Molecular Pathways

Molecular Pathways: Targeting the Cyclin D–CDK4/6 Axis for Cancer Treatment

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Abstract

Cancer cells bypass normal controls over mitotic cell-cycle progression to achieve a deregulated state of proliferation. The retinoblastoma tumor suppressor protein (pRb) governs a key cell-cycle checkpoint that normally prevents G_1-phase cells from entering S-phase in the absence of appropriate mitogenic signals. Cancer cells frequently overcome pRb-dependent growth suppression via constitutive phosphorylation and inactivation of pRb function by cyclin-dependent kinase (CDK) 4 or CDK6 partnered with D-type cyclins.

Three selective CDK4/6 inhibitors, palbociclib (Ibrance; Pfizer), ribociclib (Novartis), and abemaciclib (Lilly), are in various stages of development in a variety of pRb-positive tumor types, including breast cancer, melanoma, liposarcoma, and non–small cell lung cancer. The emerging, positive clinical data obtained to date finally validate the two decades-old hypothesis that the cyclin D–CDK4/6 pathway is a rational target for cancer therapy. *Clin Cancer Res; 21(13); 2905–10. ©2015 AACR.*

Background

The mitotic cell cycle is a highly orchestrated process involving the sequential activation of several distinct cyclin–CDK complexes. The timing of key cell-cycle phase transitions is controlled by cell-cycle checkpoints, which prevent cells from advancing inappropriately or prematurely to the next phase of the cell cycle (1, 2). A particularly critical checkpoint occurs in G_1-phase and governs cellular commitment to enter S-phase and initiate DNA synthesis. Passage through this "restriction point" (3) is normally orchestrated by growth factor–dependent mitogenic signals, delivered at a level in excess of the threshold needed to drive cells into S-phase and on to mitosis.

Predictably, loss of restriction point control over cell proliferation is commonly selected for in evolving cancer cells (3). A key effector of this G_1 checkpoint is pRb, which inhibits the expression of genes required for S-phase entry and progression by suppressing the functions of certain E2F transcription factors (Fig. 1; refs. 4, 5). Hypophosphorylated pRb enforces the restriction point, and CDK-dependent pRb hyperphosphorylation overrides this tumor-suppressive function of pRb (6–8). The initial phosphorylation events leading to pRb inactivation are normally dependent on cyclin D–CDK4/6 complexes, which accumulate during G_1-phase in response to growth factor–dependent mitogenic signals (9–11).

The canonical mechanisms of pRb phosphorylation and subsequent functional inactivation have been the subject of several detailed reviews (12–14) and are not discussed in detail here. As mentioned above, cancer cells need to overcome the pRb-dependent restriction point, and commonly do so through alterations that lead to constitutive cyclin D–CDK4/6 activation, or through loss of pRb itself. Examples of genetic mechanisms promoting inappropriate cyclin D–CDK4/6 activity include deregulated transcription and/or amplification of the *CCND1* gene, which encodes the cyclin D1 isoform (15, 16). An alternative and very common avenue to achieve constitutive activation of the cyclin D–CDK4/6 holoenzyme involves genetic and/or epigenetic inactivation of the *CDKN2A* locus, whose product, p16[INK4A], inhibits cyclin D–CDK4/6 kinase activity (16–19).

The principal function of cyclin D–CDK4/6 activity is to phosphorylate pRb at sites that prevent its binding to members of the E2F family of transcription factors, which control the expression of genes that support DNA synthesis and S-phase progression. More than 12-amino acid residues in pRb are targeted by cyclin D– and/or cyclin E–associated CDKs. A long-standing model posited that cyclin D–CDK4/6 executed a limited set of modifications that primed pRb for full phosphorylation and inactivation by cyclin E–CDK2 complexes, which accumulate during late G_1-phase, due in part to the E2F-dependent transcriptional activation of the cyclin E–encoding *CCNE* gene. However, recent data support a refined model of pRb regulation, which suggests that cyclin D–CDK4/6 primes pRb for hyperphosphorylation by modifying only one of the available phosphoacceptor sites, and that cyclin E–CDK2 executes all remaining, inactivating modifications of pRb (20). These findings may help to explain the observation that cycling cells (bearing activated cyclin–CDK2 kinases) in culture require prolonged (24 hours) exposure to a selective CDK4 inhibitor in order to return pRb to its maximally dephosphorylated state (21–23). This time lag to cell-cycle arrest also reflects the fact that after passage through the G_1 restriction point, dephosphorylation of pRb no longer inhibits cell-cycle progression. Thus, cells treated with a CDK4 inhibitor during post-G_1 phase must cycle back to the subsequent G_1 phase in order for pRb to exert its antiproliferative effect.

