosed by the other author.

Grant Support

C.J. Sherr is supported by the Howard Hughes Medical Institute, by NIH Cancer Center core grant CA-21765, and by ALSAC of St. Jude Children's Research Hospital. G.I. Shapiro is supported by NIH grants R01-CA090687 and P50-CA168504 and Susan G. Komen investigator-initiated research grant IIR12223953, and receives research funding from Pfizer.

Received July 26, 2015; revised September 30, 2015; accepted October 9, 2015; published OnlineFirst December 11, 2015.

REFERENCES

1. 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.
2. VanArsdale T, Boshoff C, Arndt KT, Abraham RT. Molecular pathways: targeting the cyclin D-CDK4/6 axis for cancer treatment. Clin Cancer Res 2015;21:2905–10.
3. Dickson MA. Molecular pathways: CDK4 inhibitors for cancer therapy. Clin Cancer Res 2014;20:3379–83.
4. Norbury C, Nurse P. Animal cell cycles and their control. Annu Rev Biochem 1992;61:441–70.
5. Xiong Y, Connolly T, Futcher B, Beach D. Human D-type cyclin. Cell 1991;65:691–9.
6. Matsushime H, Roussel MF, Ashmun RA, Sherr CJ. Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. Cell 1991;65:701–13.
7. Matsushime H, Roussel MF, Sherr CJ. Novel mammalian cyclins (CYL genes) expressed during G1. Cold Spring Harb Symp Quant Biol 1991;56:69–74.
8. Motokura T, Bloom T, Kim HG, Juppner H, Ruderman JV, Kronenberg HM, et al. A novel cyclin encoded by a bcl1-linked candidate oncogene. Nature 1991;350:512–5.
9. Inaba T, Matsushime H, Valentine M, Roussel MF, Sherr CJ, Look AT. Genomic organization, chromosomal localization, and independent expression of human cyclin D genes. Genomics 1992;13:565–74.
10. Xiong Y, Menninger J, Beach D, Ward DC. Molecular cloning and chromosomal mapping of *CCND* genes encoding human D-type cyclins. Genomics 1992;13:575–84.
11. Matsushime H, Ewen ME, Strom DK, Kato JY, Hanks SK, Roussel MF, et al. Identification and properties of an atypical catalytic subunit ($p34^{PSK-J3}$/Cdk4) for mammalian D type G1 cyclins. Cell 1992;71:323–34.
12. Meyerson M, Harlow E. Identification of G1 kinase activity for cdk6, a novel cyclin D partner. Mol Cell Biol 1994;14:2077–86.
13. Ubersax JA, Woodbury EL, Quang PN, Paraz M, Blethrow JD, Shah K, et al. Targets of the cyclin-dependent kinase Cdk1. Nature 2003;425:859–64.
14. Kato J, Matsushime H, Hiebert SW, Ewen ME, Sherr CJ. Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. Genes Dev 1993;7:331–42.
15. Ewen ME, Sluss HK, Sherr CJ, Matsushime H, Kato J, Livingston DM. Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. Cell 1993;73:487–97.
16. Burkhart DL, Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat Rev Cancer 2008;8:671–82.
17. Anders L, Ke N, Hydbring P, Choi YJ, Widlund HR, Chick JM, et al. A systematic screen for CDK4/6 substrates links FOXM1 phosphorylation to senescence suppression in cancer cells. Cancer Cell 2011;20:620–34.
18. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995;81:323–30.
19. Sherr CJ. Cancer cell cycles. Science 1996;274:1672–7.
20. Buchkovich K, Duffy LA, Harlow E. The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. Cell 1989;58:1097–105.

21. DeCaprio JA, Ludlow JW, Lynch D, Furukawa Y, Griffin J, Piwnica-Worms H, et al. The product of the retinoblastoma susceptibility gene has properties of a cell cycle regulatory element. Cell 1989;58:1085–95.
