

The Cell-Cycle Regulator CDK4: An Emerging Therapeutic Target in Melanoma

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Abstract

The recent clinical success of targeted therapies in melanoma directed at the oncogene BRAF validates the concept of targeting oncogenes. The p16-cyclin D-CDK4/6-retinoblastoma protein pathway (CDK4 pathway) is dysregulated in 90% of melanomas, and is, therefore, an obvious therapeutic target for this disease. The main outcome of CDK4 activation is the phosphorylation and, thus, inhibition of the retinoblastoma protein leading to G₁-S cell-cycle transition. In addition, CDK4 directly phosphorylates other proteins that promote cell-cycle progression and inhibit both cell senescence and apoptosis. In preclinical studies, the response to CDK4 inhibition correlates with genomic changes that increase CDK4 activity, most notably where the tumor suppressor *CDKN2A* (p16^{INK4A}) is deleted. A central question is whether melanomas with activating events in the CDK4 pathway have become "addicted" to this signaling pathway, in which case inhibition of CDK4 would not simply induce cell-cycle arrest but induce cell death and tumor regression. Recently, a number of selective CDK4/6 inhibitors have entered clinical trials, and these compounds are showing great promise in that they are well tolerated and show clinical benefit. This review discusses the CDK4 pathway, its dysregulation in melanoma, the consequences of CDK4 pathway inhibition, and potential novel combinational strategies for the treatment of melanoma. *Clin Cancer Res*; 19(19); 5320–8. ©2013 AACR.

Introduction

Cyclin-dependent kinases (CDK) drive cell-cycle progression, control transcriptional processes, and, thus, regulate cell proliferation. Considering that aberrant entry into the cell cycle and uncontrolled cell proliferation is a hallmark of cancer (1), it is not surprising that dysregulation of CDKs plays a central role in tumorigenesis. The p16-cyclin D-CDK4/6-retinoblastoma protein (RB1) pathway (CDK4 pathway) promotes G₁-S cell-cycle transition, and this pathway is commonly dysregulated in most cancers. In melanoma, the CDK4 pathway is associated with activating genomic alterations in more than 90% of cases (2, 3), and, in both human and mouse models of melanoma, activation of the CDK4 pathway potentially cooperates with mutant BRAF or NRAS in transformation of melanocytes (4–6). Furthermore, the

RAS/RAF/MEK/ERK pathway, which is dysregulated in 65% to 90% of metastatic melanoma (7), enhances CDK4 pathway signaling through increasing cyclin D1 expression (8, 9), and this increase is implicated in the transforming ability of both RAS and RAF (10).

The recent clinical success of targeted therapies directed at the oncogene BRAF in melanoma (11, 12) validate the concept of targeting oncogenes in this disease and pave the way to potentially target other activated signaling pathways. The prevalence of activating events in the CDK4 pathway in melanoma highlights it as a potential therapeutic target. A central question discussed further here is whether melanomas with these activating events are "addicted" to the pathway, such that inhibition of CDK4 specifically targets cancer cells by inducing G₁-arrest or alternately leading to other cellular outcomes. Currently, there are several inhibitors with varying selectivity for specific members of the CDK family, but CDK4/6 inhibitors have emerged as the most attractive therapeutic option because of their ability to inhibit cell proliferation and minimal toxic effects. The pyridopyrimidine PD0332991 (PD991: palbociclib) is the most advanced in clinical development, and studies to date with this compound have shown favorable pharmacokinetics, absence of significant off-target toxicities, and, most importantly, evidence of target modulation in humans (13–15).

This review discusses the CDK4 pathway, its dysregulation in melanoma, the consequences of CDK4 pathway inhibition, and potential novel combinational strategies for the treatment of melanoma.

