Cyclin D as a therapeutic target in cancer

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Abstract | Cyclin D1, and to a lesser extent the other D-type cyclins, is frequently deregulated in cancer and is a biomarker of cancer phenotype and disease progression. The ability of these cyclins to activate the cyclin-dependent kinases (CDKs) CDK4 and CDK6 is the most extensively documented mechanism for their oncogenic actions and provides an attractive therapeutic target. Is this an effective means of targeting the cyclin D oncogenes, and how might the patient subgroups that are most likely to benefit be identified?

INK4 family
This family of CDK inhibitor proteins specifically prevent the activation of CDK4 and CDK6, generally by inhibiting cyclin D association.

CIP and KIP family
This family of CDK inhibitor proteins bind cyclin–CDK complexes and are potent inhibitors of cyclin E–CDK2 and cyclin A–CDK2. They act as assembly factors for cyclin D–CDK4 and cyclin D–CDK6, but can also inhibit the activity of these kinases.

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The cyclins were named because of their periodic, cell cycle-dependent pattern of expression. The synthesis of individual cyclins, and consequent cyclin-dependent kinase (CDK) activation at specific cell cycle stages, coordinates the sequential completion of DNA replication and cell division[1,2]. These kinases also underlie the checkpoints that halt cell cycle progression in response to DNA damage and defects in the mitotic spindle. Consequently, CDK activity is tightly regulated at multiple levels through several mechanisms. These include the abundance of the regulatory cyclin subunits; their association with the catalytic CDK subunit; activating and inhibiting phosphorylation events; and the abundance of members of two families of CDK inhibitory proteins — the INK4 family, which comprises INK4A (also known as p16), INK4B (also known as p15 and CDKN2B), INK4C (also known as p18 and CDKN2C) and INK4D (also known as p19 and CDKN2D), and the CIP and KIP family, which comprises p21 (also known as CDKN1A), p27 (also known as CDKN1B) and p57 (also known as CDKN1C)[1,2].

Cyclin D1, cyclin D2 and cyclin D3 are closely related G1 cyclins (BOX 1). They activate CDK4 and CDK6, which are phylogenetically distinct from the canonical CDKs, CDK1 and CDK2, and have different substrate specificity[3,4]. Extracellular signals, including growth factor receptor activation and integrin-derived adhesion signalling, influence cyclin D transcription, translation and protein degradation, thereby integrating mitogenic, differentiation and attachment signalling with the cell cycle machinery[5]. The deregulation of cyclin expression or CDK activation can directly lead to some of the hallmarks of cancer[6] by causing proliferation that is independent of normal extracellular cues, or by overriding checkpoints that ensure genomic integrity and stability[7]. Since their discovery, mammalian cyclins and CDKs have been the focus of widespread attention as potential oncogenes, and there is an extensive body of literature documenting their deregulation in cancer and oncogenic capacity in experimental models (reviewed in REF. 7).

This Review focuses on the role of D-type cyclins as oncogenes and their potential as therapeutic targets in human cancer. The emphasis is on cyclin D1, because of the weight of evidence for its widespread role in human cancer and the greater depth of its functional characterization compared with cyclin D2 and cyclin D3. We summarize the progress that has been made with inhibitors of the cyclin D-associated kinases CDK4 and CDK6 as potential cancer therapeutics and we evaluate other means of targeting cancers with altered cyclin D expression, and how the patient subgroups that are most likely to respond to therapeutic interventions that target cyclin D might be identified.

Biological functions of D-type cyclins

CDK activation. The earliest known and best-understood function of cyclin D is to promote cell proliferation as a regulatory partner for CDK4 or CDK6. Extensive research into the underlying mechanisms led to the RB pathway model (reviewed in REF. 7), in which the activation of cyclin D–CDK4/CDK6 initiates the release of the RB-dependent cell cycle-inhibitory 'brake' that governs cell cycle transitions during quiescence, senescence and differentiation (FIG. 1). The specific inhibition of cyclin D–CDK4/CDK6 through the induction of INK4A promotes RB-dependent cell cycle arrest in some circumstances, such as during senescence[8]. The simple model in which RB phosphorylation by CDKs, including cyclin D–CDK4/CDK6, promotes the activation of E2F-responsive

At a glance

- Cyclin D–cyclin-dependent kinase 4 (CDK4) or CDK6 activation promotes cell cycle progression through the phosphorylation of substrates, including RB and transcription factors with roles in proliferation and differentiation. These kinase complexes also target substrates with roles in centrosome duplication, mitochondrial function, cell growth, cell adhesion and motility, and cytoskeletal modelling.
- D-type cyclins have non-catalytic roles in which interactions with chromatin-modifying enzymes and diverse transcription factors, including steroid hormone receptors, leads to the transcriptional regulation of suites of genes that are involved in proliferation and differentiation. Independently of CDK activation, the D-type cyclins also facilitate efficient DNA repair and indirectly activate CDK2 through the sequestration of CDK inhibitors.
- *CCND1* is an established human oncogene that is commonly overexpressed through copy number alterations, or more rarely by mutation, or as a consequence of the deregulation of mitogenic signalling downstream of oncogenes such as *ERBB2*. *CCND1* overexpression causes a number of potentially oncogenic responses in experimental models and is associated with poor patient outcome.
- Cyclin D1 and its associated CDKs are potential therapeutic targets. Promising results from early CDK inhibitors in experimental systems were not followed by evidence for efficacy in clinical trials. Possible reasons for this disappointing outcome include poor pharmacokinetics, suboptimal dosing schedules and clinical testing in unselected patient populations. Second-generation, more selective inhibitors of CDK4 and CDK6 are now undergoing clinical testing.
- Possible alternative approaches to targeting cyclin D1 include the use of compounds that affect *CCND1* transcription or cyclin D1 protein turnover, and the use of combination therapies that simultaneously target multiple end points of cyclin D1 action. Central to the effective use of these novel approaches is the better selection of patient subgroups that are likely to respond.

genes that are necessary for DNA synthesis has subsequently been elaborated to take into account factors such as combinatorial interactions between RB family proteins and various members of the E2F family, which includes both activator and repressor proteins, as well as some atypical E2Fs that repress transcription independently of RB family members (reviewed in REF. 9).

Evidence that cyclin D1 is not necessary for proliferation in cultured cells in the absence of functional RB suggested that RB is the principal substrate for cyclin D1–CDK4, and presumably for other cyclin D-associated CDKs[10], at least in terms of this specific end point of cyclin D1 action. However, cyclin D1 has functions other than facilitating the initiation of DNA synthesis, and these observations do not exclude the possibility that these functions might depend on substrates other than RB. Indeed, more recent studies have shown that cyclin D–CDK4 has physiologically relevant substrates in addition to RB family members (FIG. 1). These include transcription factors, such as the transforming growth factor-β (TGFβ)-responsive transcriptional modulator SMAD3 (REF. 11); members of the RUNX family[12,13]; GATA4 (REF. 14) and the MEF2 family (REF. 15), which have roles in the proliferation and differentiation of specific cell lineages; and BRCA1 (REF. 16), which coordinates DNA damage repair, ubiquitylation and transcriptional regulation to maintain genomic stability. Other CDK4 targets have roles in processes that are tightly linked to, and coordinated with, chromosomal DNA replication and segregation, such as centrosome duplication and separation[17], mitochondrial function[18] and cell growth. Cyclin D–CDK4 regulation of cell growth occurs through effects on both protein synthesis (through tuberous sclerosis complex (TSC) regulation of mTOR)[19] and ribosome biogenesis (through phosphorylation of MEP50, a co-activator of the arginine methyltransferase PRMT5, which in turn methylates ribosome proteins)[20,21]. With the exception of TSC regulation of mTOR, whether these substrates are targets for cyclin D–CDK complexes other than cyclin D1–CDK4 has not been examined in detail. CDK4 and CDK6 have overlapping, but not identical, substrate specificity *in vitro*[22]. The cyclin component of the kinase complex also confers some substrate specificity[22], and functional differences between cyclin D1 and cyclin D2 have been identified[23]. Consequently, these roles may not be characteristic of D-type cyclin–CDK complexes other than cyclin D1–CDK4.

