

# *Potential Clinical Uses of CDK Inhibitors: Lessons from Synthetic Lethality Screens*

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**Abstract:** Developments in genetic and genomic technology have produced vast quantities of data that are gradually yielding new insights into fundamental cellular and molecular processes. In particular, they have revealed some differences between normal and transformed cells that could potentially be exploited to develop targeted, personalized cancer therapies with unprecedented efficiencies. This review summarizes recent findings from synthetic lethality (SL) screens against cyclin-dependent kinases (CDKs) that can be targeted with small molecule kinase inhibitors. SL screens can be used to identify cancers sensitive to CDK inhibitors. Several SL partners of specific CDKs have been identified, including MYC, K-Ras, VHL, PI3K, and PARP, all of which are discussed in the review. CDK inhibitors have been in clinical trials for nearly 20 years and it has become clear that effective therapy using these compounds will require careful selection of patients with respect to the specific molecular phenotype of their disease. © 2015

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## **1. SYNTHETIC LETHALITY AND THE EVOLUTION OF TARGETED THERAPY**

Anticancer chemotherapeutics developed over the past century have saved many lives. However, there are still some bottlenecks that hinder their use in vivo. One of the main problems is their low therapeutic index, that is, the narrow concentration range within which they kill cancers without harming healthy tissues.<sup>1</sup> Most clinically used anticancer drugs kill rapidly growing cells nonspecifically, with the result that they target not only cancer cells but also certain healthy dividing cells such as hematopoietic bone marrow progenitor cells, hair follicle cells, and gastrointestinal mucosal epithelial cells.<sup>2</sup> Many normal nondividing cells are also sensitive to

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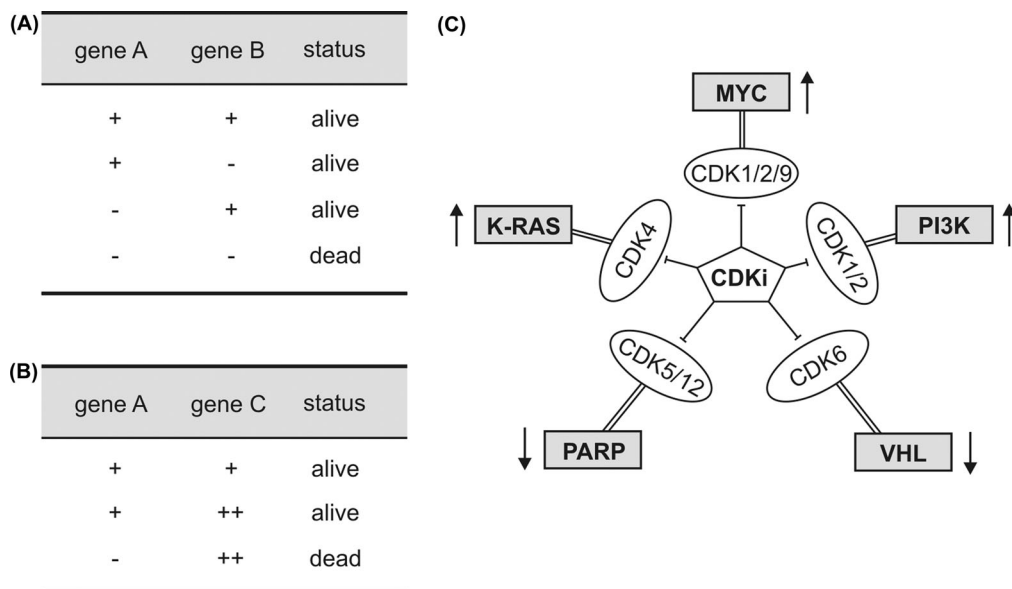
classical chemotherapeutics.<sup>3–5</sup> Consequently, there is an urgent need for a better understanding of the differences between normal and cancer cells. Such an understanding would enable the identification of targets that are only essential for the viability of tumor cells, leading to the development of new drugs targeting cancers as specifically as possible.

Tumorigenesis is a complex multistep process that often takes many years, during which cells acquire a set of genetic lesions that ultimately yield a cancerous state. It has been stated that cancers are often self-sufficient in the production of growth factors, less sensitive to growth-inhibitory signals and induction of apoptosis, unlimited in their replicative potential, and capable of inducing both angiogenesis and metastases.<sup>6</sup> These properties are typically conferred by loss of function mutations in tumor suppressor genes and gain of function mutations in oncogenes that are collectively critically important for cancer development. While they are essential for the induction of the cancerous state, these attributes can also be regarded as weaknesses that could be exploited therapeutically because they define the ways in which transformed cells differ from their normal counterparts.

A pivotal milestone in the development of molecular anticancer therapeutics was the discovery of imatinib mesylate, a potent kinase inhibitor targeting a protein encoded by a fused breakpoint cluster region (BCR)-Abelson murine leukemia viral oncogene homologue (ABL). This oncogene is activated by a translocation between chromosomes 9 and 22 in hematopoietic stem cells, and induces chronic myeloid leukemia (CML).<sup>7</sup> Imatinib proved to be strikingly effective in the treatment of CML patients and was approved for this purpose in 2001. Since then, several small molecule and monoclonal antibody inhibitors of oncogenic enzymes have been approved for therapeutic use, and many others are in various stages of clinical development.<sup>8,9</sup> While most of these agents have acceptable therapeutic indices, it is well known that they target both oncoproteins present in cancer cells and other proteins found in normal cells. Although several of the newer agents exhibit improved selectivity toward cancers, many fail in cancer treatment due to the emergence of distinct patterns of resistance based on general multidrug resistance, metabolism, compartmentalization or target-specific mutations such as point mutations, and mutations that induce oncogene overexpression. It therefore seems necessary to identify new strategies in order to overcome issues of drug resistance and find ways of targeting cells with loss of function mutations.

Over the past few years, intensive studies on cancer genetics have yielded new insights into gene–gene interactions. As a result, conventional strategies that target oncogenic pathways regardless of their impact on normal cell signaling have been outperformed by new alternatives discovered as a result of the ongoing development of genomic technologies. A very promising anticancer drug discovery method that was first described in 1922 is the so-called synthetic lethal (SL) approach.<sup>2</sup> SL is a genetic property whereby the presence of one gene allows an organism to tolerate genetic changes in a second gene that would be lethal in the absence of the first (Fig. 1A).<sup>10</sup> In some cases, simultaneous mutations in both genes may only reduce cellular fitness, resulting in a condition known as synthetic sickness. Strategies based on synthetic sickness and lethality could potentially solve a major problem of anticancer drug development by permitting the specific targeting of cancer cells with loss of function mutations in tumor suppressor genes. The products of a pair of synthetic lethal genes may be components of the same multiprotein complex, participate in parallel redundant pathways, belong to the same linear pathway, or even act in two separate pathways essential for cell viability.

Many synthetic lethal interactions have been mapped out in a range of model organisms using RNA interference. However, the field as a whole is rooted in studies on the budding yeast *Saccharomyces cerevisiae*. In 1999, a yeast knockout library was generated in which each open reading frame was replaced with a genetic marker and tagged with two specific molecular TAGs or barcodes (upstream and downstream)—20-base oligomer sequences that serve as



**Figure 1.** (A) Synthetic lethality is a genetic property whereby the presence of gene A allows a cell to tolerate mutation in gene B that would otherwise be lethal (and vice versa). (B) In an alternative version of synthetic lethality, gene A may interact with the third gene C such that increasing the expression or activity of C would be synthetically lethal when paired with the downregulation of gene A. (C) Schematic depiction of known SL interactions involving CDKs. Arrows pointing downward and upward indicate decreased and increased gene activity, respectively.

strain identifiers.<sup>11</sup> Synthetic lethal or sick interactions were then identified by crossing strains carrying mutations of interest with the array of deletion mutants after mating and sporulation. The desired interactions were readily identified because they produce haploid double mutants. However, another technique was required to quantify the double mutations effects on growth rates. To this end, the molecular barcodes in each mutant strain were flanked by universal priming sites, enabling the amplification of the tag sequences by PCR. The amplified products were hybridized to oligonucleotide arrays so that the intensity of the amplified signal could be determined.<sup>12</sup> However, many cancer-related genes do not have yeast orthologs, so it was also necessary to conduct similar studies using alternative metazoan models. The most widely used organisms for this purpose were *Caenorhabditis elegans* and *Drosophila melanogaster*, both of which enabled the use of more sophisticated RNA interference strategies than were previously possible. Unfortunately, the siRNA sequences used to induce interference in simpler model organisms elicit antiviral responses in mammalian cells, so they were replaced with shRNA encoded by plasmids or viral vectors.<sup>13,14</sup> This improved RNAi system has become a staple of novel screening strategies that enable the systematic identification of gene–gene interactions in human cells. There are two main approaches used by geneticists to map out SL relationships, referred to as the forward and reverse approaches. The forward tactic relies on the genetic variability of cancer cell lines characterized by a common mutation in a gene of interest, while the reverse strategy uses an isogenic cell line pair created by a single specific genetic change.