22. Chen PL, Scully P, Shew JY, Wang JYJ, Lee WH. Phosphorylation of the retinoblastoma gene product is modulated during the cell cycle and cellular differentiation. Cell 1989;58:1193–8.
23. Mihara K, Cao XR, Yen A, Chandler S, Driscoll B, Murphree AL, et al. Cell cycle dependent regulation of phosphorylation of the human retinoblastoma gene product. Science 1989;246:1300–3.
24. Whyte P, Buchkovich KJ, Horowitz JM, Friend SH, Raybuck M, Weinberg RA, et al. Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature 1988;334:124–9.
25. Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16 E7-oncoprotein is able to bind to the retinoblastoma gene product. Science 1989;243:934–7.
26. DeCaprio JA, Ludlow JW, Figge J, Shew JY, Huang CM, Lee WH, et al. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell 1988;54:275–83.
27. Ludlow JW, DeCaprio JA, Huang CM, Lee WH, Paucha E, Livingston DM. SV40 large T antigen binds preferentially to an underphosphorylated member of the retinoblastoma susceptibility gene product family. Cell 1989;56:57–65.
28. Matsushime H, Quelle DE, Shurtleff SA, Shibuya M, Sherr CJ, Kato JY. D-type cyclin-dependent kinase activity in mammalian cells. Mol Cell Biol 1994;14:2066–76.
29. Dyson N. The regulation of E2F by pRB-family proteins. Genes Dev 1998;12:2245–62.
30. Trimarchi JM, Lees JA. Sibling rivalry in the E2F family. Nat Rev Mol Cell Biol 2002;3:11–20.
31. Nevins JR. Toward an understanding of the functional complexity of the E2F and retinoblastoma families. Cell Growth Differ 1998;9:585–93.
32. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific-inhibition of cyclin-D/Cdk4. Nature 1993;366:704–7.
33. Lukas J, Parry D, Aagaard L, Mann DJ, Bartkova J, Strauss M, et al. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. Nature 1995;375:503–6.
34. Medema RH, Herrera RE, Lam F, Weinberg RA. Growth suppression by p16ink4 requires functional retinoblastoma protein. Proc Natl Acad Sci U S A 1995;92:6289–93.
35. Koh J, Enders GH, Dynlacht BD, Harlow E. Tumour-derived p16 alleles encoding proteins defective in cell-cycle inhibition. Nature 1995;375:506–10.
36. Hannon GJ, Beach D. p15(Ink4b) is a potential effector of Tgf-beta-induced cell-cycle arrest. Nature 1994;371:257–61.
37. Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, et al. A cell cycle regulator potentially involved in genesis of many tumor types. Science 1994;264:436–40.
38. Hall M, Peters G. Genetic alterations of cyclins, cyclin-dependent kinases, and Cdk inhibitors in human cancer. Adv Cancer Res 1996;68:67–108.
39. Peeper DS, Bernards R. Communication between the extracellular environment, cytoplasmic signalling cascades and the nuclear cell-cycle machinery. FEBS Lett 1997;410:11–6.
40. Marshall CJ. Small GTPases and cell cycle regulation. Biochem Soc Trans 1999;27:363–70.
41. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev 1999;13:1501–12.
42. Ewen ME. Relationship between Ras pathways and cell cycle control. Prog Cell Cycle Res 2000;4:1–17.
43. Cheng M, Sexl V, Sherr CJ, Roussel MF. Assembly of cyclin D-dependent kinase and titration of p27Kip1 regulated by mitogen-activated protein kinase kinase (MEK1). Proc Natl Acad Sci U S A 1998;95:1091–6.
44. Diehl JA, Yang W, Rimerman RA, Xiao H, Emili A. Hsc70 regulates accumulation of cyclin D1 and cyclin D1-dependent protein kinase. Mol Cell Biol 2003;23:1764–74.