Some CDK4/CDK6 substrates have roles in cellular processes that are less directly involved in cell cycle control, in particular cell motility, cell adhesion and cytoskeletal remodelling[24]. Fibroblasts, epithelial cells and macrophages have increased adhesion and reduced migration in the absence of cyclin D1 (REFS 25–27), and thymocyte adhesion is decreased in the absence of either CDK4 or cyclin D3 (REF. 28). In fibroblasts and epithelial cells, the expression of a cyclin D1 point mutant (K112E) (BOX 1) that cannot activate CDK4 or CDK6 does not decrease adherence or enhance motility, unlike the wild-type protein[26,27], indicating that these effects are dependent on CDK activity.

Non-catalytic functions. Not all actions of cyclin D–CDK4/CDK6 depend on substrate phosphorylation. One major non-catalytic function of the D-cyclins is transcriptional regulation. Cyclin D1 is tethered to the promoters of many genes during normal development, probably through interactions with various transcription factors[29]. It also binds regulators of histone acetylation and methylation[30–32] (FIG. 2), seeming to act as a bridge that links DNA-bound transcription factors with chromatin-modifying enzymes and the transcriptional machinery in order to regulate cell proliferation and differentiation (reviewed in REFS 29,33,34). Cyclin D1-responsive genes also include some that promote migration and invasion, such as thrombospondin and the Rho effector ROCK2 (REF. 26). Cyclin D–transcription factor interactions commonly occur through motifs in regions that are poorly conserved between the D-cyclins, pointing to specificity in the transcriptional effects of the individual D-type cyclins, although this has not been widely tested and much of the published literature examines only cyclin D1. However, ectopic expression of each of the D-type cyclins in hepatocytes led to distinct transcriptional profiles[35].

Multiple members of the steroid hormone receptor superfamily and their co-regulators interact with cyclin D1 (reviewed in REFS 33,34) (FIG. 2). Cyclin D1 enhances oestrogen receptor-α (ERα) activity, through interactions with ERα and its co-regulators SRC1 (also known as NCOA1) and AIB1 (also known as NCOA3). Cyclin D2 and cyclin D3 interact poorly with ERα[36,37]. In contrast to its activation of ERα signalling, cyclin D1

DNA damage response
A global cellular response that halts cell cycle progression while damaged DNA is repaired, or that triggers cell death by apoptosis if the damage is too extensive for repair.

Cyclin box
A domain that is characteristic of cyclins and has high sequence conservation across the cyclin family. It mediates cyclin–CDK binding.

inhibits the activity of the androgen receptor (AR), thyroid hormone receptor-β and peroxisome proliferator activated receptor-γ (PPARγ), which has a crucial role in fatty acid metabolism, energy homeostasis and adipogenesis. Cyclin D3 interacts with cellular retinoic acid-binding protein 2 (CRABP2) and retinoic acid receptor-α (RARα); it is the only D-cyclin to interact with these proteins[38] but, like cyclin D1, cyclin D3 also inhibits AR and PPARγ[39,40]. Both direct cyclin D3–AR binding and cyclin D3–CDK11 phosphorylation of AR have been implicated in these effects[40,41].

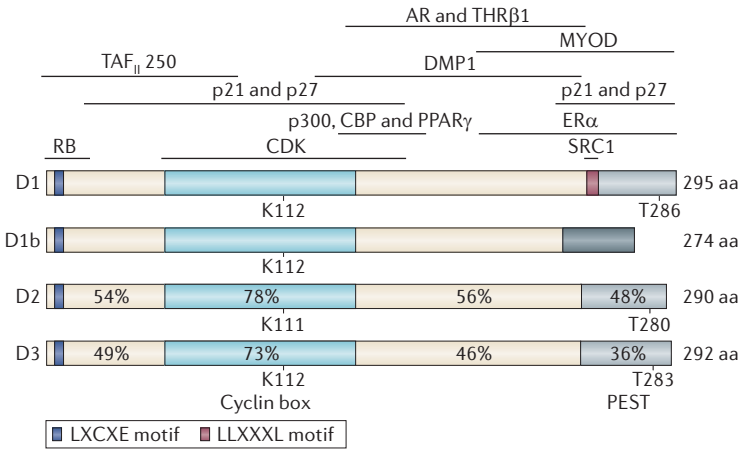
A second well-described non-catalytic role of cyclin D is the sequestration of p21 and p27 by cyclin D–CDK4/CDK6, leading to the indirect activation of CDK2 (REF. 42). The interaction with p21 and p27 has a key role in coordinating CDK activity during G1 phase of the cell cycle, but may also be important for other end points. Cyclin D1 cannot promote migration following p27 knockdown[26], and p27 has CDK-independent effects on cell migration through RHOA and stathmin[43], suggesting that cyclin D1 association with p27 could contribute to cyclin D1 effects on migration independently of CDK4. Similarly, cyclin D1 interaction with p21 contributes to its emerging non-catalytic function in DNA repair.

Cyclin D1 regulates the expression of genes that are involved in DNA replication and the DNA damage checkpoint, and it also interacts with a number of proteins that are involved in the DNA damage response[44,45]. By binding BRCA2 and the recombinase RAD51, cyclin D1 facilitates the recruitment of RAD51 to sites of DNA damage and so promotes homologous recombination-mediated DNA repair[44,45]. Both the ability of cyclin D1 to enhance the DNA damage response and the formation of RAD51 foci require p21 (REFS 45,46), suggesting that p21 may also be present in the cyclin D1–RAD51–BRCA2 complex. Importantly, decreased cyclin D1 expression, but not treatment with a CDK4/CDK6 inhibitor, impairs DNA repair even in cells that lack RB and so do not require cyclin D1 for proliferation[44]. In addition, both the cyclin D1 K112E mutant and wild-type cyclin D1 can restore an efficient DNA damage response in cells lacking all three D-type cyclins[44]. Thus, cyclin D1 facilitation of DNA repair is independent of CDK4/CDK6 activation and distinct from cyclin D1 regulation of proliferation.

Many of the non-catalytic effects of the D-type cyclins have been elucidated using cell culture models, and simultaneous knockout of all three D-type cyclins has a very similar phenotype to knockout of both *Cdk4* and *Cdk6* (REFS 47,48), leading to questions over the degree to which non-catalytic effects contribute to the normal cellular functions of cyclin D1. However, defects in retinal and mammary gland development in mice lacking *Ccnd1* are largely restored when the K112E cyclin D1 point

Box 1 | D-type cyclins

The three D-type cyclins — cyclin D1, cyclin D2 and cyclin D3 — are closely related. Overall, the human cyclin D2 and cyclin D3 proteins are 62% and 51%, respectively, identical to human cyclin D1, and 62% identical to each other (see the Figure). A second human cyclin D1 isoform, cyclin D1b, arises through alternative splicing. It is identical to the full-length, canonical cyclin D1 protein for the first 240 amino acids but diverges at the carboxy-terminal and therefore lacks some key interaction domains[69].



The greatest homology between the D-cyclins occurs in the cyclin box that mediates cyclin-dependent kinase (CDK) binding and is necessary for interaction with the CDK inhibitors p21, p27 and p57. All three D-type cyclins share an RB-binding LXCXE motif at the extreme amino-terminal, a C-terminal PEST domain that is rich in proline, glutamate, serine and threonine and that is characteristic of proteins that are rapidly turned over, and a threonine residue near the C terminus (T286 in cyclin D1) that, when phosphorylated, triggers ubiquitin-mediated degradation. T286 phosphorylation also promotes cyclin D1 nuclear export. Mutations within these highly conserved regions have been widely used to probe cyclin D1 functions; for example, the T286A mutant, which is stable and constitutively located in the nucleus, and the K112E mutant, which does not activate cyclin-dependent kinase 4 (CDK4) or CDK6. The K112E mutant retains the ability to bind CDK4 and sequester CDK inhibitors in some experimental models[49], but not others[27].

The region between the cyclin box and the C terminus contains domains that are responsible for transcription factor–cyclin D1 interactions and is relatively poorly conserved. One key interaction domain is the C-terminal leucine-rich motif of cyclin D1 (LLXXXL), which binds an LXXLL motif in the steroid receptor co-activators SRC1 and AIB1 (REF. 176). The corresponding region in cyclin D2 and cyclin D3 does not contain an LLXXXL motif, although it is leucine-rich.

aa, amino acids; AR, androgen receptor; ERα, oestrogen receptor-α; PPARγ, peroxisome proliferator activated receptor-γ; THRβ1, thyroid hormone receptor β1.

