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Cell cycle proteins as promising targets in cancer therapy

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Preface

Cancer is characterized by uncontrolled proliferation resulting from aberrant activity of various cell cycle proteins; therefore, cell cycle regulators are considered attractive targets in cancer therapy. Intriguingly, animal models demonstrated that some of these proteins are not essential for proliferation of non-transformed cells and development of most tissues. In contrast, many cancers are uniquely dependent on these proteins and are hence selectively sensitive to their inhibition. After decades of research on the physiological functions of cell cycle proteins and their relevance for cancer, this knowledge recently translated into the first approved cancer therapeutic targeting of a direct regulator of the cell cycle. Here, we review the role of cell cycle proteins in cancer, the rationale for targeting them in cancer treatment and results of clinical trials, as well as future therapeutic potential of various inhibitors. We focus only on proteins that directly regulate cell cycle progression. Cyclin-dependent kinases with transcriptional functions, as well as PARP inhibitors, which are highly successful in targeting BRCA1/BRCA2-mutant tumours, are not covered by this review.

Introduction

The mammalian cell cycle is a highly organized and regulated process that ensures duplication of genetic material and cell division. This regulation involves growth-regulatory signals as well as signals by proteins monitoring the genetic integrity to ascertain the absence of any genetic damage. Proliferation depends on progression through four distinct phases of the cell cycle (G0/G1, S, G2 and M), which is regulated by several cyclin-dependent kinases (CDKs) that act in complex with their cyclin partners. The activity of CDKs involved in cell cycle regulation is tightly controlled; it is induced by mitogenic signals and can be inhibited by activation of cell cycle checkpoints in response to DNA damage (FIG. 1).

Cancer is characterized by aberrant cell cycle activity. This occurs either as result of mutations in upstream signalling pathways or by genetic lesions within genes encoding cell cycle proteins. Aberrant activation of CDKs, which is frequently seen in human cancers, provided a rationale for designing synthetic inhibitors of CDKs as anticancer drugs.

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Cell cycle proteins and their role in physiology and cancer

The biology of the CDK4/CDK6-RB pathway

In most adult tissues, cells are residing in a cell cycle arrested state termed G0 phase, which can be either transient (quiescence) or permanent (upon terminal differentiation or senescence). Quiescent cells can be triggered to reenter the cell cycle through stimulation with mitogenic factors. Most of these factors activate cascades of intracellular signalling networks and impinge on CDK4 and CDK6 to drive cell cycle progression from G0/G1 into S phase, in which DNA replication occurs (FIG. 2a). CDK4 and CDK6 are highly homologous serine/threonine kinases that are expressed in a tissue-specific manner. CDK4 and CDK6 phosphorylate a largely overlapping set of target proteins[1]. Indeed, gene knockout experiments supported a significant redundancy between CDK4 and CDK6 in most tissues[2]. Apart from that, CDK6 was shown to possess some unique, cyclin-independent transcriptional roles in haematopoietic cells[3]. The activity of CDK4 and CDK6 is controlled by several mechanisms: positively by association with D-type cyclins (D1, D2 and D3) and negatively by binding to CDK inhibitors of the INK4 family ($p16^{INK4A}$, $p15^{INKB}$, $p18^{INK4C}$ and $p19^{INK4D}$)[4].

Cyclin D-CDK4/6 complexes promote cell cycle progression by two major mechanisms[5]. First, they sequester $p21^{CIP1}$ and $p27^{KIP1}$, two CDK inhibitors that bind and prevent activation of cyclin E-CDK2 kinase (BOX 1). Second, active cyclin D-CDK4/6 complexes phosphorylate a variety of cellular targets, most importantly the retinoblastoma tumour suppressor protein (RB) and its closely related proteins p107 and p130, thereby enabling E2F transcription factors to activate transcription of a plethora of genes involved in cell cycle progression from G1 into S phase, DNA replication, chromatin structure, chromosome segregation and mitotic spindle assembly checkpoint. Among the E2F transcriptional targets are cyclins E1 and E2, which bind and activate CDK2. Cyclin E-CDK2 complexes further phosphorylate RB, thereby initiating a positive feedback loop. In addition to these canonical cell cycle functions, D-type cyclins, CDK4 and CDK6 were shown or postulated to perform a number of non-canonical functions, some of which may be relevant for regulation of proliferation[6].

Box 1

Cyclin-dependent kinase inhibitor proteins and their role in cancer

The activity of cyclin-dependent kinases (CDKs) is also regulated by their association with cyclin-dependent kinase inhibitor proteins (CKIs). These include members of the INK4 family ($p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$ and $p19^{INK4D}$), which bind to CDK4 and CDK6 and block their association with D-type cyclins, thereby extinguishing the kinase activity of CDK4 and CDK6. In contrast, CKIs from the CIP/KIP family ($p21^{CIP1}$, $p27^{KIP1}$ and $p57^{KIP2}$) bind to all CDK complexes and inhibit the kinase activity of CDK2 and CDK1.

As expected from their role as negative regulators of the cell cycle, CKIs display certain tumour-suppressive properties. Expression of INK4 proteins, in particular $p16^{INK4A}$ and $p15^{INK4B}$ (encoded by *CDKN2A* and *CDKN2B*), is silenced in human tumours by

genomic deletions, loss-of-function point mutations or promoter methylation (FIG. 3c). Furthermore, $p27^{KIP1}$ is frequently downregulated as a result of enhanced protein degradation in human tumours, an event associated with poor survival[246–248], although deletion of its genomic locus (*CDKN1B*) is only rarely observed[249].

Several mouse models were generated to address the role of CKIs in tumorigenesis. For example, mice deficient for $p16^{INK4A}$ spontaneously developed tumours and exhibited increased susceptibility to carcinogen-induced neoplasia[250]. Similarly, mice deficient for $p21^{CIP1}$ exhibited an increased frequency of spontaneous tumour formation in a variety of tissues[47]. Interestingly, mice heterozygous for *Cdkn1b* (encoding $p27^{KIP1}$) displayed increased susceptibility to tumorigenesis following exposure of animals to gamma radiation or to chemical carcinogens, but did not exhibit the loss of the remaining wild-type allele, indicating a haplo-insufficient tumour suppressor role of this CKI[46]. These findings illustrate that CKIs generally function as tumour-suppressors, presumably by restricting uncontrolled CDK activity and thereby serving as an additional barrier to malignant transformation.

The role of the CDK4/CDK6-RB pathway in cancer

Components of the CDK4/6-RB pathway are commonly mutated in human cancers (FIG. 3a, 3c, 3d). For example, the cyclin D1 gene (*CCND1*) represents the second most frequently amplified locus among all human cancer types[7]. *CDK4* is amplified in 50% of glioblastomas[8] and constitutively activated by a point mutation (R24C, which renders CDK4 insensitive to inhibition by INK4 family members) in melanomas[9]. Similarly, CDK6 is activated by genomic translocations in splenic marginal zone lymphomas[10]. Furthermore, the *CDKN2A* gene (which encodes the tumour suppressors $p16^{INK4A}$ and $p14^{ARF}$) represents the most frequently deleted locus in human cancers and its expression is also commonly silenced by promoter methylation[7]. Finally, deletion of the retinoblastoma gene (*RB1*) occurs frequently in many tumour types and allows proliferation independently of cyclin D-CDK4/6 activity[7].

To test the role of D-type cyclins and their catalytic partners CDK4 and CDK6 in tumorigenesis and tumour maintenance, a variety of genetically engineered mouse models were developed (FIG. 4). For instance, introduction of the CDK4 point mutation found in human melanoma (R24C) into the mouse *Cdk4* locus caused tumorigenesis in various tissues[11] and increased susceptibility to carcinogen-induced melanoma formation[12]. Furthermore, transgenic mice engineered to overexpress cyclin D1 in mammary glands developed mammary hyperplasia and mammary carcinomas[13]. These results highlighted the oncogenic properties of D-type cyclins, CDK4 and CDK6. Surprisingly, however, carcinogen-induced skin tumorigenesis was compromised by transgenic overexpression of cyclin D3 or CDK6 in mice[14, 15], whereas cyclin D1, D2 or CDK4 overexpression enhanced skin tumorigenesis as expected[14, 16, 17].

In contrast, mice lacking cyclin D1 were resistant to mammary cancer formation induced by specific oncogenes (such as *v-Hras* or $Erbb2^{V664E}$)[18, 19]; at least for Erbb2, this critically depended on the kinase activity of CDK4[20–22]. *Ccnd3*-null mice were resistant to

Notch1^{ICD}-driven T-cell acute lymphoblastic leukaemia[23], whereas *Cdk6* knockout mice were resistant to lymphoma formation induced by constitutively active AKT[24]. Intriguingly, lung cancer driven by oncogenic *Kras^{G12V}* exhibited selective sensitivity to CDK4 inhibition, since acute deletion (i.e. conditional deletion after tumour formation) of *Cdk4,* but not of *Cdk6 or Cdk2,* induced senescence and prevented tumour progression[25]. Similarly, an acute and global ablation of *Ccnd1* or pharmacological inhibition of CDK4 and CDK6 kinase activity in mice bearing Erbb2^{V664E}-driven mammary tumours blocked cancer progression and triggered tumour cell-specific senescence without having any obvious effect on normal tissues[26]. Surprisingly, an acute and ubiquitous deletion of *Ccnd3* or inhibition of CDK4 and CDK6 in mice with Notch1^{ICD}-induced T-cell acute lymphoblastic leukaemia provoked tumour cell-specific apoptosis, rather than senescence, although the mechanism for this response has not yet been elucidated[26, 27]. Collectively, these analyses revealed that individual D-type cyclins, CDK4 and CDK6 are required for tumour initiation, and that their continued expression is critical for tumour maintenance. This is in stark contrast to normal non-transformed tissues, in which shutdown of individual D-type cyclins or inhibition of CDK4 and CDK6 catalytic activity had no major effects[26]. Collectively, these studies illustrate that tumours are frequently dependent on individual cyclins and CDKs and hence susceptible to their targeted inhibition, which is in noticeable contrast to the redundancy observed in most normal, non-transformed tissues[5].

CDK2

This CDK is activated through its association with E-type or A-type cyclins. In the absence of mitogens CDK2 complexes are inhibited by association with the CDK inhibitors p27^{KIP1} or p21^{CIP1}. During the late G1 phase, CDK2 activity increases as a result of E2F-mediated transcription of cyclin E genes, cyclin D-CDK4- and cyclin D-CDK6-mediated sequestration of p27^{KIP1} and p21^{CIP1}, as well as ubiquitin-mediated proteolysis of p27^{KIP1} and p21^{CIP1} following their phosphorylation by CDK2. In addition, CDK2 activity is inhibited by WEE1-mediated phosphorylation at Tyr-15, and this inhibitory phosphorylation is removed by the CDC25 family of phosphatases such as CDC25A and CDC25B[28]. Cyclin E-CDK2 complexes phosphorylate a variety of proteins required for cell cycle progression, DNA replication and centrosome duplication[29, 30]. During S phase, cyclin E is rapidly degraded following FBXW7-mediated ubiquitination[31, 32] and CDK2 associates with newly synthesized cyclin A2 to form active cyclin A-CDK2 complexes.

CDK2 mutations are rarely found in human cancers; however the catalytic activity of CDK2-containing complexes is hyperactivated via several mechanisms. The *CCNE1* locus is frequently amplified, for example in ovarian and breast cancers (FIG. 3b)[33, 34]. In some tumour types cyclin E overexpression occurs as a result of loss-of-function mutations within the gene encoding FBW7 (*FBWX7*), a ubiquitin ligase component involved in cyclin E degradation[35, 36]. Alternatively, certain tumours express a hyperactive, truncated form of cyclin E1[37]. Similarly, cyclin A is frequently overexpressed, sometimes as a result of genomic amplification, for example in hepatocellular carcinomas[38], colorectal[39] and breast cancers[40]. In some tumours CDK2 activity is enhanced following reduced expression of the CDK inhibitor p27^{Kip1}, e.g. due to increased SKP2-mediated degradation[41]. In addition, CDC25A and CDC25B are overexpressed in various tumours[42–44].

These different mechanisms of CDK2 activation have been validated using mouse cancer models. Thus, transgenic overexpression of cyclin E1 in mammary glands led to mammary cancer formation[45]. Increased activity of cyclin E-CDK2 resulting from deletion of genes encoding CDK inhibitor proteins $p27^{KIP1}$ or $p21^{CIP1}$ also increased the susceptibility to tumour formation (BOX 1)[46, 47]. Transgenic overexpression of CDC25A or CDC25B enhanced v-HRAS-induced, $ERBB2^{V664E}$-induced and carcinogen-induced mammary cancer formation[48, 49]. Conversely, heterozygous deletion of *Cdc25a* delayed v-HRAS-induced and $ERBB2^{V664E}$-induced mammary tumorigenesis[50].

It is not clear whether CDK2 activity is required for tumour initiation and maintenance. Several human cancer cell lines were shown to proliferate despite inhibition of CDK2 activity[51]. Likewise, mice lacking CDK2 displayed unperturbed tumorigenesis in several tissues[25, 52–54]. However, MYC-overexpressing tumours were shown to require CDK2-mediated phosphorylation of MYC to suppress senescence[55, 56]. Indeed, deletion of *Cdk2* delayed tumour formation in a mouse model of MYC-overexpressing B-cell lymphoma (*Eμ-Myc*)[55]. Moreover, CDK2 depletion suppressed cell cycle progression in melanoma cells[57]. Also, mouse cancer models showed that CDK2 is critically required for mammary cancer formation induced by overexpression of $Erbb2^{V664E}$ or a cancer-associated truncated cyclin E1 isoform[37, 58]. Hence, CDK2 function may be required in specific cancer types.

CDK1

CDK1 represents the only CDK that is essential for cell cycle progression[59]. During G2 phase CDK1 binds and becomes activated by cyclins A2 and B. Upon entry into mitosis, cyclin A2 is degraded and CDK1 activity is maintained in complexes with B-type cyclins; CDK1 kinase activity is required for mitotic entry and several mitotic events. B-type cyclins are degraded by the anaphase-promoting complex (APC^{CDC20} and APC^{CDH1}) in late mitosis[60]. This attenuates CDK1 activity and allows chromosome separation and completion of mitosis and cytokinesis. In addition to regulation by its cyclin partners, CDK1 activity is inhibited by phosphorylation at Thr-14 and Tyr-15, mediated by kinases MYT1[61] and WEE1[62], respectively; this phosphorylation is relieved by CDC25 phosphatases (FIG. 2b)[62].

Interestingly, CDK1 activity is not commonly deregulated in cancer; one of the few examples being *CCNB3* gene amplifications in neuroendocrine prostate cancers[63]. Transgenic overexpression of cyclins B1 or B2 increased susceptibility to carcinogen-induced skin and lung tumours, revealing a potential role for elevated CDK1 activity in tumorigenesis[64]. CDK1 was shown to be required for tumour formation and progression. For example, liver-specific ablation of *Cdk1* conferred resistance to $NRAS^{G12V}$-induced liver tumorigenesis[65], while CDK1 inhibition blocked the growth of *KRAS*-mutant (G12V, G12D or G12S) colorectal cancer xenografts[66]. However, CDK1 activity is essential for proliferation also in normal, non-transformed cells[59], arguing against inhibition of CDK1 as a viable therapeutic strategy. Intriguingly, inhibition of CDK1 triggered apoptosis of MYC-overexpressing mouse lymphomas and liver tumours[67], as well as human basal-like triple-negative breast cancer cells[68]. These findings raise a possibility that CDK1 inhibition might specifically kill tumour cells, while causing only a transient cell cycle arrest in normal tissues, a notion that requires further investigation using genetic mouse models.