45. Stepanova L, Leng X, Parker SB, Harper JW. Mammalian p50Cdc37 is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. Genes Dev 1996;10:1491–502.
46. Parry D, Mahony D, Wills K, Lees E. Cyclin D-CDK subunit arrangement is dependent on the availability of competing INK4 and p21 class inhibitors. Mol Cell Biol 1999;19:1775–83.
47. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997;88:593–602.
48. LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, et al. New functional activities for the p21 family of CDK inhibitors. Genes Dev 1997;11:847–62.
49. Blain SW, Montalvo E, Massague J. Differential interaction of the cyclin-dependent kinase (Cdk) inhibitor p27Kip1 with cyclin A-Cdk2 and cyclin D2-Cdk4. J Biol Chem 1997;272:25863–72.
50. Cheng M, Olivier P, Diehl JA, Fero M, Roussel MF, Roberts JM, et al. The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. EMBO J 1999;18:1571–83.
51. Soos TJ, Kiyokawa H, Yan JS, Rubin MS, Giordano A, DeBlasio A, et al. Formation of p27-CDK complexes during the human mitotic cell cycle. Cell Growth Differ 1996;7:135–46.
52. Grimmler M, Wang Y, Mund T, Cilensek Z, Keidel EM, Waddell MB, et al. Cdk-inhibitory activity and stability of p27Kip1 are directly regulated by oncogenic tyrosine kinases. Cell 2007;128:269–80.
53. Huang Y, Yoon MK, Otieno S, Lelli M, Kriwacki RW. The activity and stability of the intrinsically disordered Cip/Kip protein family are regulated by non-receptor tyrosine kinases. J Mol Biol 2015;427:371–86.
54. Baldin V, Lukas J, Marcote MJ, Pagano M, Draetta G. Cyclin D1 is a nuclear protein required for cell cycle progression in G1. Genes Dev 1993;7:812–21.
55. Diehl JA, Zindy F, Sherr CJ. Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. Genes Dev 1997;11:957–72.
56. Diehl JA, Cheng M, Roussel MF, Sherr CJ. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. Genes Dev 1998;12:3499–511.
57. Alt JR, Cleveland JL, Hannink M, Diehl JA. Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. Genes Dev 2000;14:3102–14.
58. Lin DI, Barbash O, Kumar KG, Weber JD, Harper JW, Klein-Szanto AJ, et al. Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF(FBX4-alphaB crystallin) complex. Mol Cell 2006;24:355–66.
59. Aggarwal P, Lessie MD, Lin DI, Pontano L, Gladden AB, Nuskey B, et al. Nuclear accumulation of cyclin D1 during S phase inhibits Cul4-dependent Cdt1 proteolysis and triggers p53-dependent DNA rereplication. Genes Dev 2007;21:2908–22.
60. Li Y, Chitnis N, Nakagawa H, Kita Y, Natsugoe S, Yang Y, et al. PRMT5 is required for lymphomagenesis triggered by multiple oncogenic drivers. Cancer Discov 2015;5:288–303.
61. Augello MA, Berman-Booty LD, Carr R 3rd, Yoshida A, Dean JL, Schiewer MJ, et al. Consequence of the tumor-associated conversion to cyclin D1b. EMBO Mol Med 2015;7:628–47.
62. Malumbres M, Sotillo R, Santamaria D, Galan J, Cerezo A, Ortega S, et al. Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. Cell 2004;118:493–504.
63. Sherr CJ, Roberts JM. Living with or without cyclins and cyclin-dependent kinases. Genes Dev 2004;18:2699–711.
64. Kozar K, Ciemerych MA, Rebel VI, Shigematsu H, Zagozdzon A, Sicinska E, et al. Mouse development and cell proliferation in the absence of D-cyclins. Cell 2004;118:477–91.
65. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer 2009;9:153–66.
66. 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.