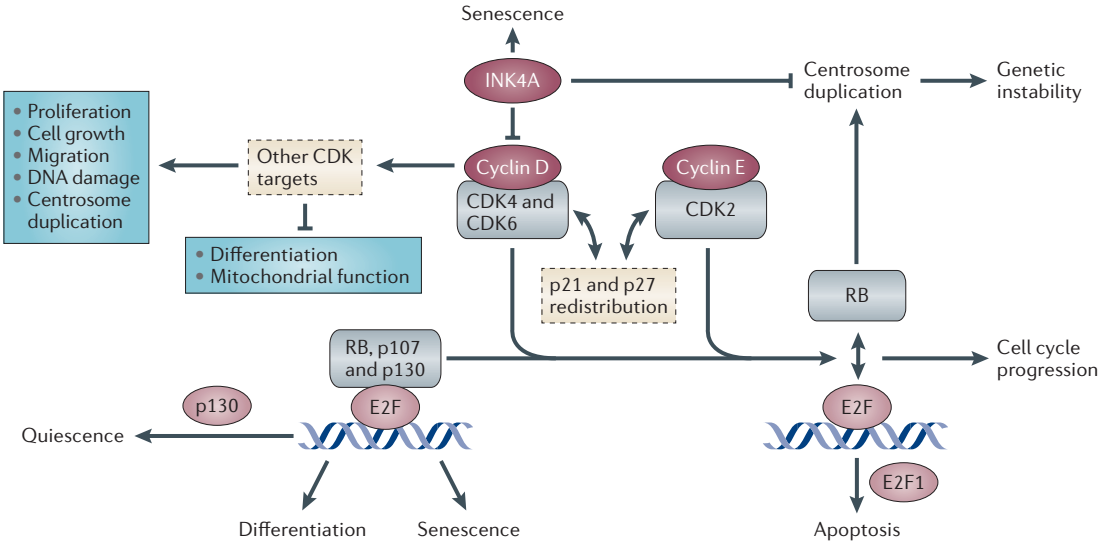


Figure 1 | **CDK-dependent functions of cyclin D.** The activation of cyclin D–cyclin-dependent kinase 4 (CDK4) or CDK6 initiates the phosphorylation of the tumour suppressor protein RB and other RB family members (such as p107 and p130), resulting in the release of E2F transcription factors. In turn, this leads to the transcriptional activation of E2F-responsive genes that are essential for DNA synthesis, including cyclin E and cyclin A, which further promote RB phosphorylation by activating CDK2. Cyclin D–CDK4/CDK6 complexes also indirectly activate cyclin E–CDK2 by sequestering the CDK inhibitors p21 and p27. Both INK4A and RB also affect cellular processes other than cell cycle progression, such as centrosome replication, so that their loss can lead to centrosome amplification and genomic instability. Similarly, cyclin D–CDK4/CDK6 phosphorylates substrates in addition to RB, thereby regulating a diverse set of end points (shown in the blue boxes).

mutant is knocked in to the *Ccnd1* locus[49]. Similarly, deletion of the gene encoding p27 (*Cdkn1b*) can rescue many of the developmental defects that are observed following the deletion of *Ccnd1* or *Cdk4* (REFS 50,51). Thus, the ability of the cyclin D1–CDK4 complex to sequester p27 is required during development, but the kinase activity of this complex is not essential. This provides strong support for the idea that non-catalytic functions of cyclin D1, particularly its ability to sequester CDK inhibitors, are physiologically relevant.

Oncogenic consequences of cyclin D deregulation. More than 100 proteins that interact with cyclin D1 in human cancer cell lines have been identified[44]. Proteins that are involved in cell cycle control and transcriptional regulation are prominent among these interactors. However, proteins that are involved in DNA repair, RNA metabolism, protein folding, cell structure and cell organization are also enriched in the list of cyclin D1-interacting proteins[44]. Consequently, the deregulation of cyclin D1 will not only promote mitogen-independent proliferation, but may also affect other cellular processes, both directly and indirectly, in ways that have potentially oncogenic consequences. These consequences include angiogenesis, through the regulation of vascular endothelial growth factor (VEGF) expression[52], centrosome duplication[53] and the DNA damage response. Nuclear cyclin D1 (but not cyclin D2) is rapidly degraded after DNA damage or replication stress as part of the S phase DNA damage checkpoint[54], but the remaining low levels contribute to efficient DNA repair[44]. High levels of cyclin D1 prime cells for an enhanced DNA damage response[45], perhaps acting as a 'safety net' for rapidly proliferating cells, but sustained cyclin D1–CDK4 activity following DNA damage leads to the inappropriate re-replication of DNA and chromosomal damage[20,55,56]. Collectively, these observations raise the question of which molecular functions of cyclin D1 are crucial during oncogenesis.

Cells lacking all three D-type cyclins are resistant to transformation by various oncogenes *in vitro*, and mice lacking cyclin D1 are resistant to the effects of some, although not all, mammary oncogenes[47,57]. The CDK activation function of cyclin D1 is largely dispensible during mammary development, but it is required for mammary oncogenesis, as mice lacking cyclin D1 or CDK4, or expressing the CDK4/CDK6-specific inhibitor INK4A or the cyclin D1 K112E mutant, are resistant to mammary cancers induced by ERBB2 (also known as HER2 and Neu)[49,57–59]. The targeted deletion of either *Cdk6* or *Ccnd3* causes resistance to lymphomagenesis[60,61], germline deletion of *Cdk4* prevents carcinogen- and MYC-induced skin cancer[7,62], and deletion of *Ccnd2* prevents colorectal adenomas in adenomatous polyposis coli (*Apc*)-deficient mice[63]. The frequent similarities in phenotype between the deletion of D-type cyclins and the deletion of CDK4 or CDK6 indicate that CDK activation makes a substantial contribution to the requirement for the D-type cyclins during oncogenesis. However, it is important to note that these models of tumour initiation do not address the functions of the D-type cyclins that may be required for the continued proliferation of established tumours or their metastatic spread. Similarly, germline deletion of

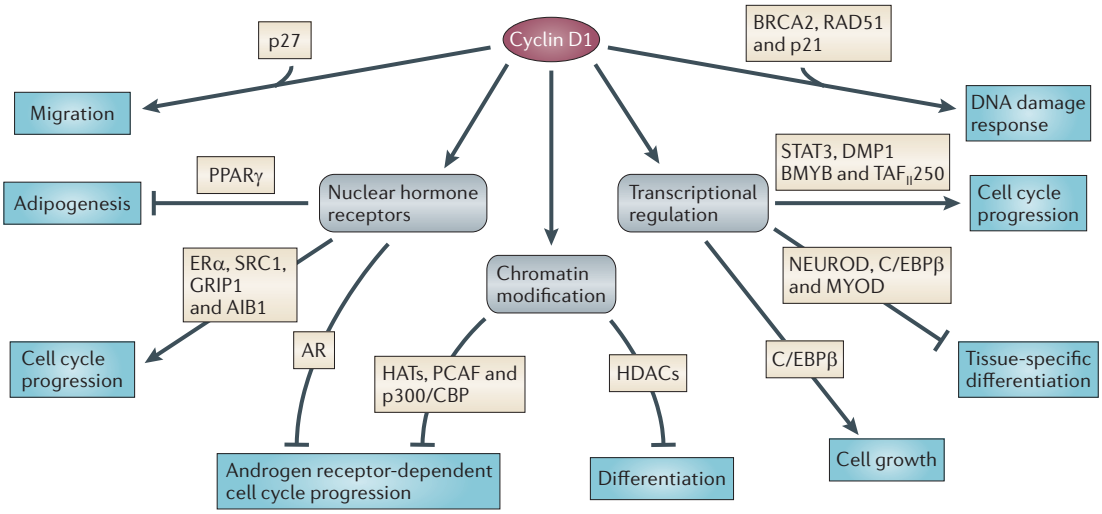


Figure 2 | **CDK-independent functions of cyclin D1.** Although p21 and p27 are constituents of cyclin D–cyclin-dependent kinase 4 (CDK4) or CDK6 complexes, cyclin D1 can bind p21 or p27 independently of CDK4 or CDK6 binding, leading to effects on migration[71] and the DNA damage response[45], respectively. It also has effects on the DNA damage response through interactions with RAD51 and BRCA2 (REF. 44). Cyclin D1 regulates cell proliferation, cell growth and differentiation by binding representatives of several transcription factor families. These include nuclear hormone receptor family members (oestrogen receptor-α (ERα), androgen receptor (AR) and peroxisome proliferator-activated receptor-γ (PPARγ)) and their co-activators (SRC1, GRIP1 and AIB1), MYBB and the MYB-related transcription factor DMP1, as well as the helix–loop–helix transcription factors neurogenic differentiation factor 1 (NEUROD1), MYOD and C/EBPβ (reviewed in REFS 29,33,34). In addition, cyclin D1 binds chromatin-modifying enzymes, including histone acetyltransferases (HATs) such as P/CAF[30], p300/CBP[31] and histone deacetylases (HDACs)[32]. More general effects on transcription can also result from cyclin D1 binding to TAF$_{II}$250 (also known as TAF1)[177], a subunit of the basal transcriptional machinery. STAT3, signal transducer and activator of transcription 3.

these genes is not necessarily informative regarding the likely therapeutic success of inhibiting function rather than expression.