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CDK4 pathway

The mammalian cell cycle is regulated by two key classes of molecules—the regulatory cyclins and the catalytic CDKs that form active heterodimers leading to phosphorylation of target proteins. CDK4 and its close homolog CDK6 are serine/threonine kinases that form heterodimers with D-type cyclins and are central regulators of G₁–S transition of the cell cycle (Fig. 1). Together with cyclin E–CDK2 complexes, cyclin D–CDK4/6 complexes sequentially phosphorylate the retinoblastoma protein (RB1), diminishing its ability to suppress RNA polymerase I and III activity (16, 17) and gene transcription (Fig. 2). Hypophosphorylated RB1 modulates gene transcription through its action on suppressing the E2F family of transcription factors and by recruiting histone deacetylases to the promoters of genes required for S-phase entry (18). These inhibitory actions of hypophosphorylated RB1 lead to the modulation of ribosome biogenesis, which has an impact on protein synthesis and transcription factors that coordinate subsequent cell-cycle progression, nucleotide biosynthesis, DNA replication, mitotic progression, and DNA damage repair (19). Thus, CDK4/6-mediated deactivation of RB1 is critical for

cell-cycle progression. This process is negatively regulated by the tumor suppressor p16^{INK4A}, which specifically inhibits the assembly and activation of cyclin D–CDK4/6 complexes. Further control of this pathway is via a negative feedback loop whereby CDK4/6 inactivation of RB1 relieves RB1-mediated suppression of p16^{INK4A} (20), leading to increased p16^{INK4A} and subsequent reduction in CDK4/6 activity. Thus, this negative feedback loop effectively works as a natural brake on the activation of this pathway (Fig. 1). Increased p16^{INK4A} expression due to loss of RB1 is also seen in HPV-infected cells, where the E7 protein leads to RB1 degradation, and in cells in which there is a genetic loss of RB1 (21). These data confirm that the regulatory feedback from RB1 to p16^{INK4A} exists under both physiologic and pathologic conditions. Under these circumstances where there is a permanent loss of RB1, this loss renders the increased p16^{INK4A} expression incapable of inhibiting the cell cycle. In normal cells, p16^{INK4A} levels are usually low, allowing cell-cycle progression. Increased p16^{INK4A} expression upon oncogenic signaling, DNA damage, and physiologic aging (22, 23) inhibits cell proliferation, and these three processes also trigger cellular senescence, indicating

