HHS Public Access

Author manuscript

Nat Rev Urol. Author manuscript; available in PMC 2022 July 22.

Published in final edited form as:

Nat Rev Urol. 2022 May ; 19(5): 305–320. doi:10.1038/s41585-022-00571-8.

Therapeutic potential of CDK4/6 inhibitors in renal cell carcinoma

Rebecca A. Sager[1,2], **Sarah J. Backe**[1,2,3], **Elham Ahanin**[1,2,3], **Garrett Smith**[1,2], **Imad Nsouli**[1,2,4], **Mark R. Woodford**[1,2,3], **Gennady Bratslavsky**[1,2,3], **Dimitra Bourboulia**[1,2,3], **Mehdi Mollapour**[1,2,3,4,✉]

[1]Department of Urology, SUNY Upstate Medical University, Syracuse, NY, USA.

[2]Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY, USA.

[3]Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY, USA.

[4]Syracuse VA Medical Center, Syracuse, NY, USA.

Abstract

The treatment of advanced and metastatic kidney cancer has entered a golden era with the addition of more therapeutic options, improved survival and new targeted therapies. Tyrosine kinase inhibitors, mammalian target of rapamycin (mTOR) inhibitors and immune checkpoint blockade have all been shown to be promising strategies in the treatment of renal cell carcinoma (RCC). However, little is known about the best therapeutic approach for individual patients with RCC and how to combat therapeutic resistance. Cancers, including RCC, rely on sustained replicative potential. The cyclin-dependent kinases CDK4 and CDK6 are involved in cell-cycle regulation with additional roles in metabolism, immunogenicity and antitumour immune response. Inhibitors of CDK4 and CDK6 are now commonly used as approved and investigative treatments in breast cancer, as well as several other tumours. Furthermore, CDK4/6 inhibitors have been shown to work synergistically with other kinase inhibitors, including mTOR inhibitors, as well as with immune checkpoint inhibitors in preclinical cancer models. The effect of CDK4/6 inhibitors in kidney cancer is relatively understudied compared with other cancers, but the preclinical studies available are promising. Collectively, growing evidence suggests that targeting CDK4 and CDK6 in kidney cancer, alone and in combination with current therapeutics including mTOR and immune checkpoint inhibitors, might have therapeutic benefit and should be further explored.

The current era of targeted therapy and precision medicine for cancer treatment, including renal cell carcinoma (RCC), was unimaginable only a few decades ago. Patients with early-stage RCC have a good prognosis and are often cured with surgery alone, but advanced

✉ mollapom@upstate.edu .

Author contributions

M.M., R.A.S., G.E.S. and I.N. researched data for article. M.M., R.A.S., S.J.B., G.B. and D.B. made substantial contribution to the discussion of content. M.M., R.A.S., S.J.B., E.A. and M.R.W. wrote the article. M.M., R.A.S. and S.J.B. reviewed/edited the manuscript before submission

Competing interests

The authors declare no competing interests.

and metastatic disease have a much worse prognosis, with low 5-year survival (~13%)[1]. Traditional chemotherapeutics and radiotherapy have never shown considerable efficacy in metastatic RCC[2], and the first real therapeutic advance came in the mid-1990s with the success of high-dose IL-2, which suggested that harnessing the immune system might be effective in RCC treatment[3]. Unfortunately, IL-2 treatment in metastatic RCC caused extreme, limiting adverse events including hepatic and renal dysfunction[3]. In the early 2000s, the success of tyrosine kinase inhibitors (TKIs), such as sunitinib, and mechansitic target of rapamycin (mTOR) inhibitors, such as everolimus, started a new era of targeted therapy for the treatment of metastatic RCC and these agents quickly became the gold standard in RCC therapy[4,5]. Many of the genes implicated in RCC development and progression are involved in tyrosine kinase signalling pathways, including MET, vascular endothelial growth factor receptor (VEGFR), and fibroblast growth factor receptor (FGFR) pathways[6] and the mTOR pathway[7], but limited success in predicting responsiveness to TKI and mTOR inhibitors has been observed, owing to a lack of effective biomarkers[8]. Another advance in the treatment of metastatic RCC was made with the testing and FDA approval of novel immune checkpoint inhibitors (ICIs) including those targeting programmed cell death 1 (PD1) or programmed cell death 1 ligand 1 (PDL1) and cytotoxic T lymphocyte associated protein 4 (CTLA4) in the 2010s[8–10]. Checkpoint inhibitors ultimately induce T cell activation in order to promote immune-mediated killing of cancer cells[11]. Checkpoint inhibitors in combination with TKI treatment increased disease stability and improved both overall survival and progression-free survival (PFS) in patients with RCC[8–10]; however, intrinsic and acquired resistance to these agents, as well as the paucity of biomarkers for RCC[8] make it difficult to predict patients' response to treatment and identify the best sequence of therapies for individual patients.

In the mid-2010s, when checkpoint inhibitors were gaining FDA approval for the treatment of RCC, therapeutics specifically targeting cyclin-dependent kinase 4 (CDK4) and CDK6 were approved by the FDA for the treatment of breast cancer[12–14]. CDK4 and CDK6 are involved in cell-cycle entry at the G1/S checkpoint, and they require chaperoning by the CDC37–heat shock protein 90 (HSP90) chaperone system for stabilization and activation[15,16]. The holoenzyme complexes containing D-type cyclins and CDK4 and CDK6 canonically phosphorylate the tumour suppressor retinoblastoma (Rb), leading to the release of E2F transcriptional repression needed for G1/S transition[15].

Non-specific CDK inhibitors were tested in clinical trials in haematological and solid malignancies but induced high levels of toxic effects[17,18]; thus, the development of inhibitors specifically targeting both CDK4 and CDK6 provided a new potential clinical therapeutic window in cancer[17,18]. FDA approval has been given to three specific CDK4 and CDK6 inhibitors (referred to here as CDK4/6 inhibitors) — palbociclib (ibrance), ribociclib (KISQALI) and abemaciclib (verzenio) — for the treatment of hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative (HR^+ and $HER2^-$) breast cancer and are currently under clinical investigation in many other cancers[19,20]. Many of the regimens approved in breast cancer are CDK4/6 inhibitors administered in combination with anti-oestrogen therapies[12–14,21,22], and results from preclinical and clinical studies demonstrated that CDK4/6 inhibitors work remarkably well in combination with hormone therapy in breast cancer, or with kinase inhibitors (such as TKIs and mTOR

inhibitors) in a variety of cancers, owing to cross-regulation between CDK and mTOR signalling pathways[23]. Additional work highlighted a role for CDK4 and CDK6 inhibition in augmenting tumour immunogenicity and antitumour immune response[23]. Several ongoing clinical trials use CDK4/6 inhibitors in combination with immune checkpoint blockade in prostate, breast, head and neck cancer and liposarcoma[24–28]. The success of CDK4/6 inhibitors in combination with mTOR[29–35] and ICIs[24–28,36–40] in preclinical and clinical models suggests that these inhibitors could be ideal therapeutics in RCC, as, in this setting, they might also work synergistically with therapeutics currently used in RCC treatment[4,5].

In this Perspective, we discuss the relevant preclinical and clinical knowledge on CDK4 and CDK6 function and inhibition to support the idea that further exploration of the role of these therapeutics in kidney cancer is warranted.

CDK4 and CDK6 structure and role in the cell cycle

CDKs are necessary for the ordered progression of the cell cycle and function in the integration of cellular signals to control cell-cycle checkpoints[41]. As the name suggests, the function of CDKs requires the formation of holoenzyme complexes with cyclin proteins[42]. The concentration of cyclins varies throughout the cell cycle and their binding to CDKs promotes CDK kinase activation[42]. CDK4 and the closely related CDK6 both depend on D-type cyclins, the expression of which increases in early G1 in response to various mitogenic stimuli[43]. CDK4 and CDK6 are largely functionally homologous and mice lacking either gene individually are viable[43–46]. Notable differences in CDK4 and CDK6 function also exist: CDK4 is involved in hypothalamic–pituitary axis signalling, fertility and insulin production, whereas *CDK6*-null animals have mild haematopoietic defects[44–46]. In general, most studies focus on one protein or the other, with the bulk of the literature concentrating on CDK4; studies usually do not differentiate between CDK4 and CDK6 when non-specific inhibitors are used. CDK4, in complex with D-type cyclins, phosphorylates Rb[47] (FIG. 1). Rb directly binds to the activation domain of the transcription factor E2F and inactivates it, thereby preventing cells from entering S phase[41]; however, phosphorylation of Rb disrupts its interaction with E2F1, enabling E2F-mediated transcription of its target genes and consequent exit from the G1 phase of the cell cycle[47]. The cyclin D–CDK4 complex recognizes and binds two distinct sites in the C terminus of Rb, leading to Rb phosphorylation[48]. CDK4-mediated phosphorylation of Rb prevents its cleavage by caspase 3 (as part of the apoptotic cascade) antagonizing the induction of apoptosis[49]. CDK4 and CDK6 activity also promotes the assembly of the pre-replication complex (needed to coordinate DNA replication in S phase) through the regulation of Rb–E2F-mediated transcription of target genes[50]. Additionally, CDK4 and CDK6 delay senescence, thereby extending cellular lifespan in a kinase-activity-dependent manner through an as-yet unidentified mechanism[51,52].

Sustained replicative potential and loss of cell-cycle control is a hallmark of cancer[53] and a mutual requirement for CDK4 and the transcription factor MYC exists in malignant transformation[54] (FIG. 1). CDK4 is a direct transcriptional target of MYC and is necessary for MYC-driven cell-cycle entry[55], which is in part dependent on the regulation of cyclin-dependent kinase inhibitor 1B (p27), an endogenous cell cycle inhibitor[56,57]. Elevated p27

levels in *Myc*$^{-/-}$ cells were shown to make it impossible for cyclin D–CDK4 complexes to be activated, probably owing to alterations in the stoichiometry and relative abundance of cyclin D and p27 (REF.[56]). The function of CDK4 in regulating S phase entry is partially mediated by the control of p27 activity: CDK4 sequesters p27, preventing p27-mediated inhibition of the cyclin E–CDK2 complex and enabling G1/S transition[57]. *Cdk4*$^{-/-}$*p27*$^{-/-}$ double knockout mouse embryonic fibroblasts (MEFs) displayed partially restored kinetics of the G0/S transition[57]. *Cdk4*$^{-/-}$ MEFs displayed similar growth to their wild-type counterpart under continuous growth conditions but their ability to re-enter S phase after a period of serum starvation was considerably impaired[57]. However, investigations carried out in *Drosophila*, which only has single CDK4 and cyclin D genes, demonstrated that cell-cycle progression is not impaired in the absence of *Cdk4*, but cell and organismal growth were compromised[58]. Hypoxia-inducible factor-prolyl hydroxylase (Hph) is an oxygen-dependent enzyme that adds hydroxyl groups to proline residues on target proteins (such as hypoxia-inducible factor (HIF)), which allows them to be recognized by the E3-ubiquitin ligase Von Hippel–Lindau (VHL) and subsequently ubiquitinated and targeted for degradation[59]. Loss-of-function mutations in Hph specifically suppressed the cell-growth function of CDK4–cyclin D complexes in *Drosophila*[60], showing that Hph is an important mediator of cyclin D–CDK4 signalling and a regulator of cell growth[60], although the effectors of Hph in this context have not been identified. This study provided an early link between the oxygen-sensing mechanism regulating HIF levels (deregulation of which is central to RCC development)[6,7] and CDK4 (REF.[60]) (FIG. 1). In conclusion, CDK4 and CDK6 control cell-cycle progression through several distinct mechanisms.