Cancer-associated mutations in cyclin D1 that lead to constitutive nuclear localization and impaired degradation increase the transforming activity of cyclin D1 in cell culture and reduce the latency of cyclin D1-driven tumour development in animal models[64]. This has led to the view that the oncogenic actions of cyclin D1 are predominantly nuclear. However, in some cancers, such as lung, prostate and ovarian cancer, overexpressed cyclin D1 is exclusively cytoplasmic in a significant proportion of cases[65–67]. Cytoplasmic cyclin D1 inhibits apoptosis following low-level DNA damage[68], suggesting that cytoplasmic localization of cyclin D1 may also be relevant to its role as an oncogene.

The cyclin D1b isoform (BOX 1) is present at low levels in many normal cells, but can be overexpressed in human cancer and has biological properties that are different from those of full-length cyclin D1 (reviewed in REF. 69). Cyclin D1b is constitutively localized to the nucleus, is deficient in promoting RB phosphorylation and elicits a transcriptional response that only overlaps with that of full-length cyclin D1 by 33%. It also has a more potent transforming ability than full-length cyclin D1, perhaps because high levels of cyclin D1b do not trigger a DNA damage response[45,69–71]. Cyclin D1b does not bind ERα[72], and although it does bind AR in androgen-dependent prostate cancer cells, it stimulates proliferation, in contrast to the inhibition of androgen-stimulated proliferation by full-length cyclin D1 (REF. 73).

Cyclin D overexpression in cancer

CCND1 is a well-established human oncogene: a recent census concluded that there was substantial evidence for the involvement of *CCND1* amplification and overexpression in breast cancer and significant evidence for its involvement in lung cancer, melanoma and oral squamous cell carcinomas[74]. The criteria used included deregulated expression, particularly when correlated with clinical outcome, and biological consequences of altered expression; for example, the demonstration that cyclin D1 overexpression in the mammary gland is tumorigenic, albeit with a long latency and incomplete penetrance[75]. As illustrated by the examples in TABLE 1, many common cancers have *CCND1* amplification rates of 15–40%, and higher rates of *CCND1* mRNA and protein overexpression. A translocation that juxtaposes *CCND1* with the immunoglobulin heavy chain locus (*IGH*), leading to cyclin D1 overexpression, is diagnostic of mantle cell lymphoma (MCL) (TABLE 1), and the small proportion (<10% of MCLs) that lack this translocation often display overexpression of *CCND2* or *CCND3* (REF. 76). *CCND2* or *CCND3* amplification has been reported, but is rare compared with *CCND1* amplification. *CCND2* amplification is present in only 2% of gliomas[77], and this is the only cancer in which the evidence for cyclin D2 involvement is classed as significant[74], although *CCND1* amplification is one of the most common copy-number alterations in human cancer[78]. Although cyclin D2 is overexpressed in some cancers, *CCND2* is frequently methylated, with loss of cyclin D2 expression in pancreatic, breast and

Table 1 | **Cyclin D1 deregulation in cancer**

Mechanism of deregulation	Tumour type	Frequency	Refs
Amplification and overexpression			
CCND1 amplification	Head and neck squamous cell carcinoma	26–39%	112,178
Cyclin D1 overexpression	Head and neck squamous cell carcinoma	20–68%	112,178
CCND1 amplification	Non-small-cell lung cancer	5–30%	65,74
Cyclin D1 overexpression	Non-small-cell lung cancer	18–76%	65,74,88
CCND1 amplification	Endometrial cancer	26%	179,180
Cyclin D1 overexpression	Endometrial cancer	40–56%	179,180
CCND1 amplification	Melanoma	0–25%	181
Cyclin D1 overexpression	Melanoma	30–65%	181
CCND1 amplification	Pancreatic cancer	25%	182
Cyclin D1 overexpression	Pancreatic cancer	42–82%	182
CCND1 amplification	Breast cancer	15–20%	74,102
Cyclin D1 overexpression	Breast cancer	50–70%	74,102
CCND1 amplification	Colorectal cancer	2.5%	183
Cyclin D1 overexpression	Colorectal cancer	55%	184
Chromosomal rearrangement and overexpression			
CCND1: *IGH* translocation t(11;14)(q13;q32)	Mantle cell lymphoma	>90%	76
Cyclin D1 overexpression	Mantle cell lymphoma	>90%	76
CCND1: *IGH* translocation t(11;14)(q13;q32)	Multiple myeloma	16%	185
Cyclin D1 overexpression	Multiple myeloma	30–50%	185
Splice variants and transcript aberrations			
3′ UTR rearrangements, microdeletions or point mutations	Mantle cell lymphoma	4–10%	82,104
Cyclin D1b overexpression	Breast cancer	22%*	89,91
Cyclin D1b overexpression	Prostate cancer	27%*	90
Mutations affecting nuclear export and proteolysis			
Cyclin D1 T286R; Δ266–295	Oesophageal cancer	4%	83
Cyclin D1 P287S; P287T; Δ289–292	Endometrial cancer	4%	84
FBXO4 S8R, S12L, P13S, L23Q, G30N and P76T	Oesophageal cancer	14%	85

FBXO4, F-box 4; IGH, immunoglobulin heavy chain locus; UTR, untranslated region.*Cyclin D1b overexpression without overexpression of full-length cyclin D1.

prostate cancer[79–81], pointing to a potential role as a tumour suppressor rather than as an oncogene.

Mutations have also been implicated in aberrant cyclin D1 expression (TABLE 1), although they have rarely been investigated, and thus the importance of their role is not yet clear. Mutations in the 3′ untranslated region that result in the stabilization of the *CCND1* mRNA have been reported in MCL[82], and mutations and deletions clustering around T286 have been reported in oesophageal and endometrial cancers[83,84]. Phosphorylation at this site governs the turnover and nuclear export of the cyclin D1 protein, and mutations in F-box 4 (FBXO4), an SCF E3 ubiquitin ligase that targets T286-phosphorylated cyclin D1 protein for degradation, have also been detected in endometrial cancers[85]: up to 20% of endometrial cancers may display nuclear overexpression of stable cyclin D1 through mutations in *CCND1* or *FBXO4*. An initial study has also indicated that cyclin D1 stabilization might be important in breast cancer[86].

Many polymorphisms have been identified within the *CCND1* locus, but the G/A870 polymorphism is the only one that has been investigated in any detail[69]. The A870 allele is present in a large proportion of the population (AA, 25.0%; AG, 50.0% in Caucasians) and is associated with a significant, but small, increase in cancer risk[87]. It favours the production of cyclin D1b. In some cancers, such as lung cancer, cyclin D1b is coordinately overexpressed with full-length cyclin D1, although the absolute expression level of cyclin D1b is much lower[88]. In other cancers, cyclin D1b is independently overexpressed[89–91]. The G/A870 polymorphism is not the only determinant of increased cyclin D1b expression, and although factors that affect cyclin D1 splicing have been identified[92], the mechanisms for the cancer-specific overexpression of cyclin D1b are not known (reviewed in REF. 69).