DNA damage checkpoint kinases and WEE1

Cells have checkpoints to halt cell cycle progression in response to DNA damage, thereby allowing time for DNA repair. Several DNA damage checkpoints exist and they impinge on the activity of specific CDK complexes (FIG. 1). Depending on the type of DNA damage, ATR or ATM protein kinases phosphorylate and activate checkpoint kinase 1 (CHK1, encoded by the *CHEK1* gene)[69]. Similarly, ATM can also activate CHK2 (encoded by *CHEK2*), which in turn participates in the activation of p53[70]. Activation of p53 transcriptionally induces expression of the CDK inhibitor $p21^{CIP1}$, leading to inhibition of cyclin E-CDK2 complexes and a G1 arrest (FIG. 2a)[71]. Activated CHK1 mediates a temporary S phase arrest by phosphorylating and inactivating CDC25A and a G2 checkpoint arrest by phosphorylating CDC25A, CDC25B and CDC25C[72, 73]. These events prevent dephosphorylation of Tyr-15 on CDK2 and CDK1, thereby rendering these CDKs inactive. CHK1 also activates WEE1 via direct phosphorylation, leading to enhanced inhibitory Tyr-15 phosphorylation of CDK2 and CDK1 and subsequent cell cycle arrest in G2 phase (FIG. 2b)[74]. In summary, CHK1 is an essential mediator of DNA damage-induced cell cycle arrest in S and G2 phases, particularly in cancer cells with inactivated p53, which depend on G2 checkpoint to halt cell proliferation.

The role of CHK1 and WEE1 in cancer development is controversial. CHK1 was initially regarded as a tumour suppressor. Indeed, heterozygous loss-of-function mutations of the *CHEK1* locus were detected in breast[75] and gastric cancer[76]; however, no homozygous loss-of-function mutations have been identified so far. Consistent with these findings, heterozygous deletion of *Chek1* in mice enhanced mammary tumorigenesis induced by the *Wnt* oncogene or by heterozygous deletion of *Trp53* (which encodes p53 in mice)[77, 78]. In contrast, tissue-specific homozygous deletion of *Chek1* in mice inhibited mammary tumorigenesis induced by p53 loss[78] and prevented carcinogen-induced skin tumour formation[79]. Consistent with an oncogenic role for CHK1, this protein is overexpressed in many cancers, such as triple-negative breast cancers, hepatocellular and cervical cancers[80–82]. Furthermore, an extra allele of *Chek1* protected mouse fibroblasts from replicative stress and enhanced $HRAS^{G12V}$-induced transformation by reducing DNA damage-associated apoptosis *in vitro*[83]. Collectively, these observations suggest that although reduced CHK1 levels (resulting from heterozygous deletion) may enhance tumorigenesis, CHK1 is required for tumour cell growth and survival by allowing DNA damage repair. In contrast to CHK1, CHK2 is thought to play mostly a tumour-suppressive role, since several loss-of-function mouse models exhibited enhanced tumorigenesis. Hence, CHK2 does not seem to represent a suitable target for cancer therapy (FIG. 4b).

WEE1 kinase is overexpressed in several cancer types, for instance in hepatocellular carcinoma[84], glioblastoma[85] and melanoma[86]. In contrast, heterozygous deletion of *Wee1* in the mammary gland induced spontaneous development of mammary cancers in a small percentage of older mice, while no tumours were observed upon homozygous deletion[87]. Hence, tumorigenesis may be incompatible with complete loss of WEE1 activity, similar to CHK1. Despite these contradictory results, WEE1 is generally considered to be an oncogene and a potential target in cancer therapy.

Polo-like kinases

The family of Polo-like kinases consists of five members, of which PLK1 has been studied in most detail. During G2 phase PLK1 participates in the maturation of centrosomes by regulating the centrosomal localization of Aurora A[88]. Moreover, PLK1 plays an important role in activation of cyclin B-CDK1 complexes by at least two mechanisms. First, it activates CDC25C phosphatase, which in turn removes the inhibitory Tyr-15 phosphorylation of CDK1[89]. Second, PLK1 induces phosphorylation-dependent degradation of WEE1, thereby preventing further phosphorylation of CDK1 at Tyr-15 (FIG. 2b)[90]. Subsequently, PLK1 is involved in triggering chromosome segregation during the metaphase-anaphase transition and plays important roles in cytokinesis. PLK1 is also crucial for mitotic entry following recovery from DNA damage-induced G2 phase arrest, providing a rationale for its exploitation as a target in cancer therapy.

The role of PLK1 in cancer is not clear. PLK1 expression is frequently elevated in tumours, correlates with poor prognosis and is thought to contribute to tumorigenesis by compromising cell cycle checkpoints and inducing genetic instability[91–93]. In contrast, a few cancer cell lines exhibited mutations that reduce PLK1 stability[94]. Furthermore, heterozygous deletion of *Plk1* in mice increased the incidence of spontaneous tumours, suggesting a potential tumour-suppressive role for PLK1[95]. Despite these conflicting results, PLK1 is generally considered oncogenic and a potential target in cancer therapy.

Aurora kinases

Aurora kinases are serine/threonine kinases that play major roles in mitosis and cytokinesis. Aurora A localizes to the centrosomes starting in S phase and is essential for centrosome maturation, spindle assembly and spindle orientation. Furthermore, Aurora A phosphorylates and activates PLK1, thereby promoting CDK1 activation and mitotic entry, especially after DNA damage checkpoint-dependent G2 phase arrest[96, 97]. Aurora A also stabilizes the transcription factor N-MYC (encoded by *MYCN*) by preventing its proteasomal degradation, thereby promoting G1-S progression[98]. Aurora B is found at chromosomes and at the mitotic spindle during mitosis where it constitutes a part of the chromosomal passenger complex. Aurora B controls chromosome condensation and orientation as well as proper execution of cytokinesis.

Ectopic overexpression of Aurora A caused inactivation of DNA damage checkpoint during the G2 phase[99] and inactivation of the spindle assembly checkpoint during mitosis[100], leading to tetraploidy and centrosome amplification, especially in cells with defective p53-dependent DNA damage checkpoint[101]. Also, overexpression of Aurora B caused defective chromosome separation leading to aneuploidy[102]. Analyses of human tumours support oncogenic roles for Aurora A and Aurora B. The gene encoding Aurora A is frequently amplified in prostate[103] and breast cancers[104], while several other cancer types express elevated levels of Aurora A protein[105]. Aurora B is also found overexpressed in several cancer types, although its genomic locus is rarely amplified, e.g. in only 5% of myelodysplastic syndromes[106]. Importantly, transgenic overexpression of Aurora A in mouse mammary epithelium induced tetraploidy and centrosome amplification leading to mammary cancer formation[107]. Likewise, mice ubiquitously overexpressing Aurora B

spontaneously developed lymphomas[102]. Surprisingly, despite their role as oncogenes, heterozygous deletion of the genes encoding Aurora A or Aurora B in mice also increased tumour incidence in various organs suggesting some tumour-suppressive roles[108, 109].

Rationale for targeting specific cell cycle proteins

Cell cycle proteins are frequently overactive in cancer cells leading to uncontrolled proliferation. As we described earlier, genetic ablation of individual cyclins or CDKs, or inhibition of cyclin-CDK kinase activity in tumour-bearing mice selectively blocked tumour initiation and progression of specific cancer types driven by particular oncogenic insults, without having major effects on normal tissues. This suggests that tumour cells are dependent on (or “addicted” to) specific CDKs, depending on genetic lesions they carry, and hence CDK inhibition may selectively target cancer cells while sparing normal tissues. In some instances, inhibition of CDK activity in mouse cancer models not only led to cell cycle arrest but also provoked tumour cell senescence or apoptosis. This indicates that tumours carrying particular genetic lesions critically depend on specific cell cycle proteins to inhibit tumour-suppressive programs such as senescence and apoptosis, thereby selectively sensitizing cancer cells to inhibition of these proteins.

In contrast, inhibition of cell cycle proteins critical for checkpoint function, such as CHK1 and WEE1, follows an opposite strategy. Cell cycle checkpoints are essential to halt cell cycle progression in response to DNA damage, thereby allowing time for DNA repair. Inhibition of CHK1 or WEE1 in cancer cells prevents cell cycle arrest during S or G2 phase and allows cell proliferation despite accumulation of DNA damage. This can lead to cell death during mitosis by a process sometimes referred to as “mitotic catastrophe”. This strategy particularly applies to cancer cells with compromised G1 checkpoint due to loss of p53 function; these cancer cells critically depend on the G2 checkpoint, especially in the presence of DNA damage-inducing drugs. For this reason, inactivation of p53 selectively renders cancer cells sensitive to inhibition of CHK1 or WEE1, an example of the so-called “synthetic lethality”.

Targeting CDKs in cancer therapy

Development of pan-CDK inhibitors

Most of the early compounds exhibited little specificity towards individual CDKs and are therefore commonly referred to as pan-CDK inhibitors. The first generation of these inhibitors include flavopiridol, (R)-roscovitine and olomoucine.

Flavopiridol is a semisynthetic flavone targeting many CDKs and represents the most extensively studied CDK inhibitor with over 60 clinical trials initiated since 1997 (TABLE 1). It causes cell cycle arrest in G1 and G2 phases[110]. Administration of flavopiridol induced apoptosis in several mouse tissues leading to organ atrophy[111], an effect attributed to inhibition of cyclin T1-CDK9 (P-TEFb) kinase[112]. Although flavopiridol exhibited significant anti-tumour activity in preclinical studies[111], clinical phase II studies reported insufficient efficacy for solid cancers. However, some evidence for clinical activity was observed in haematological malignancies (TABLE 2)[113, 114].

In contrast, pan-CDK inhibitors (R)-roscovitine and olomoucine did not show promising anti-tumour activities in preclinical and clinical studies; nevertheless, (R)-roscovitine is still under clinical investigation. In general, first-generation pan-CDK inhibitors suffered from a low therapeutic index leading to toxicities at concentrations necessary to inhibit their targets. To circumvent these limitations, second-generation pan-CDK inhibitors were developed; these include dinaciclib, AT7519, milciclib, TG02, CYC065 and RGB-286638 (TABLE 1).

Dinaciclib is a CDK inhibitor with over 100-fold higher potency in inhibiting RB phosphorylation and a more than ten-fold higher therapeutic index than flavopiridol (TABLE 1)[115]. It was shown to block proliferation of tumour cells in xenograft models of ovarian and pancreatic cancers, paediatric ALL and *NRAS*-mutant melanoma[115–118]. Unfortunately, the first clinical trials in several cancer types demonstrated little clinical activity[119–122]. However, recent results from a phase I/II clinical trial showed promising results with partial responses in 11% of patients with relapsed multiple myeloma (TABLE 2)[123]. Moreover, a recent phase I clinical trial reported partial responses in 54% of patients with relapsed or refractory CLL[124]. Interestingly, dinaciclib synergized with the AKT inhibitor MK-2206 and caused strong tumour growth inhibition in xenografts of pancreatic cancer[125], a treatment strategy currently investigated in a phase I clinical trial[126]. Finally, dinaciclib treatment may be efficacious in MYC-overexpressing triple-negative breast cancers and MYC-driven B-cell lymphomas since it caused tumour regression and enhanced survival in preclinical mouse models[68, 127]. Currently, an ongoing phase I study investigates the utility of dinaciclib in treating patients with MYC-overexpressing solid cancers[128].

Development and clinical success of CDK4/CDK6-selective inhibitors

Following promising results from genetic and preclinical studies, the first group of CDK-selective compounds that entered the clinics were CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib[129].

Palbociclib was originally developed by David Fry and Peter Toogood in 2001, although it took many years until its potential therapeutic value became appreciated, and phase II clinical trials eventually started in 2009[130]. Palbociclib potently inhibits both CDK4 and CDK6 kinase activity whereas other kinases are barely affected (TABLE 1)[131]. As expected, palbociclib prevents RB phosphorylation by CDK4/6 and causes cell cycle arrest in G1 phase[131]. Consistent with the notion that RB represents the major rate-limiting target of CDK4/6 during cell cycle progression, cells that lost RB did not respond to palbociclib[132]. Analyses of human cancer xenografts demonstrated a strong anti-tumour activity against glioblastomas[131], colorectal cancers[131], rhabdomyosarcomas[133], multiple myelomas[134], AML[135], ALL[136] and dermatofibrosarcomas[137]. Noteworthy, systemically administered palbociclib crossed the blood-brain barrier and blocked tumour progression in an orthotopic xenograft model of glioblastoma[138]. In search for cancer types particularly sensitive to palbociclib, Finn and colleagues demonstrated that luminal-type breast cancer cells expressing oestrogen receptor (called ER+), including luminal-type cells with amplification of the HER2 (ERBB2) receptor (referred to as HER2+), were significantly more sensitive to palbociclib than ER-negative (ER-) breast cancer cells with basal-like histology[139]. Moreover, palbociclib sensitivity was increased upon loss of $p16^{INK4A}$, $p15^{INK4B}$ or E2F1,

low cyclin E1 expression or high androgen receptor levels; on the other hand, loss of RB abolished and amplification of the *CCNE1* gene or overexpression of E2F2 decreased the sensitivity[132, 140–142]. The effect of *CDK4* amplification on CDK4/6 inhibitor sensitivity remains controversial; although it enhanced sensitivity in liposarcoma[143], it caused resistance in glioblastoma[144] and rhabdomyosarcoma[145]. Importantly, palbociclib also induced senescence in glioblastoma [138], melanoma[1], breast cancer[146] and liposarcoma cells[147]. This is of clinical relevance since senescence induction could trigger an immune response leading to tumour clearance in patients[148]. However, it is not clear what determines whether palbociclib induces transient quiescence or permanent senescence. Recently, it was suggested that in *CDK4*-amplified liposarcoma, proteolytic degradation of the ubiquitin ligase MDM2 upon palbociclib treatment is required for senescence induction *in vitro* and represents a predictor of good clinical response in patients[147]. It remains to be seen how generally applicable this is, whether MDM2 degradation causes accumulation of a senescence mediator, and whether any of the involved factors can be exploited as biomarkers of clinical outcome.

The first promising clinical study of palbociclib focussed on mantle cell lymphoma (TABLE 2), a tumour type often harbouring a *CCND1-IGH* translocation that juxtaposes the *CCND1* gene to the immunoglobulin heavy chain enhancer, thereby driving cyclin D1 overexpression[149]. The study demonstrated complete or partial responses in 18% and stable disease in 41% of patients. Further studies with preliminary signs of efficacy included patients with *CDK4*-amplified liposarcoma[150], germ cell tumours[151] and non-small-cell lung carcinoma (NSCLC)[152]. In all these studies, adverse effects of palbociclib included neutropaenia (reduced number of neutrophil granulocytes) and thrombocytopaenia. The former likely represents an on-target effect, as genetic ablation of *Ccnd3*, the major D-type cyclin in haematopoietic cells, resulted in severe neutropaenia in mice[153].

