Review

Clinical Cancer Research

To Cycle or Fight—CDK4/6 Inhibitors at the Crossroads of Anticancer Immunity

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Abstract

Dysregulation of cell division resulting in aberrant cell proliferation is a key hallmark of cancer, making it a rational and important target for innovative anticancer drug development. Three selective cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors are FDA and European Medicines Agency (EMA) approved for hormone receptor–positive/HER2-negative advanced breast cancer. A major emerging appreciation is that these inhibitors not only are cytostatic, but also play critical roles in the interaction between tumor cells and the host immune response. However, to trigger an effective immune response, lymphocytes must also proliferate. This review aims to assimilate our emerging understanding on the role of CDK4/6 inhibitors in cell-cycle control, as well as their biological effect on T cells and other key immune cells, and the confluence of preclinical evidence of augmentation of anticancer immunity by these drugs. We aim to provide a framework for understanding the role of the cell cycle in anticancer immunity, discussing ongoing clinical trials evaluating this concept and challenges for developing rational combinations with immunotherapy.

Introduction

The mammalian cell cycle is a highly organized and regulated process that ensures duplication of genetic material and cell division (1). Key features of this process are cascades of growth-regulatory signals and signaling proteins that monitor genetic integrity. Proliferation depends on progression from the quiescent state (G_0) through four distinct phases: G_1 (the first gap phase), S-phase (DNA synthesis), G_2 (the second gap phase), and M (mitosis)—which is controlled at checkpoints by cyclins and their associated cyclin-dependent kinases (CDK; ref. 2). CDKs 4 and 6 (CDK4/6) are fundamental drivers of the cell cycle and required for entry into, and progression through, G1.

Unsurprisingly, this intricate process is disrupted in most cancers (3), either as a result of mutations in upstream signaling pathways or by defects in genes encoding cell-cycle proteins (reviewed in ref. 4). Specific inhibitors of CDK4/6 have been touted as paradigm-shifting with recent FDA and European Medicines Agency (EMA) approval for three orally available inhibitors—palbociclib (PD-0332991; Ibrance; Pfizer), ribociclib (LEE011; Kisqali; Novartis), and abemaciclib (LY2835219; Verzenio; Lilly; refs. 5–7). In contrast to traditional chemotherapeutic agents, CDK4/6 inhibitors arrest progression through G_1, promoting transient quiescence or inducing senescence, and have shown significant clinical benefit in combination with aromatase inhibitors; the selective estrogen receptor (ER) degrader fulvestrant; and tamoxifen (8).

Translational outputs from these ongoing trials have unexpectedly revealed effects of CDK4/6 inhibitors in several critical roles underpinning the interactions of cancer cells with the host immune system (9–11). The cell-cycle cascade couples two processes that are required for the generation of an effective adaptive immune response: clonal expansion and differentiation, and consequently, CDK inhibitors have the potential to participate in the decision between tolerance, anergy, and the promotion of antitumor immunity.

In this review, we discuss the biological functions of the CDK4–CDK6–Retinoblastoma (CDK4/6–Rb) axis—both when pathologically hijacked in cancer and physiologically in immune cells, with a view to providing a framework for understanding the role of the cell cycle in anticancer immunity. We discuss emerging preclinical and clinical data showing effects of CDK4/6 inhibition on promoting various aspects of antitumor immunity including enhancing antigen presentation, depleting immunosuppressive regulatory T cells, and ultimately shifting the balance toward the generation of an efficient antitumor immune response. We also ponder the challenges faced by ongoing clinical trials attempting to therapeutically target these together with immunotherapy.

The Biology of the Cyclin D–CDK4/6–Rb Axis

Numerous detailed reviews of this pathway are available (4, 12). This review will therefore focus on the major principles of this axis. Quiescent cells in G_0 can be triggered to re-enter the cell cycle through stimulation by a variety of mitogenic factors that activate intricate intracellular signaling networks that are "*sensed*" by the holoenzyme complex of Cyclin D and CDK4 and/or CDK6 (ref. 4; Fig. 1). Evolutionarily highly conserved, there are three mammalian Cyclin Ds that have overlapping functions in a cell-lineage–specific manner. These allosterically bind to and regulate CDK4/6—two highly homologous serine/threonine kinases that have unique functions that are cell-type specific as well as being tightly developmentally and temporally regulated (13, 14). CDK6 is expressed at high levels in hematopoietic cells (14), and *Cdk6* deficiency is characterized by subtle defects in the hematopoietic system, such as defects in thymocyte development (13, 15). CDK6 is also a more robust kinase (as compared with CDK4) with