Non-canonical role of CDK4 and CDK6 in metabolism

CDK4 and CDK6 are also involved in the control of metabolism, including insulin signalling and glucose metabolism[46,61–64] (FIG. 2). The role of CDK4 and CDK6 in metabolism might be crucial in RCC, which is often considered to be a metabolic disease[7]. Distinct subtypes of hereditary and sporadic RCC tumorigenesis arise from mutations in tumour suppressors such as folliculin, hamartin, tuberin and fumarate hydratase, which are also involved in cellular metabolism and nutrient sensing pathways such as mTOR signalling[7]. The first indication that CDK4 might have a role in metabolism came from *Cdk4*-deficient mice that are viable, but small, and develop insulin-deficient diabetes as a result of decreased pancreatic β-islet cells[46]. The expression of a CDK4 mutant that blocks interaction with the protein p16 (encoded by *CDKN2A*), which binds to and inhibits CDK4 kinase activity[65], in this mouse model resulted in increased CDK4 activity and subsequently led to abnormal β-cell proliferation and pancreatic hyperplasia[46]. These effects can potentially be explained by defective insulin signalling, as mice with disrupted insulin signalling are phenotypically similar to CDK4-deficient mice[46]. CDK4 has also been shown to regulate insulin secretion from β-cells in a mouse model through E2F1-regulated expression (mediated by CDK4) of *Kir6.2*, a component of the K_{ATP} channel involved in insulin secretion[66]. The role of CDK4 in glucose metabolism has been shown to be independent of its role in cell-cycle regulation in a mouse model of diabetes, both in mouse hepatocytes and in whole animals[61]. In this mouse model, in response to insulin, cyclin D–CDK4 was shown to phosphorylate and activate the acetyltransferase general control non-repressed protein 5

(GCN5)[61]. GCN5 acetylates and suppresses hepatic glucose production via inhibition of the transcriptional co-activator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α)[61], which, therefore, links insulin signalling to the expression of glucose and lipid metabolic genes[61]. Furthermore, cyclin D3–CDK6 was found to phosphorylate and inhibit 6-phosphofructokinase and pyruvate kinase M2, two important regulatory glycolytic enzymes, to redirect glycolytic intermediates to the pentose phosphate and serine pathways in a cellular model of T cell acute lymphoblastic leukaemia[62]. Treatment with CDK4/6 inhibitors in this model reduced the flux through the pentose phosphate and serine pathways, leading to depletion of NADPH and glutathione and a subsequent increase in reactive oxygen species and apoptosis of tumour cells[62]. Taken together, several independent lines of evidence support a role for CDK4 in the regulation of cellular metabolic pathways (FIG. 2).

CDK4 has also been shown to be involved in the regulation of lysosomal function, autophagic flux and fatty acid oxidation (FAO) through the regulation of the mTOR pathway[67]. The nutrient-sensing kinase 5′ adenosine monophosphate-activated protein kinase (AMPK) inhibits mTOR signalling[68]. Treatment of several human cell lines with CDK4/6 inhibitors in combination with an AMPK activator induced autophagy and impaired lysosomal function, leading to cell death and tumour regression owing to the lack of metabolic intermediates needed to synthetize macromolecules to survive[67]. CDK4 has also been shown to repress FAO by directly phosphorylating AMPKα2, which controls FAO through phosphorylation of acetyl-CoA-carboxylase[69]. Pharmacological inhibition or knockout of *CDK4* in a mouse model caused an increase in oxidative metabolism and exercise capacity[69]; this effect is dependent on AMPK, as CDK4 inhibition failed to increase FAO in AMPK-knockout cells[69]. The involvement of CDK4 and CDK6 in the regulation of metabolic processes further supports the idea of inhibiting these kinases in RCC, in which both proliferation and metabolic signalling are dysregulated[7], particularly through alterations in oxygen and nutrient sensing signalling pathways.

Regulation of CDK4 and CDK6 function

CDK4 and CDK6 function is regulated by multiple factors, including endogenous interacting partners, post-translational modifications (PTMs) and the molecular chaperone HSP90[15,70–72] (FIGS 1,2).

Inhibition of CDK4 and CDK6 by endogenous proteins.

Many levels of regulation of CDK4 and CDK6 function, including endogenous interacting partners and PTMs, have been reported[15,70] (FIG. 2). CDK4/6–cyclin D complex activation and inhibition are controlled by two distinct classes of regulatory subunits: the CDK interacting protein/kinase inhibitory protein (Cip/Kip) family, including $p21^{Cip1}$ (*CDKN1A*)[73–76], $p27^{Kip1}$ (*CDKN1B*)[77–79] and $p57^{Kip2}$ (*CDKN1C*)[80,81]; and the INK4 family, including $p15^{INK4b}$ (*CDKN2B*)[82], $p16^{INK4a}$ (*CDKN2A*)[65], $p18^{INK4c}$ (*CDKN2C*)[83,84] and $p19^{INK4d}$ (*CDKN2D*)[84,85]. Cip/Kip family members can act on a broad spectrum of CDK–cyclin complexes, inhibiting CDK1, CDK2, CDK4 and CDK6, whereas INK4 proteins exclusively inactivate CDK4–cyclin D and CDK6–cyclin D complexes[65,86–89]. INK4 proteins function as tumour suppressors, and loss-of-function

mutations in these proteins cause carcinogenesis[90]. For example, *CDKN2A* maps to a region on chromosome 9p21 (REF.[91]) that was found to be deleted in an array of various cancer types, including RCC, suggesting a tumour suppressive function for *CDKN2A*[92]. Structural work using circular dichroism and nuclear magnetic resonance (NMR) spectroscopy showed that purified p16 has low structural stability and a highly dynamic structure[93]. Interaction of p16 with CDK4 occurs between ankyrin-like repeats in p16 (structural domains frequently implicated in mediating protein–protein interaction)[93], and the N-terminal portion of CDK4, which overlaps with the binding site for cyclin D[94]. Binding of p16 to CDK4 inhibits CDK4 kinase activity[65]. A point mutation in CDK4 (CDK4–R24C) is sufficient to abrogate its binding with p16 (REF.[95]), suggesting that the R24 site of CD4K is involved in binding with p16. Additional CDK4 sites important for p16 binding were identified using scanning mutagenesis that changed charged residues to neutral alanines and included residues 22, 25, 97 and 281 of CDK4 (REF.[95]). Mice homozygous for the *CDK4–R24C* mutation develop multiple tumours and are at increased susceptibility to chemical carcinogens such as 7,12-dimethylbenz[a]anthracene (DMBA)[96,97]. MEFs from homozygous $CDK4^{R24C}$ mice are susceptible to oncogene-induced transformation[96,97], have an increased proliferation rate and escape both replicative senescence and contact-mediated growth inhibition, demonstrating the importance of p16-mediated inhibition of CDK4 in cell growth[97].

Specific alterations in the cyclin D–CDK4–Rb axis are frequent events in many cancers[98,99], including RCC[99]. One study analysing 42 glioblastoma tissue samples found deletions, mutations or loss of heterozygosity in either *RB1* (encoding Rb) or *CDKN2A* in 86% of samples, and changes in these two genes were generally mutually exclusive, implying that the CDK4–Rb axis is a crucial regulatory pathway in glioblastoma[100]. Mutation and deletion of *CDKN2A*, including germline mutations, are also frequent events in melanoma[101] and were shown to correlate with sensitivity to the CDK4/6 inhibitor palbociclib in a panel of 47 melanoma cell lines, whereas *RB1* loss predicted resistance to palbociclib[101], again indicating the mutual exclusivity of these pathways. Interestingly, an increased risk of melanoma has been observed in patients diagnosed with RCC and vice versa[102]. Kidney cancer is often diagnosed after melanoma and is generally of a lower stage at diagnosis in patients with a history of melanoma than in those without[102]. In a study including 42 patients with both RCC and melanoma, the renal tumour was diagnosed concomitantly or after melanoma in 83% of patients[102], but germline *CDKN2A* mutations were only identified in patients with a known familial melanoma syndrome (2 of 42 total patients), suggesting that a potential genetic predisposition underlying melanoma and RCC coexistence is partially independent from *CDKN2A*[102]. However, somatic *CDKN2A* mutation, deletion or hypermethylation was found in 15.8% of renal tumours overall in The Cancer Genome Atlas (TCGA) database ($n = 894$ total samples) and was altered in all examined histological subtypes[103]. *CDKN2A* alterations were also associated with worse overall survival across different types of RCC on Kaplan–Meier analysis ($P < 0.0001$)[103]. Collectively, these results suggest that the regulation of CDK4 activity by the tumour suppressor p16 is important in RCC, and CDK4 activity modulation could be harnessed in RCC treatment.

Several germline mutations causing inherited genetic syndromes can predispose patients to developing RCC[7], including Von Hippel–Lindau syndrome (*VHL*), Birt–Hogg–Dubé syndrome (*FLCN*), hereditary papillary RCC (*MET*) and hereditary leiomyomatosis (*FH*)[7,104–108]. Germline missense mutations in *CDKN2B* (encoding the endogenous CDK4 inhibitor protein $p15^{INK4B}$) were identified in one family with features of hereditary RCC in which none of the gene mutations known to predispose to RCC was found[109]. Functional evaluation in colony formation and cell-growth assays demonstrated that these mutations impaired the tumour suppressive function of *CDKN2B*[109].

p16 expression has been demonstrated to increase under hypoxic conditions in a monkey kidney cell line (CV-1), where p16 binds CDK4 under hypoxia, but not under aerobic conditions in non-transformed cells[110]. Regulation of p16 expression under hypoxia is of particular interest for RCC, as the most frequent RCC subtype (clear cell RCC (ccRCC)) is characterized by a dysregulation of oxygen sensing regulatory pathways[7]. Furthermore, p16 is regulated by phosphorylation, and phosphorylated p16 preferentially interacts with CDK4 (REF.[111]). Identified phosphorylation sites on p16 include serines 7, 8, 140 and 152, which are located outside the conserved CDK4 interaction domain, and are instead positioned within the regions mutated in sporadic and inherited cancers[111]. All deletions and mutations affecting p16 expression or function ultimately abolish p16-mediated inhibition of CDK4, generating uncontrolled, pro-tumorigenic CDK4 activity[99]. Control of CDK4 activity by the tumour suppressor p16 is the most widely studied endogenous CDK4 inhibition strategy, and further work is necessary to understand the functional spectrum of CDK4-inhibitory proteins.

