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INVITED REVIEW

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The role of CDK6 in cancer

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Abstract

The regulation and function of cyclin-dependent kinase 6 (CDK6)- and cyclin-dependent kinase 4 (CDK4)-cyclin complexes are commonly altered with enhanced kinase activity found in hematopoietic malignancies, breast cancer and melanoma making CDK4 and CDK6 attractive targets for therapeutic interference. Although dual CDK4/6 inhibitors have revolutionized treatment of breast cancer patients and reveal promising results in several solid tumors and hematological malignancies, there is a need for novel compounds targeting the versatile kinase-independent functions of CDK6 to improve cancer treatment. The following review summarizes the latest findings on CDK6 in cancer development and discusses novel therapeutic approaches to selectively inhibit CDK6s function as a transcriptional regulator.

KEYWORDS
cancer, CDK4, CDK4/6 inhibitors, CDK6

1 | INTRODUCTION

The family of cyclin-dependent kinases (CDKs) covers 13 different serine/threonine kinases that become catalytically active when bound to their respective regulatory subunits, the cyclins. CDKs, in complex with cyclins, regulate various critical cellular processes including cell-cycle progression as well as transcription. Disordered cell cycle regulation, due to aberrant kinase activation, often leads to uncontrolled cell proliferation and results in cancer development. The importance of CDKs in promoting cancer initiation as well as progression has made them an attractive target for pharmacological inhibition.[1]

The present review gives an overview of the current knowledge on the versatile regulatory functions of CDK6 as a cell cycle-dependent kinase and transcriptional regulator under homeostatic conditions as well as in malignant transformed settings. (Figure 1) In particular, the role of CDK6 in normal hematopoiesis, hematologic malignancies, breast cancer and melanoma as well as current and novel therapeutic approaches for targeting CDK6 will be discussed.

2 | REGULATION OF CDK6

CDK6 and the highly homologous enzyme CDK4 are known as classic cell cycle kinases that facilitate the progression of cells through the early G1 phase of the cell cycle by forming complexes with D-type cyclins (D1, D2 and D3). CDK4/6-cyclin D complexes enable the

Abbreviations: AML, acute myeloid leukemia; ATP, adenosine triphosphate; AURK, aurora kinase; BCR/ABL, break point cluster region/Abelson; Cdc37, cell division cycle 37; CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; CDKN2A, cyclin-dependent kinase inhibitor 2A; CDKs, cyclin-dependent kinases; CK2, casein kinase 2; CKIs, CDK inhibitors; DN, double-negative; DNMT1, DNA methyltransferase 1; Egr1, early growth response protein 1; ERV, endogenous retroviral elements; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; FDA, Food and Drug Administration; FLT3, fms-related tyrosine kinase 3; FOXM1, forkhead box M1; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; HSCs, hematopoietic stem cells; Hsp90, heat shock protein 90; IFN, interferon; IL2, interleukin 2; LSCs, leukemic stem cells; LSK, Lin-Sca-1 + c-Kit+; Mdm4, transformed mouse 3 T3 cell double minute 4; MDS, myelodysplastic syndrome; MEK, mitogen-activated protein kinase; MLL, mixed-lineage leukemia; NFAT, nuclear factor of activated T-cells; NRTKs, nonreceptor tyrosine kinases; NSCLC, nonsmall cell lung cancer; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; PFS, prolonged progression-free survival; Ph+, Philadelphia-positive; PP5, serine/threonine phosphatase protein phosphatase 5; Ppm1d, protein phosphatase 1D magnesium-dependent, delta isoform; Prmt5, protein arginine methyltransferase 5; PROTACs, proteolysis-targeting chimeras; RB, retinoblastoma; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; SPOP, cullin3-speckle-type POZ protein; STAT3, signal transducer and activator of transcription 3; T-ALL, T-cell acute lymphoblastic leukemia; T-LBL, T-cell lymphoblastic lymphoma; TReg, regulatory T cells; TP53, tumor protein 53; VEGFA, vascular endothelial growth factor A.