These approaches led to the identification of several new synthetic lethal interactions. For example, many cancers are characterized by oncogenic mutations in Ras, which is difficult to inhibit directly with small molecule inhibitors and was therefore targeted for synthetic lethal screening. It was demonstrated that cells expressing mutant K-Ras are highly dependent on the expression of TANK-binding kinase 1 (TBK1), mitotic polo-like kinase 1 (PLK1), and the

transcriptional repressor SNAIL2.<sup>15–17</sup> In a similar way it was shown that deficiencies of the tumor suppressor Rb, which are often responsible for malignant conversion, can be overcome by inactivating tuberous sclerosis complex 2 (TSC2); that overexpression of the serine/threonine-protein kinase PIM1 in prostate cancer cells can be overcome by PLK1 inhibition; and that p53 deprivation can be relieved by silencing telomerase reverse transcriptase (TERT).<sup>18–20</sup>

While novel screening strategies have revealed many new SL interactions, the limited overlap between the results obtained in different SL screening campaigns targeting the same gene indicates that there are important weaknesses in existing methodologies. The identification of three different SL partner proteins in three separate K-Ras screens is a case in point, and suggests that the genetic backgrounds of the tested cells can have significant effects on the observed lethality. The limited overlap between the results of different SL screens targeting the same protein may be due to the use of different cell lines in different studies, imperfections in RNAi-mediated gene knockdown, or off-target effects. Regardless of its causes, this variability makes it essential to thoroughly validate newly identified SL interactions using multiple independent models in order to provide a sound basis for rational patient–drug pair selection.

Except of experimental studies, many predictive approaches have been proposed, but these primarily focus on extending experimentally derived SL networks rather than de novo prediction of interactions, limiting their utility for cancer.<sup>21</sup> However, a new computational algorithm DAISY (data mining synthetic lethality identification pipeline) that aims to facilitate the large-scale identification of SLs in cancer has been described recently.<sup>22</sup> Importantly, cancer SL networks identified by DAISY included known SL partners of tumor suppressors and oncogenes. In addition, it has been shown to be useful in predicting gene essentiality, drug efficacy, and clinical prognosis.

It is not yet clear whether discovered SL interactions will ever translate into efficient therapeutics. However, the basic concept of genetic screens for SL interactions has already been applied in drug discovery screening studies. In these investigations, an inhibitor of a given protein is screened against a panel of viable cells bearing mutations in different genes to identify cases where the combination of the mutation and inhibitor results in cell death but neither is fatal by itself.<sup>23</sup> This approach has revealed SL effects of many compounds in specific cancer cell lines. At present, the most important clinical application of an SL relationship is probably the use of synthetic poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors such as olaparib or iniparib to treat breast cancers featuring deletions of BRCA-1 or BRCA-2.<sup>24</sup> BRCA-1 and 2 are necessary for the repair of DNA double-strand breaks (DSB) by homologous recombination (HR). PARP-1 is implicated in the repair of single-strand breaks (SSB) via autopoly(ADP-ribosylation), in which it serves as a docking site for other proteins involved in the repair process. PARP-1 inhibition prevents the docking of these proteins, leading to the formation of multiple SSB; these SSB in turn give rise to DSB at replication forks. The DSBs would normally be repaired by HR, but this is not possible in the absence of BRCA-1 or BRCA-2 so the DNA lesions are instead repaired by nonhomologous end joining (NHEJ), leading to extensive chromosomal alterations and cell death.

The identification of cyclin-dependent kinases (CDKs) as cell cycle regulators prompted the development of several small molecule CDK inhibitors, many of which have shown promising results in the context of anticancer therapy and could be potentially exploitable in combinatorial experiments as discussed in the next section.

## **2. DEREGLATION OF CDKS IS A FREQUENT HALLMARK OF CANCER DISEASES**

The CDKs are a family of 20 serine/threonine protein kinases that are generally classified as regulators of the cell cycle (CDK1, 2, 4, 6) or transcription (CDK7, 8, 9, 11, 20). However, in the

last few years they have been shown to have diverse functions including the regulation of angiogenesis, senescence, exocytosis, spermatogenesis, and neuronal development.<sup>25</sup> CDK activity is highly dependent on the binding of regulatory subunits called cyclins, whose name derives from their oscillatory expression: they are produced and degraded during different phases of the cell cycle. To be fully activated, most CDKs must be phosphorylated by CDK7 at specific residues in their so-called T-loops.<sup>26</sup> The timing of CDK activity is also subject to negative regulation mediated by the binding of natural CDK inhibitors (INK4, Cip/Kip), and by inhibitory phosphorylation catalyzed by the Wee1 and Myt1 kinases.<sup>26,27</sup> These phosphorylations can be reversed by the cdc25 phosphatases.

The uncontrolled upregulation of CDK activity has been identified as a hallmark of cancer and several CDK hyperactivity-inducing mechanisms have been identified. Many of these mechanisms involve loss of function mutations (deletions, silencing, or point mutations) affecting genes encoding natural CDK inhibitors or the overexpression of CDK-activating cyclins. For example, excessive production of cyclin D1 has been detected in breast, bladder, esophageal, and squamous cell carcinoma.<sup>28</sup> Similarly, overproduction of cyclin E has been detected in colon, lung, and breast cancers as well as acute lymphoblastic and myeloid leukemias,<sup>29–33</sup> and cyclin A overproduction has been observed in lung carcinoma.<sup>30</sup> In addition, some breast malignancies are promoted by shortened hyperactive forms of cyclin E that are generated by proteolysis.<sup>34</sup> However, in some cases, especially those involving CDK4 and 6, hyperactivity is caused by the amplification or overexpression of the CDK gene itself.<sup>35–37</sup> Alternatively, mutations in CDK genes may affect the corresponding proteins' sensitivity to negative regulators. For example, in melanoma the R24C point mutation in CDK4 was found to cause insensitivity to inhibition by p16INK4a without affecting the variant protein's ability to bind cyclin D and form an active kinase.<sup>38</sup> Finally, CDK activation requires the removal of inhibitory phosphates by Cdc25 phosphatases, which are present at unusually high levels in certain tumors.<sup>39,40</sup> For these reasons, CDKs and their natural modulators have become important targets for anticancer drug development in recent years. Most efforts in this area have focused on small molecule inhibitors.