Overexpression of cyclin D1 is much more common than can be accounted for by copy number or mutational events that affect *CCND1* (TABLE 1). Another route to cyclin D1 overexpression is as a consequence

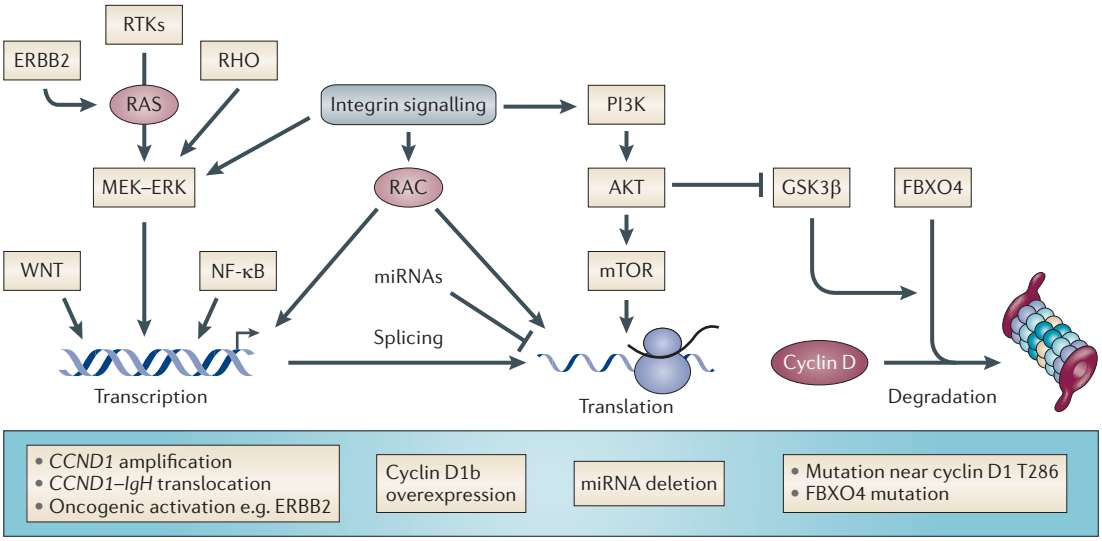


Figure 3 | **Oncogenic activation of cyclin D1.** Cyclin D1 abundance is regulated at multiple levels, each of which can be affected during oncogenesis (shown in the blue box). In addition to activating mutations that target *CCND1*, several other oncogenic events can affect cyclin D1 abundance. These include the activation of signalling through receptor tyrosine kinases (RTKs), and the MEK–ERK, WNT and nuclear factor-κB (NF-κB) pathways that lead to increased *CCND1* transcription. Post-transcriptional effects on cyclin D1 include protein translation through the PI3K–mTOR pathway and effects on cyclin D1 protein turnover through phosphorylation of cyclin D1 at T286 by kinases, including glycogen synthase kinase 3β (GSK3β), that promote F-box 4 (FBXO4)-mediated proteolysis. miRNA, microRNA.

of oncogenic activation of mitogenic signalling pathways. Many signalling intermediates, including the RAS–MEK–ERK and PI3K pathways, regulate cyclin D1 expression[5] (FIG. 3). Overexpression of cyclin D1 through these pathways could have oncogenic consequences in addition to deregulation of mitogenic signalling, as RAS-induced centrosome amplification is dependent on CDK4 (REFS 17,93). ERBB2-driven or RAS-driven mammary cancers in mice express high levels of cyclin D1 (REFS 94,95), and most ERBB2-positive human breast cancers display moderate or strong cyclin D1 expression (for example, REFS 58,96,97). A newly emerging alternative mechanism for cyclin D1 overexpression is the loss of microRNAs (miRNAs) that target *CCND1*, such as miR-15a and miR-16. Their expression is inversely correlated with cyclin D1 expression in the small series of prostate and lung cancers that have been examined to date[98,99] and an *mir-15A* and *mir-16-1* cluster is located at a common site of deletion (13q14.3) in several malignancies, including prostate cancer. There is, however, a second *mir-15A* and *mir-16-1* cluster at 3q26.1, and there are also several other miRNAs that target cyclin D1 (REF. 100), so it is not yet clear how important miRNA deletion might be as a cause of cyclin D1 overexpression.

Correlation with proliferation. The cyclin D–RB–E2F pathway model depicted in FIG. 1 predicts that cyclin D1 overexpression leads to increased CDK4/CDK6 activity, RB phosphorylation, activation of E2F-responsive genes and so increased proliferation. Although this model does not take into account complicating issues such as RB interactions with a large number of cellular proteins, combinatorial interactions between the different E2Fs and RB family members, and distinct roles for individual E2Fs[7–9], there is a substantial body of experimental evidence in support of its predictions. Data from clinical studies are less conclusive. This probably partly reflects the need to use surrogate markers for key end points, such as proliferation, CDK4/CDK6 activity, RB status and E2F activity, in clinical material. However, it is also possible that some of the non-catalytic functions of cyclin D1 contribute to its role in human cancer[101,102].

In MCL *CCND1* mRNA expression correlates with a proliferation signature that is comprised of genes that are expressed at higher levels in dividing cells than in quiescent cells, with the highest levels of proliferation or cyclin D1 expression associated with the poorest overall survival[103]. The expression of this proliferation signature correlated with other markers of proliferation (such as, Ki67 expression and mitotic index)[103]. Although MCL is distinguished by almost universal overexpression of cyclin D1, concomitant deletions of the *CDKN2A* locus (encoding INK4A and ARF), *CDK4* amplification or microdeletions in *RB1* leading to loss of RB protein expression, also occur and are associated with more proliferative, aggressive disease[104]. Increasing cyclin D1 expression in the context of *CDKN2A* deletion is associated with particularly poor patient outcome in both MCL and head and neck cancer[103,105]. One possibility is that multiple lesions in the cyclin D1–RB–E2F pathway may cooperate to increase pathway activation and hence the likelihood of increased proliferation. Another is that the contribution of each component to processes other than cell cycle control (FIG. 1) may offer an additional selective advantage during oncogenesis.

The correlation between high cyclin D1 expression and markers of increased proliferation in MCL is echoed in many large studies in carcinomas. However, in a

large panel of different cancers there was no correlation between cyclin D1 expression and a signature of genes responsive to E2F1 and E2F2 (REF. 106). There was, however, a correlation between cyclin D3 and the signature of E2F1- and E2F2-responsive genes[106]. Furthermore, a study of 1,740 ER-positive breast cancers found that cyclin D1 expression was only moderately correlated with a signature of RB inactivation that contained proliferation-related genes[107], and another large study of 779 breast cancers from multiple cohorts found that although expression of cyclin B1 and cyclin E1 was consistently correlated with Ki67 expression, cyclin D1 expression was not[108]. Overall, these observations suggest that cyclin D1 action in breast cancer, and perhaps other cancers, might not simply be a consequence of increased proliferation, which is consistent with experimental evidence showing that genes regulating various cellular processes in addition to proliferation are responsive to cyclin D1 (REFS 18,109,110) and that cyclin D1 expression is not correlated with CDK4 activity in breast cancer cell lines[111]. C/EBPβ, a transcription factor that regulates cellular differentiation, could be involved in these effects, as cyclin D1 overexpression antagonized C/EBPβ repression of genes that comprise a cyclin D1 signature in breast cancer[106]. However, the relationship between genes with promoters that are bound by cyclin D1 and the expression of genes that correlate with cyclin D1 overexpression in cancer has only been examined for selected cyclin D1-responsive genes[109,110]. One recent study was able to distinguish breast cancer subgroups that displayed overexpression of cyclin D1 alone, rather than in combination with cyclin B1 overexpression, which was closely associated with proliferation[108]. The subgroup with both cyclin D1 and cyclin B1 overexpression had a significantly poorer outcome. The difference in phenotype implies a difference in intrinsic biology and suggests that it may be informative to distinguish cyclin D1 overexpression that either is or is not associated with increased proliferation.

Relationship with patient outcome. Cyclin D1 overexpression is associated with shorter patient survival in many cancers and is often associated with increased metastasis (REFS 104,112, for example), which is consistent with the ability of cyclin D1 to enhance migration and invasion. In lung and breast cancer this relationship has been addressed in multiple large cohorts but remains unclear, at least partly owing to confounding technical and reagent issues, and the presence of different isoforms and subcellular localization of cyclin D1 (REFS 65,88,89,113,114). *RB1* mutation or deletion results in reduced cyclin D1 expression[115], so the highest and lowest extremes of cyclin D1 expression are both markers of RB pathway deregulation. Consequently, in populations in which both *RB1* inactivation and cyclin D1 overexpression are common, it is difficult to draw definitive conclusions without separately comparing high and low cyclin D1 expression to intermediate, presumably normal, levels of expression. Like cyclin D1 overexpression, RB loss does not have a clear-cut association with patient outcome[116]. However, a gene signature of RB loss seems to be more consistently associated with poor patient outcome[116], suggesting that it may be useful to assess the effects of cyclin D1 deregulation using other parameters in addition to cyclin D1 expression.