Post-translational regulation of CDK4 and CDK6.

CDK4 function is in part regulated by tyrosine phosphorylation (FIG. 2), which is important for the control of DNA damage checkpoints[112]. CDK4 was found to be tyrosine phosphorylated in quiescent, arrested rat fibroblasts and then dephosphorylated upon cell cycle entry[112]. Furthermore, CDK4 catalytic activity requires phosphorylation on T172, which is independent of assembly with cyclin D[113]. This phosphorylation is mediated by CDK7 (REF.[114]) and can also be mediated by JUN N-terminal kinases (JNKs)[115]. CDK4-T172 phosphorylation increases in response to mitogenic stimuli in part through the mTORC1 signalling[116,117], which links CDK4 and CDK6 activity with nutrient sensing and cell metabolism.

HSP90-mediated chaperoning of CDK4 and CDK6.

Many proteins require assistance by chaperone proteins for proper folding and activation[118]. HSP90 is an abundant molecular chaperone involved in the conformational maturation, folding and activation of its target proteins, including many oncogenic kinases such as CDK4 (REFS[16,119,120]) (FIGS 1,2). A large cohort of co-chaperones facilitates protein interaction with HSP90 and regulates HSP90 ATPase activity, thereby promoting target protein folding and activation[121–123]. The kinase-specific co-chaperone CDC37 is highly specialized for promoting conformational development of kinase target proteins including CDKs, such as CDK4 (REFS[124,125]). CDC37 decelerates the ATPase activity of HSP90 to enable protein kinases to interact with the chaperone[122]. CDK4 and CDC37 directly interact

and CDC37–HSP90 chaperoning mediates stabilization of CDK4, preferentially associating with the apo-fraction of CDK4 not bound to cyclin D[71,72]. However, CDC37 also helps mediate the assembly of cyclin D–CDK4 complexes[126,127]. Furthermore, cyclin D1 stability is regulated by the molecular chaperone HSP70 (REF.[128]). Cyclin D1 binding to HSP70 is increased by CDK-dependent phosphorylation of HSP70 in response to changes in cellular conditions such as DNA damage, leading to cyclin D1 degradation[128] (FIG. 1).

Structural analysis of CDK4 in complex with CDC37 and HSP90 has provided a great deal of insight into the chaperoning and activation of this and other kinase clients[125,129–133]. The first structural view of HSP90 interacting with a client protein (CDK4) was solved using single-particle electron microscopy and suggested that HSP90 induces a conformational change of the bound kinase through its ATPase activity[131]. The 3.9 Å cryogenic electron microscopy (cryoEM) structure of full-length HSP90, CDK4 and CDC37 enabled visualization of this multi-protein complex assembly and provided a detailed picture of the interactions within the complex[129]. The cryoEM structure of the HSP90–CDC37–CDK4 complex revealed that CDK4, partially unfolded, is stabilized and protected by CDC37 and HSP90 (REF.[129]); the authors of the study proposed that ATP hydrolysis and subsequent opening of the HSP90 structure gives CDK4 the opportunity to properly fold and, in the case of failure, another chaperone cycle can commence[129]. Using this information, molecular modelling studies have demonstrated reciprocal changes in kinase protein and chaperone stability and suggest that the CDC37–HSP90 machinery protects proteins from degradation by exploiting areas of inherent instability in the kinase structure[124].

CDC37–HSP90 chaperoning of kinases, including CDK4, is also regulated by post-translational modifications[134]. Casein kinase 2-mediated phosphorylation of CDC37 on S13 (CDC37-S13) is a prerequisite for CDC37 association with CDK4, as well as other kinases[135]; phosphorylated CDC37 can then recruit CDK4 to HSP90 for chaperoning, leading to CDK4 stability and kinase activity[135,136]. Notably, in the cryoEM structure, CDC37 is phosphorylated on S13 and, therefore, is stabilized in a kinase-interacting conformation[125,129]; subsequent dephosphorylation of CDC37-S13 by the phosphatase co-chaperone protein phosphatase 5 (PP5) is then required for full kinase activation and release[132,137]. A series of tyrosine phosphorylation events on CDC37 is also important for CDK4 chaperoning[138]: tyrosine phosphorylations of CDC37 (CDC37-Y4 and CDC37-Y298) were shown to be involved in the dissociation of CDK4 from CDC37 after recruitment to HSP90, followed by phosphorylation of HSP90 on Y197, which released CDC37 from the kinase–HSP90 complex, as demonstrated in immunoprecipitation assays[138,139]. Finally, phosphorylation of HSP90 on Y627 led to the dissociation of CDK4 and remaining co-chaperones from HSP90, as shown in transfected COS7 cells[138].

Overall, CDC37 acts as a protein quality-control checkpoint and the chaperone machinery helps to stabilize and regulate the activity of CDK4 and other kinases[125,129,140]. Thus, CDC37 and HSP90 both support oncogenic processes and, in fact, CDC37 has been shown to promote colorectal cancer growth specifically through CDK4 activation in human colon cancer cell lines and xenografts[141]. The HSP90–CDC37 chaperone machinery, therefore, provides an additional target for the allosteric inhibition of CDK4 in cancer therapy[141]. Disruption of the HSP90–CD37–kinase complex with an HSP90 inhibitor or *CDC37*-

knockdown leads to cell-cycle arrest, apoptosis and decreased proliferation in various tumour cell lines[140,142,143]. Furthermore, short peptides specifically targeting the CDC37–HSP90 interaction have been developed using a computational approach[144] and have been shown to efficiently disrupt CDC37–HSP90 interaction and compromise the stability of CDK4 in HEK293 cells, although they exhibited limited cytotoxicity[144]. Using a similar strategy, peptides mimicking CDK4 specifically disrupted its interaction with HSP90 and led to the induction of apoptosis in ccRCC 786-O cells[145].

CDK4/6 inhibition in murine and multiple myeloma cell lines was also effectively induced by promoting their E3 ubiquitin ligase-mediated degradation, through molecules known as proteolysis-targeting chimaeras (PROTACs)[146]. Taken together, these studies highlight the importance of molecular chaperones in mediating stability and function of CDK4 and how targeting this machinery could provide an adjunctive mechanism for CDK4 inhibition. However, the most successful and well-studied strategy for CDK4 inhibition to date is the competition with ATP binding at the catalytic site[147].

Development of CDK4/6 inhibitors

The initially developed non-specific CDK inhibitors had high levels of toxic effects, in large part owing to inhibition of CDK2 and lack of specificity[17,18]. Some of these agents were used in phase I and phase II clinical trials but had high levels of dose-limiting toxic effects including deep venous thrombosis, severe diarrhoea and electrolyte abnormalities[17,18]. Considering that the CDK4–cyclin D–Rb axis is frequently mutated across tumour subtypes[15], specific inhibitors for these kinases were sought. One of the first specific small-molecule inhibitors of CDK4 tested preclinically was CINK4 (a chemical inhibitor of CDK4)[148]. CINK4 was able to specifically inhibit CDK4 in in vitro kinase assays, cause growth arrest and decrease Rb phosphorylation in osteosarcoma and colon cancer cells, and slow the growth of colon cancer xenografts[148]. Further attempts were made to refine CDK4-specific inhibitors, and using CDK4 structural information as guidance led to success[149]. Obtaining a crystal structure of CDK4 to aid inhibitor design was initially challenging, as purifying and crystallizing the protein was difficult[149]. However, the crystal structure of the related family member CDK2 had been solved[150,151]; thus, a CDK4 mimic was created by swapping the CDK4 ATP-binding pocket into CDK2, which revealed that CDK4 has a larger binding pocket than CDK2, enabling the design of slightly larger compounds that were able to specifically bind and inhibit CDK4, but did not fit the binding pocket of CDK2 (REF.[152]). Computational methods further helped define inhibitor selectivity for CDK4 over CDK2 and other related kinases[153,154]. Different molecular modelling strategies were used to understand the differences between CDK4 and CDK2 that impart selectivity for CDK4 inhibitors (including CINK and PD-0183812, another small-molecule CDK4/6-specific inhibitor[154]); upon binding of either CINK or PD-0183812 to CDK4, the motion of a disordered loop within the protein was reduced, whereas loop flexibility was largely unaffected in CDK2, suggesting a stronger structural effect of these inhibitors on CDK4 than CDK2 (REF.[154]). In this study, the selectivity of these compounds for CDK4 was also the result of reduced solvent accessibility at the active site of CDK4 compared with CDK2, in addition to the stability of the hydrogen bonds formed between the inhibitor and a lysine residue within the active site binding pocket of CDK4 (REF.[154]).

With the knowledge obtained by studies on CDK4 inhibitor specificity, several classes of compounds have been tested as specific CDK4 inhibitors[155].

Among CDK4 inhibitors that progressed to FDA approval are palbociclib and ribociclib (approved in 2015 and 2017, respectively), both of which are pyrimidine derivatives[156,157]. Structure-based modification led to the refinement of palbociclib (PD-0332991), which showed remarkable selectivity for CDK4/6 compared with 36 other protein kinases, including the closely related CDK2, and demonstrated promising pharmacokinetics, with efficacy at daily dosing and >50% oral bioavailability[156,157]. Initial studies demonstrated that palbociclib treatment led to G1 arrest, decrease in Rb phosphorylation and regression of colon cancer tumour xenografts[156]. Ribociclib (LEE011) also showed good selectivity for CDK4/6 over other related kinases, as well as efficacy in both enzymatic and cell-based assays[158]. The third CDK4/6 inhibitor to receive FDA approval, abemaciclib (LY2835219, approved in 2017), has been shown to inhibit CDK4/6 with low nanomolar potency and lead to G1 arrest specifically in Rb-proficient cells, suggesting that Rb status could be used to help predict responsiveness to this inhibitor[159]; moreover, abemaciclib was also effective and well tolerated with oral administration in mouse xenograft studies[159–162]. Unlike palbociclib and ribociclib, abemaciclib is more selective for CDK4 than CDK6 and also possesses inhibitory selectivity towards CDK9, although limited work delineating unique features specifically based on this selectivity is available[23,163]. Differences in the selectivity of these inhibitors towards CDK4/6 compared with other kinases could underlie subtle differences in their intended and off-target effects, as well as predict the effectiveness of these drugs in combinations. In general, literature rarely draws a distinction between CDK4 and CDK6 in discussing the function of these proteins or small-molecule inhibitors targeting CDK4 or CDK6. Many of the features predicted to impart selectivity of small-molecule inhibitors for CDK4 or CDK6, such as the non-conserved aspects of the ATP-binding pocket, were seen in co-crystal structures of these compounds bound to monomeric CDK6[163] (FIG. 3). Differences in structure and potency exist between palbociclib and abemaciclib, but common gene expression features create a composite signature of response to CDK4/6 inhibition[164]. Overall, the structure-guided development of CDK4/6 specific inhibitors (palbociclib, ribociclib and abemaciclib) enabled the progression of these inhibitors to clinical approval, as they were better tolerated than the previously developed non-specific CDK inhibitors.