Over the past 20 years, many CDK inhibitors have been developed using different approaches, and around 24 have entered clinical trials (Table I, Fig. 2).<sup>41–43</sup> Most CDK inhibitors are pan-selective and block the transcriptional regulators CDK7 and CDK9 in addition to the cell cycle regulating CDKs. It was demonstrated that these compounds induce cell cycle arrest and activate apoptosis by inhibiting transcription, which is most effective in cells that are strongly dependent on the expression of antiapoptotic proteins with short half-lives such as myeloid cell leukemia 1 (Mcl-1). Many groups have demonstrated that early inhibitors such as roscovitine and flavopiridol are effective against multiple myeloma and other malignancies that depend on continuous mRNA synthesis and Mcl-1 expression.<sup>44–46</sup> Inhibitors of the transcriptional CDKs also influence the stabilization of the tumor suppressor p53, probably by downregulating its target genes; these include the ubiquitin ligase Mdm2, which negatively regulates p53.<sup>47–49</sup> On the basis of various in vitro studies, it has been suggested that the simultaneous inhibition of multiple CDKs (i.e., CDK1, 2, and 9) could be a desirable feature of clinical drug candidates.<sup>50</sup> The justification for targeting multiple CDKs at once comes from studies on genetic models;<sup>51</sup> cells lacking one or more interphase CDKs can proliferate because most CDKs are redundant and capable of standing in for one another if one is disabled somehow. The only CDK whose functions cannot be fulfilled by some other member of the CDK family is CDK1.<sup>51,52</sup>

Although the simultaneous inhibition of several CDKs may be more efficient than selectively blocking a single CDK in many cases, there has been considerable interest in developing inhibitors specific to individual CDK isoforms over the last few years.<sup>42,53</sup> It was recently shown that many cancers are heavily dependent on the activity of a single CDK—breast cancer on

**Table 1.** Some Small Molecular CDK Inhibitors in Clinical Trials

Compound (alternative name)	Target	Trial phase	Condition	References
AT7519	CDK1, CDK2, CDK4, CDK6, CDK9	I/II	Lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma	90
BAY 1000394 (roniciclib)	CDK1, CDK2, CDK3, CDK4, CDK7, CDK9	I/II	Neoplasms, small cell lung carcinoma	91
Flavopiridol (alvociclib, HMR 1275, L86-8275)	CDK1, CDK2, CDK4, CDK6, CDK7, CDK9	I/II	Hematologic malignancies	92,93
LEE011 (ribociclib)	CDK4, CDK6	I/II	Solid tumors, lymphomas, malignant rhabdoid tumors, neuroblastoma, melanoma, breast cancer	www.cancer.gov
LY2835219 (abemaciclib)	CDK4, CDK6	I/II	Advanced cancer, mantle cell lymphoma, lymphoma, breast neoplasms, non-small cell lung cancer	www.cancer.gov
P1446A-05	CDK4	I	Solid tumors, hematologic malignancy	www.cancer.gov
P276-00 (riviciclib)	CDK1, CDK4, CDK9	I/II	Multiple myeloma, mantle cell lymphoma	94
PD-0332991 (palbociclib)	CDK4, CDK6	I/II/III	Solid tumors, hematologic malignancies	95
PHA-848125 (miliciclib)	CDK2, CDK4, CDK7	I/II	Thymic carcinoma, solid tumors	96
Roscovitine (seliciclib, CYC202)	CDK1, CDK2, CDK5, CDK7, CDK9	I/II	Breast cancer, advanced solid tumors, non-small cell lung carcinoma	97,98
SCH-727965 (dinaciclib)	CDK1, CDK2, CDK5, CDK9	I/II/III	Hematologic malignancies, solid tumors	99
SNS-032 (BMS-387032)	CDK2, CDK7, CDK9	I	Hematologic malignancies, tumors	100
R-547	CDK1, CDK2, CDK4	I	Neoplasms	101
TG02 (SB1317)	CDK1, CDK2, CDK7, CDK9	I	Hematologic malignancies	102

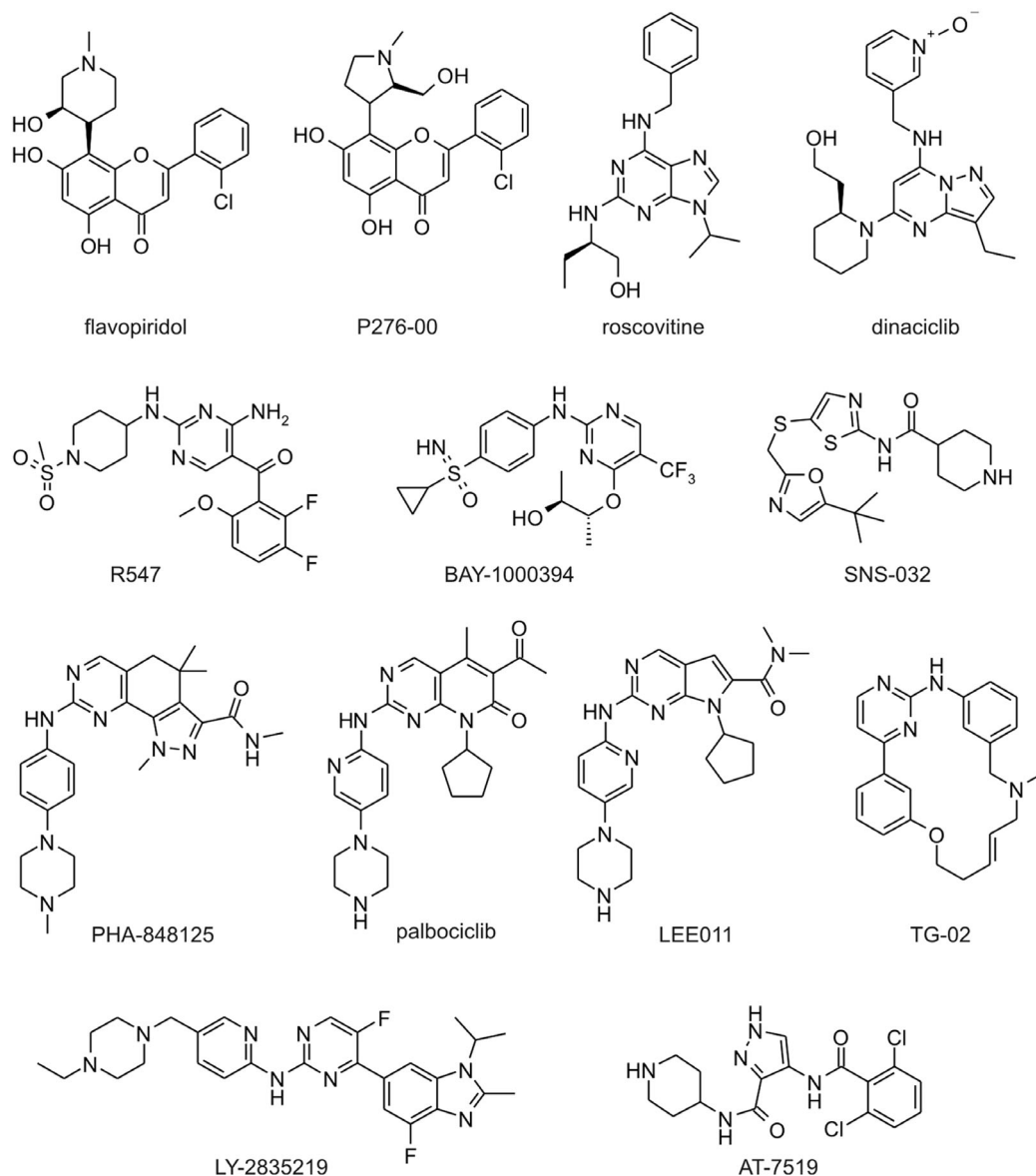


Figure 2. Chemical structures of some CDK inhibitors in clinical development.

CDK4, pancreatic cancer on CDK5, and bladder cancer on CDK6, for instance.<sup>54–56</sup> Several specific inhibitors have been designed, often with the assistance of molecular modeling. These agents include BS-181 and EXEL-8647, which target CDK7 and CDK9, respectively,<sup>57,58</sup> as well as three compounds targeting CDK4/6 that are currently undergoing clinical trials: LEE011, LY2835219, and palbociclib (granted accelerated approval by FDA in 2015).<sup>59–61</sup>

While there are currently many CDK inhibitors in clinical trials, several problems with their therapeutic use remain to be addressed. In particular, it is not straightforward to determine which patients are likely to be most sensitive to specific therapies and many current compounds have low therapeutic indices while exhibiting strong general cytotoxicity. These problems could potentially be avoided by exploiting SL.

### 3. CDK INHIBITORS CAN ENHANCE THE EFFECTIVENESS OF CURRENT CHEMOTHERAPEUTICS

Although classical chemotherapeutics continue to dominate the clinical treatment of cancer, their limited efficacy, side effects, and susceptibility to drug resistance collectively complicate their use. Most of these compounds inhibit the functioning of the mitotic spindle, block DNA synthesis, or induce DNA damage. All of these processes lead to the activation of checkpoints followed by cell cycle arrest, during which the damage they cause may be repaired and drug resistance may be induced. Combination therapies are generally believed to avoid these problems, and in recent years a number of studies have demonstrated that combination therapies involving CDK inhibitors can have remarkable effects. Several studies examining different drug combinations have revealed synergistic effects that can be enhanced by precisely controlling the sequence and schedule on which the various agents are administered.