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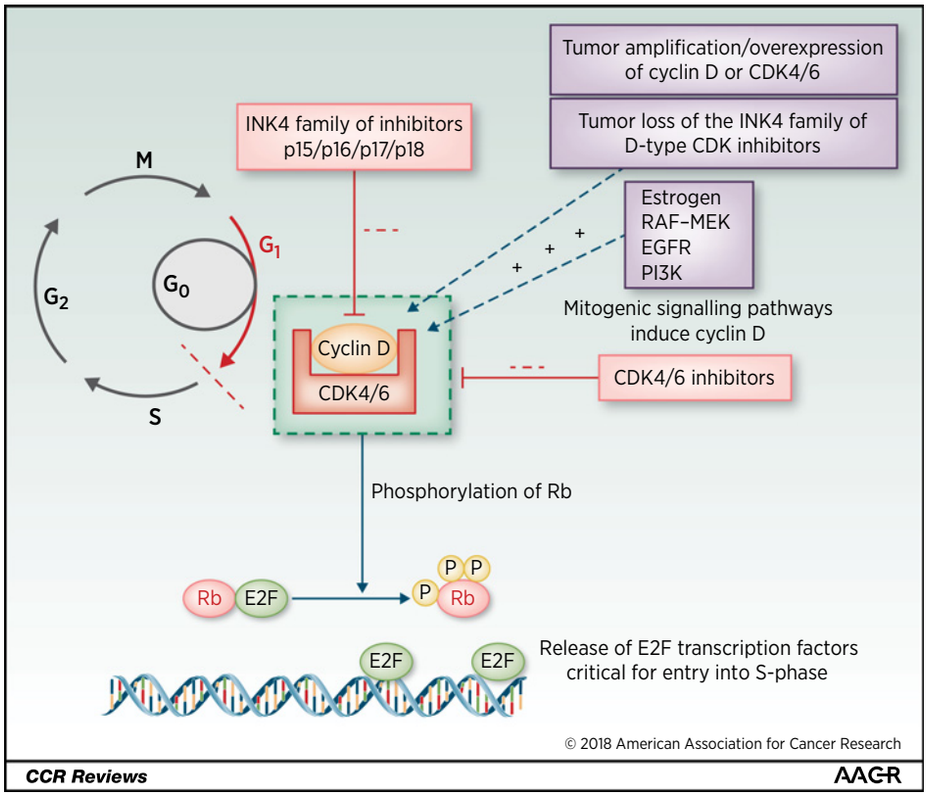

Figure 1.

Regulation and functions of cyclin D–CDK4/6 kinases. The Cyclin D–CDK4/6 holoenzyme complex (green boxe) acts as an environmental sensor responding dynamically to mitogenic signals (e.g., estrogen, Ras–Raf–MEK, EGFR, and PI3K signaling pathways), cytokines, and other cues. Upon stimulation, D-type cyclins accumulate in early G_1 phase through both transcriptional and posttranscriptional mechanisms. The activated cyclin D–CDK4/6 complexes initiate the phosphorylation of pRb releasing E2F transcription factors, thereby driving the expression of genes required for cellular commitment to enter S-phase and ultimately mitotic cell division. Growth-inhibitory signals antagonize G_1–S progression by upregulating CDK inhibitors of the INK4 family ($p16^{INK4A}$, $p15^{INKB}$, $p18^{INK4C}$, and $p19^{INK4D}$). Amplification of the *CCND1* gene encoding for Cyclin D or its overexpression, loss of stoichiometric inhibitors of cyclin D–CDK4/6 (members of the INK4A family), or the loss of Rb in tumors aberrantly activate the Cyclin D–CDK4/6 complex, thereby driving dysregulated cell-cycle progression. Depending on the cell type, and other mitogenic transforming signals, Rb-positive cells undergo either quiescence or senescence when treated with CDK4/6 inhibitors. In contrast, cells without functional Rb are refractory to arrest by chemical inhibitors of CDK4/6.

distinct affinities for specific modulatory client proteins, and together, these two Cyclin D–dependent CDKs allow for the creation of a network of finely tuned interactions to regulate cell-cycle progression (16). Activation of the Cyclin D–CDK4/6 complex promotes progression from the G_1 phase into S-phase by phosphorylating several cellular targets, of which the Rb protein is key (17). Rb phosphorylation attenuates its inhibition of transcription by the E2F family of transcription factors, leading to the commitment of the cell to DNA replication and progression through the cell cycle (18).