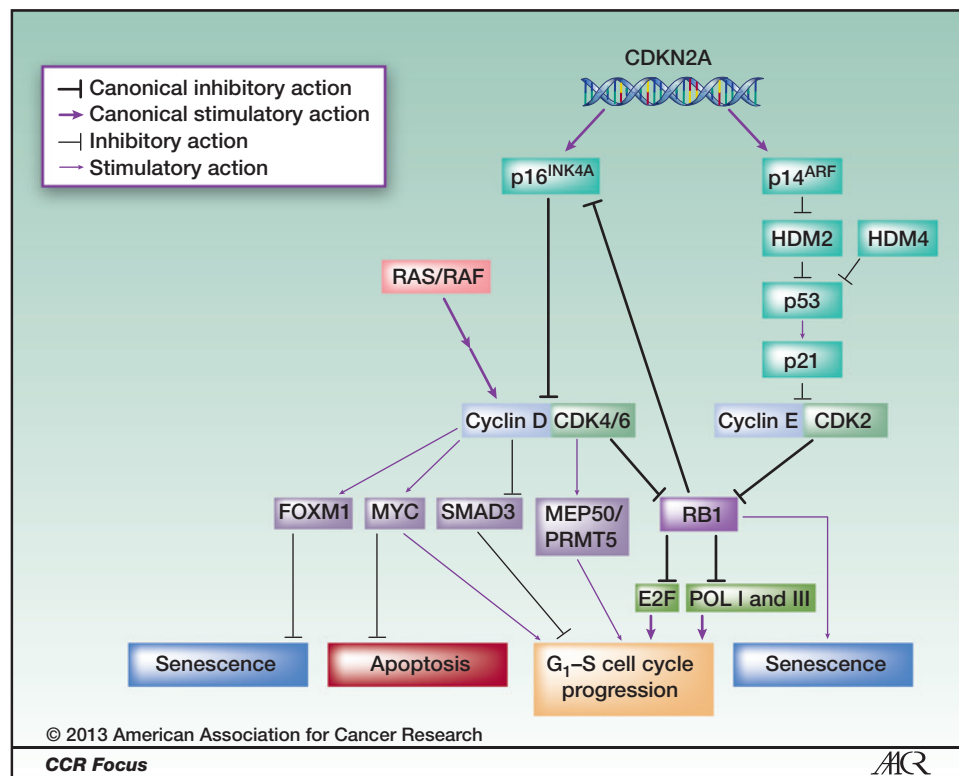


Figure 1. CDK4 pathway. In response to mitogenic signaling, CDK4 and CDK6 associate with cyclin D to form active complexes that phosphorylate the retinoblastoma protein (RB1). Subsequent phosphorylation of RB1 by CDK2–cyclin E complex allows transcription of E2F regulated genes and transcription of both ribosomal and small RNAs by RNA polymerases. Phosphorylation of RB1 also inhibits its ability to induce senescence. p16^{INK4A} and p14^{ARF} are transcribed from the same gene (*CDKN2A*) and regulate cell-cycle progression by inhibiting CDK4/6 and CDK2 activity, respectively. Induction of p14^{ARF} inhibits HDM2-mediated degradation of p53, thus increasing expression of p21, which then inhibits CDK2/cyclin E activity. p16^{INK4A} negatively regulates the cell cycle by specifically inhibiting the assembly and activation of cyclin D–CDK4/6 complexes. Further control of this pathway is via a negative feedback loop whereby CDK4/6 inactivation of RB1 relieves RB1-mediated suppression of p16^{INK4A}. In addition to the canonical CDK4 pathway, active CDK4/6–cyclin D complexes can directly phosphorylate and regulate transcription factors, including Smad3, MYC, and FOXM1 as well as MEP50, a PRMT5 coregulatory factor, resulting in RB1-independent regulation of cell-cycle progression, senescence, and apoptosis.

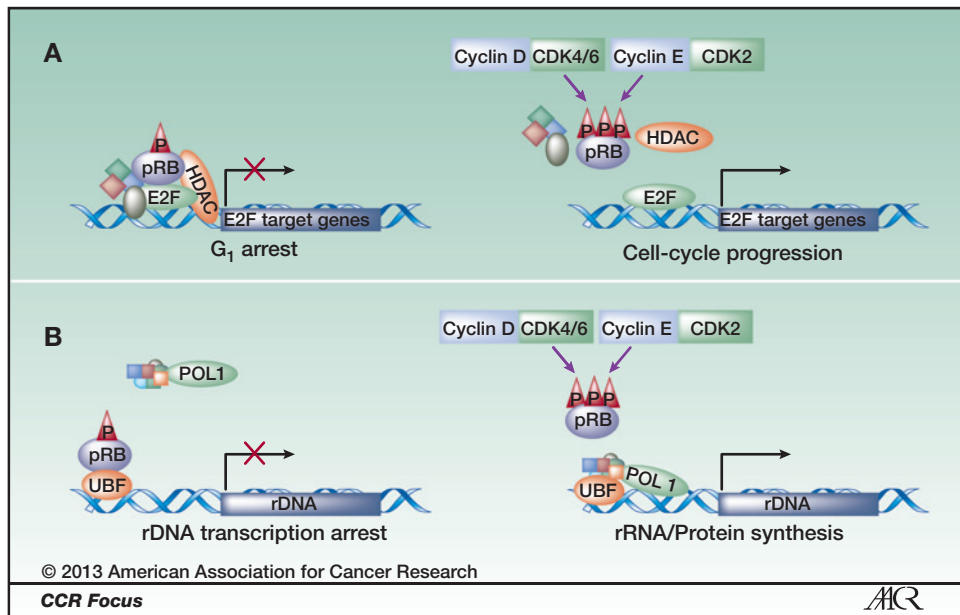


Figure 2. Retinoblastoma protein regulation of E2F target gene transcription and ribosomal RNA synthesis. A, activated hypophosphorylated RB1 binds to E2F transcriptional regulators, recruiting histone deacetylases, corepressors, and chromatin remodeling enzymes and, thus, suppressing target gene transcription. Phosphorylation of RB1 by activated CDK4/6 and CDK2 relieves inhibition and allows E2F target gene transcription. B, activated hypophosphorylated RB1 binds to the RNA polymerase I-specific factor UBF, inhibiting its association with the SL-1-RNA polymerase I complex, thereby inhibiting rRNA transcription. Phosphorylation of RB1 by activated CDK4/6 and CDK2 relieves inhibition and allows UBF to recruit the SL-1 complex and RNA polymerase I to initiate rDNA transcription.

that p16^{INK4A}-mediated cell-cycle arrest is likely necessary for induction of senescence.