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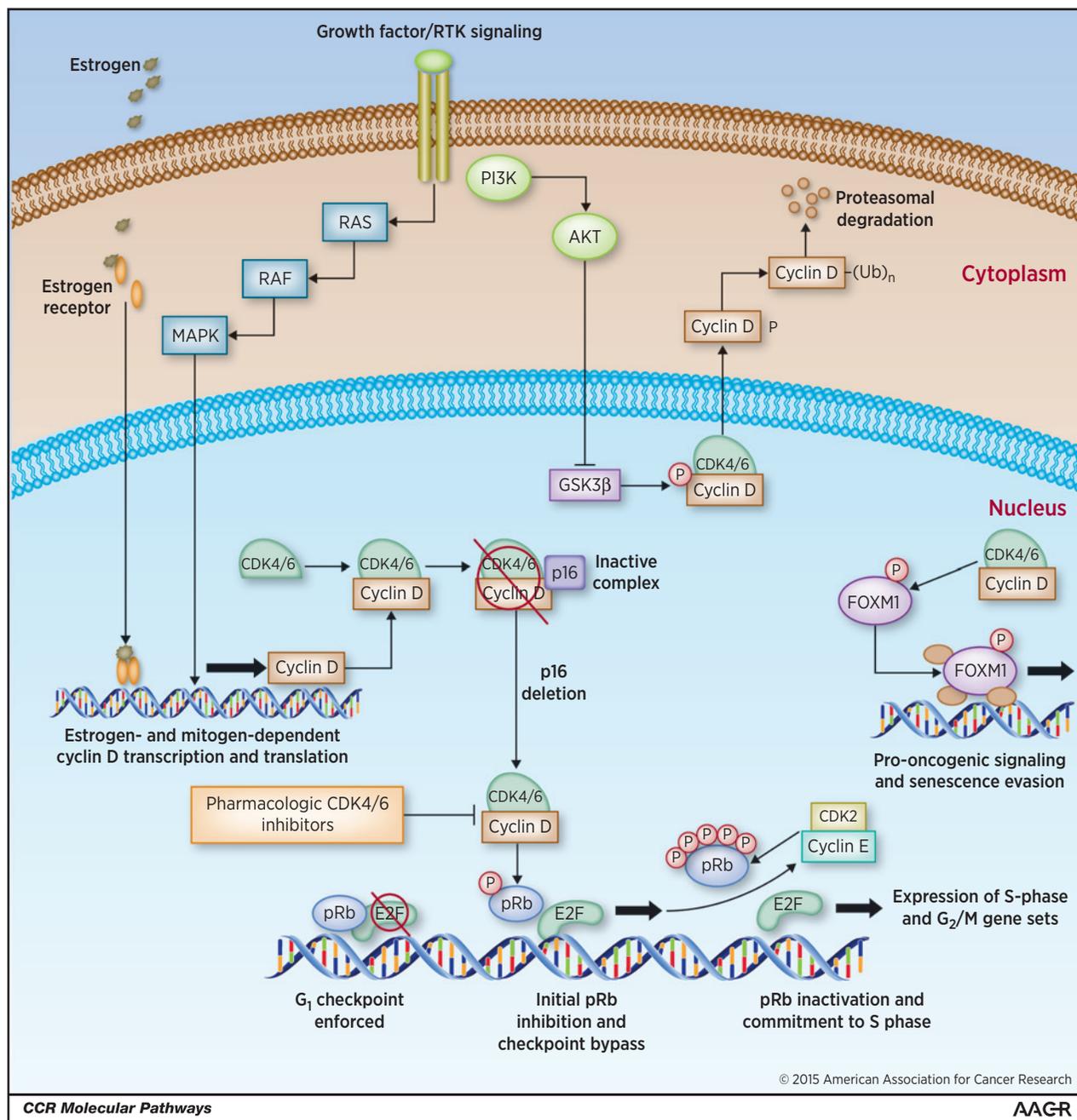
Figure 1.