Flavopiridol was the first pan-selective CDK inhibitor and the most extensively studied. It has been tested in combination with diverse classical chemotherapeutic agents, resulting in the identification of some combination therapies that are currently undergoing clinical trials. For instance, it was shown to enhance the anticancer effect of paclitaxel, a microtubule-interfering agent that inhibits mitosis.<sup>62</sup> Paclitaxel monotherapy induced a transient increase in cyclin B1 expression and CDK1 activation followed by mitotic exit without cytokinesis. Subsequent inhibition of CDK1 using flavopiridol accelerated mitotic exit, activated caspase-3, and induced PARP cleavage. Interestingly, the two drugs had antagonistic effects if the order of treatment was reversed by applying flavopiridol before paclitaxel because flavopiridol pretreatment prevented mitotic entry. This finding demonstrates the importance of applying combination therapies in the correct sequence.<sup>62</sup> Similar results were obtained when using flavopiridol in combination with docetaxel, a semisynthetic paclitaxel analogue.<sup>63</sup> In another example, SN-38, an active metabolite of the DNA topoisomerase I inhibitor CPT-11, induced p21 expression and G2 arrest in the HCT-116 gastric cancer cell line without activating apoptosis; this failure of apoptotic induction could be overcome by subsequent treatment with flavopiridol,<sup>64</sup> which caused the activation of caspase-3 and the cleavage of p21 and X-linked inhibitor of apoptosis (XIAP). A third example is the combination of flavopiridol with gemcitabine, a ribonucleotide reductase inhibitor. In several cases, gemcitabine monotherapy has led to resistance due to up-regulation of the mRNA and protein expression of the ribonucleotide reductase M2 subunit.<sup>65</sup> Flavopiridol treatment suppressed this resistance by downregulating the expression of the transcription factor E2F-1 in gemcitabine-treated cells, causing a reduction in the expression of the ribonucleotide reductase M2 subunit.<sup>65</sup>

Based on this body of evidence, clinical trials on a range of combination therapies involving various CDK inhibitors have been initiated (see Supporting Information Table I). The extensively investigated CDK inhibitor flavopiridol has been the subject of several such studies, usually in combination with DNA targeting agents with which it exhibited strong synergistic effects in preclinical settings. Given the known involvement of certain CDKs in DNA damage repair processes,<sup>66</sup> it is tempting to speculate that these synergies could be due to cell death arising from the blockage of DNA repair. There have also been several clinical trials involving the use of microtubule-interfering agents in conjunction with CDK inhibitors because such combinations have yielded promising results in animal models.<sup>62,67</sup>

In addition to classical chemotherapeutics, CDK inhibitors are being tested as components of therapeutic cocktails featuring more recently developed molecularly targeted drugs (including biologics) for which clear mutual potentiation has been observed *in vitro* or *in vivo*. The striking results obtained in these models support the hypothesis that simultaneously blocking multiple signaling pathways may confer superior clinical efficacy. Therefore, various combinations of CDK inhibitors with inhibitors of mitogen transducing kinases (both receptor and cytoplasmic

kinases), proteasome inhibitors, or antiestrogens have been designed for clinical evaluation (see Supporting Information Table I).

The examples mentioned above clearly show that CDK inhibitors can potentiate the activity of current chemotherapeutic agents. However, more effective anticancer strategies could potentially be developed by specifically targeting individual cancer-related genes in order to exploit SL interactions in patients whose genetic background is known.

#### **4. SYNTHETIC LETHALITY OF CDK INHIBITORS IN THE TREATMENT OF SPECIFIC TUMOR DISEASES**

##### **A. CDK1/2 and Phosphatidylinositol-3'-Kinase (PI3K)**

Glioblastoma multiforme is the most common and aggressive astrocytoma, and has poor prognostic outcomes despite the availability of several multimodal therapies. Almost half of all astrocytomas are characterized by an amplification of the epidermal growth factor receptor (EGFR), which subsequently overactivates PI3K leading to a deregulation of the protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway.<sup>68</sup> The malignant conversion is also influenced by gain of function mutations in PI3K $\alpha$  and loss of function mutations in tumor suppressor phosphatase and tensin homolog (PTEN), which negatively regulates PI3K activity.<sup>69</sup> Early efforts to develop targeted glioblastoma treatments largely focused on small molecule inhibitors of EGFR, PI3K, or mTOR. This approach yielded disappointing results, inducing cytostatic effects rather than cell death. However, the screening of inhibitors of PI3K isoforms led to the identification of the imidazopyridine PI-75, which effectively induced apoptosis in glioma cell lines expressing wild-type PTEN without affecting PTEN mutant cell lines.<sup>70</sup> Treatment of a wild-type PTEN cell line with the PTEN inhibitor bisperoxovanadium in combination with PI-75 caused increased phosphorylation of Akt and attenuated cell death without affecting G2/M arrest. Surprisingly, computational studies indicated that PI-75 is also a strong inhibitor of CDK1 and 2.<sup>70</sup> While the inhibition of single CDKs (CDK1 or CDK2) or CDK1 and PI3K $\alpha$  had no impact on apoptosis in glioma cells expressing wild-type PTEN, combined CDK2 and PI3K $\alpha$  inhibition increased cell death, albeit to a lesser extent than was observed following PI-75 treatment. This finding was confirmed by siRNA experiments, in which the silencing of CDK1 or 2 alone after treatment with a PI3K $\alpha$  inhibitor did not influence apoptosis in glioma cells expressing wild-type PTEN. This may indicate that CDK1 can compensate for the absence of CDK2 and vice versa.<sup>71</sup> However, the simultaneous silencing of both CDKs significantly reduced the viability of cells treated with the PI3K $\alpha$  inhibitor. In keeping with this finding, overexpression of CDK1 and 2 attenuated apoptosis in glioma cells expressing wild-type PTEN. Similarly, a combination of the CDK1/2 inhibitor roscovitine with a PI3K $\alpha$  inhibitor reduced tumor size in mice xenografts more effectively than monotherapy with either agent alone. All of these results suggest that it should be possible to use combination therapies based on CDK1/2 and PI3K inhibitors to treat patients with gliomas expressing wild-type PTEN.

##### **B. CDK1/2/9 and MYC**

Neuroblastomas are embryonal tumors that arise from the sympathetic nervous system and are the second most common cause of cancer-related deaths in children.<sup>72</sup> They are associated with a range of molecular changes including MYCN amplification, which is found in 20–30% of all neuroblastomas and is linked to advanced disease with bad prognosis.<sup>73</sup> As a ligand-independent transcription factor, MYCN is very challenging to drug. Interestingly, CDK2 was shown to have a strong effect on the viability of MYCN-amplified neuroblastomas: its silencing

using siRNA or shRNA induced apoptosis in MYCN-amplified neuroblastoma cell lines.<sup>74</sup> However, the simultaneous silencing of MYCN and CDK2 had no impact on cell viability, suggesting that these two proteins have an SL relationship. Subsequent experiments demonstrated that both roscovitine and the related compound CR8 are potent inducers of apoptosis in MYCN-positive cells but have no effects in MYCN-negative neuroblastoma lines.<sup>74,75</sup>

Various other cancers overexpress the closely related *MYC* oncogene, which encodes a transcription factor that regulates the expression of genes controlling cell growth, division, and apoptosis.<sup>76</sup> Using a panel of fibroblast human cell lines expressing nine common oncogenes, it was shown that MYC-overexpressing cells were highly sensitive to the induction of apoptosis by purvalanol A (a CDK inhibitor related to roscovitine).<sup>77</sup> Importantly, this sensitivity correlated well with the strength of the cells' expression of MYC. Artificially induced Bcl-2 overexpression prevented cell death in both normal and MYC-overexpressing cells treated with purvalanol A, demonstrating that the apoptosis observed in drug-treated cells was due to mitochondrial depolarization. This effect was attributed to a drug-induced destabilization of survivin, an inhibitor of apoptosis whose activity depends on phosphorylation by CDK1.<sup>78</sup> The anticancer efficacy of purvalanol A was also confirmed in mouse models of lymphoma and hepatoblastoma, further supporting the proposed interaction between CDK1 and MYC.<sup>77</sup>