Although RB1 is considered the major substrate of the cyclin D-CDK4/6 complex, the RB1-related proteins p107 and p130 are also substrates and have a similar function to RB1 in regulating the cell cycle (24). In addition, there is increasing evidence that CDK4/6 independent of RB1 can regulate the cell cycle, survival, and senescence via direct phosphorylation of transcription factors, including Smad3 (25), MYC, and FOXM1 (26), or through targeting transcriptional coregulators including MEP50, a PRMT5 coregulatory factor (27).

CDK4 pathway in human melanoma

The early indications that the CDK4 pathway was likely a critical oncogenic pathway in melanoma were that the inhibitory mutations in *CDKN2A* or activating mutations in CDK4 in the germ line of humans led to an increase in the risk of developing melanoma well above any other cancer type (28). Loss of p16^{INK4A} occurs in approximately 80% to 90% of metastatic tumors, most commonly via gene deletion (50–60%; ref. 29) but also via mutation (~10%) and promoter methylation (20–30%; refs. 30–32). The loss of p16^{INK4A} protein expression appears to progress from benign nevi being approximately 70% to 100% positive for p16^{INK4A} protein, primary melanoma 30% to 80% positive, and metastatic disease only being 10% to 20% positive (33). The sequential loss of p16^{INK4A} from benign nevi to metastatic melanoma indicates that this loss is vital in disease establishment and progression

and reinforces the idea that activation of the CDK4 pathway is an early event and likely essential in melanoma progression.

P16^{INK4A} specifically inhibits the assembly and activation of both cyclin D-CDK4 and cyclin D-CDK6 complexes; thus, the loss of p16^{INK4A} in melanoma results in increased CDK4/6 signaling (34). In most cases, however, mutations in p16^{INK4A} lead to decreased binding to CDK4 but not CDK6 (35, 36), underscoring a predominant role of CDK4 over CDK6 in the development of melanoma. Mutations in CDK4 (R24C) originally identified in melanoma-prone families (28) abolish the interaction with p16^{INK4A} and, thus, render CDK4 constitutively active (37), further highlighting the importance of CDK4 activation in melanoma. Although CDK6 is not mutated in melanoma (38), and there is no focal amplification of the gene, CDK6 does lie within a large region that is amplified in at least 62% of tumors (29), suggesting it may play a role in this disease. Both CDK4 and CDK6, when complexed with cyclin D1, promote cell-cycle progression through the phosphorylation and deactivation of RB1. Cyclin D1 is a known oncogene in many tumor types, including melanoma. It is amplified in 18% of melanomas (29, 39), and, more importantly, increased signaling in the RAS/RAF/MEK/ERK pathway in BRAF- and NRAS-mutant melanomas triggers the expression of cyclin D1 through both gene transcription and protein stabilization (8, 9, 40). Finally, germ line inactivation of the RB1 gene also predisposes patients to melanoma (28). In summary, there are multiple gene deletions and mutations along the CDK4 pathway that

increase signaling, leading to cell-cycle progression and an increased risk of developing malignant melanoma.

Models of melanoma with disrupted CDK4 pathway

The association of melanoma with loss of the *CDKN2A* gene prompted studies assessing the relative importance of the two distinct proteins, p16^{INK4A} and p14^{ARF} (p19^{ARF} in mouse), that are encoded by this gene. Whereas p16^{INK4A} inhibits CDK4/6 activity by maintaining RB1 in its hypo-phosphorylated, antiproliferative state (41), p14^{ARF} inhibits HDM2-mediated ubiquitination and subsequent degradation of p53 (42); thus, loss of p14^{ARF} inactivates p53 (Fig. 1). In mice with targeted deletion of the *CDKN2A* gene, which eliminates both p16^{INK4A} and p19^{ARF} (mouse homolog of p14^{ARF}), spontaneous tumors developed at an early age, and these mice are highly sensitive to carcinogenic agents (43). Mice lacking p19^{ARF} but expressing functional p16^{INK4A} also develop tumors early in life (44), indicating disruption of p19^{ARF} alone predisposes mice to tumorigenesis. Loss of p16^{INK4A} in mice is not sufficient to induce melanoma but does mimic the melanoma predisposition seen in humans (45, 46). In these p16^{INK4A} null mice, susceptibility to spontaneous melanoma was low and, similar to p19^{ARF} loss, tumor incidence in these mice increased in response to carcinogen.