CDK4/6 inhibition in breast cancer — road to FDA approval

CDK4/6 inhibitors were first used clinically in the treatment of HR^+ and $HER2^-$ advanced breast cancer[21]. This cancer is primarily treated with anti-oestrogens, similar to the antiandrogen therapies used in prostate cancer[165,166], and CDK4 inhibitors were found to work best in combination with these therapies[167]. Preclinical studies found that CDK4/6 inhibitors preferentially killed oestrogen-receptor positive (ER^+) breast cancer cell lines and worked synergistically with anti-oestrogens[167]. Several factors can mediate this synergism, including evidence that ER^+ breast cancers generally retain an Rb expression signature[168] and that cyclin D1 transcription is oestrogen responsive[169]. The phase II and III clinical trials PALOMA[12,21], MONALEESA[13,22] and MONARCH[14] led to the approval of palbociclib, ribo ciclib and abemaciclib, respectively, in combination with

anti-oestrogen therapy, either aromatase inhibitors or fulvestrant, between 2015 and 2018 (REFS[12–14,21,22]) (FIG. 4). Abemaciclib is the only inhibitor approved as a single agent and administered continuously, as both palbociclib and ribociclib demonstrated dose-limiting neutropenia[19,170] and are, therefore, administered continuously for 3 weeks, followed by 1 week off[19]. Meta-analyses of these pivotal trials demonstrated increased PFS (HR 0.55, $P < 0.00001$, $n = 4{,}580$ patients[171]) as well as overall survival (HR 0.79, $P = 0.004$, $n = 4{,}580$ patients[171]) in patients treated with CDK4/6 inhibitors in combination with anti-oestrogen therapy compared with anti-oestrogen therapy alone[171–174]. Results from the pivotal trial PALOMA, testing the efficacy of palbociclib plus letrozole (an aromatase inhibitor), showed a better PFS in patients treated with the combination therapy (24.8 months (95% CI 22.1 to not estimable)) than in patients treated with letrozole alone (14.5 months (95% CI 12.9–17.1 months))[21]. Pooled analysis of results from phase III studies highlighted that CDK4/6 inhibitors have a positive effect on PFS in patients with breast cancer if used as first-line combination therapy (HR 0.55 (95% CI 0.49–0.62)), or as a second-line and beyond combination therapy (HR 0.56 (95% CI 0.49–0.64))[175]. Furthermore, several trials, including NATALEE[176] and MonarchE[177], examining the role of CDK4/6 inhibition in the adjuvant setting, are ongoing[178]. Preliminary results from several clinical trials indicate that CDK4/6 inhibition could also have efficacy in $HER2^+$ and triple-negative breast cancer (TNBC)[19,179]. The success of CDK4/6 inhibitors in ER^+HER2^- cancer led to FDA approval of these small molecules for breast cancer treatment in combination with anti-oestrogen therapy, but the efficacy of CDK4/6 inhibitors has also been consistently explored in several other cancers[180–185].

CDK4/6 inhibition in other cancers

Palbociclib, ribociclib and abemaciclib have been tested in preclinical models of several cancers, many of which have causative alterations in the CDK4/CDK6 pathway, and clinical investigations are currently ongoing[180–185]. CDK4/6 inhibitors have not found notable success as single agents, but the relationship between alterations in CDK4/6 signalling and response to therapy provides an interesting framework for examination of these agents in RCC, as has been done in other cancers[180–185]. Germline alterations in the CDK4/6 pathway, including nonsense mutations, missense mutations and splice variants, have been documented in familial melanoma[186–188], and palbociclib was found to induce cytostasis and delay tumour growth in a xenograft model of melanoma[189]. Mantle cell lymphoma frequently results from a translocation involving *CCND1* (encoding cyclin D1) and has shown sensitivity to palbociclib in one phase I trial, with PFS >1 year in 5 of 17 patients[190–192]. CDK4 is also frequently mutated in liposarcoma, with over 90% of liposarcomas harbouring CDK4 amplifications[193]. A phase II study of palbociclib in patients with progressive liposarcoma ($n = 29$ evaluable for the primary end point) exceeded the primary end point and reported 66% PFS (90% CI 51–100%) at 12 weeks, suggesting that CDK4/6 inhibition is promising in this setting[194]. Additionally, preclinical efficacy of CDK4/6 inhibitors, particularly palbociclib, was observed with a dose-dependent decrease in the viability of adrenocortical carcinoma cell lines[195]. Taken together, these studies exemplify the broad applicability of CDK4/6 inhibitors in cancer treatment.

Owing to its tumour suppressive role and its frequent loss in cancer, p16 has been examined as a stratifying marker to predict responsiveness to CDK4/6 inhibitors and has been used as a selection criterion in some preclinical and clinical studies[30,196–198]. A preclinical study using cellular and xenograft models of gastric cancer demonstrated that palbociclib selectively inhibited proliferation and migration in *CDKN2A*-mutated cells but not in cells with wild-type *CDKN2A*[196], indicating that *CDK2NA* status might predict sensitivity to CDK4/6 inhibition. Additionally, in a phase II trial of palbociclib in previously treated patients with advanced, progressive non-small-cell lung cancer with p16-null disease (assessed using immunohistochemistry), half of the patients achieved stable disease for 4–10.5 months[30]. CDK4/6 inhibitors have shown only modest benefit in unselected patients with serous ovarian cancer[197], but results from a case report demonstrated ongoing response to palbociclib and letrozole treatment in a heavily pre-treated patient with homozygous *CDKN2A* deletion, with ongoing decrease of lesion size (46% decrease from baseline) on CT scan after 1 year of therapy[197]. Homozygous *CDKN2A* deletion is a rare genetic event in ovarian cancer, but these observations suggest that patient selection could be of paramount importance in predicting response to treatment[197].

Results of a small phase II study of palbociclib in patients with metastatic, platinum-refractory urothelial carcinoma were disappointing[198]; patients were selected for p16 loss and intact Rb expression using immunohistochemistry and only 2 of 12 patients met the primary end point of PFS at 4 months, whereas the overall survival in the study cohort was 6.3 months[198]. The results of this trial suggest that robust preclinical studies to identify tumour characteristics, such as genetic alterations, are needed in urothelial as well as other cancers for optimal patient selection and rational choice of combination therapies. Examination of CDK4/6 inhibitors in various cancers demonstrates that the identification of mechanisms or expression signatures that predict responsiveness in preclinical settings must be taken with caution into the clinical arena owing to the complexity of intended and possible off-target effects. These studies also highlight the need to understand the mechanisms of resistance to single-agent therapy and how it can be overcome with combination therapies.

Resistance to CDK4/6 inhibitors

Intrinsic and acquired resistance to CDK4/6 inhibitors has been documented and examined, similar to other targeted cancer therapeutics, but this work is still largely preclinical[18]. An intact Rb pathway is thought to be required for CDK4/6 inhibitor efficacy, and Rb-null status is predictive of intrinsic resistance, as observed in a mouse model of mammary carcinoma[23,36]. One systematic screening of hundreds of different cancer cell lines found that genomic alterations leading to elevation of D-type cyclin levels were associated with enhanced sensitivity to abemaciclib, whereas *CDKN2A* alterations were less predictive of abemaciclib sensitivity[199]. Palbociclib does not bind to CDK4 in cells insensitive to the drug, in part because CDK4–p16 interaction precludes palbociclib binding[200]. Results from a retrospective analysis of cultured breast cancer cell lines[167] showed that high p16 levels were predictive of resistance to CDK4/6 inhibition, but low p16 levels did not necessarily predict sensitivity to these inhibitors, suggesting that additional determinants of CDK4/6 inhibitor sensitivity might exist[200]. Results of another preclinical study in

breast cancer cell lines showed that CDK6 overexpression and low CDK4 expression were associated with ribociclib resistance and that increased p21 levels positively correlated with ribociclib sensitivity, indicating a role for p21 in the development of ribociclib resistance[201]. Accordingly, stabilization of p53 via co-treatment with a murine double minute 2 (MDM2) antagonist resulted in p21 accumulation and increased sensitivity to CDK4/6 inhibitors in both resistant patient-derived xenografts and a mouse melanoma model[202]. Interestingly, upregulation of cyclin D1 secondary to CDK4/6 inhibitor treatment was shown to sequester p21 and reduce the ability of p21 to inhibit CDK2 (REF.[202]), which is necessary for proliferation in CDK4/6 inhibitor-treated cells, indicating that CDK2 is the effector of p21 function in mediating sensitivity to CDK4/6 inhibitors[202]. Cyclin E overexpression has also been observed concomitantly with CDK4/6 inhibitor resistance in breast cancer cell lines and patient samples, and cyclin E expression likely contributes to this resistance[203]. Cyclin E activates CDK2; therefore, resistance to CDK4/6 inhibitors can be overcome by CDK2 blockade[203], although no CDK2-specific inhibitors are currently approved. Furthermore, PTMs of CDK4, including the activating T172 phosphorylation (which is a central rate-limiting step for cell-cycle initiation)[204], was also found to be predictive of palbociclib sensitivity in breast cancer cell lines and primary tumour samples[205].

Crosstalk between the CDK4–cyclin D–Rb axis and other signalling pathways also has an important role in mediating sensitivity and resistance to CDK4/6 inhibition[206]. A rapid adaptive resistance mechanism to CDK4/6 inhibition has been seen preclinically in a KRAS-driven pancreatic cancer model[117]. This response was the result of KRAS-dependent post-transcriptional upregulation of cyclin D1 and cyclin E1, which also occurs in an mTOR and mitogenic signalling-dependent manner[117] and could be attenuated by mTOR inhibition across a variety of pancreatic cancer patient-derived xenograft lines[117]. In this study, CDK2 activation and the formation of the cyclin D–CDK4 complex, even in the presence of CDK4/6 inhibition, were hypothesized to be implicated in resistance to CDK4/6 inhibitors[117]. Alterations and rewiring of various kinase signalling pathways are important contributors to CDK4/6 inhibitor resistance and are new potential therapeutic targets to exploit[207]. In a CRISPR–dCas9 screen aimed at examining the effect of various gene deletions in the T24 bladder cancer cell line, several signalling pathways were identified as potential mediators of palbociclib resistance[208], including receptor tyrosine kinases (RTKs), phosphoinositide 3-kinase (PI3K)–AKT–mTOR, Ras–mitogen-activated protein kinase (MAPK), Janus kinase (JAK)–signal transducer and activator of transcription (STAT), and Wnt[208]. Importantly, the combination of palbociclib and inhibitors targeting these kinases demonstrated a reduction in cell growth in cell culture and xenograft models of bladder cancer, highlighting the potential of combination therapies in preclinical studies[208].

Several CDK4/6 inhibitor resistance mechanisms have been identified[199–203], but the pharmacological development of inhibitors targeting these mechanisms is still lacking. The identification of target proteins responsible for CDK4/6 inhibitor resistance might be essential to the clinical success of these therapeutics; thus, further elucidation of biomarkers of cellular resistance to CDK4/6 inhibitors is necessary.