67. Toogood PL, Harvey PJ, Repine JT, Sheehan DJ, VanderWel SN, Zhou H, et al. Discovery of a potent and selective inhibitor of cyclin-dependent kinase 4/6. J Med Chem 2005;48:2388–406.
68. 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.
69. Schwartz GK, LoRusso PM, Dickson MA, Randolph SS, Shaik MN, Wilner KD, et al. Phase I study of PD0332991, a cyclin-dependent kinase inhibitor, administered in 3-week cycles (Schedule 2/1). Br J Cancer 2011;104:1862–8.
70. 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 PD0332991, administered using a 21-day schedule in patients with advanced cancer. Clin Cancer Res 2012;18:568–76.
71. Ciemerych MA, Kenney AM, Sicinska E, Kalaszczynska I, Bronson RT, Rowitch DH, et al. Development of mice expressing a single D-type cyclin. Genes Dev 2002;16:3277–89.
72. Sicinska E, Lee YM, Gits J, Shigematsu H, Yu Q, Rebel VI, et al. Essential role for cyclin D3 in granulocyte colony-stimulating factor-driven expansion of neutrophil granulocytes. Mol Cell Biol 2006;26:8052–60.
73. Vaughn DJ, Flaherty K, Lal P, Gallagher M, O'Dwyer P, Wilner K, et al. Treatment of growing teratoma syndrome. N Engl J Med 2009;360:423–4.
74. Vaughn DJ, Hwang WT, Lal P, Rosen MA, Gallagher M, O'Dwyer PJ. Phase 2 trial of the cyclin-dependent kinase 4/6 inhibitor palbociclib in patients with retinoblastoma protein-expressing germ cell tumors. Cancer 2015;121:1463–8.
75. 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.
76. Williams ME, Swerdlow SH. Cyclin D1 overexpression in non-Hodgkin's lymphoma with chromosome 11 bcl-1 rearrangement. Ann Oncol 1994;5(Suppl 1):71–3.
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. Sharpless NE, Sherr CJ. Forging a signature of in vivo senescence. Nat Rev Cancer 2015;15:397–408.
79. Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol 2013;75:685–705.
80. Kovatcheva M, Liu DD, Dickson MA, Klein ME, O'Connor R, Wilder FO, et al. MDM2 turnover and expression of ATRX determine the choice between quiescence and senescence in response to CDK4 inhibition. Oncotarget 2015;6:8226–43.
81. Finn RS, Dering J, Conklin D, Kalous O, Cohen DJ, Desai AJ, et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res 2009;11:R77.
82. DeMichele A, Clark AS, Tan KS, Heitjan DF, Gramlich K, Gallagher M, et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. Clin Cancer Res 2015;21:995–1001.
83. 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.
84. 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.
85. Rane SG, Dubus P, Mettus RV, Galbreath EJ, Boden G, Reddy EP, et al. Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in beta-islet cell hyperplasia. Nat Genet 1999;22:44–52.
86. Tsutsui T, Hesabi B, Moons DS, Pandolfi PP, Hansel KS, Koff A, et al. Targeted disruption of CDK4 delays cell cycle entry with enhanced p27(Kip1) activity. Mol Cell Biol 1999;19:7011–9.
87. Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, Bonner-Weir S, et al. p16INK4a induces an age-dependent decline in islet regenerative potential. Nature 2006;443:453–7.
88. 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.
89. Kim S, Loo A, Chopra R, Caponigro G, Huang A, Vora S, et al. Abstract PRO 2: LEE011: an orally bioavailable, selective small molecule inhibitor of CDK4/6 reactivating Rb in cancer. Mol Cancer Ther 2013;12:11S:PRO2.
90. Infante JR, Shapiro GI, Witteveen PO, Gerecitano JF, Ribrag V, Chugh R, et al. Abstract 276: phase 1 multicenter, open-label, dose-escalation study of LEE011, an oral inhibitor of cyclin-dependent kinase 4/6, in patients with advanced solid tumors or lymphomas. Mol Cancer Ther 2013;12:A276.