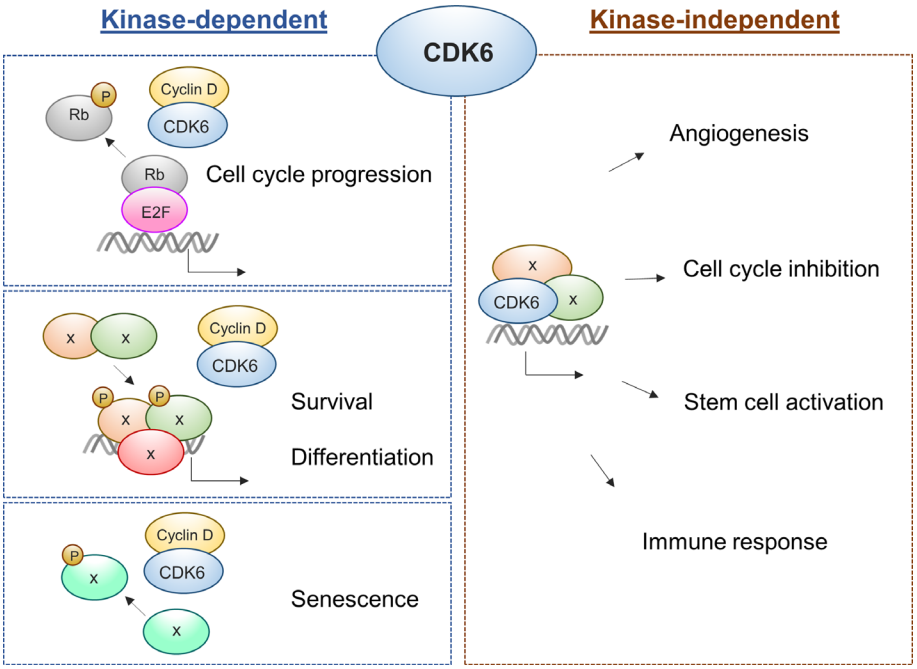

FIGURE 1 Schematic overview of CDK6-mediated kinase-dependent and kinase-independent functions in cancer. Cyclin-dependent kinase 6 (CDK6) acts as a chromatin-bound cofactor that in a kinase-independent manner induces transcription of genes regulating angiogenesis, cell cycle inhibition, stem cell activation and immune response. The function of CDK6 in cell cycle progression, survival, differentiation and senescence requires binding to D-type cyclin to enable kinase-dependent protein phosphorylation

phosphorylation of members of the retinoblastoma (RB) protein family resulting in the release of E2F transcription factors from RB-mediated inhibition. (Figure 1) Subsequently, E2F-dependent gene activation enables G1 to S phase progression and DNA synthesis.[2-4] The activation and inhibition of CDK4/6-cyclin D complexes are controlled by two distinct classes of regulatory subunits, the Cip/Kip family, comprising $p21^{Cip1}$ (CDKN1A), $p27^{Kip1}$ (CDKN1B) and $p57^{Kip2}$ (CDKN1C), and the INK4 family, including $p15^{INK4b}$ (CDKN2B), $p16^{INK4a}$ (CDKN2A), $p18^{INK4c}$ (CDKN2C) and $p19^{INK4d}$ (CDKN2D). Cip/Kip family members are able to act on a broader spectrum of CDK-cyclin complexes, to inhibit CDKs 1, 2, 4 and 6, whereas INK4 proteins exclusively inactivate CDK4/6-cyclin D complexes.[5-8]

Proteins of the Cip/Kip family are defined as double-faced regulators based on their ability to operate as both positive and negative regulators of the cell cycle, depending on their phosphorylation status.[6,9] Nonreceptor tyrosine kinases (NRTKs) phosphorylate Cip/Kip proteins being part of a trimeric holoenzyme together with CDK4/6 and Cyclin D. Phosphorylation of the Cip/Kip subunit subsequently adapts the mode of action from an inhibitor to a noninhibitor.[6] Binding of the Cip/Kip proteins to the CDK4/6-containing complex sequesters them from other CDKs, such as CDK2, and thus prevents them from inhibiting. This enables the activation of CDK-cyclin complexes that act later during cell cycle progression.[4,10,11] The importance of Cip/Kip proteins as regulatory subunits is underscored by their versatile function contributing to assembly, stabilization and nuclear translocation of CDK4/6-cyclin D complexes.[5,9,11] Further studies are needed to fully appreciate the potential of Cip/Kip proteins in terms of CDK regulation.