Regulation and functions of cyclin D–CDK4/6 kinases. Mitogenic signals (e.g., the Ras–MAP kinase and PI3K signaling pathways) stimulate the accumulation of D-type cyclins in early G_1 phase through both transcriptional and posttranscriptional mechanisms. PI3K-dependent AKT activation leads to inhibition of glycogen synthase kinase 3β (GSK3β), which, when activated, phosphorylates cyclin D1, triggering its nuclear export and proteasomal degradation. In breast cancer cells, cyclin D expression is constitutively enhanced by ligand- or mutationally activated estrogen receptors, which bind directly to the *CCND1* promoter. Aberrant activation of fully assembled cyclin D–CDK4/6 holoenzymes is further enhanced by genetic events involving either amplification of the *CCND1* gene or loss of p16[INK4A], a stoichiometric inhibitor of cyclin D–CDK4/6 activity. The activated cyclin D–CDK4/6 complexes initiate the phosphorylation of pRb and collaborate with cyclin E–CDK2 complexes (which begin to accumulate in mid/late G_1 phase) to provoke full hyperphosphorylation and functional inactivation of pRb. The subsequent release of E2F transcription factors drives the expression of genes required for cellular commitment to enter S-phase, and ultimately mitotic cell division. Cyclin D–CDK4/6 complexes also phosphorylate the transcription factor, FOXM1, leading to FOXM1-dependent expression of genes that support cellular proliferation and suppress senescence induction. In ER+ breast cancer, effective restoration of pRb-dependent tumor suppressor activity requires drug-induced inhibition of both ER signaling and cyclin D–CDK4/6 activity.

The cyclin D–CDK4/6–pRb axis unequivocally plays a pivotal role in the regulation of cellular proliferation and transformation. However, recent studies have identified additional cyclin D–CDK4/6 substrates that likely contribute to the proliferative drive imposed by cyclin D–CDK4/6 activation (24, 25). A particularly relevant example is the transcription

Figure 2.
Structures of selective CDK4/6 inhibitors in clinical development.
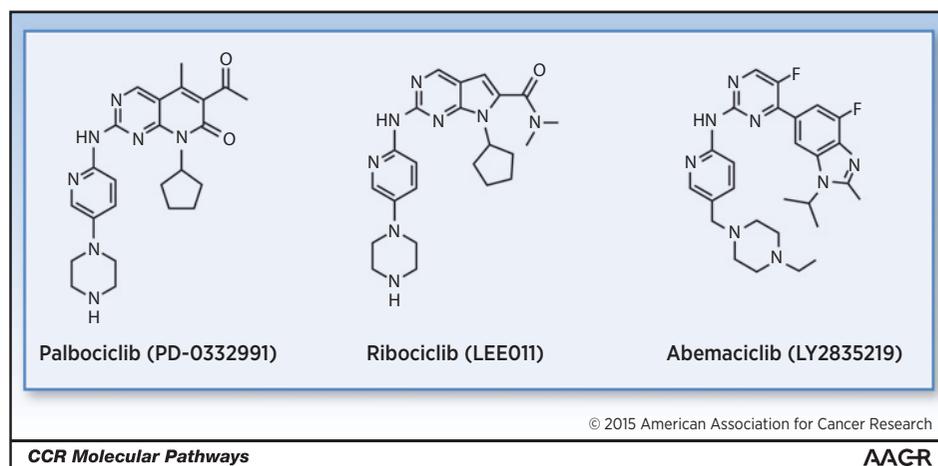
factor FOXM1, which was identified as a cyclin D–CDK4/6 substrate in an unbiased proteomic screen. Multisite phosphorylation of FOXM1 by cyclin D–CDK4/6 protects FOXM1 from proteasome-mediated degradation and increases the expression of FOXM1-dependent target genes, including the genes encoding cyclin E2, MSH6, and SKP2 (24). Pharmacologic inhibition of CDK4/6 promoted FOXM1 degradation and triggered a senescent phenotype in melanoma cell lines (24). This observation appears to explain why constitutive cyclin D–CDK4/6 signaling overrides the induction of senescence induced by oncogenic BRAF signaling in melanoma nevi (26). These findings have implications beyond melanoma; for example, both FOXM1 and cyclin D–CDK4 suppress cellular senescence induced by oncogenic RAS, and both are critical for KRAS-induced tumorigenesis in a mouse model of lung cancer (27–29). Further studies of the contribution of increased FOXM1 activity to the therapeutic effects of cyclin D–CDK4/6 inhibitors in human cancers are clearly warranted.