The randomized phase II clinical trial PALOMA-1 compared treatment with palbociclib and letrozole (a standard-of-care inhibitor of aromatase, an enzyme responsible for a key step in oestrogen biosynthesis) versus treatment with letrozole alone for postmenopausal women with previously untreated ER+ HER2 non-amplified (HER2-) advanced breast cancer. Addition of palbociclib strongly increased the median progression-free survival (PFS) from 10.2 months to 20.2 months[154]. The median overall survival also showed an improvement in the combination treatment, although a larger study needs to evaluate whether this is statistically significant. Based on these results, palbociclib received accelerated (i.e. provisional) approval by the U.S. Food and Drug Administration (FDA) in February 2015[155]. A phase III study (PALOMA-2) was initiated to validate the clinical benefit of this treatment (see Supplementary Information S1 (table)). Furthermore, a randomized, double-blind, placebo-controlled phase III trial PALOMA-3 compared treatment with palbociclib and fulvestrant (an ER antagonist) to treatment with placebo and fulvestrant for women with ER+ HER2- metastatic breast cancer that have relapsed or progressed during prior hormone therapy, including a substantial portion of patients (33%) with prior chemotherapy for metastatic disease. The interim analysis of this study demonstrated a significantly improved median PFS (9.5 months versus 4.6 months, respectively)[156, 157]. Although an analysis of overall survival is not yet possible, this second-line treatment for metastatic breast cancer received approval by the FDA in February 2016[158]. Palbociclib is currently studied in over

50 clinical trials involving a wide variety of cancer types (TABLE 1). Improvement of the clinical outcome will depend on identification of predictive biomarkers. So far, only ER (in breast cancer) and RB expression have shown some value to predict positive outcome and are used in clinical trials, whereas *CCND1* amplification[154, 159], *CDKN2A* loss[154, 159], *PIK3CA* mutation[157] and RB localization[159] were not informative.

Ribociclib also selectively inhibits CDK4 and CDK6 with high potency (TABLE 1)[160]. Similar to palbociclib, it blocks RB phosphorylation and causes cell cycle arrest of RB-positive tumour cells[161]. Furthermore, it showed anti-tumour activity in neuroblastoma (including senescence induction)[161], liposarcoma[143], rhabdomyosarcoma[145] and Ewing sarcoma xenografts[162]. The first clinical phase I trial involving various advanced RB-positive cancers reported partial responses in a patient with *CCND1*-amplified, *PIK3CA*-mutated breast cancer and a patient with *CCND1*-amplified melanoma; the major dose-limiting toxicities were neutropaenia and thrombocytopaenia[163]. Ribociclib was then studied in combination with hormonal therapy for postmenopausal women with ER+ HER2- advanced breast cancer and exhibited preliminary signs of clinical activity (TABLE 2)[164]. These phase Ib results await validation in a large phase III study (MONALEESA-2) (see Supplementary Information S1 (table)). Ribociclib is currently investigated in over 30 clinical trials involving several tumour types (TABLE 1).

Abemaciclib inhibits not only CDK4 and CDK6 but also a number of other kinases with lower potency, including CDK9 and PIM1 (TABLE 1)[165]. Similar to palbociclib and ribociclib, it inhibits RB phosphorylation and causes cell cycle arrest in the G1 phase. It demonstrated anti-tumour activity in xenograft models of colorectal cancer[165], AML[165] and melanoma[166]. Furthermore, systemically administered abemaciclib crossed the blood-brain barrier more efficiently than palbociclib and blocked tumour progression in an orthotopic glioblastoma xenograft model[167]. The first phase I trial for patients with various advanced cancer types reported responses in three patients (ovarian cancer, *KRAS*-mutant NSCLC and *CDKN2A*-negative, *NRAS*-mutant melanoma)[168]. The major adverse effects were fatigue, neutropaenia and diarrhoea. Further studies evaluated abemaciclib as monotherapy for patients with advanced NSCLC that have relapsed or progressed during previous treatment (TABLE 2). Partial responses were observed in only 2% of patients but an additional 49% achieved stable disease[169]. Whether this improves progression-free and overall survival is currently under investigation in a large phase III trial (JUNIPER, see Supplementary Information S1 (table)). In another study, 23% of metastatic breast cancer patients showed partial responses to abemaciclib monotherapy[170], leading to “breakthrough therapy” designation for abemaciclib by the FDA in October 2015[171]. As expected from previous preclinical studies, all responses occurred in patients with ER+ breast cancers whereas no tumour regression was observed in patients with HER2+ or triple-negative breast cancers. Moreover, combination of abemaciclib and aromatase inhibitors (letrozole or anastrozole) demonstrated partial responses in 6% and stable disease in 61% of patients with ER+ HER2- metastatic breast cancers[172]. The clinical benefit of this combination treatment is currently being validated in a large phase III trial (MONARCH 3).

Several studies investigated the value of combining CDK4/6 inhibitors with additional compounds. A phase I study investigated the utility of combining palbociclib with

paclitaxel, a microtubule stabilizer, and showed partial responses in 40% of patients with RB-positive metastatic breast cancers[173]. The combined inhibition of MEK and CDK4 had a synergistic effect and led to tumour regression in several preclinical mouse models of *NRAS*-mutant melanoma [174]. The potential value of this combination was then evaluated in a small clinical phase Ib/II trial[175]. Indeed, combination of ribociclib and the MEK inhibitor binimetinib resulted in partial responses in 43% of patients with *NRAS*-mutant melanoma[175]. Furthermore, resistance of *PIK3CA*-mutant breast cancer to PI3Kα inhibition was attributed to increased CDK4/6 activity[176]. Indeed, combination of PI3Kα and CDK4/6 inhibition caused synergistic tumour regression in several *PIK3CA*-mutant breast cancer xenograft models[176]. The efficacy of combining ribociclib with a PI3Kα inhibitor (BYL719) and an aromatase inhibitor (letrozole) is currently being investigated in a phase Ib study for advanced ER+ breast cancer[164]. Finally, following an increase in efficacy revealed in preclinical studies, combination of ribociclib with an mTOR inhibitor (everolimus) and an aromatase inhibitor (exemestane) is also currently under clinical investigation in ER+ breast cancer[177].

Paradoxically, combination of palbociclib with conventional chemotherapeutics decreased their anti-tumour activity[178, 179]. These results were observed only in RB+ tumours and can be explained by the fact that palbociclib induces G1 arrest of cancer cells, thereby protecting them from the cytotoxic action of chemotherapeutics. These results caution against combining CDK4/CDK6 inhibitors with chemotherapy for RB+ tumours. Importantly, administration of CDK4/CDK6 inhibitors was shown to protect normal bone marrow cells from the effects of cytotoxic drugs or radiation, by reducing the proliferation of haematopoietic progenitor cells[178, 180]. Hence, this “chemo-protective” effect of CDK4/CDK6 inhibition might be valuable in reducing haematological toxicities of chemotherapy or radiotherapy in patients bearing CDK4/CDK6-independent (e.g. RB-negative) tumours and is currently investigated in a clinical trial[181].

It is expected that tumour cells will eventually develop resistance to CDK4/CDK6 inhibition. While the molecular basis is currently unknown, possible mechanisms include the loss RB, overexpression of cyclin D1, CDK4 or E2F, hyperactivation of cyclin E-CDK2 kinase via cyclin E overexpression or the loss of CDK inhibitors $p21^{CIP1}$ or $p27^{KIP2}$, as well as overexpression of certain ABC transporters.

Targeting of other cell cycle proteins

Inhibitors of CHK1 and WEE1

During the last decade, a number of CHK1 and WEE1 inhibitors have been developed and tested in preclinical and clinical studies. Currently, three of them seem promising: the CHK1 inhibitors MK-8776 and LY2606368 and the WEE1 inhibitor AZD1775 (TABLE 1).

MK-8776 exhibits high potency and selectivity for CHK1[182]. Treatment of cancer cells with MK-8776 caused accumulation of DNA double-strand breaks leading to apoptotic cell death *in vitro*. Furthermore, it synergized with gemcitabine, hydroxyurea and cytarabine in causing apoptosis of AML and breast cancer cells *in vitro*, as well as with gemcitabine in ovarian and pancreatic cancer xenografts[182–184]. Based on these studies, the first clinical phase I trial

with MK-8776 in combination with gemcitabine was initiated for patients with advanced solid tumours. The trial showed preliminary activity and little toxicity[185]. Another phase I clinical trial investigated the sequential administration of cytarabine and MK-8776 in patients with relapsed or refractory acute leukaemias. This combination achieved complete remission in 33% of patients (TABLE 3)[186] and is currently being evaluated in a phase II trial for patients with relapsed AML[187].

LY2606368 is a recently developed inhibitor with higher selectivity for CHK1 than CHK2 (TABLE 1)[188]. As expected, it causes activation of CDC25A in cancer cells, leading to increased CDK2 activity. The inappropriate activation of the CDC25A-CDK2 axis promotes S phase progression with increased number of replication forks, resulting in DNA double-strand breaks at stalled replication forks (termed “replication catastrophe”), chromosome fragmentation and eventually mitotic cell death[188]. LY2606368 reduced tumour growth in a xenograft model of lung cancer[188]. The first clinical trial demonstrated anti-tumour activity in the subgroup of patients with metastatic squamous cell carcinoma of the anus with responses in 15% of patients (TABLE 3)[189]. This agent will be further investigated in several clinical studies that are currently recruiting participants.

AZD1775 specifically targets WEE1 and (less potently) YES kinase (TABLE 1)[190]. Inhibition of WEE1 blocks DNA-damage induced inhibitory phosphorylation of CDK1 and CDK2 at Tyr-15. This causes cells with damaged DNA to prematurely enter mitosis, triggering mitotic arrest and apoptosis[190]. AZD1775 synergized with a variety of chemotherapeutic compounds as well as radiation and was particularly active against tumour cells with a defective DNA damage checkpoint in G1 phase due to loss of p53 function, an example of synthetic lethality[190–192]. Treatment with AZD1775 (either alone or together with chemotherapeutics or gamma-radiation) achieved promising anti-tumour activity in xenograft models of pancreatic cancer, NSCLC, AML and glioma[193–196]. Moreover, WEE1 and PARP inhibition synergistically increased radiosensitivity in a xenograft model of pancreatic cancer[197]. AZD1775 also acted synergistically with inhibitors of histone deacetylases (HDACs) both *in vitro* and in xenograft models of AML and pancreatic cancer[198, 199]. Furthermore, combined WEE1 and mTOR inhibition achieved tumour regression in a mouse model of *KRAS*^{G12D}-induced lung cancer as well as in *KRAS*^{A18D}-mutant AML xenografts[200]. Finally, combined WEE1 and CHK1 inhibition induced DNA damage and apoptosis in the absence of chemotherapeutics and inhibited tumour growth in neuroblastoma xenografts[201, 202]. A phase II trial investigated AZD1775 in combination with carboplatin for treatment of p53-mutant ovarian cancer and showed partial responses in 27% of patients (TABLE 3)[203]. Subsequently, a randomized phase II trial compared the combination of AZD1775 with chemotherapeutics (carboplatin and paclitaxel) versus chemotherapeutics alone for patients with recurrent, platinum-sensitive, p53-mutant ovarian cancer, and reported an improved median PFS of 43 weeks versus 35 weeks[204]. Currently, AZD1775 is being studied in over 20 clinical trials involving a variety of cancer types, including combinations with chemotherapeutics, HDAC and PARP inhibitors.

Inhibitors of Polo-like kinases

Development of Polo-like kinase inhibitors has mainly focussed on PLK1. Currently, two promising PLK1 inhibitors, rigosertib and volasertib, are under clinical investigation (TABLE 1).

Rigosertib is a multi-kinase inhibitor with highest affinity for PLK1[205]. Rigosertib caused tumour regression in a xenograft model of head and neck squamous cell carcinoma[206] and (in combination with gamma-radiation) in xenografts of cervical cancer[207]. Clinical trials mainly focussed on pancreatic cancer and myelodysplastic syndromes (MDS). Whereas treatment with rigosertib and gemcitabine did not improve survival of pancreatic cancer patients (TABLE 3), the outcomes for MDS patients were more promising. In patients with higher-risk MDS, an analysis of four clinical phase I/II trials reported bone marrow responses in 40% and cytogenetic responses in 6% of patients[208]. Rigosertib was then compared to best supportive care for patients with higher-risk MDS, who have relapsed after, failed to respond to, or progressed during treatment with hypomethylating agents (HMAs), in a randomized phase III trial (ONTIME). Prolonged median overall survival was observed for patients with primary HMA failure (8.6 months versus 4.5 months)[209], as well as for patients with very poor prognosis (7.6 months versus 3.2 months)[210]. The clinical benefit of this treatment is currently being validated in another phase III trial (INSPIRE, see Supplementary Information S1 (table)).

Volasertib is a highly selective Polo-like kinase family inhibitor with highest potency against PLK1 (TABLE 1)[211]. Similar to rigosertib, it causes cell arrest and apoptosis. In contrast to rigosertib, volasertib induces oncogenic AKT and ERK signalling and synergizes with AKT or mTOR inhibition *in vitro*[212]. Volasertib demonstrated impressive anti-tumour activity in xenograft models of neuroblastoma (as monotherapy)[213], ALL (as monotherapy and in combination with cytarabine or quizartinib)[213, 214], breast cancer (in combination with fulvestrant)[215] and rhabdomyosarcoma (with vincristine)[216]. A clinical phase II trial compared volasertib in combination with low-dose cytarabine versus cytarabine alone for older patients with AML (TABLE 3). This combination achieved an improved complete response rate (31% versus 13%), improved median event-free survival (5.6 months versus 2.3 months) and improved median overall survival (8.0 months versus 5.2 months)[217]. The clinical benefit is currently being validated in a phase III trial (POLO-AML-2, see Supplementary Information S1 (table)). In contrast, several phase II clinical trials for patients with solid tumours showed disappointing results with lack of sufficient clinical activity[218–220]. Future clinical application will depend on identification of biomarkers that can predict clinical response. Based on *in vitro* data it was suggested that p53-negative cancers would be particularly sensitive to PLK1 inhibition[221]. Importantly, overexpression of the ABC transporter ABCB1 (P-glycoprotein) conferred resistance to volasertib treatment *in vitro*, supporting future co-administration of ABCB1 inhibitors to improve clinical responses[222].

Inhibitors of Aurora kinases

A number of inhibitors targeting the major family members Aurora A or Aurora B, such as alisertib, ENMD-2076, danusertib and AMG-900, have been developed and are under

clinical investigation (TABLE 1). In contrast, a selective Aurora B inhibitor, barasertib (AZD1152), was discontinued after a number of clinical phase II trials showed no substantial clinical benefit.

Alisertib exhibits high selectivity for Aurora A[223]. Treatment of cancer cells with alisertib was shown to induce mitotic arrest and polyploidy, and resulted in senescence or apoptosis[224, 225]. Monotherapy with alisertib showed tumour regression in preclinical mouse models of neuroblastoma[226], ALL[226] and lymphoma[223]. Interestingly, Aurora A inhibition using alisertib triggered degradation of N-MYC and hence caused tumour regression in a MYCN-driven mouse model of neuroblastoma[227]. Furthermore, combination of alisertib with chemotherapeutics induced tumour regression in mouse models of AML[228], oesophageal[229] and gastric cancer[230]. Alisertib also synergized with inhibitors of BCR-ABL (in CML)[231], CD20 (in mantle cell lymphoma[232] and diffuse large B-cell lymphoma[233]), MEK (in colorectal cancer)[234] and BCL2 (in neuroblastoma)[235], as well as with an agonist of the cell death receptor DR5 (in melanoma)[236]. The first clinical phase I trial involving alisertib monotherapy started in 2007 and reported a partial response in a patient with refractory ovarian cancer[237]. Although alisertib monotherapy did not achieve sufficient clinical activity in a subsequent phase II trial for patients with platinum-resistant/refractory ovarian cancer[238], an ongoing phase I/II study reported an encouraging response rate of 29% in combination with paclitaxel for patients with recurrent ovarian cancer (TABLE 3)[239]. Furthermore, another phase II study for solid tumours showed promising activity for patients with small cell lung carcinoma (response rate 21%) and breast cancer (response rate 18%)[240]. Combination with docetaxel demonstrated good preliminary activity in castration-resistant prostate cancer (response rate approx. 50%)[241] and in combination with bortezomib (a proteasome inhibitor) in multiple myeloma (response rate 27%)[242]. Moreover, two clinical phase II studies reported promising anti-tumour activity in relapsed/refractory B-cell and T-cell non-Hodgkin lymphoma (response rate 27%)[243] and in relapsed/refractory peripheral T-cell lymphoma (response rate 30%)[244]. The potential clinical benefit for patients with peripheral T-cell lymphoma is currently being investigated in a phase III trial (see Supplementary Information S1 (table)). Alisertib is currently studied in over 30 clinical trials involving a wide variety of cancers (TABLE 1).