The Role of the Cyclin D–CDK4/6–Rb Pathway in Cancer

In cancer, multiple components of the CDK4/6–Rb axis are commonly dysregulated (19). The Cyclin D1 gene (*CCND1*) represents the second most frequently amplified locus among all human cancer types (20), with the highest prevalence in well-differentiated and dedifferentiated liposarcoma (21), glioblastomas (22), breast cancer (23–25), non–small cell lung cancer (NSCLC), endometrial cancers, and pancreatic cancers (23). Copy-number variation or overexpression in at least one component of the cyclin D–CDK4/6 pathway is also common and seen in up to 75% of melanoma (26) and gliomas (27). Loss of the negative regulators of the pathway either by genomic deletions, loss-of-function point mutations, or promoter methylation are also frequent, with $p16^{INK4A}$ most commonly lost in breast cancers (28) and head and neck cancers (29). However, other than in mantle cell lymphoma, which is defined by a translocation involving *CCND1* resulting in cyclin D1 overexpression (30), mutations in genes encoding for the pathway are less common than copy-number changes (31).

Transcription of Cyclin D and its assembly with CDK4/6 is highly dependent on mitogenic signaling and is therefore an important mechanism of Cyclin D–CDK4/6 upregulation in cancer (31). ER signaling upregulates cyclin D1 levels as well as other signaling pathways, which largely culminate in the upregulation of CDK4/6 activity (6, 32, 33). Other upstream oncogenic signal transduction pathways including the PI3K–AKT–mTOR, wnt/β-catenin, MAPK, and NF-κB pathways also significantly lead to the induction of cell-cycle proteins, and D-type Cyclins in particular (6, 34).

Single-agent CDK4/6 inhibitors *in vitro* are fundamentally cytostatic, causing downregulation of E2F target genes, loss of proliferation markers, and cell-cycle arrest in G_1 (35). It can therefore be hypothesized that cancer cells that are addicted to mitogenic signaling pathways and have functional Rb are strongly dependent on Cyclin D–CDK4/6 and thereby more vulnerable to CDK4/6 inhibition; this has been elegantly demonstrated *in vitro* (36). However, only modest clinical benefit has been reported in the various unselected early-phase trials of single-agent CDK4/6 monotherapy including in NSCLC, glioblastoma, melanoma, colorectal, and ovarian cancers as well as in mantle cell lymphoma and advanced liposarcoma. One exception to this is abemaciclib (which at clinically efficacious doses also inhibits CDK9) and has shown potentially useful single-agent activity in breast cancer leading to licensing as monotherapy in previously treated advanced breast cancer patients (37). Furthermore, the randomized JUNIPER study (NCT02152631) will evaluate its monotherapy efficacy in NSCLC.

Given the striking dependence of activated Cyclin D–CDK4/6 complex on mitogenic signals, there has been substantial work developing synergistic combinations of signal transduction inhibitors together with CDK4/6 inhibitors. The most advanced combinations are those in with endocrine therapies in estrogen-positive breast cancer, which led to the first FDA approvals. The pivotal phase II PALOMA-1 (NCT 00721409) study randomized postmenopausal women with advanced $ER^+/HER2^-$ breast cancer to either letrozole, an aromatase inhibitor that prevents estrogen induction of Cyclin D, in combination with palbociclib, or letrozole alone (38). The significant improvement of progression-free survival in the combination arm (20.2 months vs. 10.2 months for letrozole alone; HR, 0.48; $P < 0.001$) led to early provisional FDA approval of palbociclib. Subsequent larger phase III trials have not only confirmed these results (7, 39, 40), but also extended the proof of principle of synergy in combining other CDK4/6 inhibitors with the selective ER degrader, fulvestrant (41, 42), or the antiestrogen, tamoxifen (40, 43).

The concept that combinatorial therapy with signal transduction inhibitors will amplify the effectiveness of a CDK4/6 inhibitor is now being extended to other mitogenic pathways and other tumor types. For example, preclinical evidence to suggest CDK4/6 inhibitors enhance the effect of RAS–RAF–MEK pathway inhibition in RAS-driven NSCLC (44) and RAS/RAF-resistant malignant melanoma (45) has led to early-phase trials of combination therapy in KRAS-mutant NSCLC (NCT02022982) and NRAS- and BRAF-mutant melanoma (46, 47). Hyperactivation of the PI3K pathway has also been shown to stabilize the Cyclin D protein and the Cyclin D–CDK4/6 complex (48), and CDK4/6 inhibitors have been shown preclinically to sensitize *PIK3CA*-mutant breast cancer to PI3K inhibitors (49). Triplet combinations of CDK4/6 inhibitors together with hormone therapies and PI3K inhibitors are also ongoing in breast cancer (NCT03006172; refs. 50–52).