Synergism with mTOR inhibitors

Activation of the mTOR pathway and the upstream RTK and PI3K–AKT pathways have been implicated in resistance to CDK4/6 inhibition as well as in the regulation of these kinases[206]. Agents specifically targeting mTOR and other signalling kinases are approved for the treatment of various cancers[209] and have been a mainstay in the treatment of advanced and metastatic RCC[210,211]. Combination of CDK4/6 inhibitors with mTOR inhibition has, therefore, been an area of great research interest in cancer treatment (FIG. 5).

CDK4 activity is regulated by mTOR, through mTOR-dependent phosphorylation of CDK4-T172 (REFS[116,212]). Insights into the mechanism behind the synergy of mTOR with CDK4/6 inhibition came from studies in cell culture and mouse models using abemaciclib, and suggest that CDK4 and CDK6 can activate mTOR by phosphorylating the tumour suppressor tuberous sclerosis complex 2 (TSC2) on S1217 and S1452 (REF.[63]). TSC2 normally exerts inhibitory control on mTORC1; thus, CDK4 or CDK6 phosphorylation of TSC2 releases mTOR from TSC2-mediated endogenous inhibition, much like Rb phosphorylation by CDK4 or CDK6 releases E2F[63]. Cyclin D was also found to be a binding partner of TSC2 (REF.[213]). Co-overexpression of cyclin D and CDK4 or CDK6 in human embryonic kidney fibroblasts increased TSC2 phosphorylation and decreased TSC2 levels[213], compromising TSC2 function[213]; following TSC2 inhibition, mTOR signalling was initiated, leading to increased phosphorylation of the downstream targets of mTOR, 4E-binding protein 1 (4EBP1) and S6 kinase (S6K), and promoting cell growth[213].

Taken together, these results indicate that the effects of CDK4/6 inhibitors on the suppression of cell proliferation and metabolism partially depend on the inhibition of mTOR signalling. Interestingly, in TNBC cells, palbociclib alone led to upregulation of AKT and mTOR signalling[29]; moreover, synergistic effects of sustained combination treatment with palbociclib and PI3K–mTOR inhibitors in these cells showed increased cell death[29]. In the same study, downregulation of glucose metabolism was observed in TNBC cells treated with palbociclib and this effect was enhanced by the addition of the PI3K inhibitor BYL719 under both normoxic and hypoxic conditions. These data indicate that inhibition of CDK4/6 also negatively affects the function of HIF1 (REF.[29]). The mTOR pathway is crucial for nutrient sensing and metabolic signalling[214]; thus, the synergism and crosstalk of the mTOR and CDK4/6 pathways highlights the importance of integrating nutrient status and cellular metabolism with cell-cycle control, which is of particular interest considering the biology of RCC.

Results of a growth inhibition screen in p16-null non-small-cell lung cancer cell lines in which several targeted and cytotoxic agents were combined with palbociclib showed that only mTOR inhibitors had a synergistic effect with palbociclib[30]. Preclinical work in ER^+ breast cancer cell lines and xenografts demonstrated the most robust growth arrest and delay in resistance development when mTOR inhibition was combined with CDK4/6 inhibition and hormonal therapy[31]. In these models, the addition of the mTOR inhibitor AZD2014 enhanced the effects of CDK4/6 inhibition on E2F-mediated transcription[31] (FIG. 5); furthermore, CDK4/6 inhibitor-resistant MCF7 cell lines reactivated the CDK–Rb–E2F pathway but retained sensitivity to mTOR inhibition[31]. These data suggest that mTOR

inhibition is a therapeutic option in the case of resistance to CDK4/6 inhibitors. Synergism between palbociclib and the second-generation pan-mTOR inhibitor MLN0128 has also been shown in ER^- breast cancer and TNBC cell lines[32]. In a cell-culture model of T cell acute lymphoblastic leukaemia, treatment with mTOR inhibitors and the glucocorticoid dexamethasone acted in synergy with ribociclib, whereas many drugs traditionally used in relapsed T cell acute lymphoblastic leukaemia, such as methotrexate and doxorubicin, which act on rapidly proliferating cells, behaved as ribociclib antagonists[215]. These data indicate that the drug mechanism of action is essential to identify efficacious combination therapies. Synergism between palbociclib and the mTOR inhibitor MLN0128 was also shown in a mouse model of intrahepatic cholangiocarcinoma, in which either agent alone reduced tumour growth and the combination therapy led to tumour regression[33]. In this context, mTOR inhibition counteracted the upregulation of cyclin D seen with palbociclib alone[33]. Similarly, palbociclib in combination with the mTOR inhibitor everolimus showed enhanced ability to disrupt metabolism and cell growth in preclinical models of glioblastoma compared with the single agents[34]. Additionally, everolimus treatment enhanced the blood–brain barrier permeability of palbociclib in mice, which is promising for the treatment of intracranial metastases in several malignancies, including RCC[34]. Combination of the mTOR inhibitor temsirolimus and palbociclib also showed a synergistic effect in cell-line models of diffuse intrinsic pontine glioma, a particularly lethal paediatric brain tumour[35].

In summary, inhibition of CDK4/6 in combination with mTOR inhibitors shows improved efficacy when compared with the respective monotherapies. These findings are of particular relevance to RCC given the prominent role of mTOR hyperactivation in the pathogenesis of this disease and the use of mTOR inhibitors in RCC treatment[7,9].

Role of CDK4 and CDK6 in immune modulation and checkpoint inhibitor response

CDK4 is well known to have a role in T cell proliferation and cytokine responsiveness[216,217], and several studies have explored the role of CDK4 and CDK6 in antitumour immunity as well as the synergism of CDK4/6 inhibition with ICIs (FIGS 2,5). Results of one study using a variety of preclinical models of solid tumours including cell lines and xenografts showed that, in addition to tumour cell cycle arrest, treatment with CDK4/6 inhibitors triggered antitumour immunity primarily via two mechanisms, both of which are associated with decreased DNA methyltransferase 1 (DNMT1) levels, a downstream target of the CDK–Rb–E2F transcriptional axis[36]. First, activation of endogenous retroviral elements resulting from decreased DNA methylation enhances intracellular levels of double-stranded RNA and antigen presentation through a phenomenon known as ‘viral mimicry’[36], and, second, CDK4/6 inhibition suppresses the proliferation of regulatory T cells[36]. Together, these effects promote tumour cell clearance through T cell response, which is enhanced by checkpoint-blockade therapy[36]. Furthermore, transcriptomic analysis of serial biopsy samples obtained from patients ($n = 23$) with breast cancer treated with CDK4/6 inhibitors showed the activation of antitumour immunity[36].

CDK4/6 inhibitors have also been found to enhance the activation of effector T cells and infiltration of T cells into tumours, thereby augmenting the response to anti-PD1 therapy[37–39]. This effect was, at least in part, mediated by CDK6-dependent regulation of the activity of the transcription factor nuclear factor of activated T cells (NFAT), which induces T cell activation[218]; the combination of CDK4/6 inhibitors with anti-PD1 blockade was also associated with high production of the T cell-activating cytokine IL-2, although the mechanism remains to be elucidated[37]. In another study, abemaciclib alone delayed tumour growth in a mouse syngeneic colon carcinoma tumour model and was associated with a T cell inflammatory signature[38], whereas combination with anti-PDL1 therapy led to persistent complete tumour regression in 2 of 10 animals[38]. In mouse models of colon and mammary carcinoma, responses to combined therapy were more effective and robust with phased administration of abemaciclib and anti-PDL1, than sequential administration of abemaciclib and anti-PDL1 (REF.[38]), suggesting that the immunomodulatory effect of CDK4/6 inhibition does not persist after treatment discontinuation. Combination treatment with CDK4/6 inhibitors and anti-PD1 therapy in melanoma xenograft models demonstrated enhanced growth inhibition compared with monotherapy with either agent and was associated with increased tumour immune cell infiltration[39]. Additionally, in a cohort of patients with advanced melanoma, copy number gain of *CDK4* was associated with innate resistance to checkpoint inhibitor therapy, although the mechanisms were not elucidated[39].

Experiments in cell models of cervical, breast, colon and prostate cancer have demonstrated that PDL1 expression is affected by CDK4/6 inhibitor treatment[40,219]. PDL1 undergoes ubiquitination and degradation by the E3-ligase cullin 3–speckle-type POZ protein (SPOP)[40]. In preclinical mouse models of colon carcinoma CDK4/6 inhibition decreased the phosphorylation of SPOP, promoting its degradation and ultimately increasing the stability of PDL1 (REF.[40]). In this study, tumour regression and overall survival were improved in mice treated with ICIs in combination with CDK4/6 inhibitors[40]. At the end of the study, 8 of 12 mice treated with combination therapy were alive with minimalto-no tumour burden, whereas the same response was observed only in 3 of 14 mice in the ICIs-alone group, and no survivors were reported in the untreated group[40]. This effect could, in part, result from the increase in PDL1 expression caused by CDK4/6 inhibitors, which often correlates with tumour responsiveness to ICIs[220,221]. Other mechanisms have been proposed to contribute to the increase in PDL1 expression upon CDK4/6 inhibitor treatment[219]. PDL1 is a transcriptional target of NF-κB that is selectively upregulated upon *RB1* knockdown or CDK4/6 inhibition[219] through CDK4/6-mediated phosphorylation of Rb (Rb-S249/T252), which promotes its interaction with NF-κB[219]. In a cell model of prostate adenocarcinoma, the binding of phosphorylated Rb-S249/T252 to NF-κB has been shown to block the transcription of PDL1 (REF.[219]); treatment of prostate adenocarcinoma cells with a phosphomimetic Rb peptide suppressed the upregulation of PDL1 induced by radiotherapy, which promotes immunostimulation via induction of immunogenic cell death[219,222].

Overall, several mechanisms contribute to the good response to combination therapy with CDK4/6 inhibitors and immune checkpoint blockade, and much more remains to be discovered, including whether these mechanisms are cell-cycle dependent or independent. Several clinical trials examining the efficacy of combination therapy with CDK4/6 inhibitors

and ICIs are ongoing in various cancers, including prostate, breast, and head and neck cancer[24–28]. Additionally, as checkpoint inhibitors have seen clinical success in RCC[8–10], combination of CDK4/6 inhibition with checkpoint blockade could prove very effective in RCC.

Potential of CDK4/6 as targets in RCC

No dedicated trials to test CDK4/6 inhibitors in kidney cancer have been completed so far, but one is ongoing[223] and a great deal of evidence suggests the potential utility of these drugs in RCC. Standard therapeutics for systemic therapy in RCC include TKIs (such as sunitinib)[5], mTOR inhibitors (such as everolimus)[4] and ICIs (such as nivolumab, ipilimumab and pembrolizumab)[8–10] (FIG. 5). Unfortunately, an understanding of how to predict response to therapy and how to rationally combat intrinsic and acquired resistance is still limited. CDK4/6 inhibitors have been successful in a variety of cancers and particularly promising in preclinical studies in combination with mTOR and other kinase inhibitors, as well as with checkpoint inhibitors[31,33,34,36–40,219], suggesting an opportunity to optimize the use of these therapeutics in RCC.