91. Infante JR, Shapiro GI, Witteveen P, Gerecitano JF, Ribrag V, Chugh R, et al. A phase 1 study of the single-agent CDK4/6 inhibitor LEE011 in patients with advanced solid tumors and lymphomas. J Clin Oncol 2014;32:suppl; abstr 2528.
92. Shapiro GI, Rosen LS, Tolcher AW, Goldman JW, Gandhi L, Papadopoulos KP, et al. A first-in-human phase 1 study of the CDK4/6 inhibitor, LY2835219, for patients with advanced cancer. J Clin Oncol 2013;31:suppl; abstr 2500.
93. Raub TJ, Gelbert LM, Wishart GN, Sanchez-Martinez C, Kulanthaivel P, Staton BA, et al. Abemaciclib (LY2835219) is an oral inhibitor of the cyclin-dependent kinases 4/6 that crosses the blood-brain barrier and demonstrates In vivo activity against intracranial human brain tumor xenografts. Drug Metab Dispos 2015;43: 1360–71.
94. Tate SC, Cai S, Ajamie RT, Burke T, Beckmann RP, Chan EM, et al. Semi-mechanistic pharmacokinetic/pharmacodynamic modeling of the antitumor activity of LY2835219, a new cyclin-dependent kinase 4/6 inhibitor, in mice bearing human tumor xenografts. Clin Cancer Res 2014;20:3763–74.
95. Patnaik A, Rosen LS, Tolaney SM, Tolcher AW, Goldman JW, Gandhi L, et al. Abstract CT232: clinical activity of LY2835219, a novel cell cycle inhibitor selective for CDK4 and CDK6, in patients with metastatic breast cancer. Cancer Res 2014;74:CT232.
96. Tolaney SM, Rosen LS, Beeram M, Goldman JW, Gandhi L, Tolcher AW, et al. Abstract P5-19-13: clinical activity of abemaciclib, an oral cell cycle inhibitor, in metastatic breast cancer. Cancer Res 2015;75:PS-19-13.
97. Patnaik A, Rosen LS, Tolaney SM, Tolcher AW, Goldman JW, Gandhi L, et al. LY2835219, a novel cell cycle inhibitor selective for CDK4/6, in combination with fulvestrant for patients with hormone receptor positive (HR+) metastatic breast cancer. J Clin Oncol 2014;32:suppl; abstr 534.
98. Yu QY, Geng Y, Sicinski P. Specific protection against breast cancers by cyclin D1 ablation. Nature 2001;411:1017–21.
99. Yu QY, Sicinska E, Geng Y, Ahnstrom M, Zagozdzon A, Kong YX, et al. Requirement for CDK4 kinase function in breast cancer. Cancer Cell 2006;9:23–32.
100. Landis MW, Pawlyk BS, Li T, Sicinski P, Hinds PW. Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis. Cancer Cell 2006;9:13–22.
101. Gopalan PK, Pinder MC, Chiappori A, Ivey AM, Villegas AG, Kaye FJ. A phase II clinical trial of the CDK4/6 inhibitor palbociclib (PD0332991) in previously treated, advanced non-small cell lung cancer (NSCLC) patients with inactivated CDKN2A. J Clin Oncol 2014;32:5s; abstr 8077.
102. Goldman JW, Gandhi L, Patnaik A, Rosen LS, Hilton JF, Papadopoulos KP, et al. Clinical activity of LY2835219, a novel cell cycle inhibitor selective for CDK4 and CDK6, in patients with non-small cell lung cancer. J Clin Oncol 2014;32:suppl; abstr 8026.

103. Puyol M, Martin A, Dubus P, Mulero F, Pizcueta P, Khan G, et al. A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma. Cancer Cell 2010;18:63–73.