The majority of mutations in the *CDKN2A* gene specifically target and impair p16^{INK4A} function, whereas mutations uniquely targeting p14^{ARF} are rare. Of the mutations in the *CDKN2A* gene that pertain to both p16^{INK4A} and p14^{ARF} that have been tested, all show compromised p16^{INK4A} function and a small subset shows compromised p14^{ARF} function (47). Thus, loss of p16^{INK4A} function in the majority of melanomas highlights the importance of this protein in tumor suppression, whereas the rare loss of p14^{ARF} function raises questions about its role in this disease. Inactivation of p53 via mutation is a common event in human cancer (48); however, these events are low in melanoma (49, 50). Melanomas develop with high penetrance in mouse models where there is both loss of p53 and activation of the RAS/RAF/MEK/ERK pathway (51, 52), indicating that p53 inactivation can contribute to this disease. Further evidence for a role of p53 inactivation in melanoma progression is that high expression of HDM4 is found in 65% of melanomas. This protein inhibits p53 activity and, in a mouse model of melanoma driven by the oncogene NRAS, overexpression of MDM4 (mouse homolog of HDM4) enhanced tumorigenesis (53). Thus, p53 inactivation in melanoma in the majority of tumors is likely via HDM4 and thus negates the need for loss of p53 function through mutation or loss of p14^{ARF}.

The common mutation in CDK4 (CDK4^{R24C}) in melanoma inhibits the interaction with p16^{INK4A} and thus leads to constitutive activation. Mice with a knockin of CDK4^{R24C} display a similar phenotype to mice with p16^{INK4A} loss, in that there is a low incidence of spontaneous melanoma that is augmented in response to carcinogen (37, 54, 55). Further evidence that CDK4 pathway activation may be necessary for melanoma progression is that

both cyclin D1- and CDK4-null mice are relatively resistant to chemical-induced skin cancer (56, 57). Furthermore, in a model of human skin tissue regenerated with melanocytes, CDK4 overexpression or inhibition of p53, together with activated RAS, produced invasive human melanoma (5).

From studies in mice, it is clear that loss of p16^{INK4A} or activation of CDK4 as single events is not sufficient to induce melanoma but does predispose animals to tumor formation, which aligns with the clinical data showing increased risk of developing melanoma in patients who have these genetic changes. Furthermore, systematic interrogation of the CDK4 pathway in melanocytes has shown that activation of the pathway, together with the introduction of telomerase reverse transcriptase (TERT), can lead to immortalization of these cells but is not sufficient for transformation. P16^{INK4A}-deficient human melanocytes with inactivating mutations in both alleles of p16^{INK4A}/p14^{ARF} undergo delayed senescence but can be immortalized by the introduction of TERT (58). Furthermore, TERT, in combination with CDK4 overexpression or inhibition of RB1, immortalizes cells (59). Melanocytes are unique in that activation of the CDK4 pathway, in addition to expression of TERT, is required for immortalization, whereas introduction of TERT alone in most cell lines is sufficient.

Senescence

Similar to melanoma (60), benign nevi harbor activating mutations in BRAF (61) and, less frequently, NRAS (62). Unlike melanoma, however, nevi are composed of mainly senescent cells that are in cell-cycle arrest, have increased expression of p16^{INK4A}, and express senescence biomarkers including SA- β -galactosidase (63). In human melanocytes, expression of BRAF^{V600E} induces senescence (63) and, in mouse models, when BRAF^{V600E} is expressed in the melanocytic lineage, nevi display the senescent phenotype (64, 65). Senescence was originally defined as an irreversible cell-cycle arrest and, thus, oncogene-induced senescence in melanocytes is thought to be an effective barrier preventing melanoma. CDK4 pathway activation, p53 inactivation (16, 66, 67), and, more recently, activation of the PI3K pathway (68) have been implicated in melanocytes escaping from senescence. Whether these senescent escape event (s) occur before, concurrently, or after the initiating senescent signal is, as yet, unresolved (69).