Clinical–Translational Advances

CDK-targeted therapies in clinical development

Clinical testing of CDK inhibition as a cancer therapeutic strategy began with several pan-CDK inhibitors, including flavopiridol (alvociclib; Sanofi-Aventis), which inhibits CDK1, CDK2, CDK4, CDK6, CDK7, and CDK9. In contrast with CDKs 1, 2, 4, and 6, which regulate cell-cycle progression, CDKs 7 and 9 control RNA polymerase II–dependent transcriptional activity. Although *in vitro* breadth of efficacy data indicated encouraging activity against multiple tumor cell lines, clinical activity in phase I/II studies in solid tumors was modest (30). Despite some positive results in chronic lymphocytic leukemia and mantle cell lymphoma (31, 32), the clinical development of flavopiridol was discontinued in 2012. Roscovitine (seliciclib, Cyclacel) inhibits CDKs 1, 2, 4, 7, and 9 and also failed to show an acceptable benefit:safety ratio in patients (33). Undoubtedly, the broad inhibitory effects of these compounds on CDKs underlie the therapeutic challenges that hindered their clinical development. For example, inhibition of CDK1 results in cell-cycle arrest in mitosis, which leads to myelosuppression and other toxicities commonly associated with cytotoxic chemotherapy.

CDK4 and CDK6 specific inhibitors

Medicinal chemists eventually overcame the challenge of designing highly selective inhibitors of cyclin–CDK4/6 activities, reinvigorating the effort to restore pRb-dependent G_1 checkpoint function in tumors. Among the leaders of this new wave of CDK4/6 inhibitors are palbociclib (Ibrance, formerly termed PD-0332991; Pfizer), abemaciclib (LY2835219; Eli Lilly & Company), and ribociclib (LEE011; Novartis; Fig. 2). These molecules are potent, ATP-competitive, orally administered inhibitors of CDK4 and CDK6 that cause little or no suppression of other CDK activities at clinically achievable doses.

A crucial step in the development of palbociclib's selectivity for CDK4/6 was the introduction of a 2-aminopyridyl substituent at the *C*2-position of pyrido[2,3-*d*]pyrimidin-7-one core pharmacophore. In enzymatic assays, the ultimate clinical candidate (PD-0332991, later named palbociclib) displayed potent inhibitory activities against the cyclin D–associated CDK4 (IC_{50}, 11 nmol/L) and CDK6 (IC_{50}, 16 nmol/L) kinases (34). This compound was greater than 1,000-fold less potent as an inhibitor of cyclin-associated CDK1 activity, which, as mentioned above, is likely an undesirable off-target kinase associated with toxicity to normal tissues. In a striking validation of the pivotal role of pRb modification in cyclin D–CDK4/6 function, palbociclib effectively arrested cellular proliferation in G_1-phase only in cell populations expressing functional pRb (35).

Scientists at Eli Lilly identified the 2-anilino-2,4-pyrimidine-[5-benzimidazole] scaffold as a starting point for the generation of potent inhibitors of CDK4 and CDK6. This molecule was optimized through structure-based design, and LY2835219 (abemaciclib) was selected based on its compelling biologic and pharmacologic properties. Abemaciclib inhibits CDK4 and CDK6 with an IC_{50} of 2 nmol/L and 10 nmol/L, respectively, and is at least 160-fold less potent against the cyclin–CDK1 complex (36). The chemical structure of ribociclib (Novartis) is most similar to that of palbociclib (Fig. 1). Ribociclib inhibits CDK4 and CDK6 with IC_{50} values of 10 nmol/L and 39 nmol/L, respectively (37). Like palbociclib, ribociclib is over 1,000-fold less potent against the cyclin B/CDK1 complex. The preclinical anticancer activities of these three pioneering CDK4/6 inhibitors appear to be qualitatively similar, and time will tell if these agents will differentiate based on their individual efficacy, safety, and combinability profiles in the clinic.