The SL interaction between MYC and CDK1 could potentially be exploited in the treatment of triple-negative breast cancer, which is resistant to drugs targeting the HER2, estrogen, and progesterone receptors.<sup>79,80</sup> CDK1 silencing using siRNA decreased the viability of triple-negative breast cancer cell lines and suppressed tumor growth in mice xenografts.<sup>79</sup> Two small molecule CDK inhibitors, purvalanol A and dinaciclib, induced significant apoptosis in several triple-negative cell lines with elevated MYC expression as well as in related mouse xenograft models.<sup>80</sup> CDK1 is not the only CDK that has a synthetic lethal relationship with MYC: studies on hepatocellular carcinomas revealed that CDK9 was required for their survival and its pharmacological or shRNA-mediated inhibition caused robust antitumor effects whose magnitude correlated with MYC expression levels.<sup>81</sup>

It can be difficult to unravel synthetic lethal relationships involving CDKs because of the broad specificity patterns of established CDK inhibitors and because these proteins exhibit pronounced functional redundancy such that one CDK can often compensate for deficiencies in the activity of another. However, a remarkable study in which CDK4, CDK2, and CDK1 were inhibited specifically and separately using either RNAi or small molecule inhibitors showed that only CDK1 inhibition rapidly decreased the viability of MYC-dependent cells.<sup>82</sup> The suggested mechanism of SL between CDK1 and MYC is based on the induction of mitotic catastrophe by CDK1 depletion, which may promote MYC-induced replication stress and subsequently activate checkpoint signaling, resulting in cell death.

### C. *CDK6 and VHL*

The inactivation of the Von Hippel Lindau (*VHL*) tumor suppressor gene, which serves as a regulator of hypoxia-inducible factor  $\alpha$  (HIF- $\alpha$ ), is a frequent hallmark of clear cell renal carcinomas (RCC).<sup>83</sup> In the presence of oxygen (normoxia), HIF- $\alpha$  becomes hydroxylated at one or two prolyl residues to form a binding site for VHL, a component of the ubiquitin ligase complex that directs the polyubiquitinylation of HIF- $\alpha$ . On the other hand, a lack of oxygen leads to an accumulation of HIF- $\alpha$ , which then binds HIF- $\beta$ . The HIF- $\alpha/\beta$  heterodimer acts as a transcriptional factor of genes involved in acute or chronic adaptation to hypoxia such as vascular endothelial growth factor (*VEGF*), platelet-derived growth factor B (*PDGF-B*), tumor growth factor  $\alpha$  (*TGF $\alpha$* ), and erythropoietin.<sup>83</sup> Loss of VHL leads to an activation of kinases such as EGFR, c-Met, VEGFR, or PDGFR, which can support invasiveness, angiogenesis, and

metastasis.<sup>84</sup> Renal tumors generally do not respond to conventional treatment and therefore require novel therapies. The scope for specifically targeting VHL-negative cells was investigated using isogenic cell lines derived from RCC patients of different VHL status.<sup>84</sup> Focused silencing of individual kinases resulted in the identification of three genes that reduced the viability of VHL<sup>-/-</sup> RCC cell lines: *c-Met*, *CDK6*, and *MAP2K1*. A synthetic lethal interaction between VHL and CDK6 was then confirmed by experiments using a small molecule CDK4/6 inhibitor (CAS 546102-60-7), which only blocked the growth of VHL<sup>-/-</sup> cells. This finding suggests that CDK6 inhibitors could potentially be useful in the treatment of VHL<sup>-/-</sup> RCC.

#### **D. CDK4 and K-Ras**

Given the role of CDK4/6 in the conjunction of mitogenic signaling and cell cycle regulation, it was not a surprise when CDK4 was revealed as a promising target in cancers overexpressing K-Ras.<sup>85</sup> Inducible overexpression of K-Ras in mouse embryonic fibroblasts was found to overcome the typical replicative senescence response of cells exposed to culture shock, while CDK4 ablation restored this senescence. K-Ras-positive tumor cell lines were sensitive to CDK4 silencing while cell lines lacking K-Ras were unaffected. Moreover, the induction of K-Ras expression in murine xenograft models with a loss of CDK4 significantly reduced their tumor burden, and all of the tumors that did arise were benign. It was also demonstrated that K-Ras-positive cells with a loss of CDK4 undergo senescence in a way that is normally observed only in lung cells. Mice with induced K-Ras overexpression were treated with the CDK4-specific inhibitor PD0332991. After 30 days, less than 20% of all animals developed lesions compared to 75% for control mice. Biochemical analysis revealed a decrease in Rb phosphorylation at serines 807 and 811 in the treated mice; both of these residues are targets of CDK4. However, no senescence response was detected in cells treated with a CDK4 inhibitor, suggesting that CDK4 activity was not adequately suppressed. It would therefore be desirable to develop more potent CDK4 inhibitors and test their usefulness in the treatment of K-Ras-positive NSCLC. The synthetic lethal relationship between K-Ras and CDK4 was subsequently observed in a K-Ras overexpressing NSCLC cell line, in which CDK4 silencing reduced cell proliferation, as well as in a murine xenograft model, in which it inhibited tumor growth.<sup>86</sup>

#### **E. CDK5/12 and PARP**

As noted in the introduction the SL relationships that have been most widely exploited in the clinic are those associated with PARP inhibition. Turner et al. searched for additional SL interactions between PARP and DNA damage response proteins by performing a screen using an siRNA library targeting 779 human kinases and kinase-associated genes in a breast cancer cell line.<sup>87</sup> This approach yielded six on-target hits, the most notable of which was CDK5. The SL relationship between PARP and CDK5 was subsequently confirmed by experiments using HeLa cells treated with a PARP inhibitor: CDK5-silenced cells were more sensitive than controls to DNA-damaging agents such as camptothecin and cisplatin. CDK5 silencing in cells treated with the PARP inhibitor caused a striking increase in  $\gamma$ H2AX phosphorylation and an increase in the abundance of RAD51 foci even in the absence of exogenous DNA damage. Thus, CDK5 silencing in PARP-inhibited HeLa cells causes failures of SSB repair that lead to DSB formation but has no effect on HR or NHEJ. Interestingly, when CDK5-silenced cells were irradiated, they exhibited radiation-resistant DNA synthesis and an unusually high proportion of cells were found to be in mitosis after irradiation, suggesting that CDK5 controls an intra S-phase checkpoint that normally prevents mitotic progression in cells with DNA damage. While its precise function in the various cell cycle checkpoints remains unclear, it may act via SCF ubiquitin ligase or some noncatalytic interaction with DNA-damage kinases. In

conclusion, PARP inhibition causes the accumulation of SSBs; when this is paired with a failure of an intra-S-phase checkpoint due to the absence of CDK5, the result is an increased rate of replication fork collapse that leads to cell death. These results suggest that PARP inhibitors may be particularly effective in the treatment of patients with CDK5 loss of function mutations. They also suggest that in addition to their uses in treating patients with BRCA1 or BRCA2 deficiencies, PARP inhibitors may be useful for other malignancies when applied in combination with CDK5 inhibitors.

In an effort to identify even more genes for which loss of function might predict sensitivity to PARP inhibitors, Bajrami et al. performed a genome-wide synthetic lethal screen using the PARP inhibitor olaparib.<sup>88</sup> Their analysis showed that the cytotoxicity of olaparib is governed by the status of the DNA damage response apparatus as well as genes that proofread chromatin remodeling and regulate sister chromatid cohesion. Of the genes identified in this work, *CDK12* stands out as a potential predictive biomarker for responsiveness to PARP1/2 inhibitors. CDK12 is a regulator of RNA polymerase II and is also important in HR. High-grade serous ovarian cancer (HGS-OVCa), a disease characterized by a high frequency of familial and somatic BRCA mutations, was selected as a model in which to evaluate the synthetic lethal relationship between PARP1/2 and CDK12 based on its susceptibility to olaparib after carboplatin treatment. The loss of CDK12 function may sensitize HGS-OV cells to PARP1/2 inhibitors because it reduces the expression of key DNA repair genes such as *BRCA1*, *FANCI*, *FANCD2*, and *ATR*, rendering the cell deficient in HR.<sup>88,89</sup> Consequently, the SSBs induced by PARP1/2 inhibition are not effectively repaired, leading to cell death.