Conclusions

While basic cell cycle regulators were discovered over 30 years ago, the last decade saw a dramatic increase in our understanding of their role in cancer and their potential as targets for cancer therapy. The development of novel compounds using structure-based drug design and efficient high-throughput screening platforms allowed bringing cell cycle studies from bench to bedside. Indeed, the provisional approval of a CDK4/CDK6-selective inhibitor (palbociclib) for breast cancer treatment by the FDA represents the first successful clinical translation in this field. Other CDK4/CDK6-selective inhibitors demonstrated very encouraging results and their approval is expected within the next years. Inhibitors that selectively inhibit CDK4 but not CDK6 (and vice versa) may also be developed, and they could possibly reduce the adverse effects without compromising the therapeutic benefit. Furthermore, CDK2- and CDK1-selective inhibitors will likely be developed, as they may have clinical utility for specific cancer subtypes[55, 66–68]

The success of future cell cycle-targeted therapies will depend on development of selective and potent compounds and on identification of specific vulnerabilities of cancer cells. Cell culture-based screening approaches, patient-derived xenografts and genetically engineered mouse cancer models will likely remain essential to uncover synthetic lethal interactions between genomic lesions and selective inhibition of individual cell cycle proteins. Novel treatment modalities capable of targeting multiple components of the same pathway, such as microRNAs may also provide therapeutic benefits. Indeed, MRX34, a miR-34 mimic targeting multiple cell cycle gene transcripts, recently entered clinical phase I evaluation[245]. Moreover, combination of several selective inhibitors may substantially improve clinical activity; however, in several cases combination therapies have shown a significant increase of adverse effects and may thus not be tolerated by many patients. Furthermore, current targeted therapies suffer from the relatively low percentage of patients showing satisfactory, long-term response. Hence, genomic technologies will become an invaluable diagnostic tool to identify predictive biomarkers for responsive patient subpopulations. Finally, since many preclinical studies as well as clinical experience indicate the occurrence of resistance (i.e. relapse after an initial response), research on resistance mechanisms against current compounds will help to identify treatment options for relapsed/refractory patients, or suggest combinatorial therapies that might prevent acquisition of resistance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

GLOSSARY

Clinical breast cancer subgroups

1. ER+ or HR+ (hormone receptor-positive) breast cancer: cancer with expression of ER (oestrogen receptor) and/or PR (progesterone receptor) and with normal HER2 (human epidermal growth factor receptor 2, ERBB2) expression;
2. HER2+ breast cancer: with HER2 amplification or overexpression;
3. triple-negative breast cancer (TNBC): with low/absent expression of ER/PR without HER2 overexpression.

Cell cycle checkpoints

A number of surveillance pathways that monitor occurrence of DNA damage (DNA damage checkpoints), as well as proper assembly of the mitotic spindle (spindle assembly checkpoint), and are capable of transiently arresting the cell cycle to allow time for repair or proper assembly.

Clinical responses

Complete response (CR, complete disappearance of all tumours in a given patient), partial response (PR, tumour shrinkage by ≥30%), stable disease (SD, <30% tumour shrinkage or <20% tumour growth), progressive disease (PD, ≥20% growth).

Clinical trial phases

New agents with promising preclinical results (animal models) are first tested for safety (adverse effects), optimal dosage and preliminary signs of efficacy (phase I), then for their efficacy using the optimal dosage in a defined, small group of patients (phase II), and finally in large, randomized, double-blind study in comparison to a placebo or the current “gold standard” of treatment (phase III).

Mitotic catastrophe
A particular form of apoptosis occurring during mitosis as a result of aberrant chromosome segregation or DNA damage, typically related to inactivation of cell cycle checkpoints.

Pan-CDK inhibitor
An inhibitor of cyclin-dependent kinases (CDKs) with a broad specificity (i.e. not selective for individual CDKs).

Replication catastrophe
A form of DNA damage involving DNA double-strand breaks and chromosome fragmentation. Replication catastrophe occurs during S phase as a result of unscheduled firing of DNA replication origins that causes breakage of stalled replication forks. It is typically related to an impaired ATR- and CHK1-dependent DNA damage checkpoint.

Therapeutic index
The ratio between the drug dose causing the desired pharmacological effect and the dose causing toxicity (e.g. toxicity or lethality in 50% of patients or animals, respectively).

Xenograft
Transplantation of human cancer cells (either cancer cell lines or patient-derived primary cancer specimens) into immunocompromised mice – either under the skin (subcutaneous) or into the location of the original tumour (orthotopic).

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Biographies

Tobias Otto

Tobias Otto received his Ph.D. degree from the University of Marburg in Marburg, Germany. During his graduate studies in the laboratory of Dr. Martin Eilers, he studied the role of the MYC family of transcription factors in neuroblastoma and T-cell lymphoma. He then joined the laboratory of Dr. Piotr Sicinski in Dana-Farber Cancer Institute as a postdoctoral research fellow. He investigates the role of cell cycle-targeting microRNAs during development and cancer formation using genetically engineered mouse models.

Piotr Sicinski

Piotr (Peter) Sicinski received his M.D. and Ph.D. degrees from the Warsaw Medical School in Warsaw, Poland. He was a visiting scientist at the Medical Research Council in Cambridge, UK, before joining the laboratory of Dr. Robert A. Weinberg at the Whitehead Institute for his postdoctoral training. Peter joined the faculty of the Harvard Medical School in 1997, where he is now a Professor of Genetics. His laboratory, located in the Dana-Farber Cancer Institute, studies the roles of cell cycle proteins in physiology and in cancer.

ONLINE SUMMARY

- Many cell cycle proteins are overexpressed or overactive in human cancers, in particular D-type and E-type cyclins, cyclin-dependent kinases (CDK4, CDK6 and CDK2), Polo-like kinase 1 (PLK1) and Aurora kinases (Aurora A and B). In transgenic mice, overexpression of several of these cell cycle proteins induces or contributes to tumorigenesis, revealing their prominent oncogenic roles.
- Some of these cell cycle proteins are also required for tumorigenesis and their ablation in mice impairs tumour formation induced by specific genetic lesions or by carcinogen treatment, as demonstrated for several cyclins (D1, D2, D3 and A2) and CDKs (CDK4, CDK6, CDK2 and CDK1), as well as for checkpoint kinase 1 (CHK1). Importantly, in some cases the continued presence of a cell cycle protein has been shown to be also required for tumour maintenance and progression, e.g. for cyclin D1, D3 and CDK4, thereby providing a clear rationale for targeting these proteins in cancer treatment.
- Kinases involved in cell cycle checkpoint function such as CHK1 and WEE1 also constitute potential therapeutic targets. Their inhibition compromises checkpoint function, causes excessive DNA damage and eventually leads to apoptosis, particularly in cells with compromised p53 function.
- CDK4/6-selective inhibitors, such as palbociclib, ribociclib and abemaciclib, have shown significant benefits in clinical studies, particularly in breast cancer, but also in non-small cell lung cancer, melanoma and head and neck squamous cell carcinoma. Importantly, following demonstration of a substantial improvement in progression-free survival, combination of palbociclib and letrozole received accelerated approval for first-line treatment of patients with advanced ER+ HER2- breast cancer.
- Inhibitors of PLK1, such as rigosertib and volasertib, have also shown encouraging results in clinical phase II/III studies for patients with myelodysplastic syndromes and acute myelogenous leukaemia, respectively, and several phase III trials are currently ongoing.
- Compounds targeting Aurora A, particularly alisertib, have been extensively studied in preclinical models and demonstrated synergy with many other targeted therapies, leading to tumour regression in a variety of cancer models. Moreover, clinical studies revealed encouraging activity of alisertib in peripheral T-cell lymphoma, non-Hodgkin lymphoma, non-small cell lung cancer and breast cancer.

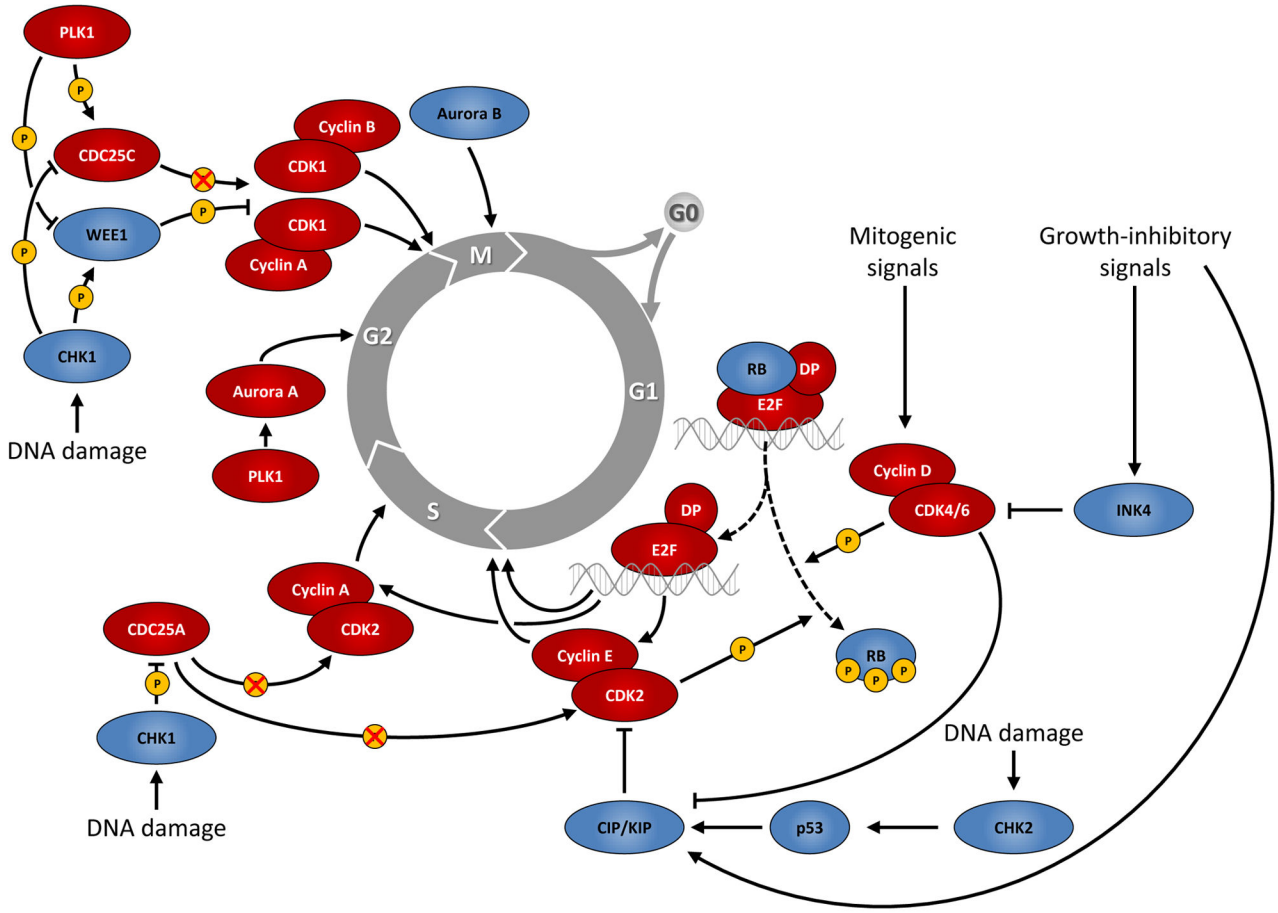


Figure 1. Cell cycle progression and major regulatory proteins

Mitogenic signals activate complexes of cyclins and cyclin-dependent kinases (CDKs) that promote progression from the G1 phase into S phase mainly by phosphorylating the retinoblastoma protein (RB) and subsequent activation of transcription by the E2F family of transcription factors. Growth-inhibitory signals antagonize G1-S progression by upregulating CDK inhibitors of the INK4 and CIP/KIP families. Progression through S phase and from G2 phase into mitosis (M phase) is also controlled by cyclin-CDK complexes, together with a variety of other proteins, such as Polo-like kinase 1 (PLK1) and Aurora kinases (Aurora A/B). Cells can also exit the cell cycle and enter a reversible or permanent cell cycle arrest (G0 phase). In addition, DNA damage is sensed by several specialized proteins and triggers cell cycle arrest via checkpoint kinase 2 (CHK2) and p53 in G1 phase or via checkpoint kinase 1 (CHK1) in S or G2 phase. Red and blue ovals denote positive and negative regulators of cell cycle progression, respectively.

CDC25, cell division cycle 25; CIP, CDK-interacting protein; G1, gap 1; G2, gap 2; INK4, inhibitor of CDK4; KIP, kinase-inhibitory protein.