In contrast to the Cip/Kip family members, INK4 proteins specifically associate with monomeric CDK4 and CDK6, forming a catalytically inactive dimeric complex. Although the INK binding site of CDKs is distant from the cyclin interaction site, INK binding alters the position of the N and C lobes of CDK4 and CDK6. This structural turn distorts the catalytic cleft, leads to allosteric changes in the cyclin binding site and subsequently weakens the CDK-cyclin interaction.[8,12] Consequently, in the absence of D-type cyclin binding, the INK4-CDK4/6 complex lacks kinase activity.

In addition to the presence of INK4 proteins in a dimeric complex, binding of INK4 proteins to a preassembled ternary holoenzyme, consisting of CDK4/6-cyclin D-Cip/Kip, has been shown.

Thereby INK4 proteins propagate conformational changes, lead to disassembly and consequently inactivation of the ternary CDK4/6-cyclin D-Cip/Kip holoenzyme.[8,11-13]

In the absence of their cognate binding partners, it has been suggested, that CDK6, as well as CDK4, tend to form larger protein complexes consisting of heat shock protein 90 (Hsp90) and its co-chaperone cell division cycle 37 (Cdc37).[14] Hsp90 is a highly conserved molecular chaperone protein that enables accurate folding, maturation, assembly and stability of a large number of proteins. It has been proposed that the absence of any interaction partner such as cyclins provokes an open and less folded state of the kinase, which can be captured by phosphorylated Cdc37 through binding to the kinase C lobe.[15] The phosphorylation of Cdc37 on Ser13 by the casein kinase 2 (CK2) is required for the appropriate function of Cdc37.[16] Once the binary Cdc37-kinase complex has formed, it binds to Hsp90. The resulting ternary complex consisting of Hsp90, Cdc37 and the so-called client protein, finally enables the loading of the client protein onto the Hsp90 ATPase-coupled chaperone machinery.[17] HSP90 inhibitors, like ganetespib, prevents loading of the client protein onto the chaperone machinery and results in aggregation of the partly unfolded kinase or degradation through the ubiquitin-proteasome system.[18] The release of the kinase from the ternary chaperone complex requires de-phosphorylation of Cdc37 on Ser13, facilitated by protein phosphatase 5 (PP5).[19] (Figure 2).

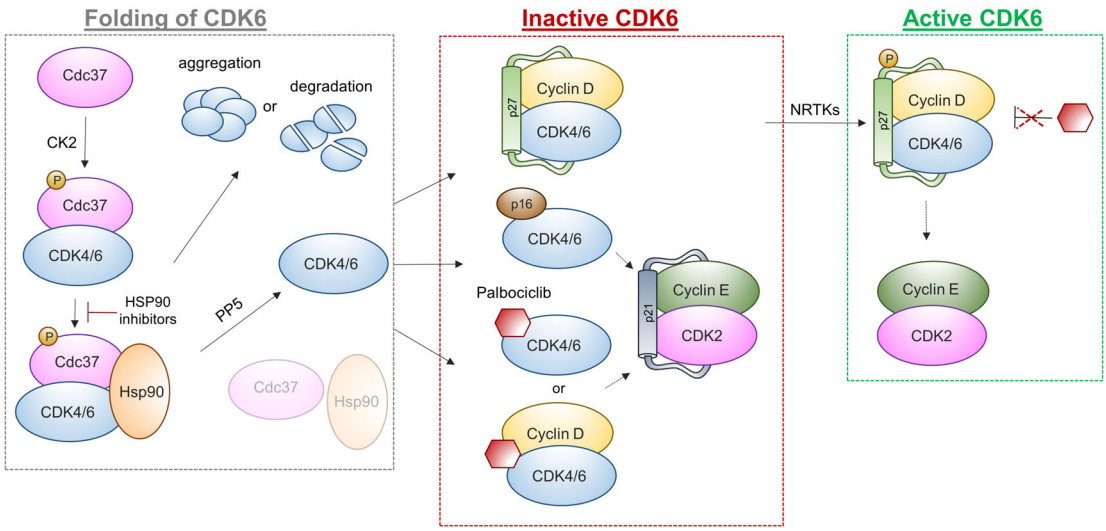

FIGURE 2 Regulation of CDK4 and CDK6. Phosphorylation of co-chaperone cell division cycle 37 (Cdc37) by the casein kinase 2 (CK2) enables binding to CDK4/6 and complex formation with heat shock protein 90 (Hsp90), which facilitates accurate protein folding. Inhibition of HSP90 leads to aggregation or degradation of unfolded kinases. Dephosphorylation of Cdc37 by phosphatase protein phosphatase 5 (PP5) releases CDK4/6 and allows complex formation with different regulatory subunits. CDK4/6, Cyclin D and Cip/Kip proteins (p27) form an inactive trimeric complex, which gets activated upon phosphorylation of p27 by nonreceptor tyrosine kinases (NRTKs) and translocates into the nucleus. Association with inhibitors, such as p16 or palbociclib, drives the formation of inactive complexes and indirectly promotes inactivation of CDK2