One really interesting observation that may be pivotal is that in addition to blocking cell proliferation, CDK4/6 inhibitors can induce senescence—an irreversible distinct cellular state characterized by the absence of proliferation markers, expression of tumor-suppressor genes, senescence-associated beta-galactosidase activity, and the presence of senescence-associated heterochromatin foci in multiple Rb-proficient cell lines (53, 54). The decision whether to transition from quiescence into senescence is the subject of much ongoing work, and the outcome appears to cell-type specific with downregulation of MDM2, redistribution of the chromatin-remodeling enzyme ATRX, repressions of oncogenes as well as upregulation of proteasomal homeostasis necessary for the shift to senescence (55–57). Senescent cells secrete a collection of inflammatory cytokines, chemokines, and proteinases, collectively referred to as the senescence-associated secretory phenotype (SASP) which recruits and activates distinct cells from the innate and adaptive immune system, such as macrophages and natural killer cells as well as T cells (58, 59). The SASP is one of the most profound features of senescence with the triggering of immune cell recruitment into the tumor (60, 61), although on the other hand, there are concerns that the inflammatory environment chronically stimulated by SASP could be protumorigenic (58).

This raises several obvious questions about the clinical effect of these inhibitors on host immune cells, and whether this would hinder, or could be leveraged for combination therapies. In the following sections, we review the role of Cyclin D–CDK4/6 in immune cell expansion and differentiation, together with the emerging learnings from the translations studies of CDK4/6 inhibitors.

The Role of the Cyclin D–CDK4/6–Rb Pathway in Immune Cell Biology

Mouse models provided the first clues to the physiologic roles of Cdk4 and Cdk6 *in vivo*, particularly with respect to the immune cell types that critically depend upon the Cyclin D–CDK4/6 pathway during development. Double-mutant mice lacking both Cdk4 and Cdk6 (*Cdk4/6*$^{-/-}$ mice) display late embryonic lethality accompanied by a defect in fetal hematopoiesis very similar to the phenotype observed in the triple *D1/2/3-cyclins*$^{-/-}$ mice, including multilineage hematopoietic abnormalities (13, 62).

Myeloid lineage

Myeloid cell development in preclinical models is entirely reliant on Cyclin D2– and Cyclin D3–driven CDK6 (13, 63), and all myeloid progenitor cells' populations were also severely reduced in *Cdk4/6*$^{-/-}$ double-mutant mice (13).

Not unsurprisingly, neutropenia has been the dose-limiting toxicity of both palbociclib and ribociclib, necessitating intermittent dosing schedules (64, 65). Abemaciclib, being a more potent inhibitor of CDK4 (as well as inhibiting CDK9 at clinically efficacious doses), seems distinct and causes much lower rates of neutropenia (Table 1) and can be dosed on a continuous schedule (66). An ongoing study of palbociclib is investigating whether a continuous dosing schedule (at 100 mg/day) is as effective and tolerable as the approved intermittent dosing schedule (NCT02630693). Clinical data on the changes in other myeloid cell subpopulations are however scarce at this time, chiefly as multiparameter data analysis of circulating immune cells or immune cells within the tumor microenvironment was not collected in the initial trials.

Lymphoid lineage

Following antigen exposure, quiescent lymphocytes require intense, prolonged, and repeated proliferation to establish a rapid immune response and generate immunologic memory. Upon stimulation, T cells exit G_0 via an NF-κB–dependent pathway (67). Cyclin D is expressed in cells during G_1, with significant upregulation of Cyclin D2 and Cyclin D3 and CDK6 during early and late G_1 (refs. 68, 69; Fig. 2). Deletion of specific cyclins and CDKs in mice has identified CDK6 and Cyclin D3 to be the key players in hematopoietic stem cells' regulation, their proliferation, and subsequent commitment to

Table 1. Myelosuppression seen in the initial early-phase trials of single-agent CDK4/6 inhibitors

		Palbociclib		Ribociclib		Abemaciclib	
IC_{50}	**CDK4**	9–11 nmol/L		10 nmol/L		2 nmol/L	
	CDK6	15 nmol/L		39 nmol/L		5 nmol/L	
Dosing		125 mg daily 3 weeks on, 1 week off		600 mg daily 3 weeks on, 1 week off		200 mg daily continuously	
Effects on immune cells		**All grades**	**$G_3/4$**	**All grades**	**$G_3/4$**	**All grades**	**$G_3/4$**
	Neutropenia	95%	**54%**[a]	46%	**29%**[a]	23%	10%
	Leukopenia	68%	23%	48%	21%	25%	10%
Trial ID		NCT00141297		NCT01237236		NCT01394016	