104. Wiedemeyer WR, Dunn IF, Quayle SN, Zhang J, Chheda MG, Dunn GP, et al. Pattern of retinoblastoma pathway inactivation dictates response to CDK4/6 inhibition in GBM. Proc Natl Acad Sci U S A 2010;107:11501–6.
105. Morschhauser F, Bouabdallah K, Stilgenbauer S, Thieblemont C, Wolf M, de Guibert S, et al. Clinical activity of Abemaciclib (LY2835219), a cell cycle inhibitor selective for CDK4 and CDK6, in patients with relapsed refractory mantle cell lymphoma. Blood 2014; ASH Annual Meeting: Abstract nr 3067.
106. Li J, Xu M, Yang Z, Li A, Dong J. Simultaneous inhibition of MEK and CDK4 leads to potent apoptosis in human melanoma cells. Cancer Invest 2010;28:350–6.
107. Johnson DB, Puzanov I. Treatment of NRAS-mutant melanoma. Curr Treat Options Oncol 2015;16:15.
108. Franco J, Witkiewicz AK, Knudsen ES. CDK4/6 inhibitors have potent activity in combination with pathway selective therapeutic agents in models of pancreatic cancer. Oncotarget 2014;5:6512–25.
109. Kwong LN, Costello JC, Liu H, Jiang S, Helms TL, Langsdorf AE, et al. Oncogenic NRAS signaling differentially regulates survival and proliferation in melanoma. Nat Med 2012;18:1503–10.
110. Lito P, Pratilas CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M, et al. Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. Cancer Cell 2012;22:668–82.
111. Lito P, Rosen N, Solit DB. Tumor adaptation and resistance to RAF inhibitors. Nat Med 2013;19:1401–9.
112. Montero-Conde C, Ruiz-Llorente S, Dominguez JM, Knauf JA, Viale A, Sherman EJ, et al. Relief of feedback inhibition of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF-mutant thyroid carcinomas. Cancer Discov 2013;3:520–33.
113. Samatar AA, Poulikakos PI. Targeting RAS-ERK signaling in cancer: promises and challenges. Nat Rev Drug Discov 2014;13:928–42.
114. Sosman JA, Kittaneh M, Lolkema MPJK, Postow MA, 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 2014;32:suppl; abstr 9009.
115. Vora SR, Juric D, Kim N, Mino-Kenudson M, Huynh T, Costa C, et al. CDK 4/6 inhibitors sensitize PIK3CA mutant breast cancer to PI3K inhibitors. Cancer Cell 2014;26:136–49.
116. Munster PN, Hamilton EP, Franklin C, Bhansali S, Wan K, Hewes B, et al. Phase Ib study of LEE011 and BYL719 in combination with letrozole in estrogen receptor-positive, HER2-negative breast cancer (ER+, HER2− BC). J Clin Oncol 2014;32:suppl; abstr 533.
117. Schwaederle M, Elkin SK, Tomson BN, Carter JL, Kurzrock R. Squamousness: next-generation sequencing reveals shared molecular features across squamous tumor types. Cell Cycle 2015;14:2355–61.
118. TCGA. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519–25.
119. Eser S, Reiff N, Messer M, Seidler B, Gottschalk K, Dobler M, et al. Selective requirement of PI3K/PDK1 signaling for Kras oncogene-driven pancreatic cell plasticity and cancer. Cancer Cell 2013;23:406–20.
120. Heilmann AM, Perera RM, Ecker V, Nicolay BN, Bardeesy N, Benes CH, et al. CDK4/6 and IGF1 receptor inhibitors synergize to suppress the growth of p16INK4A-deficient pancreatic cancers. Cancer Res 2014;74:3947–58.
121. Witkiewicz AK, Borja NA, Franco J, Brody JR, Yeo CJ, Mansour J, et al. Selective impact of CDK4/6 suppression on patient-derived models of pancreatic cancer. Oncotarget 2015;6:15788–801.