## 5. CONCLUSION

For a long time, attempts to treat patients with tumors could be likened to “tilting at windmills” due to the heterogeneity of cancer and related diseases. While treatments with classical chemotherapeutics often initially provide good outcomes, different patterns of resistance appear in many patients. In addition, these drugs are characterized by high levels of general toxicity and severe side effects. However, developments in genetics and genomic technologies have made it possible to explore the genetic basis of diverse tumors, leading to the identification of novel molecular targets whose specific inhibition offers the potential for more effective treatment that can overcome resistance. In recent years, many drugs targeting specific cancer-related proteins have been developed, several of which have been approved for clinical use. Although no CDK inhibitor has yet been approved for cancer therapy several phase III clinical trials involving such agents are underway. There has been quite a large gap between the development of the first CDK inhibitors and their use in phase II/III trials for several reasons including their low therapeutic indices (especially in monotherapy) and a lack of robust criteria for selecting patients who are likely to respond well to such therapies. Hopefully, these problems could potentially be avoided by exploiting SL. Studies on this phenomenon, which was first demonstrated in yeasts before being explored further in cell lines and model organisms, have revealed a range of gene–gene interactions that could potentially be exploited to develop novel targeted therapies that will make it possible to effectively treat previously incurable tumors and provide more effective therapies, perhaps based on CDK inhibition, with fewer side effects for other cancers.

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## REFERENCES

1. Muller PY, Milton MN. The determination and interpretation of the therapeutic index in drug development. *Nat Rev Drug Discov* 2012;11:751–761.
2. Kaelin WG. The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* 2005;5:689–698.
3. Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, Yeh ET. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med* 2012;18:1639–1642.
4. Lazarus HM, Herzig RH, Herzig GP, Phillips GL, Roessmann U, Fishman DJ. Central nervous system toxicity of high-dose systemic cytosine arabinoside. *Cancer* 1981;48:2577–2582.
5. DeLena M, Guzzon A, Monfardini S, Bonadonna G. Clinical, radiologic, and histopathologic studies on pulmonary toxicity induced by treatment with bleomycin (NSC-125066). *Cancer Chemother Rep* 1972;56:343–356.
6. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70.
7. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. *Science* 1960;132:1497–1501.
8. Fabbro D, Cowan-Jacob SW, Möbitz H, Martiny-Baron G. Targeting cancer with small-molecular-weight kinase inhibitors. *Methods Mol Biol* 2012;795:1–34.
9. Johnson LN. Protein kinase inhibitors: Contributions from structure to clinical compounds. *Q Rev Biophys* 2009;42:1–40.
10. Hartman JL, 4th, Garvik B, Hartwell L. Principles for the buffering of genetic variation. *Science* 2001;291:1001–1004.
11. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, Chu AM, Connolly C, Davis K, Dietrich F, Dow SW, El Bakkoury M, Foury F, Friend SH, Gentalen E, Giaever G, Hegemann JH, Jones T, Laub M, Liao H, Liebundguth N, Lockhart DJ, Lucau-Danila A, Lussier M, M'Rabet N, Menard P, Mittmann M, Pai C, Rebischung C, Revuelta JL, Riles L, Roberts CJ, Ross-MacDonald P, Scherens B, Snyder M, Sookhai-Mahadeo S, Storms RK, Veronneau S, Voet M, Volckaert G, Ward TR, Wysocki R, Yen GS, Yu K, Zimmermann K, Philippsen P, Johnston M, Davis RW. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 1999;285:901–906.
12. Ooi SL, Shoemaker DD, Boeke JD. DNA helicase gene interaction network defined using synthetic lethality analyzed by microarray. *Nat Genet* 2003;35:277–286.
13. Gitlin L, Karelsky S, Andino R. Short interfering RNA confers intracellular antiviral immunity in human cells. *Nature* 2002;418:430–434.
14. Paddison PJ, Hannon GJ. RNA interference: The new somatic cell genetics? *Cancer Cell* 2002;2:17–23.
15. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C, Fröhling S, Chan EM, Sos ML, Michel K, Mermel C, Silver SJ, Weir BA, Reiling JH, Sheng Q, Gupta PB, Wadlow RC, Le H, Hoersch S, Wittner BS, Ramaswamy S, Livingston DM, Sabatini DM, Meyerson M, Thomas RK, Lander ES, Mesirov JP, Root DE, Gilliland DG, Jacks T, Hahn WC. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* 2009;462:108–112.
16. Luo J, Emanuele MJ, Li D, Creighton CJ, Schlabach MR, Westbrook TF, Wong KK, Elledge SJ. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. *Cell* 2009;137:835–848.
17. Wang Y, Ngo VN, Marani M, Yang Y, Wright G, Staudt LM, Downward J. Critical role for transcriptional repressor Snail2 in transformation by oncogenic RAS in colorectal carcinoma cells. *Oncogene* 2010;29:4658–4670.
18. Gordon GM, Du W. Targeting Rb inactivation in cancers by synthetic lethality. *Am J Cancer Res* 2011;1:773–786.

19. vander Meer R, Yong Song H, Park SH, Abdulkadir SA, Roh M. RNAi screen identifies a synthetic lethal interaction between PIM1 overexpression and PLK1 inhibition. *Clin Cancer Res* 2014;20:3211–3221.
20. Xie L, Gazin C, Park SM, Zhu LJ, Debily MA, Kittler EL, Zapp ML, Lapointe D, Gobeil S, Virbasius CM, Green MR. A synthetic interaction screen identifies factors selectively required for proliferation and TERT transcription in p53-deficient human cancer cells. *PLoS Genet* 2012;8:e1003151.
21. Ryan CJ, Lord CJ, Ashworth A. DAISY: Picking synthetic lethals from cancer genomes. *Cancer Cell* 2014;26:306–308.
22. Jerby-Arnon L, Pfetzer N, Waldman YY, McGarry L, James D, Shanks E, Seashore-Ludlow B, Weinstock A, Geiger T, Clemons PA, Gottlieb E, Ruppin E. Predicting cancer-specific vulnerability via data-driven detection of synthetic lethality. *Cell* 2014;158:1199–1209.
23. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH. Integrating genetic approaches into the discovery of anticancer drugs. *Science* 1997;278:1064–1068.
24. Underhill C, Toulmonde M, Bonnefoi H. A review of PARP inhibitors: From bench to bedside. *Ann Oncol* 2011;22:268–279.
25. Malumbres M. Cyclin-dependent kinases. *Genome Biol* 2014;15:122.
26. Pavletich NP. Mechanisms of cyclin-dependent kinase regulation: Structures of Cdks, their cyclin activators, and Cip and INK4 inhibitors. *J Mol Biol* 1999;287:821–828.
27. Boutros R, Duccommun B. Asymmetric localization of the CDC25B phosphatase to the mother centrosome during interphase. *Cell Cycle* 2008;7:401–406.
28. Hall M, Peters G. Genetic alterations of cyclins, cyclin-dependent kinases, and Cdk inhibitors in human cancer. *Adv Cancer Res* 1996;68:67–108.
29. Leach FS, Elledge SJ, Sherr CJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B. Amplification of cyclin genes in colorectal carcinomas. *Cancer Res* 1993;53:1986–1989.
30. Dobashi Y, Shoji M, Jiang SX, Kobayashi M, Kawakubo Y, Kameya T. Active cyclin A-CDK2 complex, a possible critical factor for cell proliferation in human primary lung carcinomas. *Am J Pathol* 1998;153:963–972.
31. Keyomarsi K, Conte D, Jr, Toyofuku W, Fox MP. Deregulation of cyclin E in breast cancer. *Oncogene* 1995;11:941–950.
32. Scuderì R, Palucka KA, Pokrovskaja K, Björkholm M, Wiman KG, Pisa P. Cyclin E overexpression in relapsed adult acute lymphoblastic leukemias of B-cell lineage. *Blood* 1996;87:3360–3367.
33. Iida H, Towatari M, Tanimoto M, Morishita Y, Kodera Y, Saito H. Overexpression of cyclin E in acute myelogenous leukemia. *Blood* 1997;90:3707–3713.
34. Porter DC, Zhang N, Danes C, McGahren MJ, Harwell RM, Faruki S, Keyomarsi K. Tumor-specific proteolytic processing of cyclin E generates hyperactive lower-molecular-weight forms. *Mol Cell Biol* 2001;21:6254–6269.
35. Nagel S, Leich E, Quentmeier H, Meyer C, Kaufmann M, Drexler HG, Zettl A, Rosenwald A, MacLeod RA. Amplification at 7q22 targets cyclin-dependent kinase 6 in T-cell lymphoma. *Leukemia* 2008;22:387–392.
36. Faussillon M, Monnier L, Junien C, Jeanpierre C. Frequent overexpression of cyclin D2/cyclin-dependent kinase 4 in Wilms' tumor. *Cancer Lett* 2005;221:67–75.
37. Tang LH, Contractor T, Clausen R, Klimstra DS, Du YC, Allen PJ, Brennan MF, Levine AJ, Harris CR. Attenuation of the retinoblastoma pathway in pancreatic neuroendocrine tumors due to increased cdk4/cdk6. *Clin Cancer Res* 2012;18:4612–4620.
38. Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, Hayward N, Dracopoli NC. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. *Nat Genet* 1996;12:97–99.
39. Wang Z, Trope CG, Flørenes VA, Suo Z, Nesland JM, Holm R. Overexpression of CDC25B, CDC25C and phospho-CDC25C (Ser216) in vulvar squamous cell carcinomas are associated with malignant features and aggressive cancer phenotypes. *BMC Cancer* 2010;10:233.