The potential importance of CDK4/6 inhibitors in RCC is supported by work on microRNAs (miRNAs), which are short, non-coding RNAs that regulate target mRNAs by modulating their translation or stability[224]. Some miRNAs have a tumour-suppressive function, in part mediated by the targeting and inhibition of CDK4/6 and the downstream effects on the cell cycle, and are downregulated in RCC[225] (FIG. 1). The tumour suppressor miRNA miR-1 was found to be downregulated in a majority (36 of 41) of ccRCC tissue samples compared with normal tissue samples, and low miR-1 correlated with advanced cancer stage ($P = 0.013$) and poor overall survival ($P = 0.012$)[225]. Overexpression of miR-1 in cell culture and xenograft models of RCC inhibited proliferation and metastasis, whereas miR-1 silencing promoted these processes[225]. Importantly, miR-1 targets the expression of CDK4 and CDK6, as well as the proteins Caprin1 and Slug, which are involved in epithelial-to-mesenchymal transition, a process important for metastasis[225]. Another miRNA, miR-206, showed tumour-suppressive properties in RCC, and was found to be significantly downregulated in 5 ccRCC cell lines as well as 39 of 42 ccRCC tissue samples compared with paired normal tissue ($P < 0.01$)[226]. miR-206 was found to induce cell-cycle arrest directly targeting the transcripts of *CDK4*, *CDK9* and *CCND1* (REF.[226]) and low miR-206 levels correlated with increased protein expression of CDK4, CDK9 and cyclin D1 in tumour tissue samples from patients with ccRCC compared with matched normal tissue[226]. Similarly, long non-coding RNAs, which regulate translation of mRNAs and are involved in diverse cellular functions, have a role in the regulation of RCC growth through CDK4/6 signalling[227,228]. The long non-coding RNA DUXAP10 was shown to be upregulated in RCC ($n = 72$ paired RCC and adjacent normal tissues) and correlated with poor clinical prognosis in patients with RCC ($n = 88$ total patients, $P = 0.0037$)[228]. Knockdown of DUXAP10 in ccRCC cell lines correlated with decreased cell growth and downregulation of cyclin D and CDK4 (REF.[228]), indicating a role for DUXAP10-mediated regulation of RCC growth via modulation of CDK4 expression.

Mutation or loss of *CDKN2A* and *CDKN2B* are frequent events in RCC and associated with worse overall survival across various histological subtypes, including clear cell, papillary type 1 and type 2, and chromophobe RCC[103]. Mutation, hypermethylation, or deletion of *CDKN2A* are examples of molecular alterations that correlated with decreased survival across the entire cohort of RCC subtypes in one major comprehensive molecular analysis of RCC tissue samples[103]. Partial deletion of chromosome 9p, which contains *CDKN2A*, is a frequent event specifically in ccRCC (21% of 110 tumour specimens)[103]; results from an integrated proteogenomic analysis by the Clinical Proteomic Tumour Analysis Consortium showed that loss of chromosome 9p is associated with an upregulation of mTOR signalling effectors in tumour tissues from treatment-naive patients with RCC compared with adjacent normal tissue[229]. Evaluation of a panel of 25 RCC cell lines showed a wide range of sensitivity to palbociclib with IC_{50} values ranging from 25 nM to 700 nM (REF.[230]). In this study, following palbociclib treatment, RCC cells underwent G0/G1 cell cycle arrest and late apoptosis, and loss of *CDKN2A*, *CDKN2B* and E2F1 was significantly associated with palbociclib sensitivity ($P = 0.021$, $P = 0.047$ and $P = 0.033$, respectively)[230]. Varying degrees of sensitivity to ribociclib have also been shown in different RCC cell lines (76–280 nM)[228]. The mechanisms through which alterations in the CDK4 signalling axis, including *CDKN2A* loss, affect CDK4/6 inhibitor sensitivity in RCC are not completely understood and warrant further investigation.

The most common histological subtype of kidney cancer is ccRCC[2,7]. The majority of ccRCCs harbour loss-of-function mutations in *VHL*, encoding the recognition subunit of an E3 ubiquitin ligase complex[2,7]. Under normoxic conditions HIF becomes prolyl-hydroxylated, which enables recognition by VHL and subsequent proteasomal degradation of HIF[59]. Under hypoxia, HIF is no longer targeted for degradation, accumulates and induces transcription of target genes needed for angiogenesis and survival[231]. In ccRCC, VHL is non-functional[2,7] and HIF accumulates even in normoxia, transcribing its downstream targets, such as VEGF, and contributing to ccRCC survival[6]. HIF and its downstream pathway components are therapeutic targets approved or under investigation for the treatment of ccRCC[7,232]. Analysis of primary ccRCC tumour samples and matched non-malignant kidney tissue showed an upregulation of cyclin D1 and CDK4 concomitant to HIF overexpression as a result of *VHL* mutation or hypermethylation[233]. Additional work in ccRCC cells has shown that upregulation of the HIF isoform HIF2 as a consequence of *VHL* loss promotes cyclin D1 expression, suggesting a direct role for HIF in CDK4-mediated cell cycle regulation[234]. Similarly, loss of chromosome 3p, which harbours the *VHL* locus, or specific mutations in *VHL* correlate with the upregulation of the G1/S transition[229]. Additionally, inducible MYC activation in a *Vhl* and *Cdkn2A* double-knockout mouse model led to bona fide ccRCC development, whereas *Vhl* loss or MYC activation alone led to only modest clear cell changes in the developed tumours[235]. These studies highlight the importance of alterations in the CDK4/6–CDKN2A axis in ccRCC development.

Enhanced lethality in *VHL*-null ccRCC cells compared with isogenic cells with restored *VHL* was tested in a screen using short hairpin RNA vectors against a total of 88 kinases[236]. Treatment with short hairpin RNA targeting CDK6 displayed preference for *VHL*-null cells, demonstrating a selective therapeutic effect[236]. CDK6 selectivity for *VHL*-null cells was independent of HIF, and a similar enhanced lethality in *VHL*-null cells

compared with *VHL*-restored cells was observed with a derivative of SB-210878 (REF.[237]), a small-molecule CDK4/6 inhibitor[236]. These data suggest that *VHL* status can provide a predictive indication of response to CDK4/6 inhibitors. Synthetic lethality between *VHL* loss and CDK4/6 inhibition was confirmed in cultured ccRCC cell lines, as well as in an RNAi screen in *Drosophila* cells, suggesting a fundamental physiological dependency on both genes and supporting the evaluation of CDK4/6 inhibitors for ccRCC treatment[238]. The synthetic lethality between VHL and CDK4/6 was found to be HIF independent[238] despite transcriptional induction of cyclin D1 by HIF2α[238]. Combination of palbociclib with the HIF2α inhibitor PT2399 led to a synergistic anti-proliferative response in cells with constitutively upregulated HIF transcriptional activity and improved survival in a mouse xenograft model of ccRCC[238]. Mice treated with the combination of palbociclib and PT2399 had a median survival >100 days compared with the ~50 days median survival for vehicle-treated mice ($P < 0.0001$), and 3 of 11 mice in the combination arm remained tumour free and alive 175 days after initial treatment[238]. Additionally, palbociclib showed improved overall survival ($P = 0.0012$) and antitumour activity against xenografts that are not dependent on HIF transcriptional activity, although no enhanced efficacy was observed in combination with PT2399, owing to the fact that the tumour does not depend on HIF signalling[238]. Taken together, these studies clearly demonstrate a link between VHL loss, HIF activation and modulation of cell-cycle regulation via the CDK4/6–cyclin D axis. Therapeutic targeting of CDK4/6 in combination with HIF inhibition (which is also approved for treatment of patients with VHL syndrome[232]) provides additional benefit in a context of hyperactive HIF-dependent transcription, such as VHL loss[238].

The combination of TKIs and CDK4/6 inhibitors has also been explored in ccRCC[239]. Curcumin, a component of the spice turmeric was demonstrated to have anticancer activity against various cancer cells[240] and is known to exert some of its functions by acting as an inhibitor of CDK4 (REF.[241]). Moreover, addition of curcumin potentiated the growth inhibitory effect of sunitinib in 786-O ccRCC cells[242]. A similar effect was observed with the *Scutellaria baicalensis* Georgi-derived natural compound wogonin, which was found to inhibit the CDK4–Rb pathway in 786-O cells[243]. Treatment with wogonin or palbociclib was able to overcome sunitinib resistance in 786-O cells, highlighting the potential for therapeutic targeting of CDK4 in TKI-resistant RCC[243]. In another study, abemaciclib alone and in combination with the TKI sunitinib[239] decreased cellular viability and increased apoptosis in ccRCC cells[239]. Additionally, abemaciclib as a single agent or in combination with sunitinib led to regression of 786-O xenografts, and tumour regression was also observed with addition of abemaciclib following pre-treatment with sunitinib[239]. The combination of abemaciclib and sunitinib is currently under clinical investigation in a phase I trial in metastatic RCC (NCT03905889)[223]. To date, NCT03905889 is the only trial testing CDK4/6 inhibitors specifically in RCC, but other active clinical trials are ongoing for various pan-cancer indications for which patients with RCC might be eligible (TABLE 1). Many of these trials are investigating the combination of CDK4/6 inhibitors with other therapeutics, such as TKIs, and conventional chemotherapeutics[223,244–246] (TABLE 1); inclusion criteria also take into account predictive markers such as CDK4/6 amplification, and p16 or Rb expression[180–183,244,247–256] (TABLE 1). Overall, evidence suggests that use

of CDK4/6 inhibitors in RCC is promising and more preclinical and clinical examination is warranted.

Conclusions

Remarkable progress in the treatment of advanced and metastatic kidney cancer has been made over the past two decades. Despite the success of TKIs and mTOR inhibitors such as sunitinib and everolimus, as well as that of immune checkpoint blockade with agents such as nivolumab, ipilimumab and pembrolizumab, intrinsic and acquired resistance to these therapeutics is a problem. Inhibition of CDK4/6 with palbociclib, ribociclib or abemaciclib has become a standard therapy for HR^+ and $HER2^-$ breast cancer and shows great clinical promise in a wide variety of other cancers. Additionally, emerging evidence suggests that CDK4/6 inhibitors can work synergistically with TKIs, mTOR inhibitors and ICIs. The success of combination therapies, coupled with limited but promising evidence of a role for CDK4 and CDK6 in RCC, suggests that further preclinical and clinical exploration of CDK4/6 inhibition in this cancer is warranted. RCC is traditionally considered a metabolic disease, and alterations of various metabolic and nutrient sensing signalling pathways in this cancer are crucial for pathogenesis and survival. CDK4 and CDK6 are involved in the integration of metabolic and nutrient sensing signalling pathways to control cell-cycle progression. An intimate relationship between CDK4/6 and master regulators of cell growth pathways, such as mTOR and HIF, exists; thus, preclinical studies to understand the mechanisms through which CDK4/6 pathways lead to RCC progression are required. Upfront identification of regulatory mechanisms of CDK4/6 activity and markers for sensitivity and resistance to CDK4/6 inhibitors will help in the rational selection of patients and combination therapies for future clinical trials examining the efficacy of CDK4/6 inhibitors in RCC.