Recent findings by Hallett et al have shown, that the interaction of CDK6 and CDK4 with Cdc37 and the Cdc37-Hsp90 chaperone complex occurs with significantly different binding affinities; CDK6 is considered a weak client protein, whereas CDK4 is regarded as a strong interactor.[14] As a consequence D-type cyclins or INK proteins readily displace the weak client protein CDK6 from Cdc37. The binding of cyclin, INK or Cdc37 to CDK6 is thereby mutually exclusive.

In case of CDK4, only members of the INK family, but not D-type cyclins alone, are capable of displacing CDK4 from Cdc37 and Cdc37-Hsp90. The inability of D-type cyclins to sequester/partition CDK4 from Cdc37-Hsp90 changes upon binding to CDK inhibitors (CKIs). Formation of a ternary complex of CDK4/6 and cyclin D1 or cyclin D3 together with either $p21^{Cip1}$ or $p27^{Kip1}$ is resistant to displacement of the CDK by Cdc37-Hsp90.[14] Recent data in breast cancer cells by Guiley et al demonstrate that the active phosphorylated trimeric p27-CDK4-CycD1 complex is also resistant to the CDK4/6 kinase inhibitor palbociclib.[20] (Figure 2) They propose that palbociclib inhibits the kinase function in a similar manner as INK proteins by primarily targeting kinase-inactive monomers. This indirectly reduces CDK2 activity by shuttling of Cip proteins to CDK2 complexes. The capability of the small molecule inhibitor palbociclib to imitate p16 function, may partially explain the enhanced sensitivity of cells lacking p16 to this type of inhibitors.[10,20,21] The specific CDK4/6-targeting adenosine triphosphate (ATP)-competitive drug palbociclib has been approved by the US Food and Drug Administration (FDA) for therapeutic application in hormone receptor-positive breast cancer and is in clinical trials for use in various cancer types.[22-24] More details are discussed below. In summary, these data pinpoint at the highly complex and dynamic regulatory network between CDKs and their interactors that are disturbed under drug treatment. Vice versa the absence or presence of components of this regulatory network will determine the sensitivity of a cell toward inhibitor treatment.

3 | CDK6 DURING NORMAL HEMATOPOIESIS

Although CDK6 and CDK4 are described to exert redundant functions as critical regulators in cell cycle progression, findings over the last years have shown that the two kinases differ especially in cell-cycle independent tissue-specific functions. While CDK6 plays an important role in hematopoiesis, CDK4's function is crucial for pancreatic beta cells and pituitary glands.[25-27] Despite the fact that concomitant depletion of CDK6 and CDK4 results in late embryonic lethality due to defective erythropoiesis, mice lacking the one or the other are viable. Individual knockouts are hallmarked by distinct tissue-specific defects. Depletion of the CDK4 gene gives rise to defects in the proliferation of endocrine pancreatic cells and pituitary lactotrophs. Moreover, CDK4-null mice are smaller in size and infertile.[25,26]

Loss of CDK6 causes mild anemia, thymic atrophy and delayed G1 progression in lymphocytes. Female mice are marginally smaller but do not exhibit anatomical abnormalities or elevated rates of mortality.[27] In hematopoietic stem cells (HSC), the absence of CDK6 leads to prolonged exit from quiescence resulting in delayed stem cell activation.[28,29] Immature Cdk6-deficient thymocytes fail to undergo ordinary expansion, which is caused by reduced proliferation and increased apoptosis during Notch-dependent T-cell development. Additionally, $Cdk6^{-/-}$ mice are completely resistant to Akt-driven lymphomagenesis.