In breast cancer, cyclin D1 overexpression is strongly associated with the ER-positive, better prognosis subtype[102]. This probably accounts for the observation that in unstratified breast cancer samples cyclin D1 overexpression either is not significantly correlated with outcome or is associated with favourable outcome[116]. As breast cancer subtypes display marked differences in phenotype and clinicopathological features it is difficult to conclusively determine the effects of cyclin D1 overexpression unless ER-positive and ER-negative cancers are considered separately. In ER-positive patients, cyclin D1 expression was significantly associated with a shorter time to metastasis and reduced patient survival in one large study[117] (E.A.M., R.L.S. and C. M. McNeil, E. K. A. Millar and S. A. O'Toole unpublished observations). However, cyclin D1 expression did not correlate with survival in another study, although this study did find that *CCND1* amplification was a significant independent predictor of survival[118].

Not all studies of cyclin D1 overexpression have controlled for treatment effects, although in experimental models, cyclin D1 overexpression causes resistance to some cytotoxic drugs, as well as to targeted therapies such as anti-oestrogens, the selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib, and inhibitors of BRAF and MEK signalling[119–124]. It has also been implicated in acquired radioresistance[125]. Multiple studies of large cohorts with well-controlled treatment regimens have found a significant association between high cyclin D1 expression and poor outcome in women treated with tamoxifen[96,126,127], with a further study noting a borderline association[108]. However, with the exception of endocrine resistance in breast cancer, few studies have addressed the relationship between cyclin D1 expression and response to therapy in human cancer.

Targeting cyclin D as therapy for cancer

Oncoproteins are attractive therapeutic targets as they are causally related to cancer development, and cancer cells often become dependent on them for continued proliferation and survival (oncogene addiction)[128]. The D-type cyclins are generally regarded as difficult to target directly, as they lack intrinsic enzymatic activity and are intracellular, although several possible approaches have been suggested (FIG. 4). Given the increasingly routine clinical use of specific kinase inhibitors[129], a more immediately feasible approach has been to target cyclin D by inhibiting associated kinases.

Therapeutic inhibition of CDK4/CDK6. Early evidence of the high incidence of aberrant cyclin expression in myriad cancer types and the sensitivity of cancer cells to acute inhibition of cyclins or CDKs, including cyclin D1, led to programmes that aimed to develop small-molecule CDK inhibitors as therapeutics[130,131]. Initial enthusiasm was tempered by increasing experimental evidence indicating that individual CDKs, including CDK2 and CDK4, were largely dispensible during development, and that CDK2 activity was not necessary

Oncogene addiction
Heightened dependency of cancer cells on specific oncogenes, so that, despite the presence of multiple genomic alterations, inactivation of a single oncogene can be sufficient to impair proliferation and survival.

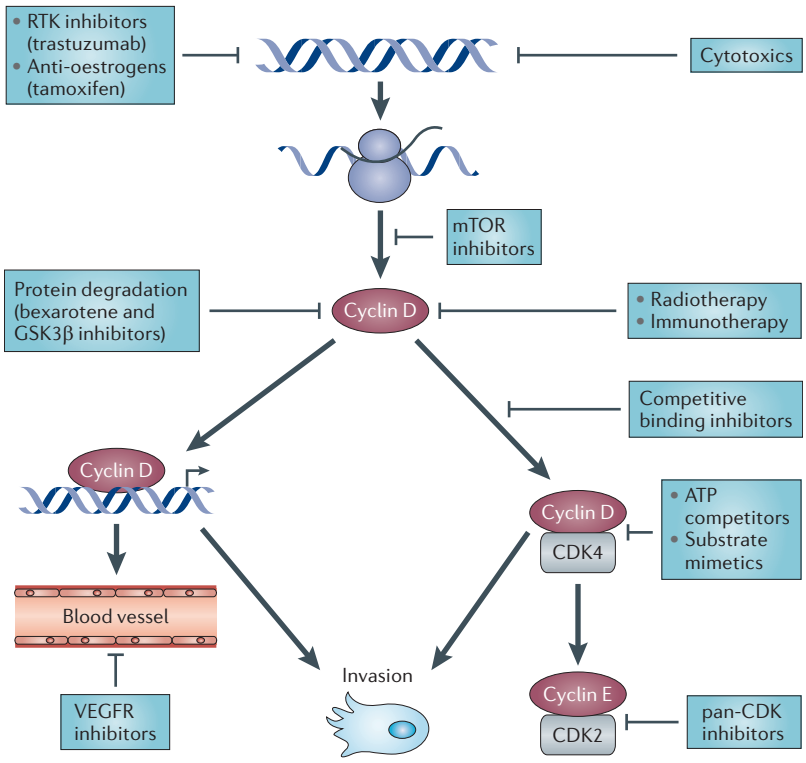


Figure 4 | **Therapeutic targeting of cyclin D1.** Possible therapeutic approaches to targeting cyclin D1-dependent cancers range from downregulating cyclin D1 to inhibiting end points of cyclin D1 action. The most immediately feasible approaches are to target proliferation through the inhibition of cyclin-dependent kinase 4 (CDK4) and CDK6, either alone or in combination with other CDKs, or to target cyclin D1 actions more generally but less directly by the use of compounds with actions that include cyclin D1 downregulation or protein degradation, such as cytotoxics, radiotherapy and targeted therapies such as trastuzumab, tamoxifen or mTOR inhibitors. Greater specificity for individual functions could be achieved by using competitive inhibitors of specific protein–protein interaction domains in cyclin D1 or by substrate mimetics. Emerging possibilities include broad-based inhibition, either by single molecules with combined actions against multiple cyclin D1 targets, or by a combination of molecules each targeting a specific function. GSK3β, glycogen synthase kinase 3β; RTK, receptor tyrosine kinase; VEGFR, vascular endothelial growth factor receptor.

in some cancer cells[130,131]. The relevance of these data to the therapeutic use of CDK inhibitors can be questioned, as the knockdown or deletion of CDKs will have consequences that are distinct from those that arise from acute inhibition of kinase activity. Conversely, the dependence of ERBB2 and other oncogenes on the ability of cyclin D to activate CDK4 and CDK6, as summarized elsewhere in this Review, provides support for the idea that CDK4/CDK6 inhibitors may be effective therapeutics. Again, whether these results imply that established tumours that display *CCND1* amplification or cyclin D1 overexpression, particularly in the context of ERBB2 amplification, are also dependent on CDK4/CDK6 activity can be questioned. Although functional mammary glands develop in the absence of CDK4/CDK6 activity, they lack the specific mammary progenitor population that is targeted by ERBB2, pointing to a developmental defect rather than a cell-intrinsic mechanism for the dependence of ERBB2-driven mammary oncogenesis on the CDK activation function of cyclin D1 (REF. 132). However, established ERBB2-driven mammary cancers are sensitive to knockdown of either cyclin D1 or CDK4 (REF. 58).

The first small-molecule CDK inhibitors inhibited CDK1, CDK2 and CDK4 at submicromolar concentrations, and often also inhibited transcriptional CDKs, which are CDK family members such as CDK7 and CDK9 that have functions in transcriptional control rather than (or in addition to) cell cycle control[7]. These pan-CDK inhibitors may also have inhibited antiproliferative CDK family members such as CDK10 and CDK11 (REF. 7). This has rarely been tested, but may be important, as both cyclin D1 and cyclin D3 bind CDK11, and cyclin D3–CDK11 has been implicated in AR regulation[29,41]. A second generation of CDK inhibitors with improved potency (IC_{50} of ~10 nM or less) and selectivity for CDK4/CDK6 in preference to other CDKs was subsequently developed, and several of these inhibitors are currently in clinical trials (TABLE 2) after showing promising antitumour activity in preclinical models. For example, the selective CDK4/CDK6 inhibitor PD0332991 causes cultured cells to arrest in G1 phase and inhibits the proliferation of xenografts of RB-positive breast, ovarian, lung, colon and prostate cancer cell lines, glioblastoma cell lines, and leukaemia, myeloma and MCL cell lines[133–139].