Acknowledgements

The authors are grateful to W. Marston Linehan (Urologic Oncology Branch, NCI) for his scientific contribution. This work was partly supported by the National Institute of General Medical Sciences with the NIH (grants R35GM139584, R01GM124256 and DoD KC190038 (M.M.), and R01GM139932 (D.B.)). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This work was also supported by funds from SUNY Upstate Medical University and the Upstate Foundation (D.B. and M.M.).

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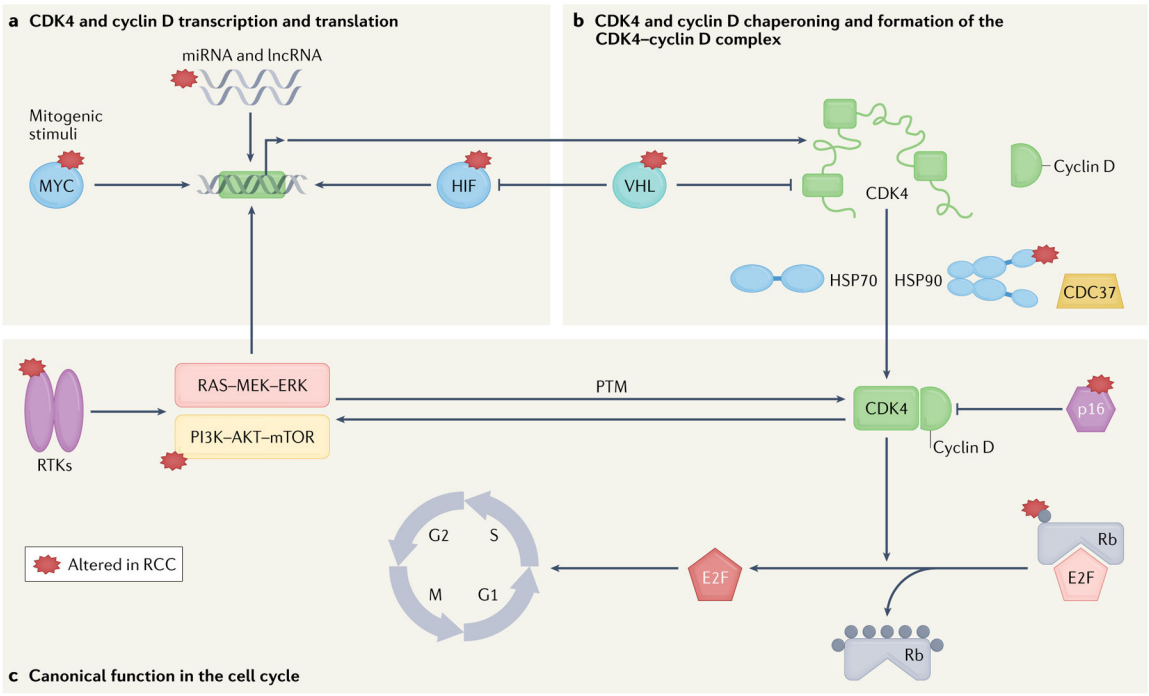

Fig. 1 |. CDK4 stabilization and canonical function in the cell cycle.

a | Cyclin D and cyclin-dependent kinase 4 (CDK4) transcription and translation can be activated by mitogenic stimuli[43] and long non-coding RNAs (lncRNAs)[227,228], or inhibited by the action of several microRNAs (miRNAs)[225,226]. **b** | CDK4–cyclin D complex assembly and stabilization require components of the chaperone machinery such as heat shock protein 90 (HSP90) in complex with the co-chaperone CDC37 (HSP90–CDC37) and HSP70, which assist folding and stabilization of cyclin D and CDK4 (REFS[71,72,124–128]). **c** | Canonically, CDK4 phosphorylates the tumour suppressor retinoblastoma (Rb; grey dots), enabling the transcription factor E2F to enter the nucleus and transcribe its target genes, promoting progression through the S phase of the cell cycle[15]. The tumour suppressor p16 is an endogenous inhibitor of CDK4 function[65]. Several signalling cascades have a role in the transcriptional regulation of CDK4–cyclin D kinase complex including MYC[55], RAS–mitogen-activated protein kinase kinase (MEK)–extracellular signal-regulated kinase (ERK)[117] and hypoxia-inducible factor (HIF)[60,234]. Von Hippel–Lindau (VHL) regulates CDK4 by controlling the expression of CDK4 and HIF[59,60]. Reciprocal activating mechanisms exist between CDK4 and the phosphoinositide 3-kinase (PI3K)–AKT–mechanistic target of rapamycin (mTOR) pathway (mTOR regulates CDK4–T172 phosphorylation, whereas CDK4 phosphorylates the endogenous mTOR inhibitor tuberous sclerosis complex 2 (TSC2))[63,116,212,213]. Many genes and proteins involved in CDK4 function and regulation are known to be altered in renal cell carcinoma (RCC, starred proteins). PTM, post-translational modification; RTK, receptor tyrosine kinase.

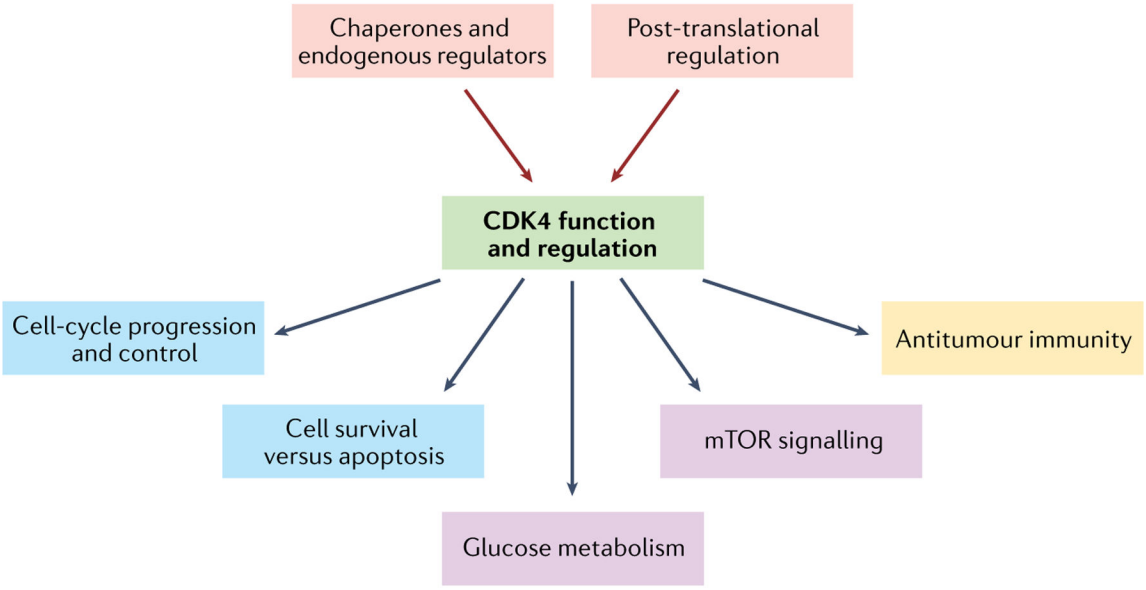

Fig. 2 |. Cellular functions and regulation of CDK4.

Cyclin-dependent kinase 4 (CDK4) activity is subject to multiple levels of regulation (red), including endogenous regulators[93,94,97,109,110] and molecular chaperones (necessary for CDK4 folding and stabilization)[71,72,124–128] as well as post-translational modifications (involved in CDK4 activation)[112,113,116,117]. Once activated, CDK4 exerts multiple functions in the cell: in addition to its canonical role in regulating cell cycle progression and cell survival (blue)[15,57], CDK4 also promotes glucose metabolism and metabolic and survival signalling pathways through the mTOR axis (purple)[62–64]. Last, CDK4 has a role in immune modulation and antitumour immunity (yellow)[23,36–40,219]. mTOR, mammalian target of rapamycin.

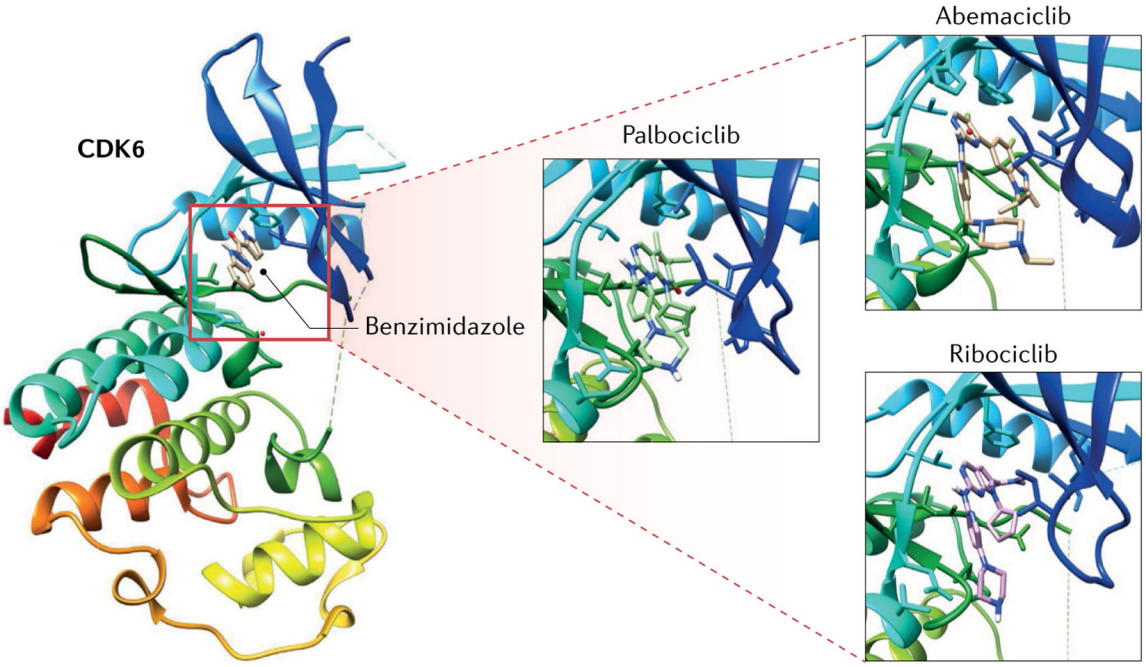

Fig. 3 |. Structure of CDK6 in complex with CDK4/6 inhibitors.