The role of both p16^{INK4A} and p53 in overcoming senescence has been addressed in several mouse models. In BRAF^{V600E} transgenic mice, progression from benign nevi to melanoma occurs with loss of both p16^{INK4A} and loss of the senescent phenotype, indicating that p16^{INK4A} is involved. In these mice, the frequency of melanoma is greatly increased when they are crossed with *CDKN2A*-null mice that have loss of both p16^{INK4A} and p19^{ARF} (64). Furthermore, in mice harboring both activated CDK4 and HRAS, there is enhanced susceptibility to melanoma compared with HRAS transgenic mice, and ultraviolet radiation increases the number of lesions and decreases the age of onset (70).

In both humans and mouse models, the loss of p16^{INK4A} is associated with loss of the senescent phenotype, indicating that p16^{INK4A} may play an active role in inducing senescence. Indeed, melanocytes overexpressing p16^{INK4A} undergo a rapid cell-cycle arrest and induction of cellular senescence that is p53-independent but pRB1-dependent (71). Inhibition of this senescence response could be induced by overexpression of either CDK4 or CDK6 but not CDK2, showing that p16^{INK4A} initiates a CDK4/6-dependent senescence program. Inhibition of CDK4/6 with the small-molecule inhibitor palbociclib induces senescence in melanoma cells (26), further supporting a role of the CDK4 pathway in overcoming senescence in melanoma. Recently, several CDK4/6 substrates were identified as possible mediators of senescence, including FOXM1 (26). FOXM1 is involved in regulating cell-cycle progression and the suppression of cellular senescence (72, 73). The CDK4/6-mediated suppression of senescence induced by FOXM1 was independent of pRB1 and due to direct phosphorylation of FOXM1, which increased its activity and stability. Senescence induced by knockdown of FOXM1, however, did not totally recapitulate the effect of the specific CDK4/6 inhibitor (26), indicating that CDK4/6 mediates robust senescence suppression through both pRB1-dependent and -independent mechanisms, which includes FOXM1 activation. The observation that interfering with the CDK4 pathway leads to a robust senescence response opens the option of targeting this pathway and exploiting senescence to induce melanoma regression (74, 75).

There is a potential pitfall in exploiting senescence induction as a therapeutic strategy for the treatment of melanoma: senescence, originally thought to be an irreversible cell state, may be reversible. In cell line models, including melanocytes, manipulating p53, cytokines, cellular metabolism, or the phosphoinositide 3 kinase (PI3K) pathway (69, 76) has been shown to reverse the senescent phenotype. Furthermore, the substantial number of melanocytic nevi contiguous with melanoma also suggests that some melanomas arise from these nevi and there is genetic evidence for this (68, 77). In addition, in a mouse model of BRAF^{V600E}-driven nevi, acute depletion of PTEN in the nevi-induced melanoma (68). Whether the cell of origin from which melanoma arise within a nevus is a fully senescent cell and whether a senescent cell that is not replicating DNA can acquire genetic and/or epigenetic defects to become malignant is still debatable.

Clinical development of CDK4 inhibitors and opportunities for combination with MEK pathway inhibitors in melanoma

Genomic data indicating a central role of dysregulation of the CDK4 pathway in human cancer led to the development of small-molecule inhibitors of the kinase activity of CDK4/6. Early compounds including the flavone flavopiridol lacked selectivity for CDK4 over other CDKs, and, in the case of flavopiridol, the molecule inhibited CDK9/pTEFb and RNA polymerase II transcription (78). Although flavopiridol has been evaluated in phase II studies in melanoma,

where it did not show clinical activity (79), it was not clear to what extent flavopiridol was able to inhibit CDK4 at the phase II dose and schedule. Furthermore, this study did not enrich for patients with genomic events known to activate CDK4. As such flavopiridol has not enabled clear conclusions to be drawn on the role of CDK4 inhibition as a treatment strategy for melanoma.