The critical role of CDK6 in Notch-Akt-dependent T-cell development and tumorigenesis cannot be compensated by CDK4.[30]

Analysis of a mouse model expressing a CDK6 kinase-dead allele (*Cdk6*^{K43M}), largely resembled the impact of *Cdk6* loss on T-cell development and resulted in reduced proliferation of thymocytes, hematopoietic stem and progenitor cells as well as defective Notch signaling. Differences between the complete loss of CDK6 and the inactivation of its kinase function were restricted to more mature thymocyte subsets, where the presence of the *Cdk6*^{K43M} allele significantly increased double-negative (DN) T-cells. In contrast, mice expressing a hyperactive CDK6 knock-in mutation (R31C), which is resistant to INK4 inhibition, display elevated levels of thymocytes and Lin^{-}Sca-1^{+}c-Kit^{+} (LSK) progenitor cells.[31]

4 | CDK6 IN HEMATOLOGIC MALIGNANCIES

Components of the CDK6-Cyclin D complexes are frequently altered in hematological malignancies. Overexpression of CDK6 has been reported in T-cell lymphoblastic lymphoma and leukemia (T-LBL or T-ALL) and in B-lymphoid malignancies. Although no mutations in CDK6 are documented, sporadic cases with chromosomal translocation involving CDK6 were identified.[32-35] Lately, a prosurvival impact on T-ALL cells through regulatory metabolic functions of CDK6 has been demonstrated.[36] Moreover, Jena et al provided evidence that a CDK6 kinase-dependent repression of CD25 is required to induce and maintain Notch1-induced T-ALL. Hematopoietic progenitor cells lacking CDK6 or its kinase-activity, due to a knock-in mutation or CDK4/6 inhibitory treatment, were resistant to leukemia induction by activated Notch. This underlines the critical role of CDK6 as a downstream target of Notch in leukemogenesis.[37]

In myeloid leukemia CDK6—but not CDK4—has been identified as a critical effector, required for progression of mixed-lineage leukemia (MLL)-rearranged acute myeloid leukemia (AML). CDK6 expression is regulated through direct binding of MLL-AF9 to the *Cdk6* locus and the resulting high CDK6 levels block myeloid differentiation and result in an immature phenotype. Pharmacological inhibition of CDK6 by the CDK4/6 inhibitor PD-0332991 (palbociclib), unlocks the blocked differentiation and reduces the leukemic phenotype in human AML cell lines. These findings proposed that CDK6 inhibition provides a clinically applicable therapeutic opportunity for MLL-rearranged leukemia.[38] A proliferation advantage of leukemic cells through MLL fusion-driven upregulation of CDK6 has also been shown in MLL-rearranged infant ALL.[39]

Studies in break point cluster region/ABL (BCR/ABL) transformed murine leukemia/lymphoma models demonstrated that enhanced expression of CDK6 may provoke accelerated as well as reduced proliferation depending on the presence or absence of the tumor suppressor protein p16^{INK4a}. The CDK6 induced p16^{INK4a} expression is considered an internal safeguard mechanism that blocks accelerated cell proliferation to prevent tumor progression. The p16^{INK4a} expression is induced by binding of CDK6, in complex together with signal transducer and activator of transcription 3 (STAT3) and Cyclin D, to the *p16*^{INK4a} promoter. Despite the presence of D type cyclins on the promoter, the transcriptional function of CDK6 is independent of its kinase function, can be induced by a kinase-dead version of CDK6 and is not shared by CDK4. Only in the absence of p16^{INK4a}, CDK6 fulfills its role as a proto-oncogene and accelerates tumor progression. Consistent with these results, an inverse correlation of CDK6 and p16^{INK4a} has been detected in most human lymphoid malignancies.[40-42]

Besides induction of p16^{INK4A}, CDK6 regulates and induces important proto-oncogenes including vascular endothelial growth factor A (*VEGFA*), fms-related tyrosine kinase 3 (*FLT3*), aurora kinase (*AURK*) and *AKT*, that are crucial for survival, proliferation and angiogenesis in ALL and AML.[41-45] (Figure 1) A role for CDK6 has been uncovered in hematopoietic stem cells (HSCs) and leukemic stem cells (LSCs) over the last years. Upon stress, CDK6 is required to release stem cells from quiescence by suppression of early growth response protein 1 (EGR1) expression. The CDK6-mediated downregulation of EGR1 is mediated by binding of CDK6 to the promoter and suppression occurs in a kinase-independent manner to allow activation of HSC and LSCs. The dependency of LSCs on CDK6 was shown in a bone marrow transplantation model where *Cdk6*$^{-/-}$ BCR-ABL^{p210+} LSKs were unable to efficiently induce disease.[28,46]