That inhibition of CDKs is feasible in a clinical setting has been shown using biomarkers such as decreases in RB phosphorylation and the expression of E2F-responsive genes[140–143]. However, the therapeutic efficacy of the first-generation pan-CDK inhibitors was modest, at least in part because of poor pharmacokinetics, dose-limiting toxicity and suboptimal dosing schedules, and several early trials were discontinued[130,131]. Improved potency and selectivity is expected to reduce the toxicity from off-target effects. Early results from Phase I trials of CDK4-specific inhibitors indicate that the side effects are tolerable[144–146], in contrast to a Phase I trial of a second-generation pan-CDK inhibitor[143]. *In vitro* resistance to long-term treatment with a CDK4/CDK6-specific inhibitor is associated with increased CDK2 activity in both breast cancer and acute myeloid leukaemia (AML) cell lines[147,148], and liver cells overexpressing cyclin D1 are particularly sensitive to loss or inhibition of CDK2 (REF. 149). Thus, it is unclear whether greater selectivity is preferable to more generalized CDK inhibition[130,131], nor is it known whether the efficacy of second-generation CDK inhibitors in preclinical models will translate to human studies. The first Phase I trials of CDK4-specific inhibitors do, however, provide preliminary evidence of antitumour activity[144–146].

The CDK inhibitors currently undergoing clinical testing are ATP competitors that block the active site of the kinase. This is only one means of impairing the activity of these kinases, but the design of inhibitors that target kinase substrates and regulatory binding sites is notoriously challenging. However, peptides that target cyclin A–CDK2 by mimicking substrate binding or p21 binding, by locking the kinase in an inactive conformation, or by blocking cyclin–CDK association have been identified[150–155]. It may also be possible to identify low-molecular-mass inhibitors of cyclin–CDK substrate interactions using high-throughput screens that are

Table 2 | **Selected second-generation CDK4/CDK6 inhibitors**

Compound (Company)	Primary targets (IC_{50})	Clinical trials*	Preclinical and clinical data
BAY1000394 (Bayer)	CDK1–cyclin B (7 nM) CDK2–cyclin E (9 nM) CDK9–cyclin T1 (<10 nM) CDK4–cyclin D1 (11 nM)	Phase I: advanced malignancies (NCT01188252)	Inhibited proliferation with a mean IC_{50} of 16 nM (8–37 nM). Activity independent of functional p53 or RB. Reduced phosphorylation of RB *in vitro* and in xenografts, indicating intracellular inhibition of CDK2 and CDK4. Reduced RB phosphorylation in paclitaxel- and cisplatin-refractory xenografts[186]
P1446-05 (Piramal Healthcare)	CDK1–cyclin B (n/a) CDK4–cyclin D1 (n/a) CDK9–cyclin T (n/a)	• Phase I: advanced refractory malignancies (NCT00840190) • Phase I: advanced refractory malignancies (NCT00772876)	No published preclinical data
PD0332991 (Pfizer)	CDK4–cyclin D3 (9 nM) CDK4–cyclin D1 (11 nM) CDK6–cyclin D2 (15 nM) CDK2–cyclin E2 (>10 μM) CDK2–cyclin A (>10 μM) CDK1–cyclin B (>10 μM) CDK5–p25 (>10 μM)[134]	• Phase I: advanced cancer (NCT00141297) • Phase I: previously treated MCL (NCT00420056) • Phase II: advanced or metastatic liposarcoma (NCT01209598) • Phase II: recurrent RB-positive glioblastoma (NCT01227434) • Phase II: refractory solid tumours (NCT01037790) • Phase I: PD0332991 plus bortezomib in relapsed MCL (NCT01111188) • Phase I/II: PD0332991 in combination with bortezomib and dexamethasone in refractory multiple myeloma (NCT00555906) • Phase I/II: letrozole plus PD0332991 and letrozole first-line treatment of ER-positive, ERBB2-negative advanced breast cancer in postmenopausal women (NCT00721409)	• Effective in RB-positive carcinoma cell lines and xenograft models[134], and MCL *in vitro*[135] • Principal and dose-limiting clinical toxicity is myelosuppression[145,146] • Preferentially inhibits the proliferation of luminal ER-positive human breast cancer cell lines *in vitro*. Synergizes with tamoxifen and trastuzumab, and increases tamoxifen sensitivity in resistant cells[136] • In combination with bortezomib, increases tumour suppression and improves survival in myeloma cell lines[169] • In combination with dexamethasone, enhances the killing of myeloma cells[170]
R547 (Hoffman-Roche)	CDK4–cyclin D (n/a) CDK2–cyclin A (0.1 nM) CDK5–p35 (0.1 nM) CDK1–cyclin B (0.2 nM) CDK2–cyclin E (0.4 nM) CDK6–cyclin D3 (4 nM) CDK7–cyclin H (171 nM) GSK3α (46 nM) GSK3β (260 nM)[187]	Phase I: advanced solid tumours (NCT00400296)	• Growth inhibitory activity *in vitro* and in xenograft models[187] • In Phase I, adverse effects were mild and manageable and included nausea, fatigue, emesis, headache and hypotension[144]
Combination therapy			
RGB-286638 (GPC Biotech/ Agennix)	CDK1–cyclin B (<5 nM) CDK2–cyclin A (<5 nM) CDK9–cyclin T (<5 nM) CDK4–cyclin D (44 nM) CDK6–cyclin D (55 nM) GSK3β, SRC, MEK and JNK (<100 nM)	Phase I: relapsed or refractory haematological malignancies (NCT01168882)	Cytotoxic in conventional drug-sensitive and resistant multiple myeloma cell lines, as well as primary cultures of multiple myeloma[188]
ZK304709 (Schering Pharma AG)	CDK2–cyclin E (4 nM) CDK9–cyclin T1 (5 nM) CDK1–cyclin B (50 nM) CDK4–cyclin D1 (61 nM) CDK7–cyclin H (85 nM) VEGFR1 (10 nM) VEGFR2 (34 nM) VEGFR3 (1 nM) PDGFRβ (27 nM)[189]	Phase I: advanced solid tumours[190]	Combined inhibition of cell cycle and angiogenesis resulted in superior efficacy compared wiht standard chemotherapeutic compounds in human tumour xenografts, as well as orthotopic human pancreatic carcinoma models[189]

CDK, cyclin-dependent kinase; ER, oestrogen receptor; GSK3β, glycogen synthase kinase 3β; JNK, JUN N-terminal kinase; MCL, mantle cell lymphoma; n/a, not applicable; PDGFRβ, platelet-derived growth factor receptor-β; VEGFR, vascular endothelial growth factor receptor. *Information from the ClinicalTrials.gov website (see Further information)

similar to those used for the discovery and preclinical testing of inhibitors of MDM2–p53 binding (nutlins)[156]. The resolution of the cyclin D1–CDK4 and cyclin D3–CDK4 crystal structures[157,158] will facilitate the development of non-ATP competitive inhibitors of CDK4/CDK6, and may also offer the possibility of targeting non-catalytic functions of cyclin D1, blocking protein–protein interactions. Conversely, mimetics of the domain of cyclin D1 involved in AR repression (BOX 1) have been proposed as a therapeutic approach in androgen-dependent prostate cancer[159]. However, the potent transforming ability of cyclin D1b, which does not bind ERα and has altered AR interactions, suggests that caution should be exercised in selectively targeting these interactions.

Phase I and II clinical trials
The first stages of clinical testing in humans. Phase I trials include tests of safety, tolerability, and pharmacokinetics; Phase II trials begin to assess efficacy.

Cyclin D1 as a therapeutic target. The multiplicity of cyclin D effects on cancer cell biology, and evidence for their CDK-independent actions on end points such as cell migration and the DNA damage response, provide an impetus for targeting cyclin D rather than, or in addition to, CDK4/CDK6 activity. However, although cyclin D1 has been identified as a target for cell-based immunotherapy in MCL[160], most currently feasible approaches to inhibiting D-type cyclins are less direct (FIG. 4), although potentially effective nonetheless. In a well-studied example, the RXR activator bexarotene enhances the effects of the EGFR inhibitor erlotinib in lung cancer, and this is thought to be due to the cooperative repression of cyclin D1 expression[161,162]. Cyclin D1 expression is a biomarker of therapeutic response to this combination, which is currently undergoing further clinical testing after promising results from Phase I and Phase II clinical trials[162–164]. Several other approaches show potential but are less well developed. The translation of *CCND1* mRNA is mTOR-dependent[5], suggesting that mTOR inhibitors might inhibit cell cycle progression partly through effects on cyclin D1 abundance. In Phase II clinical trials mTOR inhibitors were particularly effective in MCL, which is characterized by almost universal overexpression of cyclin D1, although this may not be accompanied by a reduction in cyclin D1 expression (reviewed in REF. 165). Diverse compounds, some with potential therapeutic application, lead to enhanced cyclin D1 degradation[166]. Promoting cyclin D1 degradation by knockdown of USP2, a deubiquitylating enzyme that specifically targets cyclin D1, inhibited proliferation in cancer cells overexpressing cyclin D1 but not in normal fibroblasts, suggesting that targeting cyclin D1 degradation through this mechanism could be an effective, cancer-specific therapy[167].