[a]Indicates dose-limiting toxicity.

the T-cell lineage (13, 15, 70–72). Loss of CDK6 leads to delayed G_1 progression in lymphocytes, but critically, once a cell is committed to proliferation, other Cyclin-CDKs, particularly Cyclin E and CDK2, appear to compensate. As such, despite CDK6-mutant mice having lower numbers of thymocytes early on in development, they have normal/higher than normal levels of $CD4^+/CD8^+$ cells later in development (63). This is very consistent with the modest reductions in total lymphocyte numbers seen clinically with the unique differential potency of inhibition of abemaciclib likely to be responsible for the lack of appreciable leukopenia reported clinically for abemaciclib (Table 1; ref. 66).

Commitment to specific cell fates and differentiation with transcriptional activation of specific gene expression programs are predominantly directed by CDK2 (73), and as such *in vitro* differentiation of naïve $CD4^+$ and $CD8^+$ T cells is not affected by CDK4/6 inhibitors (10). Specific T-cell subpopulations however may be much more selectively suppressed by CDK4/6 inhibition as suggested by flow cytometry analysis of circulating immune cells in preclinical models. In tumor-free mice, both abemaciclib and

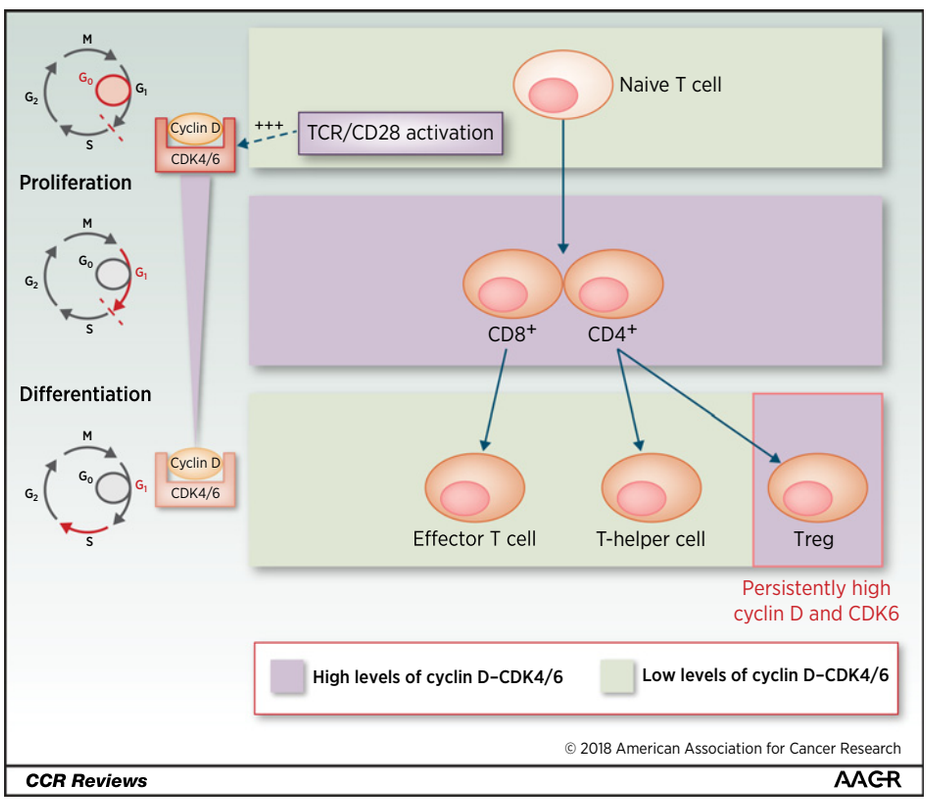

Figure 2.
Cyclin D–CDK4/6 in T-cell activation, expansion, and differentiation. The figure shows relative levels of CDK4/6 and D-type cyclins at the various stages of the cell cycle as naïve T cells respond to antigen stimulation, enter the cell cycle, and undergo clonal expansion followed by maturation and subsequent differentiation. Lilac boxes highlight cells with high levels of CDK4/6 and D-type cyclins, whereas light green indicates cells with low levels. Stimulation of the T-cell receptor (TCR) together with costimulatory signals (e.g., CD28) leads to the induction of a number of cell-cycle activators, including CDK4/6 and D-type cyclins, which set off a signaling cascade permitting progression through the G_1 phase of the cell cycle. Subsequent progression through S-phase is accompanied by downregulation of both CDK4/6 and D-type cyclins in an oscillating manner as cells undergo repeated cycles of cell division. As cells differentiate, Cyclin D remains low in the majority of T cells, with the exception of regulatory T cells (Treg), which retain high expression of both Cyclin D and CDK4/6 (74, 75). As such, although therapeutic targeting of CDK4/6 can *theoretically* slow T-cell proliferation, *in vivo* it has a preferential effect of promoting cell differentiation while specifically depleting Tregs (9, 10).