Therapeutic Applications of CDK4/6 Inhibitors

In principle, CDK4/6 inhibitors may prove useful in the treatment of a variety of cancer subtypes, based on the premise that the cyclin D–CDK4/6 complex is required to overcome the tumor-suppressive activity of pRb in cancer cells that express fully functional pRb. Although this review focuses on ER$^+$ breast cancer, the first indication in which a CDK4/6 inhibitor received full approval from FDA in 2015, the anticancer activities of CDK4/6 inhibitors are being actively explored in many other cancer subtypes, including melanoma, neuroblastoma, liposarcoma, and mantle cell lymphoma. It seems likely that CDK4/6 inhibitors will generally prove most effective when used in combination with other agents that either reinforce the cytostatic activity of CDK4/6 inhibitors or convert reversible cytostasis into irreversible growth arrest or cell death. The choice of partners for CDK4/6 inhibitors in combination settings will need to be made carefully, because CDK4/6 inhibition, and the resultant G$_1$-phase growth arrest, may antagonize cell killing by therapies that preferentially kill cancer cells in S-phase or mitosis. Preclinical studies showed that CDK4/6 inhibitor pretreatment protects both cancer cells and normal hematopoietic cells from death induced by ionizing radiation and other DNA-damaging agents (38). Similarly, antigen-stimulated T cells are dependent on CDK4/6 activity for proliferative expansion and differentiation (39), suggesting that combinations of CDK4/6 inhibitors with immune checkpoint inhibitors (e.g., anti–PD-1) will require careful evaluation for potential antagonism in preclinical models and human subjects.

One combination approach that has yielded particularly exciting results is the co-administration of CDK4/6 inhibitors with antiestrogen therapy in patients with estrogen receptor–positive (ER+) breast cancer. Palbociclib (Ibrance) was recently granted accelerated approval by the FDA to treat ER$^+$ (HER2-negative) metastatic breast cancer in combination with letrozole, an aromatase inhibitor. These clinical results were presaged by experiments in ER$^+$ breast cancer cell lines, which demonstrated striking, persistent antiproliferative effects when CDK4/6 inhibition was combined with blockade of ER signaling (40). It is noteworthy that the vast majority (>90%) of ER$^+$ breast cancer tumors retain functional pRb, making this disease indication a highly attractive target for CDK4/6 inhibitor–based therapies (41). Moreover, combinations of ER antagonists with CDK4/6 inhibitors displayed strong efficacy in ER$^+$ breast cancer models that had acquired resistance to ER antagonist monotherapy (42). Mechanistically, the combination of palbociclib with ER antagonists leads to more penetrating inhibition of pRb phosphorylation than does either agent alone. Notable downstream consequences of this combination therapy are a synergistic reduction of E2F target gene expression, increased markers of cellular senescence, and delayed reentry into the cell cycle following drug removal (43).

The therapeutic efficacy of palbociclib was first demonstrated in 165 postmenopausal women with ER$^+$, HER2-negative breast cancer who had not received previous treatment for advanced disease (44). This patient population is highly enriched for functional pRb, currently the most reliable biomarker for CDK4 inhibitor sensitivity (45). Study participants were randomly assigned to receive palbociclib in combination with letrozole (an inhibitor of estrogen biosynthesis) or letrozole alone. Participants treated with the two-drug combination lived 20.2 months without disease progression (progression-free survival), compared with 10.2 months in participants receiving letrozole monotherapy (hazard ratio, 0.488; 95% confidence interval, 0.319–0.748; one-sided $P = 0.0004$). The impact of the combination on the key metric of overall survival is not yet available. The most common side effects observed in palbociclib-treated patients were reversible, non-febrile neutropenia, leukopenia, fatigue, and anemia.

Patient tumors were screened for amplification of CCND1 or loss of p16^{INK4A}, which were predicted biomarkers for heightened CDK4/6 inhibitor sensitivity. However, these putative sensitivity biomarkers failed to enrich for patients with an enhanced therapeutic response to palbociclib, suggesting that both biomarker-positive and biomarker-negative ER$^+$ breast cancer cells are similarly reliant on cyclin D–CDK4/6 activity to overcome pRb's tumor suppressor function. Presumably, the biomarker-negative tumors inactivate pRb through deregulated signaling mechanisms that drive constitutive cyclin D–CDK4/6 activation. A prime suspect in this regard is the activated ER itself, which binds to the CCND1 promoter and stimulates cyclin D1 expression (46, 47). Therefore, the dramatic therapeutic effects of the CDK4/6–ER inhibitor combination may be attributed in part to their convergent inhibitory actions on cyclin D1–CDK4/6 activity. The interplay between the ER and cyclin D1 may be bidirectional, in that cyclin D1 has been shown to bind directly to the ER, resulting in increased ER-mediated signaling functions (48). The latter mechanism of ER activation is not dependent on cyclin D–CDK4/6 activity, and hence should not be sensitive to CDK4/6 inhibitors.