Cyclin D–CDK inhibition in combination therapy. To date, CDK inhibitors have had limited success when used as single agents, but they may find more clinical use when combined with other drugs. In addition, it may be more effective to simultaneously target multiple functions of cyclin D1, either by using combinations of therapies each targeting a specific function or by targeting multiple pathways using a single molecule. Examples of multiple pathway targeting, such as combining CDK inhibition with VEGFR inhibition to target angiogenesis as well as proliferation, are now in Phase I clinical trials (TABLE 2).

Several preclinical studies have indicated that pan-CDK inhibitors function in synergy with cytotoxic drugs (cisplatin, 5-fluorouracil, doxorubicin and pacilitaxel) especially when the cytotoxic agent is administered first[168]. This suggests that CDK inhibitors may be more effective when cells are synchronized or arrested in specific cell phases, and that one limitation of previous clinical studies might have been poor pharmacokinetics, so that the effective dose was not maintained long enough to allow a majority of the target cell population to enter the sensitive phase of the cell cycle.

CDK4/CDK6 inhibitors may also lead to enhanced efficacy when used in combination with other therapies — for example, the proteasome inhibitor bortezomib or dexamethasone in multiple myeloma[169,170] — and these combinations are currently in Phase I and Phase II clinical trials (TABLE 2). Similarly, a CDK4/CDK6 inhibitor enhanced the activity of an FLT3 inhibitor in AML cell lines that expressed a mutant form of the FLT3 receptor tyrosine kinase[148], and acted synergistically with the BCR-ABL kinase inhibitor imatinib in leukaemia cell lines[171]. Although it has been suggested that CDK4/CDK6 inhibitors could be used to induce reversible quiescence, thereby protecting normal cells from radiation-induced toxicity[172], CDK4/CDK6 inhibition enhanced the effects of radiotherapy in gliobastoma xenografts[137]. There is evidence that resistance to therapies directed at ER signalling, ERBB2, EGFR and BRAF is accompanied by increased cyclin D1 expression[121–123], suggesting that the addition of a cyclin D-targeted therapy might reduce therapeutic resistance. Consistent with this idea, the CDK4/CDK6 inhibitor PD0332991 was synergistic with both the anti-oestrogen tamoxifen and the ERBB2-targeted therapy trastuzumab in ER-positive breast cancer cell lines and was also effective in anti-oestrogen-resistant cell lines[136]. A Phase I/II trial of the combination of letrozole, which like tamoxifen targets ER signalling, with PD0332991 is in progress (TABLE 2). Finally, consistent with the CDK-independent role of cyclin D1 in homologous recombination, depletion of cyclin D1 enhanced sensitivity to radiation treatment, and to inhibition of poly(ADP ribose) polymerase 1 (PARP1), on which cells that are deficient in homologous recombination become dependent[44]. Although these studies are preliminary, they do suggest some treatment combinations that are worthy of further investigation.

Patient selection. Whole-genome transcript profiling and sequencing efforts have documented numerous distinct molecular phenotypes, often accounting for <10% of a particular cancer[173]. Consequently, it is increasingly apparent that testing novel targeted therapeutic strategies in unselected patients may underestimate efficacy, and that many candidate therapeutics, the development of which was halted because of an apparent lack of efficacy, could potentially be reassessed, revived and used effectively if responsive subgroups could be identified[173]. This may well be the case for CDK inhibitors, for which essentially all previous and ongoing trials are in unselected patients. Recent preclinical studies have begun to address this issue, by examining the relationship between cyclin D1 expression, RB pathway inactivation and response to CDK4/CDK6 inhibition, and by undertaking more global analyses of genes that are differentially expressed in sensitive and resistant cell lines[136,138,139]. CDK4/CDK6 inhibitors are generally ineffective in cells lacking RB[133,134,136,137,139]. However, even when there is no apparent dysfunction of RB itself, response to CDK4/CDK6 inhibition can be variable. In a panel of breast cancer cell lines there was a strong correlation between sensitivity to CDK4/CDK6 inhibition and the luminal, ER-positive phenotype, and with high expression of cyclin D1 and RB but low expression of INK4A[136]. Sensitivity to CDK4/CDK6 inhibition is also correlated with high expression of RB and low expression of INK4A in ovarian cancer[139], as well as deletion of both INK4A and INK4C in glioblastoma[138]. However, in

ovarian cancer cell lines there was no relationship between CDK4/CDK6 inhibitor sensitivity and expression of any of the D-type cyclins, CDK4 or CDK6 (REF. 139), and sensitivity was not correlated with CDK6 or CDK4 expression in glioblastoma[138]. These observations raise questions about whether the measurement of cyclin D levels is likely to be a generally useful biomarker of response to CDK4/CDK6 inhibitors, although theses observations do begin to identify a biomarker profile that can be used to better direct these compounds to patients who are most likely to respond.

Cancers displaying activation of specific oncogenic pathways may also be particularly sensitive to CDK4/CDK6 inhibition. The evidence for a specific dependence of ERBB2-driven carcinogenesis on CDK4/CDK6 activity, and the sensitivity of ten of 16 ERBB2-amplified breast cancer cell lines to CDK4/CDK6 inhibition, suggests that breast cancers overexpressing ERBB2 may be effectively targeted using CDK4/CDK6 inhibitors, possibly in combination with specific ERBB2-targeted therapies, such as trastuzumab[136]. KRAS-driven non-small-cell lung cancer is particularly dependent on CDK4, but not on CDK2 (REF. 174), and is also sensitive to the cyclin D1-degrading combination of bexarotene and erlotinib[164], although KRAS-mutant cancers usually respond poorly to erlotinib[175]. Thus, KRAS-mutant lung cancer is another specific disease subtype that is resistant to other targeted therapies, but which may be responsive to the inhibition of the cyclin D1–CDK4/CDK6 pathway in the clinic.

Conclusions

Several decades of work have established that the deregulation of the cyclin D–RB–E2F pathway is central to the development of most human cancers, with amplification, mutation and overexpression of cyclin D a major contributor. Cyclin D1 has both catalytic and non-catalytic roles that are important in both normal and neoplastic cells, with the implication that targeting CDK4/CDK6 activity alone may only be partially effective in cyclin D1-dependent cancers. However, much work still needs to be done to establish whether cyclin D1 itself can be effectively targeted, and if so, whether this will be more useful therapeutically than inhibiting CDK4/CDK6 activity.

The most effective use of potential therapies directed towards cyclin D1 or CDK4/CDK6 will rely on improved patient selection on the basis of genomic and/or proteomic signatures of cyclin D1 and/or CDK4/CDK6 dependence, as well as on the parallel development of biomarkers of therapeutic response. Patient subgroups that are particularly likely to benefit will also include those in which resistance to cytotoxics and targeted therapies is commonly accompanied by increased cyclin D1 and/or CDK4/CDK6 activity. Important questions include whether cancers are more or less addicted to cyclin D1 overexpression as a result of *CCND1* translocation, amplification or mutation, compared with cyclin D1 overexpression secondary to another oncogenic event. As amplification of 11q13 can involve amplicons in addition to the one harbouring *CCND1*, the effect of co-amplification of other oncogenes and overexpression of neighbouring genes is also worthy of continued investigation. Furthermore, given that the use of most targeted therapies is limited by the development of resistance, it will be important to better understand mechanisms of resistance to inhibition of cyclin D or CDK4/CDK6: for example, the identification of CDKs that compensate for CDK4/CDK6 could help to further tailor the combination of CDKs to be targeted. Similarly, a better understanding of the degree to which the deregulation of cyclin D or CDK4/CDK6 contributes to resistance to other targeted therapies could allow the rational design of therapeutic combinations that might minimize the development of resistance or that could be useful in resistant disease. The first steps have been taken towards these goals, but much remains to be achieved.

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Competing interests statement
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