palbociclib significantly reduced FOXP3$^+$ regulatory T cells (10) without affecting other cell subtypes, and this may relate to the higher levels of both Cyclin D and CDK4/6 (74, 75) or Rb1 present in these cells (76). Abemaciclib may also have additional epigenetic effects by selectively inhibiting the enzyme DNMT1 in regulatory T cells, resulting in overexpression of the negative regulator p21 (10). The effects of CDK4/6 inhibition on tumor-infiltrating lymphocytes may be more complex with both palbociclib and trilaciclib causing increased infiltration of T cells into lung tumors in an immunocompetent genetically engineered mouse model (GEMM). In this model, absolute numbers of CD4$^+$ and CD8$^+$ cells were unchanged, but proliferation of tumor-infiltrating FOXP3$^+$ regulatory cells as well as immunosuppressive myeloid cells were significantly reduced, resulting in an increased percentage of effector cells within the tumor microenvironment (9).

In summary, the data thus far reveal that although therapeutic targeting of CDK4/6 can theoretically slow T-cell proliferation, *in vivo* it has a preferential effect of promoting cell differentiation while specifically depleting regulatory T cells (refs. 9, 10; Fig. 2).

Effects of CDK4/6 Inhibition on the Tumor Microenvironment and the Tumor–Host Immune Reaction

Enhancing immune cell infiltration into tumor

Cell-cycle arrest and the induction of senescence lead to the activation of the SASP in a subset of cancer cells which can induce the recruitment of innate immune cells including macrophages, neutrophils, and natural killer cells into the tumor microenvironment where they are provoked into coordinately attacking tumors through both phagocytosis and direct cytotoxic killing (61, 66). The challenge remains in understanding how tumor cells are directed toward either reversible quiescence or a more stable senescence, as preliminary work in a small subset of abemaciclib-sensitive breast cancer cell lines suggests that genes encoding for the canonical SASP cytokines were not shown to be upregulated in the cell lines tested (11).

Enhancing antigen presentation

The initial studies suggesting links between CDK4/6 inhibition and the immune system came from an Rb-proficient transgenic mouse model of breast cancer (10). Treatment with abemaciclib caused not only cell stasis but also a significant decrease in tumor volume and reduced cell proliferation (10). Gene expression analysis has shown that in addition to downregulating genes related to cell cycle, mitotic, and E2F targets, abemaciclib also significantly upregulates genes responsible for antigen processing and presentation including MHC class I molecules (10). This was confirmed *in vitro* as well as in patient-derived xenografts. Strikingly, tumor cells treated with CDK4/6 inhibitors show a marked reduction of *DNMT1*, which decreases DNA methylation of genes that regulate immune function as well as endogenous retroviral genes. Expression of double-stranded RNA triggers "viral mimicry" stimulating the production of an IFN response (ref. 10; Fig. 3).

Further support of this model comes from data from The Cancer Genome Atlas showing that breast cancers harboring *CCND1* amplification (therefore enhanced CDK4/6 activity) display significantly lower expression of MHC class I molecules *HLA-A*, *HLA-B*, and *HLA-C* than nonamplified tumors (10).

Effects on cytokine milieu in the tumor microenvironment

In addition, Deng and colleagues used a small-molecule screen designed to identify targets that enhanced T-cell activity in the setting of PD-1 engagement and found that CDK4/6 inhibitors potently upregulated IL2 (9). Using siRNAs, they confirmed that CDK6, not CDK4, was responsible for the enhanced IL2 secretion supporting a predominant role for CDK6 in immune cell function. Careful dissection of the mechanistic basis of this effect found that CDK6 was an upstream regulator of Nuclear Factor of