More recently, a number of more selective CDK4 inhibitors have entered clinical trials. Palbociclib is the most extensively evaluated compound in patients. It is able to inhibit CDK4 as shown by a reduction in RB1 phosphorylation, inhibit uptake of 18F-fluoro-L-thymidine (cell proliferation), and induce clinical responses in patients with mantle cell lymphoma that have cyclin-D1 translocations (15). Dose-limiting toxicity associated with palbociclib was myelosuppression consistent with on-target inhibition (13–15). Other CDK4/6 inhibitors in clinical development include LEE011, LY2835219, and P276-00. All these compounds show good selectivity for CDK4 in preclinical studies (Table 1; refs. 80–83).

Single-agent activity of CDK4-inhibitors is well described in preclinical models of a variety of cancers including melanoma with inhibition of CDK4 leading to cell-cycle arrest, senescence, and/or cell death (26, 82, 84, 85). Activity appears to be greater where genomic changes increase activity of CDK4, most notably with higher activity seen where *CDKN2A* is deleted (86, 87), and resistance is associated with loss of RB1 (85). Intriguingly, preclinical studies indicate that, in some situations, inhibition of CDK4 will not only induce G₁-arrest or senescence but also induce cell death (82, 84, 88). Whether these tumor cells are "addicted" to CDK4 signaling and, thus, CDK4 inhibition is a treatment strategy capable of inducing cell death and tumor regression will ultimately have to be evaluated in clinical trials. Nonetheless, as CDK4 is activated in conjunction with other signaling pathways, and as inhibition of CDK4 should not be assumed to be sufficient to obtain tumor regression and clinical response, there is significant interest in combining CDK4/6 inhibitors with other cancer therapeutics. Preclinical studies have suggested that CDK4 inhibition may reduce the cytotoxicity of chemotherapy by inducing a G₁ arrest (89) and, therefore, greater attention has focused on combinations with inhibitors of other signaling pathways including the RAS/RAF/MEK/ERK pathway in melanoma (90) and estrogen-receptor signaling in breast cancer (86, 91, 92). The recent success of palbociclib in combination with letrozole in ER⁺/HER2⁻ advanced breast cancer supports this idea of combining palbociclib with inhibitors of other signaling pathways and biomarker-based patient selection (92).

Activating mutations in RAS or BRAF clearly activate ERK signaling and the CDK4 pathway, defining CDK4 as a component of the RAS/RAF/MEK/ERK signaling pathway. Therefore, the concept of combining BRAF or MEK inhibitors with CDK4 inhibitors is appealing. Kwong and colleagues have shown synergistic efficacy of the MEK-inhibitor GSK1120212 when combined with the CDK4-inhibitor palbociclib in NRAS-mutant melanoma (90). Furthermore,

Table 1. Activity of CDK4/6 inhibitors in clinical trials

CDK4/6 inhibitor	IC ₅₀						Target inhibition (p-RB)		Clinical trial involving melanoma patients			
	CDK4/D1	CDK6/D3	CDK1/B	CDK2/A	CDK7	CDK9/T1	In vitro	In vivo (xenografts)	Phenotype	Trial	Tumor type	References
PD0332991 (palbociclib)	++++	++++	+	+	+	+	Yes	Yes	G ₁ arrest, senescence apoptosis	- NCT01037790 Phase II (recruiting)	Recurrent and stage IV melanoma	(26, 84, 86)
LY2835219	++++	++++	?	?	?	?	Yes	Yes	G ₁ Arrest	- NCT01394016 Phase I: MTD (recruiting)	Advanced cancer (including melanoma)	(80, 81)
LEE011	++++	++++	+	+	+	+	Yes	Yes	G ₁ arrest, senescence apoptosis	- NCT01237236 Phase I: LEE011 MTD (recruiting)	Advanced solid tumors (including melanoma)	(Sudha Parasuraman, Novartis; personal communication)
										- NCT01781572 Phase Ib & II: LEE011 + MEK162 MTD (recruiting)	NRAS mutant melanoma	
										- NCT01777776 Phase I & II: LEE011 ± LGX818 MTD (recruiting)	BRAF mutant melanoma	
P276-00	++++	+++	++++	+++	++	++++	Yes	Yes	G ₁ -G ₂ arrest, apoptosis	- NCT00835419 Phase II (complete)	Melanoma positive for cyclin D1 expression	(82, 83)
Flavopiridol	++++	++++	++++	++++	+++	+	Yes	NT	G ₁ -G ₂ arrest, apoptosis	- NCT00005971 Phase II (completed; 44% SD, No OR)	Metastatic melanoma	(79, 101)
										- NCT00003690 Phase I carboplatin/ cisplatin/alvociclib + flavopiridol MTD (completed: 34%SD, no OR)	Advanced solid tumors (including melanoma)	