CDK6 is not only important upon establishment of leukemia but also determines the reaction to oncogene-induced stress. Bellutti et al showed that CDK6 acts as a prosurvival factor for preleukemic cells and is required to antagonize oncogene-induced activation of the tumor protein 53 (TP53), also known as p53. During transformation and immortalization, CDK6 induces a complex transcriptional program comprising a variety of genes counteracting p53 functions including protein arginine methyltransferase 5 (*Prmt5*), transformed mouse 3T3 cell double minute 4 (*Mdm4*) and protein phosphatase 1D magnesium-dependent, delta isoform (*Ppm1d*). In the absence of CDK6, lymphoid cells are forced to mutate or delete p53 to overcome oncogene-induced stress. Analysis of patient samples suffering from ALL, AML and myelodysplastic syndrome (MDS) confirmed the inverse correlation of CDK6 and p53 mutations.[47,48]

5 | CDK6 IN BREAST CANCER

In breast cancer, as well as normal breast epithelium, D-type cyclins and their binding partner kinases are key regulators of cell cycle progression, tumor formation and proliferation. The activity and expression of CDK4/6 and cyclin D is regulated and influenced by several mitogenic signaling pathways including the estrogen receptor, receptor tyrosine kinase (RTK) signaling pathways and the downstream PI3K-AKT-mTOR or RAS-RAF-MEK-ERK routes.[49-52]

Hyperactivation and dysregulation of components of the cyclin D-CDK4/6 axis are common in breast cancer rendering them attractive targets for therapeutic interference.[53,54] Not surprisingly, the CDK4/6 inhibitors palbociclib (PD0332991), ribociclib (LEE011) and abemaciclib (LY835219) have been approved by the US Food and Drug Administration (FDA) for the treatment of hormone

receptor-positive breast cancer. Treatment of patients with advanced breast cancer is guided by the hormone receptor (HR) status (estrogen and progesterone), and by the expression status of the tyrosine kinase human epidermal growth factor receptor 2 (HER2). In case of metastatic HR-positive, HER2-negative breast cancer patients, combinatorial treatment of CDK4/6 inhibitors with endocrine therapy, like letrozole or fulvestrant, significantly prolonged progression-free survival (PFS). Preclinical and clinical trials proved the beneficial effect of combining CDK4/6 inhibitors with drugs that reduce the estrogen levels, such as aromatase inhibitors like letrozole, or target the estrogen receptor, like tamoxifen or fulvestrant.[55-58] Strikingly, abemaciclib has also been approved as monotherapy and showed promising results as a single agent in HR+/HER2− metastatic breast cancer patients.[59] The prolonged exposure of MCF-7 breast cancer cell lines to abemaciclib provokes drug resistance accompanied by increased CDK6 expression. Similarly, the enforced expression of CDK6 in drug-sensitive cells was sufficient to mediate abemaciblib resistance and was paralleled by reduced ER and PR receptor expression, a phenomenon also observed in patients. Vice versa, knockdown of CDK6 restored sensitivity to the CDK4/6 inhibitor.[54]

6 | CDK6 AND IMMUNE FUNCTIONS

The effect of CDK4/6 inhibitors goes far beyond cell cycle arrest. One additional effect is that CDK4/6 inhibitors trigger antitumor immunity. Goel et al reported two mechanisms that account for the enhanced antitumor responses in breast cancer. CDK4/6 inhibition reduces expression of the DNA methyltransferase 1 (DNMT1) resulting in a subsequent DNA hypomethylation.[60] (Figure 3) This leads to the upregulation of endogenous retroviral elements, the expression of double-stranded RNA, a prompt type III interferon (IFN) production and enhanced antigen production resulting in enhanced tumor cell killing. Moreover, CDK4/6 inhibition preferentially reduces the proliferation of immunosuppressive regulatory T cells (T_{Reg}). Both effects are mediated by the suppression of the RB-E2F axis.[60] Inhibition of CDK4/6 increases interleukin 2 (IL2) secretion of effector T-cells via an upregulation of the nuclear factor of activated T-cells (NFAT), which further enhances antitumor activity. NFAT4, which belongs to a family of transcription factors critical for T-cell activation, is a direct substrate of CDK6, but not CDK4. Inhibition of CDK4/6 decreases the phosphorylation of NFAT, which enhances its nuclear translocation and increases its transcriptional activity.[61] (Figure 3) A distinct beneficial mechanism of CDK4/6 inhibition has been reported by Jerby-Arnon et al in melanoma cells, who showed that inhibition represses a cancer cell-induced program that is associated with T cell exclusion and immune evasion.[62] On the other hand, Zhang et al showed that CyclinD-CDK4 together with the cullin3-speckle-type POZ protein (SPOP) E3 ligase negatively regulates programmed cell death 1 ligand 1 (PD-L1) protein stability. PD-L1 expression levels inversely correlate with CDK4 activity and treatment of tumor cells with CDK4/6 inhibitors