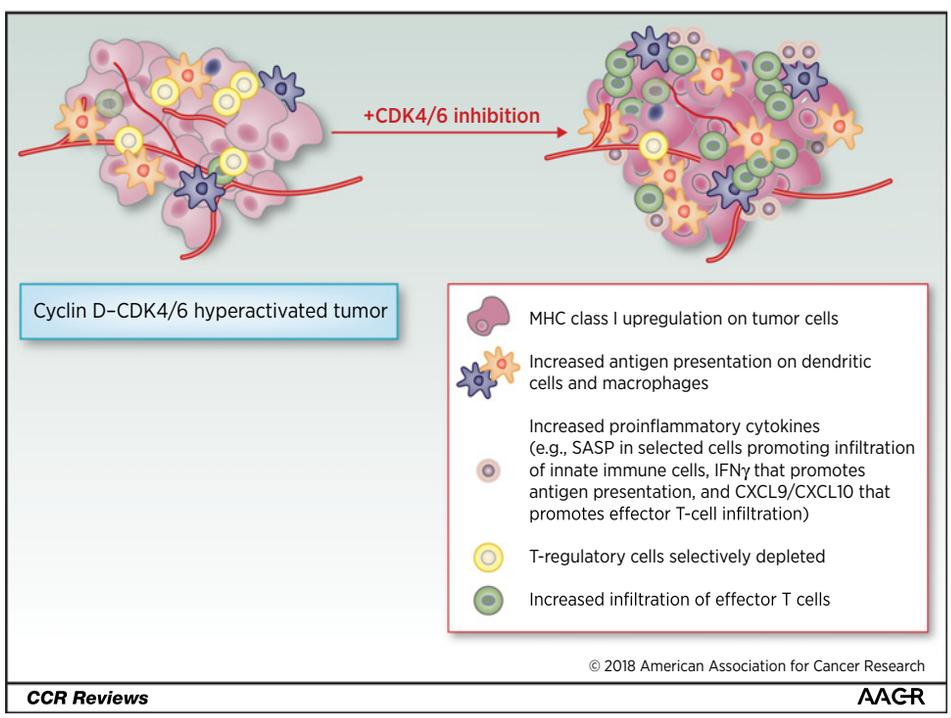

Figure 3.

To cycle or fight. Tumors with hyperactivation of the Cyclin D–CDK4/6 axis aberrantly progress through the cell cycle and effectively *hide* from the host immune system through multiple mechanisms including downregulation of MHC class I molecules. Treatment with CDK4/6 inhibitors both arrests cell cycle and promotes a "fight" mode, promoting antitumor immunity by stimulating antigen presentation through (i) the upregulation of MHC class I expression within tumors and (ii) the increase in proinflammatory cytokine secretion (e.g., IFNγ) either via inducing the SASP or other mechanism resulting in activation of dendritic cells and macrophages. CDK4/6 inhibitors also selectively deplete immunosuppressive regulatory T cells and change the cytokine milieu within the tumor microenvironment, thereby increasing effector T-cell infiltration into the tumor (9–11).

Activated T cells (NFAT) proteins which are critical in regulation of T-cell activation and function. CDK4/6 inhibition resulted in increased nuclear levels of NFAT and increased transcriptional activity ultimately resulting in a change in cytokine milieu within the tumor microenvironment and increased effector T-cell activity (9, 11). Levels of IL6, IL10, and IL23, three cytokines produced by immunosuppressive myeloid cells, were significantly reduced, whereas an increase of the Th1 chemokines CXCL9 and CXCL10 which govern the trafficking of effector cells to tumor sites was seen (9, 77).

Effects on PD-L1 and other coinhibitor molecule expression

PD-L1 protein abundance fluctuates during cell-cycle progression in multiple human cancer cell lines, peaking in M and early G_1, with a sharp reduction in latter stages of the cell cycle. This is tightly regulated by Cyclin D–CDK4-mediated phosphorylation of the speckle-type POZ protein, a core component of the Cullin3-SPOP E3 ligase responsible for the proteasomal degradation of PD-L1 (78). Inhibition of CDK4/6 in this single article increases PD-L1 expression, but only in SPOP-proficient cancer cells. The story is far from complete though, as effects of CDK4/6 inhibition on the expression of other coinhibitory molecules, particularly on immune cells, are likely to be complex. For example in the GEMM mouse model used by Deng and colleagues, levels of the coinhibitory molecular PD-1 and CTLA-4 were reduced in both $CD4^+$ and $CD8^+$ T cells after CDK4/6 inhibition (9).