In early-phase clinical studies, both abemaciclib and ribociclib have shown encouraging clinical activity in ER$^+$ breast cancer. Furthermore, the combination of ribociclib with BYL-719, a PI3Kα-specific inhibitor, delivers striking antitumor activity in pRb-positive breast cancer models that are resistant to BYL-719 alone (37). Oncogenic activation of the PI3K–mTOR pathway is also capable of stimulating cyclin D1–CDK4/6 activity, due in part to the increased translation of the cyclin D1 mRNA transcript, and to the stabilization of the cyclin D1 protein (49, 50). Activating mutations in the PI3KCA gene, which encodes the catalytic α subunit of PI3K, are observed in 30% to 40% of ER$^+$ breast cancers and represent a potential mechanism of resistance to both antiestrogen drugs and CDK4/6 inhibitors (51–54). This scenario has prompted implementation of triple combination studies involving the addition of a PI3K or mTOR inhibitor to the established antihormonal plus CDK4/6 inhibitor regimens (55). The current surge in interest regarding the development of combination therapies involving CDK4/6 inhibitors supports the notion that the introduction of these drugs represents the most exciting therapeutic advance in the ER$^+$ breast cancer field since the introduction of tamoxifen, the first antiestrogen, nearly 35 years ago (56).

Conclusions

The seminal discoveries that delineated the cyclin D–CDK4/6–pRb axis in mammalian cells were made more than 20 years ago. Although the canonical model of pRb phosphorylation by the sequential activities of the cyclin D–CDK4/6 and cyclin E–CDK2 complexes has undergone some revision in recent years, the groundbreaking research from the laboratories of Charles Sherr, David Beach, and other investigators remains a critical opening chapter in a journey that has led to the development of highly effective and safe treatments for ER$^+$ cancer (57).

The next chapter will undoubtedly extend the therapeutic activities of selective CDK4/6 inhibitors into other cancer sub-types that express a functional pRb pathway. As is the case with most targeted therapies, we can expect that intrinsic or acquired resistance mechanisms will be identified as more patients are treated with CDK4/6 inhibitors. Loss of pRb itself is one resistance mechanism that completely obviates the use of CDK4/6 inhibitors, but additional alterations, such as cyclin D or cyclin E overexpression, are expected to reduce the therapeutic responsiveness of tumors that retain functional pRb. Moreover, an orthogonal mechanism of drug resistance may affect the therapeutic activities of the cyclin D–CDK4/6 inhibitor–antiestrogen combination in ER$^+$ breast cancer. Recent studies have identified several ER mutations that hyperactivate the ER and confer resistance to ER antagonists (58, 59). Whether CDK4/6 inhibitors will resensitize breast cancer cells expressing these mutationally activated ERs to antiestrogens remains to be determined. Whatever the outcome, the long journey from the discovery of the cyclin D–CDK4/6–pRb axis to the therapeutic validation of this pathway in ER$^+$ breast cancer has been well worth the wait, with more good news for patients with cancer on the near-term horizon.

Disclosure of Potential Conflicts of Interest

K.T. Arndt has ownership interest in Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C. Boshoff, R.T. Abraham
Writing, review, and/or revision of the manuscript: T. VanArsdale, C. Boshoff, K.T. Arndt, R.T. Abraham