Figure 2a

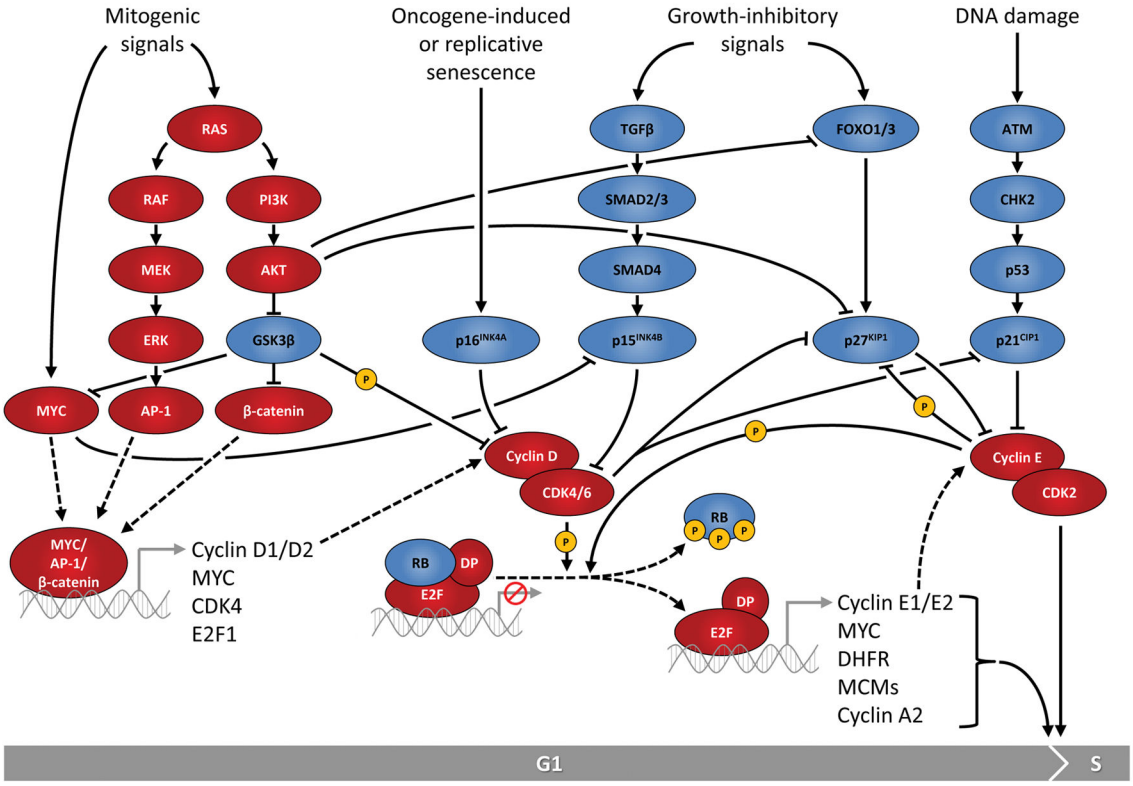


Figure 2b

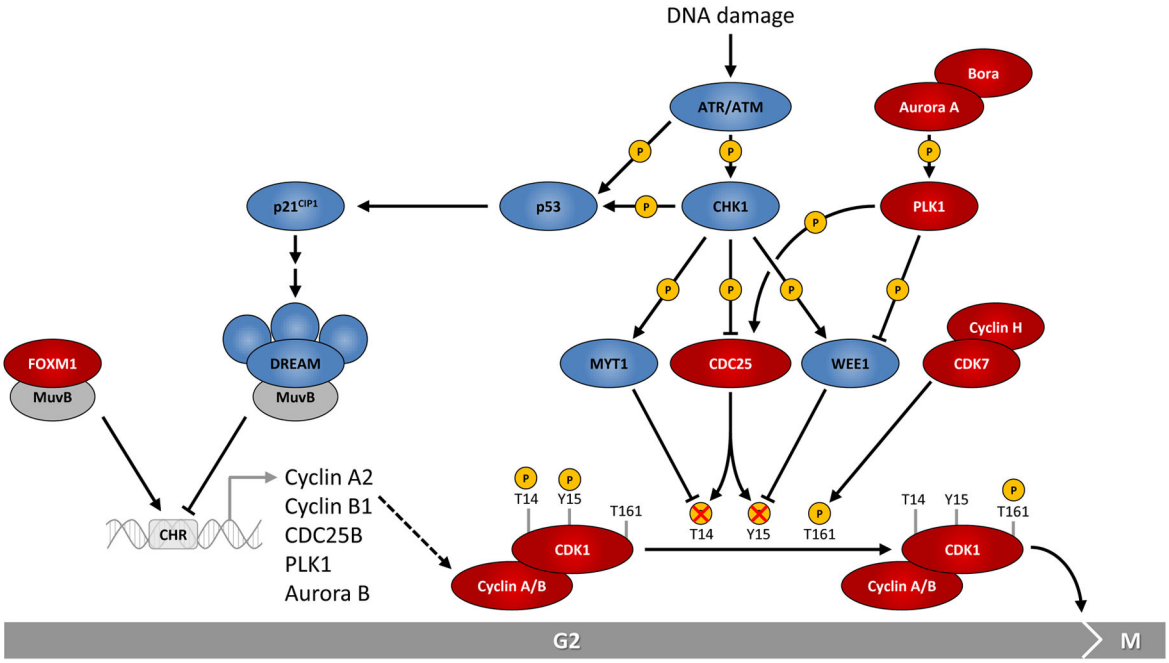


Figure 2. Regulation of G1-S and G2-M cell cycle transitions is controlled by multiple proteins and pathways

a: Entry into the cell cycle is typically induced in response to mitogenic signals that activate signalling pathways such as the RAS pathway. These pathways eventually impinge on transcriptions factors such as MYC, AP-1 or β-catenin and lead to induction of a number of cell cycle proteins including D-type cyclins. Formation of active complexes of D-type cyclins and cyclin-dependent kinases (CDKs) 4 and 6 drives phosphorylation of the RB (retinoblastoma) protein and is antagonized by the INK4 family ($p16^{INK4A}$ and $p15^{INK4B}$) in response to senescence-inducing or growth-inhibitory signals, such as the transforming growth factor β (TGFβ). Upon RB phosphorylation, E2F transcription factors are able to activate transcription of a plethora of S phase-promoting genes, including cyclins E1 and E2. Cyclin E-CDK2 complexes are kept inactive by interaction with inhibitors $p27^{KIP1}$ and $p21^{CIP1}$ that are regulated by growth-inhibitory signals and the p53-dependent G1 DNA damage checkpoint. Activation of cyclin E-CDK2 involves several mechanisms including the sequestration of $p27^{KIP1}$ and $p21^{CIP1}$ by cyclin D-CDK4/6 complexes, and phosphorylation of $p27^{KIP1}$ by cyclin E-CDK2 kinase. Active cyclin E-CDK2 complexes further phosphorylate RB, as well as many other targets culminating in S phase entry.

b: During G2 phase, the MuvB complex associates with the transcription factor FOXM1 and binds promoters containing cell cycle genes homology region (CHR) elements, thereby

inducing transcription of genes required for entry into and progression through mitosis (M phase), including B-type cyclins. Activation of cyclin B-CDK1 kinase requires phosphorylation of CDK1 at Thr-161 by the cyclin H-CDK7 complex (CAK, CDK-activating kinase) as well as dephosphorylation of Thr-14 and Tyr-15 on CDK1 by cell division cycle 25 (CDC25) family phosphatases, the latter process being antagonized by protein kinases MYT1 and WEE1. Activation of CDK1 is prevented in response to activation of the CHK1-dependent G2 DNA damage checkpoint. Upon recovery from DNA damage, Polo-like kinase 1 (PLK1) is essential to re-activate CDK1. Activation of cyclin A/B-CDK1 complexes is required and sufficient for entry into mitosis. Red and blue ovals denote positive and negative regulators of cell cycle transitions, respectively.

AKT, v-akt murine thymoma viral oncogene homolog (kinase); AP-1, activator protein 1; ATM, ataxia telangiectasia mutated (kinase); ATR, ataxia telangiectasia and Rad3 related (kinase); CHK, checkpoint kinase; DHFR, dihydrofolate reductase; DREAM, multiprotein complex consisting of p107/p130, E2F4/E2F5, DP1 and MuvB; ERK, extracellular signal-regulated kinase; FOXM1, forkhead box M1; FOXO, forkhead box O; GSK3β, glycogen synthase kinase 3 beta; MEK, mitogen-activated protein kinase kinase; MCMs, minichromosome maintenance complex component proteins (DNA helicases); MuvB, synthetic multivulva class B complex; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; SMAD, SMAD family of transcription factors.

Figure 3a

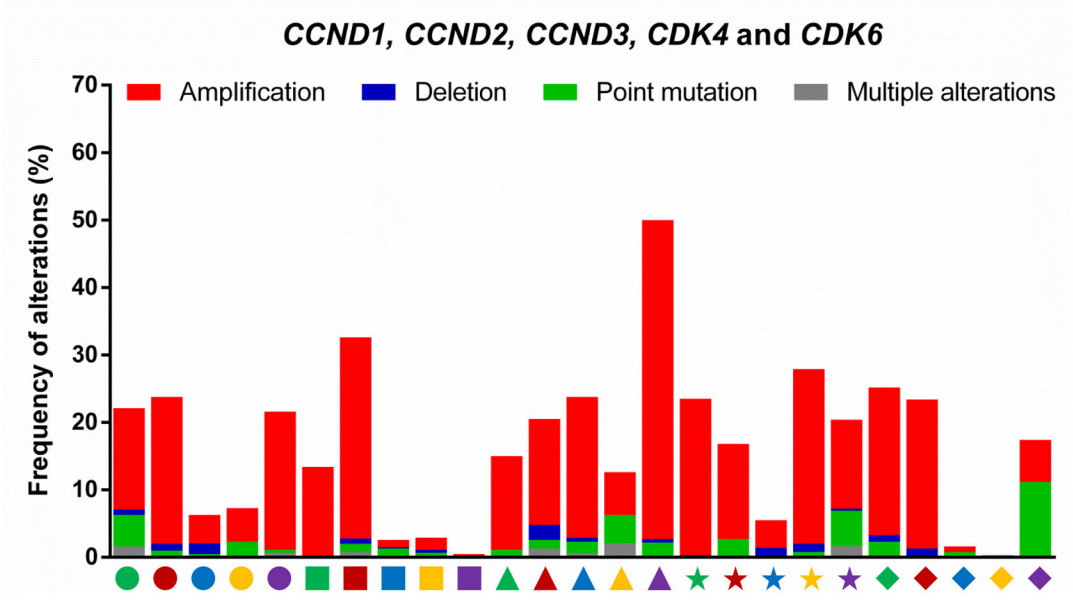


Figure 3b

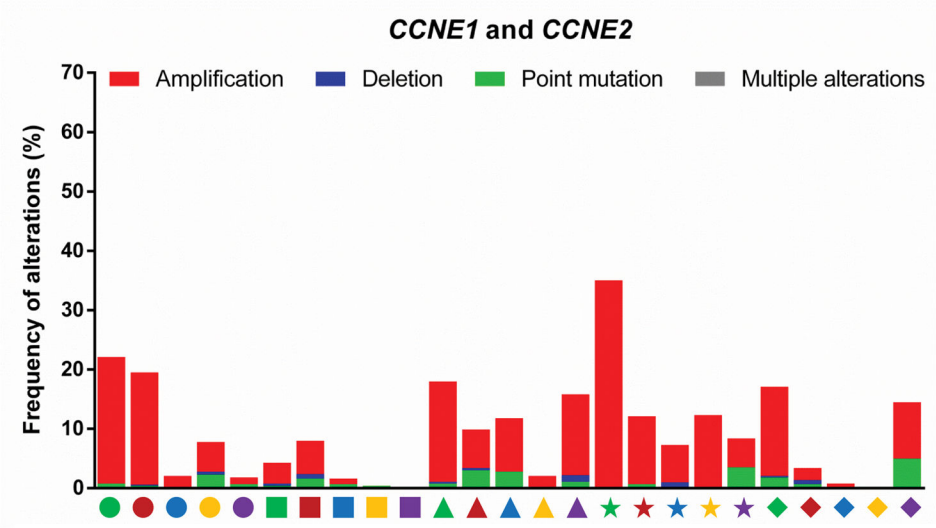


Figure 3c

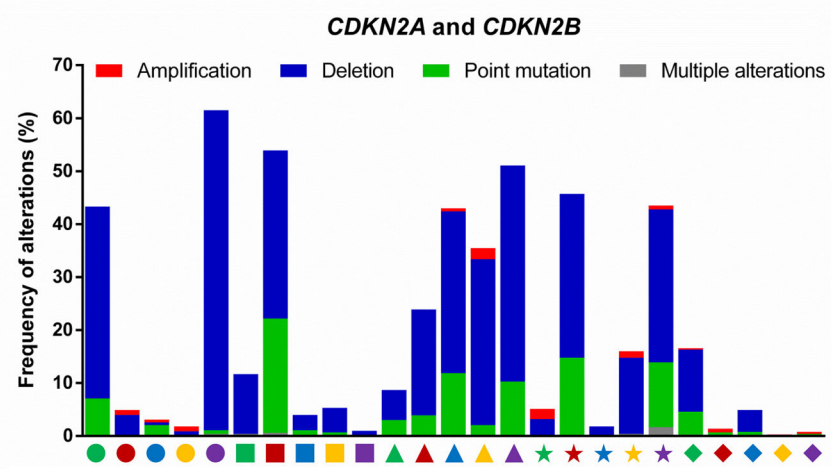


Figure 3d

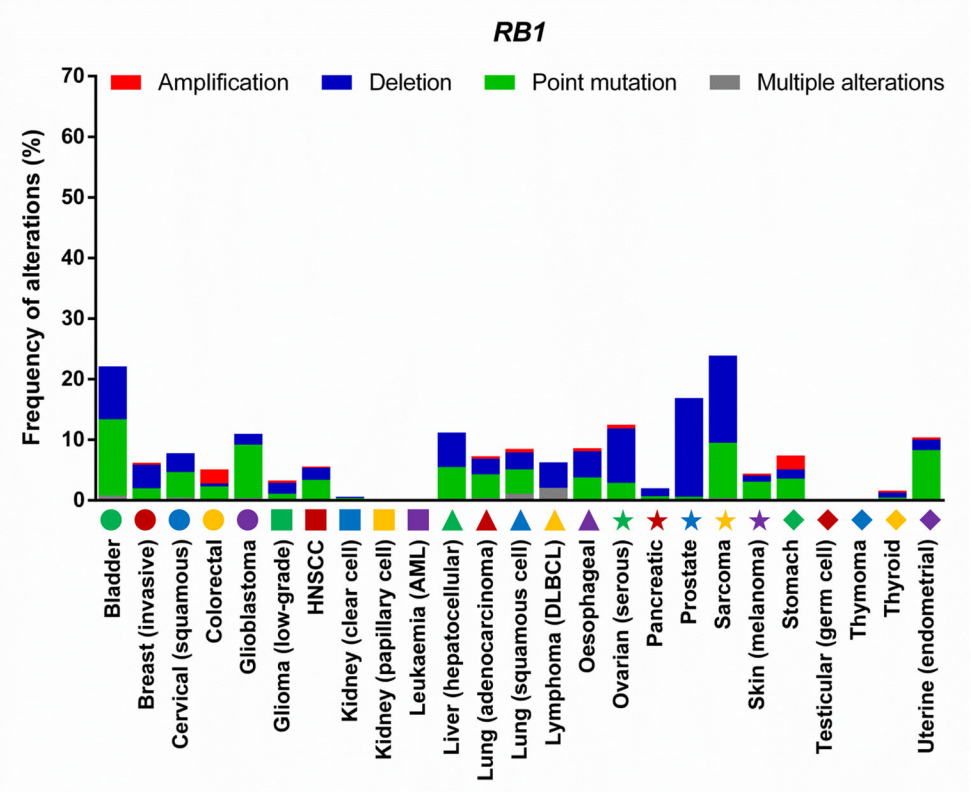


Figure 3. Deregulation of cell cycle proteins in human cancers

The frequencies of genetic alterations within genes encoding major cell cycle regulators across 25 types of human cancers. Genetic alterations include amplifications (red bars), deletions (blue bars), point mutations (green bars) and multiple alterations (grey bars). Each cancer type is denoted by a symbol with unique shape and colour below the graph (for symbol legend, see FIG. 3d). Data were obtained from The Cancer Genome Atlas (TCGA) and accessed through the cBioPortal for Cancer Genomics (http://www.cbioportal.org/) (26th August 2016). For each cancer type, the TCGA data set with the highest number of tumours was selected. The figures summarize genetic alterations from 48 (for lymphoma) to 1105 (for breast cancer) individual tumours (median of 479 individual tumours per cancer type). More detailed information for each cancer type is available via the cBioPortal for Cancer Genomics.

a: Alterations of cyclin D1 (*CCND1*), D2 (*CCND2*), D3 (*CCND3*) and cyclin-dependent kinase 4 (*CDK4*) and 6 (*CDK6*) genes.