40. Xing X, Chen J, Chen M. Expression of CDC25 phosphatases in human gastric cancer. *Dig Dis Sci* 2008;53:949–953.
41. Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. *Nat Rev Drug Discov* 2009;8:547–566.
42. Kryštof V, Uldrijan S. Cyclin-dependent kinase inhibitors as anticancer drugs. *Curr Drug Targets* 2010;11:291–302.
43. Bruyère C, Meijer L. Targeting cyclin-dependent kinases in anti-neoplastic therapy. *Curr Opin Cell Biol* 2013;25:772–779.
44. Raje N, Kumar S, Hideshima T, Roccaro A, Ishitsuka K, Yasui H, Shiraishi N, Chauhan D, Munshi NC, Green SR, Anderson KC. Seliciclib (CYC202 or R-roscovitine), a small-molecule cyclin-dependent kinase inhibitor, mediates activity via down-regulation of Mcl-1 in multiple myeloma. *Blood* 2005;106:1042–1047.
45. Gojo I, Zhang B, Fenton RG. The cyclin-dependent kinase inhibitor flavopiridol induces apoptosis in multiple myeloma cells through transcriptional repression and down-regulation of Mcl-1. *Clin Cancer Res* 2002;8:3527–3538.
46. Kitada S, Zapata JM, Andreeff M, Reed JC. Protein kinase inhibitors flavopiridol and 7-hydroxystaurosporine down-regulate antiapoptosis proteins in B-cell chronic lymphocytic leukemia. *Blood* 2000;96:393–397.
47. Lohrum MA, Ludwig RL, Kubbutat MH, Hanlon M, Vousden KH. Regulation of HDM2 activity by the ribosomal protein L11. *Cancer Cell* 2003;3:577–587.
48. Dai MS, Lu H. Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. *J Biol Chem* 2004;279:44475–44482.
49. Dai MS, Zeng SX, Jin Y, Sun XX, David L, Lu H. Ribosomal protein L23 activates p53 by inhibiting MDM2 function in response to ribosomal perturbation but not to translation inhibition. *Mol Cell Biol* 2004;24:7654–7668.
50. Cai D, Latham VM, Jr, Zhang X, Shapiro GI. Combined depletion of cell cycle and transcriptional cyclin-dependent kinase activities induces apoptosis in cancer cells. *Cancer Res* 2006;66:9270–9280.
51. Santamaria D, Barrière C, Cerqueira A, Hunt S, Tardy C, Newton K, Cáceres JF, Dubus P, Malumbres M, Barbacid M. Cdk1 is sufficient to drive the mammalian cell cycle. *Nature* 2007;448:811–815.
52. Echalié A, Cot E, Camasses A, Hodimont E, Hoh F, Jay P, Sheinerman F, Krasinska L, Fisher D. An integrated chemical biology approach provides insight into Cdk2 functional redundancy and inhibitor sensitivity. *Chem Biol* 2012;19:1028–1040.
53. Cienas J, Valius M. The CDK inhibitors in cancer research and therapy. *J Cancer Res Clin Oncol* 2011;137:1409–1418.
54. Reddy HK, Mettus RV, Rane SG, Graña X, Litvin J, Reddy EP. Cyclin-dependent kinase 4 expression is essential for neu-induced breast tumorigenesis. *Cancer Res* 2005;65:10174–10178.
55. Eggers JP, Grandgenett PM, Collisson EC, Lewallen ME, Tremayne J, Singh PK, Swanson BJ, Andersen JM, Caffrey TC, High RR, Ouellette M, Hollingsworth MA. Cyclin-dependent kinase 5 is amplified and overexpressed in pancreatic cancer and activated by mutant K-Ras. *Clin Cancer Res* 2011;17:6140–6150.
56. Wang G, Zheng L, Yu Z, Liao G, Lu L, Xu R, Zhao Z, Chen G. Increased cyclin-dependent kinase 6 expression in bladder cancer. *Oncol Lett* 2012;4:43–46.
57. Ali S, Heathcote DA, Kroll SH, Jogalekar AS, Scheiper B, Patel H, Brackow J, Siwicki A, Fuchter MJ, Periyasamy M, Tolhurst RS, Kanneganti SK, Snyder JP, Liotta DC, Aboagye EO, Barrett AG, Coombes RC. The development of a selective cyclin-dependent kinase inhibitor that shows antitumor activity. *Cancer Res* 2009;69:6208–6215.
58. Heuer TS. Discovery of selective CDK9 small molecule inhibitors: CDK9 inhibition in tumor cells is associated with inhibition of proliferation and induction of apoptosis. AACR-NCIEORTC International Conference on Molecular Targets and Cancer Therapeutics. Geneva, Switzerland; October 21–24, 2008.