References

1. Nurse P. Genetic control of cell size at cell division in yeast. Nature 1975;256:547–51.
2. Beach D, Durkacz B, Nurse P. Functionally homologous cell cycle control genes in budding and fission yeast. Nature 1982;300:706–9.
3. Pardee AB. G1 events and regulation of cell proliferation. Science 1989;246:603–8.
4. Chellappan SP, Hiebert S, Mudryj M, Horowitz JM, Nevins JR. The E2F transcription factor is a cellular target for the RB protein. Cell 1991; 65:1053–61.
5. Matsushime H, Ewen ME, Strom DK, Kato J-Y, Hanks SK, Roussel MF, et al. Identification and properties of an atypical catalytic subunit (p34PSK-J3/cdk4) for mammalian D type G1 cyclins. Cell 1992; 71:323–34.
6. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995;81:323–30.
7. 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.
8. Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell 1999;98:859–69.
9. 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.
10. Xiong Y, Connolly T, Futcher B, Beach D. Human D-type cyclin. Cell 1991;65:691–9.
11. Meyerson M, Harlow E. Identification of G1 kinase activity for cdk6, a novel cyclin D partner. Mol Cell Biol 1994;14:2077–86.
12. 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.
13. Choi YJ, Anders L. Signaling through cyclin D-dependent kinases. Oncogene 2014;33:1890–903.
14. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Disc 2015;14:130–46.
15. Erikson J, Finan J, Tsujimoto Y, Nowell PC, Croce CM. The chromosome 14 breakpoint in neoplastic B cells with the t(11;14) translocation involves the immunoglobulin heavy chain locus. Proc Natl Acad Sci U S A 1984; 81:4144–8.
16. Walker GJ, Flores JF, Glendening JM, Lin AH, Markl ID, Fountain JW. Virtually 100% of melanoma cell lines harbor alterations at the DNA level within CDKN2A, CDKN2B, or one of their downstream targets. Genes Chromosomes Cancer 1998;22:157–63.
17. Hirai H, Roussel MF, Kato JY, Ashmun RA, Sherr CJ. Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases CDK4 and CDK6. Mol Cell Biol 1995;15:2672–81.
18. 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.
19. Foulkes WD, Flanders TY, Pollock PM, Hayward NK. The CDKN2A (p16) gene and human cancer. Mol Med 1997;3:5–20.
20. Narasimha AM, Kaulich M, Shapiro GS, Choi YJ, Sicinski P, Dowdy SF. Cyclin D activates the Rb tumor suppressor by mono-phosphorylation. Elife 2014;3, doi:10.7554/eLife.02872.
21. Buchkovich K, Duffy LA, Harlow E. The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. Cell 1989;58:1097–105.
22. 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.
23. Ludlow JW, Glendening CL, Livingston DM, DeCarprio JA. Specific enzymatic dephosphorylation of the retinoblastoma protein. Mol Cell Biol 1993;13:367–72.
24. Anders L, Ke N, Hydbring P, Choi YJ, Widlund HR, Chick JM. A systematic screen for CDK4/6 substrates links FOXM1 phosphorylation to senescence suppression in cancer cells. Cancer Cell 2011;20:620–34.
25. Matsuura I, Denissova NG, Wang G, He D, Long J, Liu F. Cyclin-dependent kinases regulate the antiproliferative function of Smads. Nature 2004; 430:226–31.
26. Dhomen N, Reis-Filho JS, da Rocha Dias S, Hayward R, Savage K, Delmas V, et al. Oncogenic Braf induces melanocyte senescence and melanoma in mice. Cancer Cell 2009;15:294–303.
27. Park HJ, Carr JR, Wang Z, Nogueira V, Hay N, Tyner AL, et al. FoxM1, a critical regulator of oxidative stress during oncogenesis. EMBO J 2009; 28:2908–18.
28. Wang IC, Ustiyan V, Zhang Y, Cai Y, Kalin TV, Kalinichenko VV. Foxm1 transcription factor is required for the initiation of lung tumorigenesis by oncogenic Kras(G12D.). Oncogene 2014;33:5391–6.
29. 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.
30. Shapiro GI. Preclinical and clinical development of the cyclin-dependent kinase inhibitor flavopiridol. Clin Cancer Res 2004;10:4270s-5s.
31. Kouroukis CT, Belch A, Crump M, Eisenhauer E, Gascoyne RD, Meyer R, et al. Flavopiridol in untreated or relapsed mantle-cell lymphoma: results of a phase II study of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2003;21:1740–5.
32. Byrd JC, Lin TS, Dalton JT, Wu D, Phelps MA, Fischer B, et al. Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. Blood 2007;109:399–404.
33. Whittaker SR, Walton MI, Garrett MD, Workman P. The Cyclin-dependent kinase inhibitor CYC202 (R-roscovitine) inhibits retinoblastoma protein

phosphorylation, causes loss of Cyclin D1, and activates the mitogen-activated protein kinase pathway. Cancer Res 2004;64:262–72.

34. 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.

35. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E. 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.

36. 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 Drug 2014;32:825–37.