NOTE: IC₅₀s should not be compared between compounds given different kinase assay conditions. +++++ IC₅₀ < 100 nmol/L; +++ IC₅₀ < 500 nmol/L; ++ IC₅₀ < 5 μmol/L; + IC₅₀ > 5 μmol/L.

Abbreviations: ?, Unknown; MTD, maximum tolerated dose; NT, not tested; OR, objective response; P-RB, phosphorylated retinoblastoma protein; SD, stable disease.

decreased clinical benefit from dabrafenib in BRAF-mutant melanoma patients was associated with p16^{INK4A} deletion, indicating combination of a BRAF and CDK4 inhibitor would likely be superior to single-agent dabrafenib treatment (93). Further studies and clinical trials will determine if this approach is more broadly applicable to NRAS- and BRAF-mutant melanoma.

BRAF inhibitor resistance is associated with mechanisms that reactivate ERK signaling (94) and expression of cyclinD1 is increased in a proportion of these cases (95). These data suggest that the combination of a BRAF-inhibitor and CDK4 inhibitor is worthy of assessment in patients with acquired resistance to BRAF-inhibitors or, perhaps, even more appealing is to use the combination of BRAF/MEK and CDK4-inhibition upfront in an attempt to prevent or delay the emergence of resistance.

The concept of combining inhibitors of BRAF or MEK with inhibitors of CDK4 raises the possibility of potential antagonism. As discussed earlier, it is proposed that activation of CDK4 through loss of p16^{INK4A} is the central mechanism to overcome NRAS- or BRAF-mediated oncogene-induced senescence to allow melanomas to develop. Therefore, any reduction in ERK signaling may attenuate the induction of senescence associated with inhibition of CDK4. However, could BRAF/MEK inhibition and CDK4 inhibition be used sequentially and interleaved such that when ERK-pathway inhibition is removed CDK4 inhibition be applied to promote senescence in surviving cells? Given the recent observation that removing BRAF/MEK inhibition may lead to temporary hyperactivation of ERK-signaling (96), this approach might be successful in driving oncogene-induced senescence in advanced BRAF- and NRAS-mutant melanoma. In addition, the potential immunosuppressive effects of CDK4 inhibition (97, 98) need to be considered, especially in light of the recent clinical success of inhibitory T-cell checkpoint blockade in patients with advanced melanoma (99, 100).

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Finally, based on preclinical studies, patients most likely to respond to CDK4 inhibition are those that have increased CDK4 signaling through loss of *CDKN2A*, whereas loss of RB1 would predict resistance. The degree of CDK4 inhibition could be assessed by measuring reduction in phosphorylated RB1, cell proliferation by Ki67 staining, or 18F-fluoro-L-thymidine by PET scans, and cell senescence via β -galactosidase staining. The identification of CDK4-inhibitor-response biomarkers that correlate with clinical benefit will facilitate clinical evaluation of candidate CDK4 inhibitors and dosing regimens as monotherapy or in combination with other therapeutics.

With the introduction of a number of highly selective CDK4/6 inhibitors into clinical trials, the field is now poised to definitively assess whether CDK4, clearly a central mediator of oncogenic signaling in melanoma cells, is a drug target that is able to improve survival and quality of life in patients with melanoma.

Disclosure of Potential Conflicts of Interest

G.A. McArthur received commercial research funding from Pfizer, Novartis, and Millennium. K.E. Sheppard received commercial research funding from Pfizer.

Authors' Contributions

Conception and design: K.E. Sheppard, G.A. McArthur
Development of methodology: G.A. McArthur
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.E. Sheppard, G.A. McArthur
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.E. Sheppard, G.A. McArthur
Writing, review, and/or revision of the manuscript: K.E. Sheppard, G.A. McArthur
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