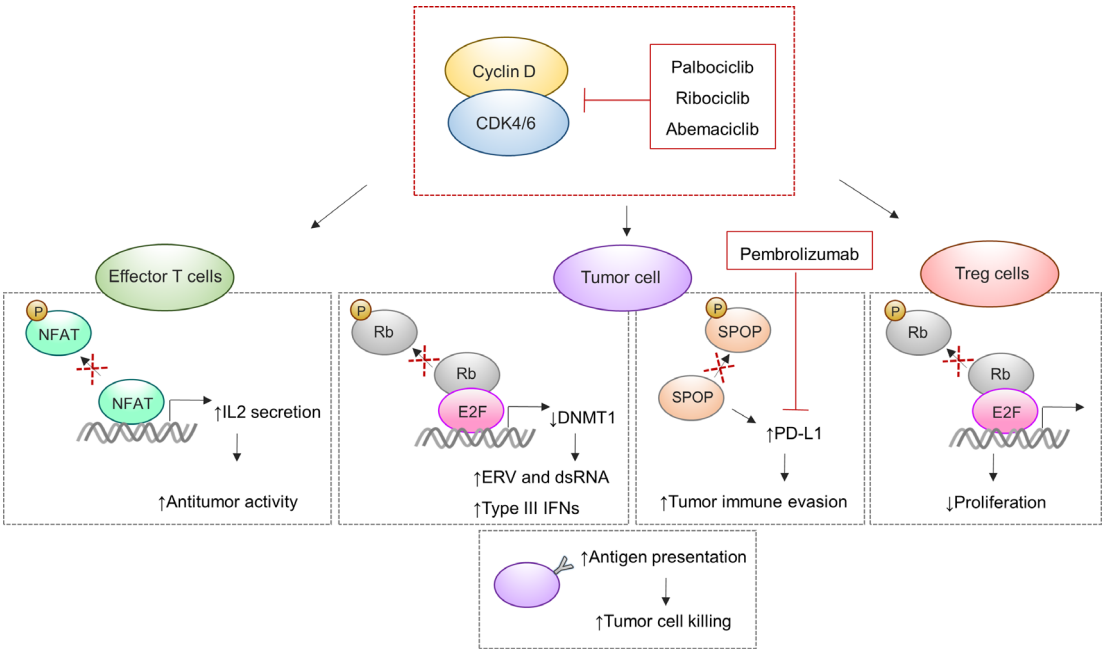

FIGURE 3 Effects of CDK4/6 inhibition on antitumor immunity. In tumor cells, inhibition of CDK4/6 leads to reduced DNA methyltransferase 1 (DNMT1) expression levels resulting in elevated antigen presentation capacity through upregulation of endogenous retroviral elements (ERV). Additionally, programmed cell death 1 ligand 1 (PD-L1) levels are upregulated upon inhibition causing sensitivity of tumor cells to immune checkpoint inhibitors, such as pembrolizumab. In effector T cells, CDK4/6 inhibition promotes nuclear factor of activated T-cells (NFAT) activity, a transcription factor critical for T-cell activation. Finally, suppression of CDK4/6 causes reduced proliferation of immunosuppressive regulatory T-cells (Treg)

increases PD-L1 protein levels, enabling enhanced tumor evasion. Hence, high PD-L1 expression on tumor cells goes along with inhibited T cell activation, allowing tumor cells to escape antitumor immune responses. On the other side of the coin, these enforced PD-L1 levels allow higher sensitivity to immune checkpoint inhibitors (Figure 3).[63,64]

Collectively, these data point at beneficial effects upon combinatorial treatment of CDK4/6 inhibitors with immune checkpoint inhibitors. in vivo experiments verified synergistic effects of CDK4/6 inhibition with programmed cell death 1 (PD-1) blockade, which lead to elevated tumor regression and better overall survival rates.[61,63] Safety and efficacy of the CDK4/6 inhibitor abemaciclib in combination with pembrolizumab (PD-1 inhibitor) is currently investigated in a phase 1b study for patients with HR+/HER2- metastatic breast cancer or advanced nonsmall cell lung cancer (NSCLC) (NCT02779751).[65]