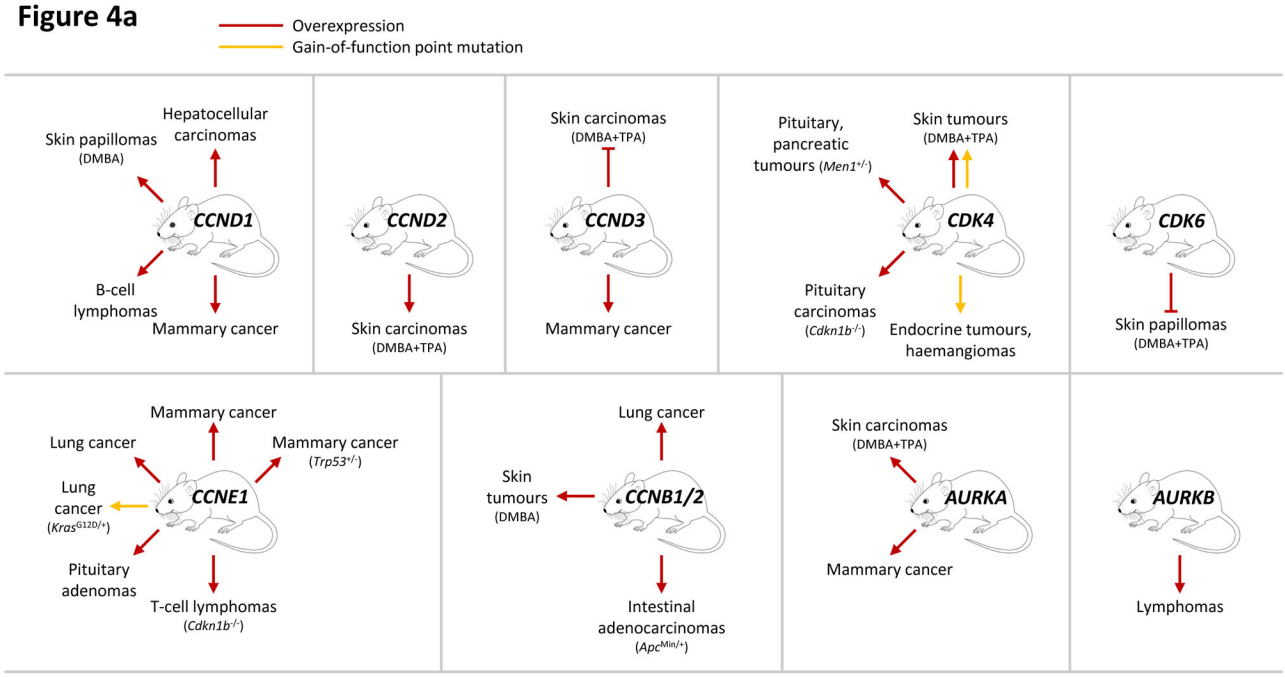
b: Alterations of cyclin E1 (*CCNE1*) and cyclin E2 (*CCNE2*) genes.

c: Alterations of genes encoding CDK inhibitors $p15^{INK4B}$ (*CDKN2B*) and $p16^{INK4A}$ (*CDKN2A*); the latter locus also encodes $p14^{ARF}$ (alternate reading frame protein), which inhibits ubiquitin ligase MDM2 and stabilizes p53.

d: Alterations of the retinoblastoma gene (*RB1*).

AML, acute myeloid leukaemia; DLBCL, diffuse large B-cell lymphoma; HNSCC; head and neck squamous cell carcinoma.

Figure 4a



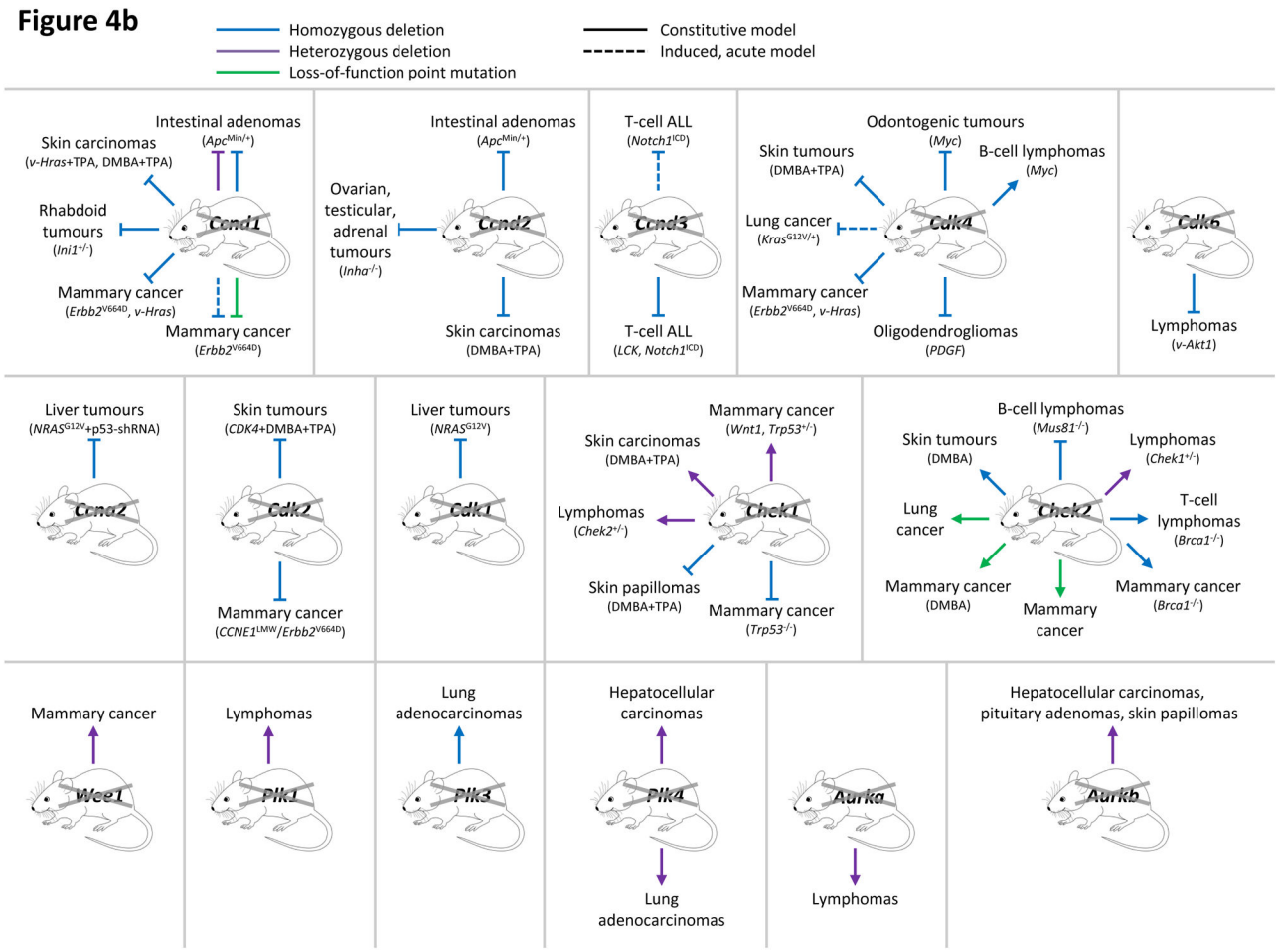


Figure 4. Analyses of cell cycle proteins in cancer using genetically engineered mouse models

This figure summarizes genetic mouse models used to investigate the role of cell cycle proteins in tumorigenesis. In case of transgenic overexpression, enhanced cancer formation is depicted by red arrows, inhibition of tumorigenesis by red inhibition symbols. Orange arrows indicate cancer formation induced by gain-of-function point mutations. In case of loss-of-function mutations (depicted by crossed out gene symbols), blue inhibition symbols indicate that homozygous ablation of a given gene prevented tumorigenesis, thereby revealing the requirement for this cell cycle protein in cancer formation. Blue dashed inhibition symbols depict an inducible, acute shutdown of *Ccnd1*, *Ccnd3* or *Cdk4*, used to demonstrate a critical role for these proteins in tumour progression. Arrows indicate that homozygous (blue) or heterozygous (violet) deletion of a cell cycle gene accelerated tumorigenesis. In case of loss-of-function point mutations (as opposed to gene inactivation by deletion described above), enhanced cancer formation is depicted by green arrows, suppressed cancer formation by green inhibition symbols. For tumours induced by a cooperating event (i.e. overexpression or mutation of oncogenes, loss of tumour suppressors or carcinogen treatment), this cooperating event is indicated in parentheses.

a: Genetic mouse models with increased activity of cell cycle proteins, i.e. cyclin D1 (*CCND1*), D2 (*CCND2*), D3 (*CCND3*), CDK4, CDK6, cyclin E1 (*CCNE1*), cyclin B1 or B2 (*CCNB1/2*), Aurora A (*AURKA*) and Aurora B (*AURKB*).

b: Genetic mouse models with reduced or abolished activity of cell cycle proteins, i.e. cyclin D1 (*Ccnd1*), D2 (*Ccnd2*), D3 (*Ccnd3*), CDK4, CDK6, cyclin A2 (*Ccna2*), CDK2, CDK1, checkpoint kinase 1 (*Chek1*) and 2 (*Chek2*), WEE1, Polo-like kinases 1 (*Plk1*), 3 (*Plk3*) and 4 (*Plk4*), Aurora A (*Aurka*) and Aurora B (*Aurka*).

AKT1, thymoma viral proto-oncogene 1; ALL, acute lymphoblastic leukaemia; APC, adenomatosis polyposis coli; BRCA1, breast cancer 1; CDKN1B, CDK inhibitor 1b ($p27^{KIP1}$); DMBA, 7,12-Dimethylbenz[a]anthracene (a carcinogen); ERBB2 (HER2), erb-b2 receptor tyrosine kinase 2; HRAS, Harvey rat sarcoma virus oncogene; INHA, Inhibin alpha; INI1 (SMARCB1), SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1; KRAS, Kirsten rat sarcoma viral oncogene homolog; LCK, lymphocyte protein tyrosine kinase; MEN1, multiple endocrine neoplasia 1; MUS81, MUS81 endonuclease homolog; MYC, myelocytomatosis oncogene; NRAS, neuroblastoma ras oncogene; p53-shRNA, short hairpin RNA targeting p53; PDGFB, platelet derived growth factor, B polypeptide; TPA, 12-O-Tetradecanoylphorbol-13-acetate (a tumour promoter); TRP53, transformation related protein 53 (p53); WNT1, wingless-type MMTV integration site family, member 1.

CCND1 gain:	Hepatocellular carcinomas[251] Skin papillomas (DMBA)[16] B-cell lymphomas[252] Mammary cancer[13]
CCND2 gain:	Skin carcinomas (DMBA+TPA)[14]
CCND3 gain:	Skin carcinomas (DMBA+TPA)[14] Mammary cancer[253]
CDK4 gain:	Skin tumours (DMBA+TPA)[12, 17] Pituitary, pancreatic tumours ($Men1^{+/-}$)[54] Pituitary carcinomas ($Cdkn1b^{-/-}$)[254] Endocrine tumours, haemangiomas[11]
CDK6 gain:	Skin papillomas (DMBA+TPA)[15]
CCNE1 gain:	Mammary cancer ($Trp53^{+/-}$)[255] Mammary cancer[45] Lung cancer[256] Lung cancer ($Kras^{G12D/+}$)[257] Pituitary adenomas[258] T-cell lymphomas ($Cdkn1b^{-/-}$)[259]
CCNB1/2 gain:	Lung cancer[64] Skin tumours (DMBA)[64] Intestinal adenocarcinomas ($Apc^{Min/+}$)[64]
AURKA gain:	Skin carcinomas (DMBA+TPA)[260] Mammary cancer[107]
AURKB gain:	Lymphomas[102]
Ccnd1 loss:	Intestinal adenomas ($Apc^{Min/+}$)[261] Skin carcinomas (*v-Hras*+TPA, DMBA+TPA)[262] Rhabdoid tumours ($Ini1^{+/-}$)[263] Mammary cancer ($Erbb2^{V664D}$, *v-Hras*)[18, 19] Mammary cancer ($Erbb2^{V664D}$)[22, 26]
Ccnd2 loss:	Intestinal adenomas ($Apc^{Min/+}$)[264] Ovarian, testicular, adrenal tumours ($Inha^{-/-}$)[265] Skin carcinomas (DMBA+TPA)[14]
Ccnd3 loss:	T-cell ALL ($Notch1^{ICD}$)[26] T-cell ALL (*LCK*, $Notch1^{ICD}$)[23]
Cdk4 loss:	Odontogenic tumours (*Myc*)[266] Skin tumours (DMBA+TPA)[267]

	Lung cancer (*Kras*$^{G12V/+}$)[25] Mammary cancer (*Erbb2*^{V664D}, *v-Hras*)[20, 21, 268] Oligodendrogliomas (*PDGF*)[269] B-cell lymphomas (*Myc*)[270]
Cdk6 loss:	Lymphomas (*v-Akt1*)[24]
Ccna2 loss:	Liver tumours (*NRAS*^{G12V}+53-shRNA)[271]
Cdk2 loss:	Skin tumours (*CDK4*+DMBA+TPA)[53] Mammary cancer (*CCNE1*^{LMW}, *Erbb2*^{V664D})[37, 58]
Cdk1 loss:	Liver tumours (*NRAS*^{G12V})[65]
Chek1 loss:	Mammary cancer (*Wnt1*, *Trp53*$^{+/-}$)[77, 78] Skin carcinomas (DMBA+TPA)[79] Lymphomas (*Chek2*$^{+/-}$)[272] Skin papillomas (DMBA+TPA)[79] Mammary cancer (*Trp53*$^{+/-}$)[78]
Chek2 loss:	B-cell lymphomas (*Mus81*$^{-/-}$)[273] Skin tumours (DMBA)[274] Lung cancer[275] Mammary cancer (DMBA)[275] Mammary cancer[276] Mammary cancer (*Brca1*$^{-/-}$)[277] T-cell lymphomas (*Brca1*$^{-/-}$)[277] Lymphomas (*Chek1*$^{+/-}$)[272]
Wee1 loss:	Mammary cancer[87]
Plk1 loss:	Lymphomas[95]
Plk3 loss:	Lung adenocarcinomas[278]
Plk4 loss:	Hepatocellular carcinomas[279] Lung adenocarcinomas[279]
Aurka loss:	Lymphomas[108]
Aurkb loss:	Hepatocellular carcinomas, pituitary adenomas, skin papillomas[109]

Table 1

Inhibitors of cell cycle proteins in clinical development

Inhibitor (synonym) [company]	Major targets (IC50)	Preclinical studies (*in vitro*, mouse models)	Clinical trials (open/active/completed)
Pan-CDK inhibitors			
Flavopiridol[≠] (alvocidib) [Tolero Pharmaceuticals]	CDK9 (6 nM), CDK1 (30–50 nM), CDK2 (70–170 nM), CDK4 (100 nM)[112, 280]	• Caused G1 arrest, G2 arrest and apoptosis *in vitro*[110, 111] • Induced tumour regression in leukaemia and lymphoma xenografts[111]	• Phase II: AML, lymphoma, AML, multiple myeloma and many others
(R)-Roscovitine[*] (seliciclib) [Cyclacel Pharmaceuticals]	CDK2 (100–710 nM), CDK7 (490 nM), CDK1 (650–2690 nM), ERK2 (1.2–14 μM)[281, 282]	• Induced G2/M arrest and cell death *in vitro*[281, 282] • Slightly inhibited growth of colorectal cancer and uterine cancer xenografts[281]	• Phase I: advanced solid tumours
Dinaciclib[≠] (SCH 727965/ MK-7965) [Merck & Co.]	CDK2 (1 nM), CDK5 (1 nM), CDK1 (3 nM), CDK9 (4 nM), CDK7 (NA), CDK6 (NA)[115]	• Induced G1 arrest, G2/M arrest and apoptosis *in vitro*[115] • Reduced migration *in vitro*[116] • Exhibited anti-tumour activity in ovarian[115] and pancreatic cancer[116], ALL[117] and *NRAS*^{Q61L}-mutant melanoma[118]	• Phase III: CLL • Phase II: melanoma, CLL, lung, breast, multiple myeloma
AT7519[≠] (AT7519M) [Astex Therapeutics]	CDK9 (<10 nM), CDK5 (13 nM), CDK2 (47 nM), GSK3β (89 nM), CDK4 (100 nM), CDK6 (170 nM), CDK1 (210 nM)[283]	• Induced mainly G2/M arrest *in vitro*[283] • Showed promising anti-tumour activity in ovarian[284] and colon cancer[283] and AML xenografts[285] • Achieved tumour regression and improved survival in *MYCN* transgenic neuroblastoma model[286]	• Phase II: CLL, mantle cell lymphoma, multiple myeloma • Phase I: non-Hodgkin's lymphoma
Milciclib[*] (PHA-848125/ PHA-848125AC) [Tiziana Life Sciences]	CDK2 (45–363 nM), TRKA (53 nM), CDK7 (150 nM), CDK4 (160 nM), CDK5 (265 nM), CDK1 (398 nM)[287]	• Induced G1 arrest and cell death via autophagy *in vitro*[287, 288] • Inhibited tumour growth of ovarian cancer[287] and glioma xenografts[288], KRAS^{G12D}-induced lung cancer[289] and DMBA-induced mammary cancer[290]; extended survival of	• Phase II: thymoma, thymic carcinoma • Phase I: advanced solid tumours