122. Miller ML, Molinelli EJ, Nair JS, Sheikh T, Samy R, Jing X, et al. Drug synergy screen and network modeling in dedifferentiated liposarcoma identifies CDK4 and IGF1R as synergistic drug targets. Sci Signal 2013;6:ra85.
123. Chiron D, Martin P, Di Liberto M, Huang X, Ely S, Lannutti BJ, et al. Induction of prolonged early G1 arrest by CDK4/CDK6 inhibition reprograms lymphoma cells for durable PI3Kdelta inhibition through PIK3IP1. Cell Cycle 2013;12:1892–900.
124. Chiron D, Di Liberto M, Martin P, Huang X, Sharman J, Blecua P, et al. Cell-cycle reprogramming for PI3K inhibition overrides a relapse-specific C481S BTK mutation revealed by longitudinal functional genomics in mantle cell lymphoma. Cancer Discov 2014;4:1022–35.
125. Johnson SM, Torrice CD, Bell JF, Monahan KB, Jiang Q, Wang Y, et al. Mitigation of hematologic radiation toxicity in mice through pharmacological quiescence induced by CDK4/6 inhibition. J Clin Invest 2010;120:2528–36.
126. Ajchenbaum F, Ando K, DeCaprio JA, Griffin JD. Independent regulation of human D-type cyclin gene expression during G1 phase in primary human T lymphocytes. J Biol Chem 1993;268:4113–9.
127. Modiano JF, Mayor J, Ball C, Fuentes MK, Linthicum DS. CDK4 expression and activity are required for cytokine responsiveness in T cells. J Immunol 2000;165:6693–702.
128. Aleem E, Arceci RJ. Targeting cell cycle regulators in hematologic malignancies. Front Cell Dev Biol 2015;3:16.
129. Roberts PJ, Bisi JE, Strum JC, Combest AJ, Darr DB, Usary JE, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. J Natl Cancer Inst 2012;104:476–87.
130. Roberts PJ, White HS, Sorrentino JA, Tadema H, Sale M, Tiessen RG, et al. Evaluation of targeted bone marrow arrest by G1T28, a CDK4/6 inhibitor in clinical development to reduce chemotherapy-induced myelosuppression. J Clin Oncol 2015;33:suppl; abstr 2529.
131. Garber K. The cancer drug that almost wasn't. Science 2014;345:865–7.
132. Weinstein B. Relevance of the concept of oncogene addiction to hormonal carcinogenesis and molecular targeting in cancer prevention and therapy. Adv Exp Med Biol 2008;617:3–13.
133. Paguero JA, Knost JA, Bauer TM, Taylor MH, Braitch FS, Eder JP, et al. Successful implementation of a novel trial model: the Signature program. J Clin Oncol 2015;33:suppl; abstr 106.
134. Wang L, Wang J, Blaser BW, Duchemin AM, Kusewitt DF, Liu T, et al. Pharmacologic inhibition of CDK4/6: mechanistic evidence for selective activity or acquired resistance in acute myeloid leukemia. Blood 2007;110:2075–83.
135. Dean JL, Thangavel C, McClendon AK, Reed CA, Knudsen ES. Therapeutic CDK4/6 inhibition in breast cancer: key mechanisms of response and failure. Oncogene 2010;29:4018–32.
136. Zhang YX, Sicinska E, Czaplinski JT, Remillard SP, Moss S, Wang Y, et al. Antiproliferative effects of CDK4/6 inhibition in CDK4-amplified human liposarcoma in vitro and in vivo. Mol Cancer Ther 2014;13:2184–93.
137. Sullivan RJ, Amaria RN, Lawrence DP, Brennan J, Leister C, Singh R, et al. Phase 1b dose-escalation study of trametinib (MEKi) plus palbociclib (CDK4/6i) in patients with advanced solid tumors. Proc AACR-NCI-EORTC 2015; PR06.