7 | CDK6 IN MELANOMA

Melanoma is characterized by a high mutational load, which includes and leads to deregulated expression of a panel of cell cycle regulatory proteins. Inhibitory mutations in the tumor suppressor $p16^{INK4a}$ are a frequent event in primary melanoma samples and melanoma cell lines. Activating germline mutations have been described in CDK4 in families that suffer from hereditary melanoma. These mutation types highly increase the risk of developing melanoma.[66-70] Combined overexpression of KIT and CDK4 has been found in a subgroup of melanomas.[71] Several preclinical studies verified the sensitivity for melanoma cells to CDK4/6 inhibition in vivo and in vitro. CDK4, cyclin-dependent kinase inhibitor 2A (CDKN2A) and RB1 expression levels are predictors of sensitivity.[72-74] A recent study points at CDK4 and CDK6 as regulators of cell-proliferation, migration and tumor-angiogenesis in melanoma. The tight balance/equilibrium between CDK4 and CDK6 expression controls the transcriptional activity of CDK6 to regulate protumorigenic genes, including *VEGF*. The strong correlation between CDK6 levels and enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) target gene expression in human melanoma samples, underscores the proangiogenic role of CDK6 in melanoma as EZH2 is regulated by VEGF.[75,76] CDK6 was also found to directly phosphorylate EZH2.[77] We thus postulate a dual influence of CDK6 on VEGF in melanoma.

Anders et al describe the forkhead box M1 (FOXM1) transcription factor as a substrate of CDK4/6-Cyclin D complexes. CDK4/6 phosphorylate FOXM1 at multiple sites and thereby regulates its stabilization and activation. As FOXM1 protects tumor cells from senescence, by reducing reactive oxygen species (ROS), a link from CDK4/6 kinase activity to senescence induction was postulated (Figure 1). Support for this idea stems from the fact that melanoma cells show increased signs of senescence upon palbociclib treatment. CDK4/CDK6 inhibition in combination with a mitogen-activated protein kinase (MEK) inhibitor leads to apoptosis as well as cell cycle arrest and show synergistic therapeutic efficacy in a NRAS mutant melanoma mouse model.[78] As a consequence CDK4/CDK6 inhibitors are used in clinical studies including combinations with mitogen-activated protein kinase (MEK) and BRAF inhibitors.[79,80]

8 | CONCLUSION AND OUTLOOK

Initially identified as cell cycle regulating kinases that enable cell cycle progression, CDK6 and its close homolog CDK4 have attracted substantial attention in cancer research over the last years. Altered expression and dysregulated function have made them attractive targets for pharmacological inhibitors in various cancer types. Dual CDK4/6 inhibitors, that target the ATP-binding pocket of the kinase, have revolutionized treatment of patients with breast cancer and showed first promising results in hematological malignancies and melanoma. None of these approved small molecule inhibitors distinguishes between CDK6 and CDK4, as they share 94% homology in their ATP-binding pocket.[81]

Moreover, kinase-independent functions, as described for the transcriptional regulator CDK6, are not targeted by dual CDK4/6 inhibitors.[28,42] To address this issue, different selective CDK6 degraders have been designed lately and showed promising in-vitro results.[81-86] CDK6-selective proteolysis-targeting chimeras (PROTACS) remarkably reduced leukemia burden in mice injected with patient-derived Philadelphia-positive (Ph+) ALL.[83]

The possibility to selectively degrade CDK6 in cancer patients could be a strategy to restore drug sensitivity or even prevent drug resistance, which is seen after prolonged CDK4/6 inhibitory treatment.[54] We also propose that compounds, that distinguish between CDK6 and CDK4, would be less harmful to normal hematopoietic progenitor cells and offer novel therapeutic options.[28,83] CDK6 specific drugs may interfere with transcriptional responses downstream of CDK6 while leaving proliferation intact which might be exploited in drug combinations using chemotherapy. First studies propose a benefit of chemotherapy and CDK4/6 inhibition depending on the sequential administration.[87] Although the versatile function of CDK6 in disease and tumor progression has been investigated in depth over the last decades, further studies are needed to improve selective cancer treatment and find novel effective synergistic combinations to prevent drug tolerance and resistance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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