Inhibitor (synonym) [company]	Major targets (IC50)	Preclinical studies (*in vitro*, mouse models)	Clinical trials (open/active/completed)
		mice bearing leukaemia[290] and intracranial glioma xenografts[288]	
TG02 * [Tragara Pharmaceuticals]	CDK9 (3 nM), CDK5 (4 nM), CDK2 (5 nM), CDK3 (8 nM), CDK1 (9 nM), LCK (11 nM), TYK2 (14 nM), FYN (15 nM), JAK2 (19 nM), FLT3 (19 nM)[291]	• Induced G1 arrest and apoptosis *in vitro*[291] • Caused tumour regression and extended survival of mice with AML xenografts[291]	• Phase I: CLL, AML, ALL, MDS, multiple myeloma
CYC065 * [Cyclacel Pharmaceuticals]	CDK2 (5 nM), CDK5 (21 nM), CDK9 (26 nM), CDK3 (29 nM), CDK7 (193 nM), CDK4 (232 nM)[292]	• Induced apoptosis in trastuzumab-resistant breast cancer cells[33] • Inhibited growth of trastuzumab-resistant breast cancer xenografts[33]	• Phase I: advanced solid tumours and lymphomas
RGB-286638≠ [Agennix]	CDK9 (1 nM), FMS (1 nM), CDK1 (2 nM), CDK2 (3 nM), GSK3β (3 nM), CDK4 (4 nM), CDK3 (5 nM), CDK5 (5 nM), TAK1 (5 nM)[293]	• Induced cell cycle arrest and apoptosis and inhibited transcription *in vitro*[293] • Inhibited tumour growth and extended survival of mice bearing multiple myeloma xenografts[293]	• Phase I: advanced solid tumours[294]
CDK4 and CDK6-selective inhibitors			
Palbociclib * (PD0332991) [Pfizer]	CDK4 (9–11 nM), CDK6 (15 nM)[131]	• Inhibited cell proliferation and induced G1 arrest in RB-positive cancer cells[131] • Inhibited growth of rhabdomyosarcoma[133], multiple myeloma[134], AML[135], ALL[136] and dermatofibrosarcoma[137] xenografts • Induced tumour regression in glioblastoma and colorectal cancer xenografts[131] • Showed synergistic anti-tumour activity with PI3K inhibition in *PI3KCA*-mutant triple-negative breast cancer xenografts[142]	• Phase III: breast, lung • Phase II: breast, lung, head and neck, multiple myeloma, AML, ALL, gastrointestinal, ovarian, hepatocellular, prostate, melanoma, liposarcoma, urothelial, lymphoma, endometrial, oligoastrocytoma, oligodendroglioma
Ribociclib * (LEE011) [Novartis]	CDK4 (10 nM), CDK6 (39 nM)[160]	• Induced G1 arrest and senescence *in vitro*[161] • Inhibited tumour growth in neuroblastoma[161], rhabdomyosarcoma[145]	• Phase III: breast • Phase II: breast, melanoma, liposarcoma, prostate, lung, uterine, gastrointestinal, ovarian, paediatric glioma,

Inhibitor (synonym) [company]	Major targets (IC50)	Preclinical studies (*in vitro*, mouse models)	Clinical trials (open/active/completed)
		and Ewing sarcoma xenografts[162] • Caused tumour regression in liposarcoma xenografts[143]	hepatocellular, teratoma, pancreatic, colorectal
Abemaciclib * (LY2835219) [Eli Lilly]	CDK4 (2 nM), CDK6 (10 nM), HIPK2 (31 nM), PIM1 (50 nM), CDK9 (57 nM), DYRK2 (61 nM), CK2 (117 nM), GSK3β (192 nM)[165]	• Induced G1 arrest *in vitro*[165] • Showed anti-tumour activity in colorectal cancer[165], AML[165], glioblastoma (orthotopic)[167] and vemurafenib-resistant melanoma xenografts[166]	• Phase III: breast, lung • Phase II: breast, lung, melanoma, mantle cell lymphoma
CHK1 and WEE1 inhibitors			
MK-8776‡ (SCH 900776) [Merck & Co.]	CHK1 (3 nM), CDK2 (160 nM), PIM1 (NA)[182]	• Induced DNA double-strand breaks, G2/M arrest and apoptosis *in vitro*[182]; sensitized cancer cells to various chemotherapeutics[183] and to histone deacetylase inhibition[295] • Combination with gemcitabine inhibited tumour growth in pancreatic cancer and induced tumour regression in ovarian cancer xenografts[182]	• Phase II: AML • Phase I: non-Hodgkin's lymphoma
LY2606368‡ (prexasertib) [Eli Lilly]	CHK1 (<1 nM), CHK2 (8 nM), RSK1 (9 nM), MELK (38 nM), SIK (42 nM), BRSK2 (48 nM), ARK5 (64 nM)[188]	• Caused DNA double-strand breaks during S phase ("replication catastrophe"), leading to fragmented chromosomes and mitotic cell death *in vitro*[188] • Inhibited tumour growth in lung cancer xenografts[188]	• Phase II: breast, ovarian, prostate, lung • Phase I: head and neck, AML, MDS
AZD1775 * (MK-1775) [AstraZeneca]	WEE1 (5.2 nM), YES (14 nM)[190]	• Sensitized p53-deficient tumour cells to apoptosis induction by DNA damaging agents and radiation[190–192] • Induced tumour regression in lung cancer[194] and (combined with gemcitabine) in pancreatic cancer xenografts[193]	• Phase II: lung, ovarian, pancreatic, stomach, AML, MDS, head and neck • Phase I: head and neck, glioma, pancreatic, cervical, CML, AML, bladder

Inhibitor (synonym) [company]	Major targets (IC50)	Preclinical studies (*in vitro*, mouse models)	Clinical trials (open/active/completed)
		• Extended survival of mice with AML[195] and high-grade glioma (intracerebral) xenografts[196] • Synergized with targeted inhibition of CHK1[201, 202, 296], histone deacetylases[198, 199], mTOR[200] and PARP[197]	
GDC-0575 * (Arry-575) [Genentech]	CHK1 (NA)[297]	• NA	• Phase I: solid tumours and lymphoma
PLK inhibitors			
Rigosertib‡ (ON 01910.Na) [SymBio Pharmaceuticals]	PLK1 (9 nM), PDGFR (18 nM), BCR-ABL (32 nM), FLT1 (42 nM), SRC (155 nM), FYN (182 nM), PLK2 (260 nM), CDK1 (260 nM)[205]	• Induced spindle abnormalities, mitotic arrest and apoptosis *in vitro*[205] • Caused tumour regression in orthotopic head and neck squamous cell carcinoma xenografts[206] • Combination with chemotherapy led to tumour regression in hepatocellular and breast carcinoma xenografts[205] • Combination with radiotherapy achieved long-lasting tumour regression in cervical cancer xenografts[207]	• Phase III: MDS, pancreatic • Phase II: MDS, AML, ALL, CMML, ovarian, squamous cell
Volasertib‡ (BI 6727) [Boehringer Ingelheim]	PLK1 (0.9 nM), PLK2 (5 nM), PLK3 (56 nM)[211]	• Induced G2/M arrest and apoptosis *in vitro*[211] • Caused tumour regression in colorectal cancer[211], neuroblastoma[213] and paediatric ALL xenografts[213] • Caused tumour regression in combination with cytarabine or FLT3 inhibitor quizartinib in AML[214] and with vincristine in rhabdomyosarcoma xenografts[216]	• Phase III: AML • Phase II: AML, lung, ovarian, urothelial, MDS
TKM-080301‡ (TKM-PLK1) [Arbutus Biopharma]	PLK1 (targeted by a lipid nanoparticle formulation of an siRNA)[298]	• NA	• Phase II: liver, adrenocortical, neuroendocrine

Inhibitor (synonym) [company]	Major targets (IC50)	Preclinical studies (*in vitro*, mouse models)	Clinical trials (open/active/completed)
			• Phase I: liver
CFI-400945 * [University Health Network, Toronto]	PLK4 (2.8 nM), ABL-T315I (5 nM), TRKA (6 nM), TRKB (9 nM), BMX (17 nM), TIE2 (22 nM)[299]	• Induced defects in centriole duplication and mitosis leading to apoptosis[299] • Caused tumour regression in carboplatin-resistant, PTEN−/− ER+ breast cancer xenografts[299]	• Phase I: advanced cancer
Aurora inhibitors			
Alisertib * (MLN8237) [Millennium Pharmaceuticals]	Aurora A (1.2 nM), EPHA2 (NA)[223]	• Induced mitotic arrest, spindle abnormalities, polyploidy, followed by senescence or apoptosis *in vitro*[224, 225] • Caused tumour regression in neuroblastoma[226], paediatric ALL[226] and lymphoma xenografts[223] • Induced tumour regression and prolonged survival in *MYCN*-driven mouse model of neuroblastoma[227] • Combination with chemotherapy induced tumour regression in AML[228], oesophageal[229] and gastric cancer xenografts[230] • Synergized with a DR5 agonist[236] and inhibitors for BCR-ABL[231], CD20[232], MEK[234] and BCL-2 in various cancer xenografts[235]	• Phase III: peripheral T-cell lymphoma • Phase II: lymphoma, lung, breast, ovarian, prostate, AML, gastroesophageal, melanoma, multiple myeloma, uterine, head and neck, mesothelioma, neuroblastoma, MDS, rhabdoid, urothelial
ENMD-2076 * [Miikana Therapeutics]	FLT3 (3 nM), Aurora A (14 nM), SRC (23 nM), VEGFR2 (40 nM), FGFR1 (93 nM), KIT (120 nM)[300]	• Induced G2/M arrest and apoptosis *in vitro*[301] • Reduced tumour vascularity, vascular permeability and perfusion *in vivo*[300] • Induced tumour regression in breast and colorectal cancer, melanoma, AML and multiple myeloma xenografts[302]	• Phase II: ovarian, breast, hepatocellular, sarcoma • Phase I: multiple myeloma

Inhibitor (synonym) [company]	Major targets (IC50)	Preclinical studies (*in vitro*, mouse models)	Clinical trials (open/active/completed)
AMG 900 * [Amgen]	Aurora C (1 nM), Aurora B (4 nM), Aurora A (5 nM), p38α (53 nM)[303]	• Induced mitotic arrest, polyploidy and apoptosis *in vitro*[303, 304] • Caused tumour regression in combination with ixabepilone in triple-negative breast cancer xenografts[304]	• Phase I: AML, advanced solid tumours

Clinical trial data obtained from http://www.clinicaltrials.gov (accessed: 26th August 2016)

* Oral.

≠ Intravenous.

ALL, acute lymphoblastic leukaemia. AML, acute myelogenous leukaemia. CLL, chronic lymphocytic leukaemia. CMML, chronic myelomonocytic leukaemia. CML, chronic myeloid leukaemia. DMBA, 7,12-Dimethylbenz[a]anthracene (a carcinogen). ER+, oestrogen receptor-expressing tumours/cells. IC50, inhibitor concentration that causes 50% inhibition of kinase activity (*in vitro* kinase assay). MDS, myelodysplastic syndromes. NA, not available. siRNA, short interfering RNA.

Table 2

Clinical trial results of selected CDK inhibitors

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)
Flavopiridol *(alvocidib)*				
AML (poor prognosis)[305]	• Phase II • N=62 • NCT00407966	• 50 mg/m^2 IV OD (days 1–3) • Combination with cytarabine (2 g/m^2 IV over 72 hours, days 6–8) and mitoxantrone (40 mg/m^2 IV on day 9) (“FLAM”)	• CR: 52% (32/62) • PR: 5% (3/62) • CR (newly diagnosed secondary AML): 75% (12/15) • Median OS: 8 months	• Tumour lysis syndrome (53%)
AML (newly diagnosed)[306]	• Phase II • N=165 • “FLAM” (N=112) vs “7+3” (N=56) • NCT01349972	• “FLAM” (see study above) • “7+3”: cytarabine (100 mg/m^2 IV daily, days 1–7) with daunorubicin (90 mg/m^2 IV daily, days 1–3), for residual leukaemia after 14 days: cytarabine (100 mg/m^2 IV daily, days 1–5) with daunorubicin (45 mg/m^2 IV daily, days 1–2)	• Median EFS: 9.7 months vs 3.4 months (HR=0.74, p=0.15) • Median OS: 17.5 months vs 22.2 months (HR=1.2, p=0.39) • CR: 70% (73/109) vs 57% (32/56) (p=0.08)	• Febrile neutropaenia (48% vs 45%), infection (35% vs 38%), hepatic dysfunction (21% vs 23%), gastrointestinal dysfunction (11% vs 9%)
CLL (relapsed)[113]	• Phase II • N=64 • NCT00098371	• 60–80 mg/m^2 IV over 4 hours (days 1, 8, 15 of 28-day cycle) • Monotherapy	• CR: 2% (1/64) • PR: 52% (33/64) • Median PFS: 8.6 months	• Neutropaenia (88%), diarrhoea (64%), tumour lysis syndrome (42%), elevated transaminases (34%), infection (31%), thrombocytopenia (27%)
CLL (fludarabine-refractory)[114]	• Phase II • N=159 • NCT00464633	• 60–80 mg/m^2 IV over 4 hours (days 1, 8, 15 of 28-day cycle)	• CR: 2% (3/159) • PR: 24% (38/159)	• Neutropaenia (34%), infections (30%), gastrointestinal (25%), tumour lysis

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)
		• Monotherapy	• SD: 33% (53/159) • Median PFS: 7.6 months • Median OS: 14.6 months	syndrome (21%) and other
Dinaciclib *(SCH 727965, MK-7965)*				
CLL (relapsed or refractory)[124]	• Phase I • N=52 • NCT00871663	• 5–17 mg/m^2 IV weekly (3 weeks on, 1 week off), RP2D: 14 mg/m^2 • Monotherapy	• PR: 54% (28/52) • SD: NA • Median PFS: 15.8 months	• Neutropaenia (75%), thrombocytopaenia (40%), increased AST (29%), anaemia (29%), hyperglycaemia (21%) and other
Multiple myeloma (relapsed after prior therapy)[123]	• Phase I/II • N=27 • NCT01096342	• 30–50 mg/m^2 IV every 3 weeks, MTD: 50 mg/m^2 • Monotherapy	• PR: 11% (3/27) • SD: 56% (15/27)	• Neutropaenia (12%), diarrhoea (12%), blurred vision (12%)
Palbociclib *(PD0332991)*				
Breast cancer (advanced, ER+ HER2-, first-line treatment, post-menopausal)[154]	• Phase II • N=165 • Palbociclib + letrozole (N=84) vs letrozole alone (N=81) • NCT00721409 (PALOMA-1)	• 125 mg PO OD (3 weeks on, 1 week off) • Combination with letrozole (2.5 mg PO OD, continuous)	• Median PFS: 20.2 months vs 10.2 months (HR=0.488, p=0.0008) • Median OS: 37.5 months vs 33.3 months (HR=0.813, p=0.42) • CR: 1% vs 1% • PR: 42% vs 32% • SD: 44% vs 37% • SD ≥ 24 weeks: 38% vs 25%	• Neutropaenia (54% vs 1%)
Breast cancer (advanced, ER+ HER2-, relapsed or progressed during prior hormone therapy)[156, 157]	• Phase III • N=521 • Palbociclib + fulvestrant (N=347) vs placebo + fulvestrant (N=174)	• 125 mg PO OD (3 weeks on, 1 weeks off) • Combination with fulvestrant (500 mg IM every 2–4 weeks)	• Median PFS: 9.5 months vs 4.6 months (HR=0.46, p<0.001) • Effect on OS yet unknown	• Neutropaenia (65% vs 1%)