37. Kim S, Loo A, Chopra R, Caponigro G, Huang A, Vora S, et al. LEE011: an orally bioavailable, selective small molecule inhibitor of CDK4/6–reactivating Rb in cancer [abstract]. In: Proceedings of the AACR–NCI–EORTC International Conference: Molecular Targets and Cancer Therapeutics; 2013 Oct 19–23; Boston, MA. Philadelphia (PA): AACR; Mol Cancer Ther 2013;12(11 Suppl):Abstract nr PR02.

38. 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.

39. 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.

40. Finn RS, Dering J, Conklin D, Kalous O, Cohen DJ, Desai AJ. 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.

41. Ertel A, Dean JL, Rui H, Liu C, Witkiewicz AK, Knudsen KE, et al. RB-pathway disruption in breast cancer: differential association with disease subtypes, disease-specific prognosis and therapeutic response. Cell Cycle 2010;9:4153–63.

42. Miller TW, Balko JM, Fox EM, Ghazoui Z, Dunbier A, Anderson H, et al. ERα-dependent E2F transcription can mediate resistance to estrogen deprivation in human breast cancer. Cancer Discov 2011;1:338–51.

43. Lee NV, Yuan J, Eisele K, Cao JQ, Painter CL, Chionis J, et al. Mechanistic exploration of combined CDK4/6 and ER inhibition in ER-positive breast cancer [abstract]. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5-9; San Diego, CA. Philadelphia (PA): AACR; Cancer Res 2014;74(19 Suppl):Abstract nr LB-136.

44. 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.

45. Lukas J, Bartkova J, Bartek J. Convergence of mitogenic signalling cascades from diverse classes of receptors at the cyclin D-cyclin-dependent kinase-pRb-controlled G1 checkpoint. Mol Cell Biol 1996;16:6917–25.

46. Foster JS, Wimalasena J. Estrogen regulates activity of cyclin-dependent kinases and retinoblastoma protein phosphorylation in breast cancer cells. Mol Endocrinol 1996;10:488–98.

47. Altucci L, Addeo R, Cicatiello L, Dauvois S, Parker MG, Truss M, et al. 17beta-Estradiol induces cyclin D1 gene transcription, p36D1-p34cdk4 complex activation and p105Rb phosphorylation during mitogenic stimulation of G(1)-arrested human breast cancer cells. Oncogene 1996; 12:2315–24.

48. Zwijsen RM, Wientjens E, Klompmaker R, van der Sman J, Bernards R, Michalides RJ. CDK-independent activation of estrogen receptor by cyclin D1. Cell 1997;88:405–15.

49. Schmidt M, Fernandez de Mattos S, van der Horst A, Klompmaker R, Kops GJ, Lam EW, et al. Cell cycle inhibition by FoxO forkhead transcription factors involves downregulation of cyclin D. Mol Cell Biol 2002;22:7842–52.

50. She QB, Chandarlapaty S, Ye Q, Lobo J, Haskell KM, Leander KR, et al. Breast tumor cells with PI3K mutation or HER2 amplification are selectively addicted to Akt signaling. PloS One 2008;3:e3065.

51. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer Biol Ther 2004;3:772–5.

52. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature 2012;486:400–4.

53. Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. J Biol Chem 2001;276:9817–24.

54. Sun M, Paciga JE, Feldman RI, Yuan Z, Coppola D, Lu YY, et al. Phosphatidylinositol-3-OH Kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor alpha (ERalpha) via interaction between ERalpha and PI3K. Cancer Res 2001;61:5985–91.

55. Bardia A, Modi S, Chavez-Mac Gregor M, Kittaneh M, Marino AJ, Matano A, et al. Phase Ib/II study of LEE011, everolimus, and exemestane in postmenopausal women with ER+/HER2-metastatic breast cancer. J Clin Oncol 32:5s, 2014 (suppl; abstr 535).

56. Cole MP, Jones CT, Todd ID. A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. Br J Cancer 1971;25:270–5.

57. Sherr CJ. G1 phase progression: cycling on cue. Cell 1994;79:551–5.

58. Jeselsohn R, Yelensky R, Buchwalter G, Frampton G, Meric-Bernstam F, Gonzalez-Angulo AM, et al. Emergence of constitutively active estrogen receptor-alpha mutations in pretreated advanced estrogen receptor-positive breast cancer. Clin Cancer Res 2014;20:1757–67.

59. Robinson DR, Wu YM, Vats P, Su F, Lonigro RJ, Cao X, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. Nat Genet 2013;45:1446–51.

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