Crystal structure of cyclin-dependent kinase 6 (CDK6) in complex with its ligand benzimidazole bound to the catalytic cleft of CDK6 (Protein Data Bank (PDB) code: 4AUA, red square in the crystal structure on the left)[265]. The catalytic cleft of CDK6 contains the ATP-binding pocket and lies between the N-domain (red, orange, yellow and green) and the C-domain (cyan and blue). Close up of CDK6 catalytic cleft (squares on the right) in complex with the ATP-competitive CDK4 and CDK6 inhibitors palbociclib (PDB code: 5L2I), abemaciclib (PDB code: 5L2S) and ribociclib (PDB code: 5L2T)[163].

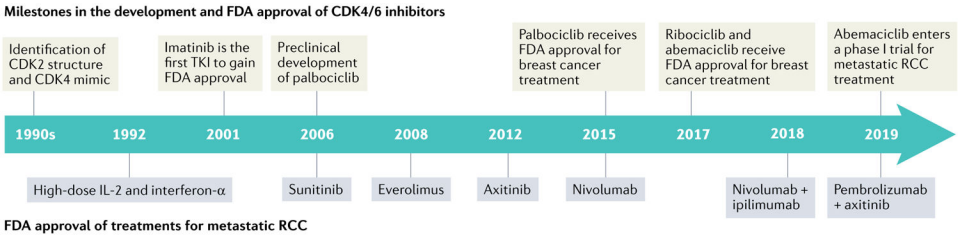

Fig. 4 |. Timeline of CDK4/6 inhibitor development and approval of systemic therapies for renal cell carcinoma.

Timeline showing milestones in the development and FDA approval of cyclin-dependent kinase 4 (CDK4) and CDK6 inhibitors (above)[12–14,21,22]. Also, FDA approval of various systemic and targeted therapies for the treatment of advanced and metastatic renal cell carcinoma (RCC, below) is shown[3–5,8–10]. TKI, tyrosine kinase inhibitor.

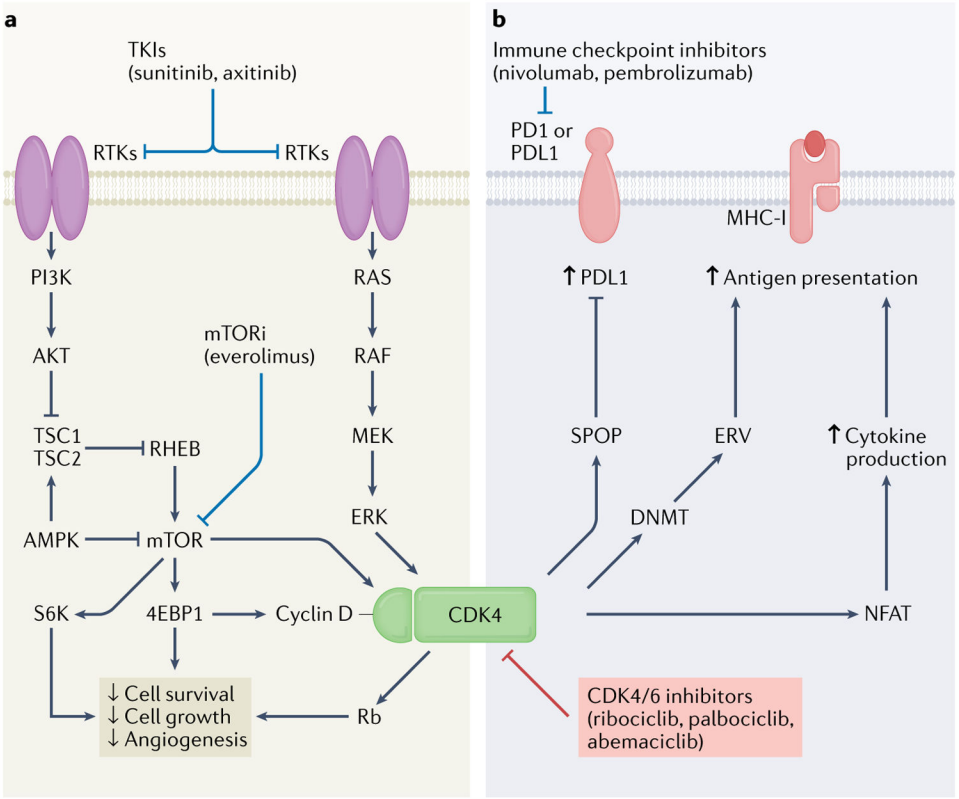

Fig. 5 |. CDK4 at the interface between mTOR signalling and immune checkpoint regulation.

Cyclin-dependent kinase 4 (CDK4)–cyclin D complex function is in part regulated through receptor tyrosine kinase (RTK) signalling axes such as RAS–mitogen-activated protein kinase kinase (MEK)–extracellular signal-regulated kinase (ERK)[117] and phosphoinositide 3-kinase (PI3K)–AKT–mechanistic target of rapamycin (mTOR)[7] and their downstream effectors. Collectively, these pathways work to affect cell and tumour growth and survival. **a** | Tyrosine kinase inhibitors (TKIs, such as sunitinib and axitinib) and mTOR inhibitors (such as everolimus) can work synergistically with CDK4/6 inhibitors (ribociclib, palbociclib and abemaciclib) to reduce cell survival, cell growth and angiogenesis[63,208]. **b** | CDK4 also alters tumour immune response[36–40,219]. CDK4 inhibitors can work synergistically with immune checkpoint inhibitors (such as nivolumab and pembrolizumab) to stimulate antitumour immune response through several mechanisms, including functional modulation of the upstream regulator of programmed cell death 1 ligand 1 (PDL1) speckle-type POZ protein (SPOP) (leading to upregulation of PDL1)[40], as well as DNA methyltransferase (DNMT)[36] (leading to increased antigen presentation) and nuclear factor of activated T cells (NFAT)[37] (leading to increased cytokine production and antigen presentation). CDK6 has not been included in the figure, owing to the discrepancy in the availability of high-quality data. 4EBP1, 4E-binding protein1; AMPK, AMP-activated protein kinase; ERV, endogenous retrovirus; MHC-I, major histocompatibility complex class I; PD1, programmed cell death 1; Rb, retinoblastoma; S6K, S6 kinase; TSC, tuberous sclerosis complex.

Table 1 |

Current CDK4/6 inhibitor trials in rCC and pan-cancer settings

CDK4/6 inhibitor	Companion drug	Companion drug target	Cancer type	Phase, study status	Altered CDK signalling genes[a]	NCT identifier	Refs
Abemaciclib	Sunitinib	VEGF	Metastatic RCC	Phase I recruiting	NA	NCT03905889	26
	None	NA	Solid tumours	Phase II recruiting	*CCND1, CCND2 CCND3, CDK4, CDK6*	NCT03310879	247
	None	NA	Advanced and/or metastatic cancers	Phase I completed	NA	NCT02919696	185,257
	Fulvestrant	ER	Advanced cancer	Phase I active not recruiting	NA	NCT01394016	184,258
	Paclitaxel	None	Advanced or metastatic solid tumours	Phase Ib/II not yet recruiting	*CCND1, CCND2 CCND3, CDK4, CDK6*	NCT04594005	244
Abemaciclib or palbociclib	None	NA	Advanced solid tumours, non-Hodgkin lymphoma, multiple myeloma	Phase II recruiting	*CDK4* or *CDK6* amplification, *CDKN2A* deletion or mutation	NCT02693535	180–182,248
Palbociclib	None	NA	Advanced malignant solid neoplasm	Phase II recruiting	*CCND1, CCND2* or *CCND3* amplification or *CDK4* or 6 amplification and *RB1*-positive	NCT02465060	183,249
	None	NA	Advanced solid tumours, non-Hodgkin lymphoma, multiple myeloma	Phase II recruiting	*CDKN2A, CDK4, CCND1, SMARCA4*	NCT03297606	250
	None	NA	Rare tumours, refractory tumours	Phase II recruiting	*CDK4* or *CDK6* mutation or amplification	NCT03239015	251
	Telaglenastat	Glutaminase	Solid tumours NSCLC, CRC	Phase I and II recruiting	*KRAS* mutation	NCT03965845	259
	Gedatolisib	PI3K–mTOR	Advanced SCLC, pancreatic cancer, head and neck cancer, solid tumours	Phase I recruiting	*CDK4 or CDK6*, PI3K activity	NCT03065062	252
	Trametinib	MEK	Solid tumours	Phase I completed	NA	NCT02065063	245
	PD-0325901	MEK	Metastatic or unresectable NSCLC and solid tumours	Phase I/II active not recruiting	*KRAS* mutation	NCT02022982	246
	5-FU, oxaliplatin	Antimetabolite, alkylating agent	Advanced solid tumours	Phase I active not recruiting	*RB1*-positive	NCT01522989	253
	None	NA	Advanced malignant solid tumours, advanced and refractory lymphomas	Phase II active not recruiting	*CCND1, CCND2, CCND3* amplification	NCT04439201	260
	Taselisib, pictilisib	PI3K	Advanced solid tumours, breast cancer	Phase I active, not recruiting	Hyperactive PI3K–AKT pathway	NCT02389842	261

CDK4/6 inhibitor	Companion drug	Companion drug target	Cancer type	Phase, study status	Altered CDK signalling genes[a]	NCT identifier	Refs
Ribociclib	Gemcitabine	Antimetabolite	Metastatic solid tumours	Phase I active not recruiting	Amplification of *CDK4, CDK6, CCND1* or *CCND3*; expression of *RB1, CDKN2A*	NCT03237390	254
	HDM201	p53–MDM2	Malignant solid tumour	Phase II recruiting	*CDK4, CDK6 CCND1* or *CCND3* amplification, *CDKN2A* deletion, *RB1*-positive, *TP53* wild type	NCT04116541	255
CS3002	None	NA	Advanced solid tumours	Phase I recruiting	NA	NCT04162301	262
HS-10342	None	NA	Advanced solid tumours	Phase I recruiting	NA	NCT04060511	263
PF-07220060	Letrozole, fulvestrant	Aromatase, ER	Liposarcoma, colorectal, prostate, breast, lung adenocarcinoma, solid tumours	Phase I not yet recruiting	*CDK4* or *CCND1* amplification for solid tumours	NCT04557449	256
TQB3303	None	NA	Advanced malignant solid tumour	Phase I not yet recruiting	NA	NCT04275050	264

CDK, cyclin-dependent kinase; CRC, colorectal cancer; ER, oestrogen receptor; MEK, mitogen-activated protein kinase kinase; MDM2, murine double minute 2; mTOR, mammalian target of rapamycin; NA, not applicable; NCT, national clinical trial; NSCLC, non-small-cell lung cancer; PI3K, phosphoinositide 3-kinase; Rb, retinoblastoma; RCC, renal cell carcinoma; SCLC, small cell lung cancer; VEGF, vascular endothelial growth factor.

[a]Altered CDK signalling genes column indicates genes in which abnormalities are required for inclusion criteria; specific types of alteration are indicated when appropriate.