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)
	• NCT01942135 (PALOMA-3)	• Additional goserelin for pre/peri-menopausal women		
Breast cancer (metastatic, RB +)[159]	• Phase II • N=37 (84% ER+ HER2-) • NCT01037790	• 125 mg PO OD (3 weeks on, 1 week off) • Monotherapy	• PR: 5% (2/37) • SD ≥ 6 months: 14% (5/37) • Median PFS: 3.7 months	• Neutropaenia (54%), lymphopaenia (30%), thrombocytopaenia (19%)
Breast cancer (metastatic, RB +)[173]	• Phase I • N=15 • NCT01320592	• 50–125 mg PO OD (on days 2–6, 9–14, 16–20 of 28-day cycle) • Combination with paclitaxel (80 mg/m^2 IV weekly)	• PR: 40% (6/15) • SD: 33% (5/15)	• Neutropaenia (67%)
Non-small cell lung cancer (previously-treated, recurrent or metastatic, RB+, with p16^{INK4A} loss)[152]	• Phase II • N=19 • NCT01291017	• 125 mg PO OD (3 weeks on, 1 weeks off) • Monotherapy	• RR: 0% (0/16) • SD: 50% (8/16)	• Neutropaenia (16%)
Head and neck squamous cell carcinoma (incurable)[307]	• Phase I • N=9 • NCT02101034	• 100–125 mg PO OD (3 weeks on, 1 week off), RP2D: 125 mg • Combination with cetuximab (250–400 mg/m^2 IV weekly)	• PR: 22% (2/9) • SD: 56% (5/9)	• None reported
Mantle cell lymphoma (relapsed, with cyclin D1 overexpression)[149]	• Phase Ib • N=17 • NCT00420056	• 125 mg PO OD (3 weeks on, 1 week off) • Monotherapy	• CR: 6% (1/17) • PR: 12% (2/17) • SD: 41% (7/17) • PFS > 1 year: 29% (5/17)	• Neutropaenia (35%), thrombocytopaenia (24%)

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)
Liposarcoma (advanced, well-differentiated or dedifferentiated, RB+, with CDK4 amplification)[150]	• Phase II • N=30 • NCT01209598	• 200 mg PO OD (2 weeks on, 1 week off) • Monotherapy	• PR: 3% (1/29) • SD: NA • PFS at 12 weeks: 66% (19/29) • Median PFS: 18 weeks	• Neutropaenia (50%), thrombocytopaenia (30%), anaemia (17%)
Germ cell tumours (incurable, refractory, RB+)[151]	• Phase II • N=29 (arm 4) • NCT01037790	• 125 mg PO OD (3 weeks on, 1 week off) • Monotherapy	• PFS at 24 weeks: 28% (8/29) • PFS at 24 weeks among patients with teratomas: 45% (5/11)	• Neutropaenia (43%), thrombocytopaenia (17%)
Ribociclib *(LEE011)*				
Breast cancer (advanced, ER+ HER2-, post-menopausal)[164]	• Phase Ib • N=10 (arm 1) • NCT01872260	• 600 mg PO OD (3 weeks on, 1 week off) • Combination with letrozole (2.5 mg PO OD, continuous)	• PR: 17% (1/6) • SD: 33% (2/6)	• Neutropaenia (50%)
Melanoma (NRAS mutant)[175]	• Phase Ib/II • N=14 • NCT01781572	• 200–300 mg PO OD (3 weeks on, 1 week off) • Combination with binimetinib (25 mg PO BD, continuous)	• PR: 43% (6/14), pending confirmation • SD: 43% (6/14)	• Various DLTs (21%)
Abemaciclib *(LY2835219)*				
Breast cancer (metastatic)[170]	• Phase I • N=47 (arm 1) • NCT01394016	• 200 mg PO BD (continuous) • Monotherapy	• PR: 23% (11/47), pending confirmation • PR among ER+: 31% (11/36)	• NA
Breast cancer (metastatic, ER+ HER2-)[172]	• Phase Ib • N=36 (parts A+B) • N=16 (part C) • NCT02057133	• 120–200 mg PO BD (continuous) • Combination with letrozole (2.5 mg PO OD) (part A) or	• PR: 6% (2/36) (parts A+B) • SD: 61% (22/36) (parts A+B)	• Diarrhoea (NA)

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)
		anastrozole (1 mg PO OD) (part B) • Combination with tamoxifen (20 mg PO OD) (part C)	• PR: 0% (0/16) (part C) • SD: 75% (12/16) (part C)	
Non-small cell lung cancer (advanced, relapsed/ progressed)	• Phase I • N=49 • NCT01394016	• 150–200 mg PO BD (continuous) • Monotherapy	• PR: 2% (1/49) • SD: 49% (24/49) • Median PFS: 2.1 months	• Rare

AML, acute myelogenous leukaemia. AST, aspartate aminotransferase. BD, twice daily. CLL, chronic lymphocytic leukaemia. CR, complete response/remission. DLT, dose-limiting toxicity. EFS, event-free survival. ER+ HER2-, oestrogen or progesterone receptor expressing (ER+) and HER2 non-amplified (HER2-) breast cancer. HR, hazard ratio. IM, intramuscular administration. IV, intravenous administration. MTD, maximum tolerated dose. N, number of patients. NA, not available. OD, once daily. OS, overall survival. p, p value of two-sided statistical test. PFS, progression-free survival. PO, oral administration. PR, partial response/remission. RB+, RB protein expressing tumours. RP2D, recommended phase II dose. RR, overall response rate (complete + partial responses). SD, stable disease. vs, versus.

Table 3

Clinical trial results of selected inhibitors of other cell cycle proteins

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)?
MK-8776 *(SCH 900776)*				
Acute leukaemias (relapsed or refractory)[186]	• Phase I • N=24 (AML: N=21) • NCT00907517	• 10–80 mg/m^2 IV OD (days 2, 3, 11 and 12), RP2D: 56 mg/m^2 • Combination with cytarabine (2 g/m^2 IV over 72 hours, days 1–3 and 10–12)	• CR/CRi: 33% (8/24)	• Hepatic dysfunction (17%)
LY2606368 *(prexasertib)*				
Anal squamous cell carcinoma (metastatic)[189]	• Phase I • N=26 (subgroup expansion) • NCT01115790	• 105 mg/m^2 IV every 14 days • Monotherapy	• CR: 4% (1/26) • PR: 12% (3/26) • SD: 42% (11/26)	• Neutropaenia (grade 4: 77%)
AZD1775 *(MK-1775)*				
Ovarian cancer (refractory or resistant, p53 mutant)[203]	• Phase II • N=24 • NCT01164995	• 225 mg PO BD (for 2.5 days in a 21-day cycle) • Combination with carboplatin (IV, day 1)	• PR: 27% (6/22) • SD: 41% (9/22)	• NA
Ovarian cancer (platinum-sensitive, p53-mutant)[204]	• Phase II • N=121 • AZD1775 + "P/C" (N=59) vs placebo + "P/C" (N=62) • NCT01357161	• 225 mg PO BD (for 2.5 days in a 21-day cycle) • Combination with "P/C": paclitaxel (175 mg/m^2 IV, day 1) and carboplatin (IV, day 1)	• Median PFS: 42 weeks vs 35 weeks (HR=0.55, p=0.03) • RR: 81% vs 76% (p=0.459)	• Various (overall: 78% vs 65%)
Rigosertib *(ON 01910.Na)*				
MDS (primary HMA failure)[209]	• Phase III • N=169 (subgroup) • Rigosertib (N=117) vs best supportive care (N=52)	• 1800 mg IV OD (for 3 days; every 2 weeks for 16 weeks, then every 4 weeks)	• Median OS: 8.6 months vs 4.5 months (HR=0.63, p=0.011)	• Anaemia (16% vs 10%), thrombocytopaenia (15% vs 6%), neutropaenia (15% vs 8%), febrile neutropaenia (13% vs 10%), pneumonia (12% vs 12%)

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)?
	• NCT01241500 (ONTIME)			
MDS (very high risk)[210]	• Phase III • N=134 (subgroup) • Rigosertib (N=93) vs best supportive care (N=41) • NCT01241500 (ONTIME)	• 1800 mg IV OD (for 3 days; every 2 weeks for 16 weeks, then every 4 weeks)	• Median OS: 7.6 months vs 3.2 months (HR=0.56, p=0.005)	• Anaemia (24% vs 11%), thrombocytopaenia (21% vs 11%), febrile neutropaenia (17% vs 11%), neutropaenia (15% vs 13%), pneumonia (12% vs 13%)
Pancreatic cancer (metastatic, first-line treatment)[308]	• Phase II/III • N=160 (subgroup) • Rigosertib + gemcitabine (N=106) vs gemcitabine alone (N=54) • NCT01360853 (ONTRAC)	• 1800 mg/m^2 IV twice per week (3 weeks on, 1 week off) • Combination with gemcitabine (1000 mg/m^2 weekly, 3 weeks on, 1 week off)	• Median PFS: 3.4 months vs 3.4 months (HR=0.96) • Median OS: 6.1 months vs 6.4 months (HR=1.24) • PR: 19% vs 13% • SD: 50% vs 56%	• Hyponatremia (17% vs 4%)
Volasertib *(BI 6727)*				
AML (patients ineligible for intensive treatment)[217]	• Phase II • N=87 • Volasertib + cytarabine (N=42) versus cytarabine alone (N=45) • NCT00804856	• 350 mg IV OD (on days 1 and 15 of 28-day cycle) • Combination with cytarabine (20 mg s.c. BD, days 1–10)	• Median EFS: 5.6 months vs 2.3 months (HR=0.57, p=0.021) • Median OS: 8.0 months vs 5.2 months (HR=0.63, p=0.047) • CR/CRi: 31% vs 13%	• Febrile neutropaenia (55% vs 16%), infections (48% vs 22%), gastrointestinal (24% vs 7%)
Alisertib *(MLN8237)*				
Peripheral T-cell lymphoma and transformed	• Phase II • N=37 • NCT01466881	• 50 mg PO BD (1 week on, 2 weeks off) • Monotherapy	Peripheral T-cell lymphoma: • CR: 7% (2/30) • PR: 23% (9/30) • SD: 17% (5/30) Transformed Mycosis Fungoides: • RR: 0% (0/7) • SD: 28% (0/7)	• Neutropaenia (32%), anaemia (30%), thrombocytopaenia (24%), lymphopaenia (22%), febrile neutropaenia (14%)
B-cell and T-cell non-Hodgkin lymphoma (relapsed or refractory)[243]	• Phase II • N=48 • NCT00807495	• 50 mg PO BD (1 week on, 2 weeks off) • Monotherapy	• CR: 10% (5/48) • PR: 17% (8/48) • SD: 33% (16/48)	• Neutropaenia (63%), anaemia (35%), thrombocytopaenia (33%), stomatitis (15%), febrile neutropaenia (13%)
Ovarian, fallopian tube, primary	• Phase I	• 10–50 mg PO BD (days	• PR: 29% (8/28)	• Neutropaenia (54%)

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)?
peritoneal and breast cancer (recurrent)[239, 309]	• N=28 (ovarian: N=20) • NCT01091428	1–3, 8–10, 15–17 in 28-day cycle), RP2D: 40 mg • Combination with paclitaxel (60–80 mg/m^2 IV BD, days 1+8+15, RP2D: 60 mg/m2)	• SD: 11% (3/28)	
Breast, small-cell lung, non-small-cell lung, head and neck squamous cell, gastro-oesophageal cancer (advanced, relapsed or refractory)[240]	• Phase II • N=249 • NCT01045421	• 50 mg PO BD (1 week on, 2 weeks off) • Monotherapy	Breast cancer: • PR: 18% (9/49) • SD: 51% (25/49) Small-cell lung cancer: • PR: 21% (10/48) • SD: 33% (16/48)	Breast cancer: • Neutropaenia (57%), stomatitis (15%), fatigue (11%) Small-cell lung cancer: • Neutropaenia (37%), anaemia (17%), thrombocytopaenia (10%)
Solid tumours (advanced) including prostate cancer (castration-resistant)[241]	• Phase I • N=35 • NCT01094288	• 10–50 mg PO BD (1 week on, 2 weeks off); RP2D: 20 mg • Combination with docetaxel (60–75 mg/m^2 IV OD, on day 1, RP2D: 75 mg/m^2)	For castration-resistant prostate cancer: • PR: 35% (6/17) • SD: 35% (6/17)	• Neutropaenia (86%), febrile neutropaenia (23%), stomatitis (14%)
Multiple myeloma[242]	• Phase Ib • N=26 • NCT01034553	• 20–50 mg PO BD (1 week on, 3 weeks off) • Combination with bortezomib (1.5 mg/m^2 IV weekly)	• CR: 4% (1/26) • PR: 23% (6/26) • SD: 38% (10/26) • Median PFS: 5.9 months • Median OS: 23.6 months	• Neutropaenia (38%), thrombocytopaenia (31%), lymphopaenia (19%), infection (15%), muscle weakness (12%)
Neuroblastoma (relapsed or refractory)[310]	• Phase I • N=22 • NCT01601535	• 45–80 mg/m^2 PO OD (days 1–7 in 21-day cycle) • Combination with irinotecan (50 mg/m^2 IV OD, on days 1–5) and temozolimide (100 mg/m^2	• CR: 23% (5/22) • PR: 9% (2/22) • SD: 50% (11/22)	• NA

Tumour type	Study characteristics, ClinicalTrials.gov Identifier	Drug dosage and combination	Efficacy	Major grade 3/4 adverse effects (≥10%)?
		PO OD, on days 1–5)		
ENMD-2076				
Ovarian cancer (recurrent, platinum-resistant)[311]	• Phase II • N=64 • NCT01104675	• 250–325 mg PO OD (continuous) • Monotherapy	• PR: 8% (5/64) • SD: 50% (32/64) • PFS at 6 months: 22% • Median OS: ≈12 months	• Hypertension (27%), fatigue (19%)
Soft tissue sarcoma (advanced)[312]	• Phase II • N=10 • NCT01719744	• 275 mg PO OD (continuous) • Monotherapy	• PR: 20% (2/10) • SD ≥ 6 months: 10% (1/10) • Median PFS: 1.8 months	• Hypertension (20%), elevated transaminases (10%), leukopaenia (10%), diarrhoea (10%)

AML, acute myelogenous leukaemia. BD, twice daily. CR, complete response/remission. CRi, complete remission with incomplete recovery. EFS, event-free survival. HMA, hypomethylating agents (azacitidine or decitabine). HR, hazard ratio. IV, intravenous administration. MDS, myelodysplastic syndromes. N, number of patients. NA, not available. OD, once daily. OS, overall survival. p, p value of two-sided statistical test. PFS, progression-free survival. PO, oral administration. PR, partial response/remission. RP2D, recommended phase II dose. RR, overall response rate (complete + partial responses). s.c., subcutaneously. SD, stable disease. vs, versus.