Taken together, these studies illustrate the complex connection between immunity and cell-cycle regulation and constitute an exciting new area of research, which is likely to lead to significant anticancer therapeutic opportunities, and pharmacodynamic and translational outputs from the ongoing clinical trials are eagerly awaited. Combining CDK4/6 inhibitors together with immune checkpoint inhibitors enhances tumor regression in a number of immunocompetent preclinical mice models (9, 78). These effects seem to be at least in part to be tumor-intrinsic, as most potent upregulation of the antigen-processing machinery at a gene expression level occurred in CDK4/6-sensitive cell lines (10). In addition, there are hints that as cancers evolve and undergo immune-editing, thus becoming more immune-refractory, they may be increasingly dependent on Cyclin D–CDK4/6. Oh and colleagues studied a highly immune-refractory cancer and found that synaptonemal complex protein 3 (SCP3) is overexpressed in immune-edited cancer cells and upregulates the pluripotency transcriptional factor NANOG by hyperactivation of the Cyclin D–CDK4/6 axis. In this model, the combination of palbociclib together with adoptive cytotoxic cell transfer showed considerable therapeutic efficacy, suggesting a niche role for CDK4/6 inhibitors in immunotherapy combinations in the resistant/refractory setting (79).

Challenges for the Future

The ongoing clinical trials testing combinations of CDK4/6 inhibitors with immune checkpoint inhibitors are listed in Table 2, but a few specific challenges in combining these are worth exploring. Understanding the temporal kinetics of pharmacodynamics effects of CDK4/6 inhibitors on the tumor microenvironment and the immune system would be key to optimizing sequencing of any combinations. Schaer and colleagues looked at the differences in antitumor responses when anti–PD-L1 therapy was given either concurrently, sequentially (after completion of CDK4/6 inhibitor), or in a phased (initiated after 1 week of CDK4/6 inhibitor) manner with abemaciclib (11). Surprisingly, concurrent administration of abemaciclib with immune checkpoint inhibitors showed no significant difference in the antitumor response compared with monotherapy. Sequential treatment was additive, but the phased regime was significantly synergistic, with complete responses seen in 2 of 10 mice (11) highlighting the importance of understanding the biology to direct scheduling of combinations. Furthermore, they analyzed the effect of transient versus continuous exposure to abemaciclib on primary T cells during T-cell receptor (TCR)–mediated expansion and found that the greatest effect in upregulating genes indicated of T-cell activation was seen with the continuous exposure. This may be pertinent when other CDK4/6 inhibitors that are given intermittently are considered for combinations. Moving forward, it will be absolutely imperative that proof-of-mechanism pharmacodynamics studies utilizing paired tumor biopsies as well as in-depth analyses of host immune responses be incorporated into these trials to maximize patient benefit.

Biomarker-driven patient selection is also likely to direct these combinations to the patients who are most likely to derive benefit; for example, if loss of MHC I expression or markers of T-cell exhaustion are seen, combinations of phased in pretreatment with CDK4/6 inhibitors given with immunotherapy may be therapeutically beneficial. Paraphrasing Shakespeare, much

Table 2. Ongoing clinical trials combining CDK4/6 inhibitors with immune checkpoint inhibitors

Trial name/ID	Phase	Patient population	Combination agents	Primary objective
Ribociclib + PDR001 in Breast Cancer and Ovarian Cancer/NCT03294694	I	HR^+HER2^- breast cancer Epithelial ovarian cancer	Ribociclib PDR001–PD-1 inhibitor Fulvestrant	MTD/RP2D
PACT/NCT02791334	I	Solid tumor Microsatellite instability-high (MSI-H) solid tumors Cutaneous melanoma Pancreatic cancer Breast cancer (HR^+HER2^-)	LY3300054—PD-1 Inhibitor Ramucirumab Abemaciclib Merestinib	DLT
A Study of Abemaciclib (LY2835219) in Participants With Non-Small Cell Lung Cancer or Breast Cancer/NCT02779751	I	NSCLC HR^+HER2^- breast cancer	Abemaciclib Pembrolizumab	AEs
PAVEMENT—A phase Ib study of palbociclib and avelumab in metastatic AR^+ triple-negative breast cancer (NCT pending)	Ib	Androgen-receptor–positive triple-negative breast cancer	Palbociclib Avelumab	R2PD

Abbreviations: AEs, adverse events; DLT, dose-limiting toxicity; RP2D, recommended phase II dose.

more remains to be learned about how a cell decides whether to cycle or to fight, and future work will reveal if the promise of combining CDK4/6 inhibitors with immunotherapy will be realized and validated.

Disclosure of Potential Conflicts of Interest

A.F.C. Okines reports receiving commercial research grants from Pfizer and speakers bureau honoraria from Roche. J.S. Lopez is a consultant/advisory board member for Basilea, Eisai, Genmab, Merck, and Novartis. No potential conflicts of interest were disclosed by the other authors.

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