59. Rader J, Russell MR, Hart LS, Nakazawa MS, Belcastro LT, Martinez D, Li Y, Carpenter EL, Attiyeh EF, Diskin SJ, Kim S, Parasuraman S, Caponigro G, Schnepf RW, Wood AC, Pawel B, Cole KA, Maris JM. Dual CDK4/CDK6 inhibition induces cell-cycle arrest and senescence in neuroblastoma. *Clin Cancer Res* 2013;19:6173–6182.
60. Tate SC, Cai S, Ajamie RT, Burke T, Beckmann RP, Chan EM, DeDios A, Wishart GN, Gelbert LM, Cronier DM. Semi-mechanistic pharmacokinetic/pharmacodynamic modeling of the antitumor activity of LY2835219, a new cyclin-dependent kinase 4/6 inhibitor, in mice bearing human tumor xenografts. *Clin Cancer Res* 2014;20:3763–3774.
61. Finn RS, Dering J, Conklin D, Kalous O, Cohen DJ, Desai AJ, Ginther C, Atefi M, Chen I, Fowst C, Los G, Slamon DJ. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. *Breast Cancer Res* 2009;11:R77.
62. Motwani M, Delohery TM, Schwartz GK. Sequential dependent enhancement of caspase activation and apoptosis by flavopiridol on paclitaxel-treated human gastric and breast cancer cells. *Clin Cancer Res* 1999;5:1876–1883.
63. Motwani M, Rizzo C, Sirotnak F, She Y, Schwartz GK. Flavopiridol enhances the effect of docetaxel in vitro and in vivo in human gastric cancer cells. *Mol Cancer Ther* 2003;2:549–555.
64. Motwani M, Jung C, Sirotnak FM, She Y, Shah MA, Gonen M, Schwartz GK. Augmentation of apoptosis and tumor regression by flavopiridol in the presence of CPT-11 in Hct116 colon cancer monolayers and xenografts. *Clin Cancer Res* 2001;7:4209–4219.
65. Jung CP, Motwani MV, Schwartz GK. Flavopiridol increases sensitization to gemcitabine in human gastrointestinal cancer cell lines and correlates with down-regulation of ribonucleotide reductase M2 subunit. *Clin Cancer Res* 2001;7:2527–2536.
66. Johnson N, Shapiro GI. Cyclin-dependent kinases (cdks) and the DNA damage response: Rationale for cdk inhibitor-chemotherapy combinations as an anticancer strategy for solid tumors. *Expert Opin Ther Targets* 2010;14:1199–1212.
67. O'Connor DS, Wall NR, Porter AC, Altieri DC. A p34(cdc2) survival checkpoint in cancer. *Cancer Cell* 2002;2:43–54.
68. Wong AJ, Bigner SH, Bigner DD, Kinzler KW, Hamilton SR, Vogelstein B. Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. *Proc Natl Acad Sci USA* 1987;84:6899–6903.
69. Sami A, Karsy M. Targeting the PI3K/AKT/mTOR signaling pathway in glioblastoma: Novel therapeutic agents and advances in understanding. *Tumor Biol* 2013;34:1991–2002.
70. Cheng CK, Gustafson WC, Charron E, Houseman BT, Zunder E, Goga A, Gray NS, Pollok B, Oakes SA, James CD, Shokat KM, Weiss WA, Fan QW. Dual blockade of lipid and cyclin-dependent kinases induces synthetic lethality in malignant glioma. *Proc Natl Acad Sci USA* 2012;109:12722–12727.
71. Ortega S, Prieto I, Odajima J, Martín A, Dubus P, Sotillo R, Barbero JL, Malumbres M, Barbacid M. Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice. *Nat Genet* 2003;35:25–31.
72. Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. *Lancet* 2007;369:2106–2120.
73. Lodrini M, Oehme I, Schroeder C, Milde T, Schier MC, Kopp-Schneider A, Schulte JH, Fischer M, DePreter K, Pattyn F, Castoldi M, Muckenthaler MU, Kulozik AE, Westermann F, Witt O, Deubzer HE. MYCN and HDAC2 cooperate to repress miR-183 signaling in neuroblastoma. *Nucleic Acids Res* 2013;41:6018–6033.
74. Molenaar JJ, Ebus ME, Geerts D, Koster J, Lamers F, Valentijn LJ, Westerhout EM, Versteeg R, Caron HN. Inactivation of CDK2 is synthetically lethal to MYCN over-expressing cancer cells. *Proc Natl Acad Sci USA* 2009;106:12968–12973.
75. Delehouzé C, Godt K, Loaëc N, Bruyère C, Desban N, Oumata N, Galons H, Roumeliotis TI, Giannopoulou EG, Grenet J, Twitchell D, Lahti J, Mouchet N, Galibert MD, Garbis SD, Meijer L. CDK/CK1 inhibitors roscovitine and CR8 downregulate amplified MYCN in neuroblastoma cells. *Oncogene* 2013;33:5675–5687.

76. Li Y, Casey SC, Felsher DW. Inactivation of MYC reverses tumorigenesis. *J Intern Med* 2014;276:52–60.
77. Goga A, Yang D, Tward AD, Morgan DO, Bishop JM. Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. *Nat Med* 2007;13:820–827.
78. Barrett RM, Osborne TP, Wheatley SP. Phosphorylation of survivin at threonine 34 inhibits its mitotic function and enhances its cytoprotective activity. *Cell Cycle* 2009;8:278–283.
79. Liu Y, Zhu YH, Mao CQ, Dou S, Shen S, Tan ZB, Wang J. Triple negative breast cancer therapy with CDK1 siRNA delivered by cationic lipid assisted PEG-PLA nanoparticles. *J Control Release* 2014;192:114–121.
80. Horiuchi D MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. *J Exp Med* 2012;209:679–696.
81. Huang CH, Lujambio A, Zuber J, Tschaharganeh DF, Doran MG, Evans MJ, Kitzing T, Zhu N, deStanchina E, Sawyers CL, Armstrong SA, Lewis JS, Sherr CJ, Lowe SW. CDK9-mediated transcription elongation is required for MYC addiction in hepatocellular carcinoma. *Genes Dev* 2014;28:1800–1814.
82. Kang J, Sergio CM, Sutherland RL, Musgrove EA. Targeting cyclin-dependent kinase 1 (CDK1) but not CDK4/6 or CDK2 is selectively lethal to MYC-dependent human breast cancer cells. *BMC Cancer* 2014;14:32.
83. Shen C, Kaelin WG, Jr. The VHL/HIF axis in clear cell renal carcinoma. *Semin Cancer Biol* 2013;23:18–25.
84. Bommi-Reddy A, Almeciga I, Sawyer J, Geisen C, Li W, Harlow E, Kaelin WG, Jr, Grueneberg DA. Kinase requirements in human cells: III. Altered kinase requirements in VHL-/- cancer cells detected in a pilot synthetic lethal screen. *Proc Natl Acad Sci USA* 2008;105:16484–16489.
85. Puyol M, Martín A, Dubus P, Mulero F, Pizcueta P, Khan G, Guerra C, Santamaría D, Barbacid M. A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma. *Cancer Cell* 2010;18:63–73.
86. Mao CQ, Xiong MH, Liu Y, Shen S, Du XJ, Yang XZ, Dou S, Zhang PZ, Wang J. Synthetic lethal therapy for KRAS mutant non-small-cell lung carcinoma with nanoparticle-mediated CDK4 siRNA delivery. *Mol Ther* 2014;22:964–973.
87. Turner NC, Lord CJ, Iorns E, Brough R, Swift S, Elliott R, Rayter S, Tutt AN, Ashworth A. A synthetic lethal siRNA screen identifying genes mediating sensitivity to a PARP inhibitor. *EMBO J* 2008;27:1368–1377.
88. Bajrami I, Frankum JR, Konde A, Miller RE, Rehman FL, Brough R, Campbell J, Sims D, Rafiq R, Hooper S, Chen L, Kozarewa I, Assiotis I, Fenwick K, Natrajan R, Lord CJ, Ashworth A. Genome-wide profiling of genetic synthetic lethality identifies CDK12 as a novel determinant of PARP1/2 inhibitor sensitivity. *Cancer Res* 2014;74:287–297.
89. Joshi PM, Sutor SL, Huntoon CJ, Karnitz LM. Ovarian cancer-associated mutations disable catalytic activity of CDK12, a kinase that promotes homologous recombination repair and resistance to cisplatin and poly(ADP-ribose) polymerase inhibitors. *J Biol Chem* 2014;289:9247–9253.
90. Squires MS, Feltell RE, Wallis NG, Lewis EJ, Smith DM, Cross DM, Lyons JF, Thompson NT. Biological characterization of AT7519, a small-molecule inhibitor of cyclin-dependent kinases, in human tumor cell lines. *Mol Cancer Ther* 2009;8:324–332.
91. Siemeister G, Lücking U, Wengner AM, Lienau P, Steinke W, Schatz C, Mumberg D, Ziegelbauer K. BAY 1000394, a novel cyclin-dependent kinase inhibitor, with potent antitumor activity in mono- and in combination treatment upon oral application. *Mol Cancer Ther* 2012;11:2265–2273.
92. Sedlacek H, Czech J, Naik R, Kaur G, Worland P, Losiewicz M, Parker B, Carlson B, Smith A, Senderowicz A, Sausville E. Flavopiridol (L86 8275; NSC 649890), a new kinase inhibitor for tumor therapy. *Int J Oncol* 1996;9:1143–1168.
93. Chao SH, Fujinaga K, Marion JE, Taube R, Sausville EA, Senderowicz AM, Peterlin BM, Price DH. Flavopiridol inhibits P-TEFb and blocks HIV-1 replication. *J Biol Chem* 2000;275:28345–28348.

94. Joshi KS, Rathos MJ, Joshi RD, Sivakumar M, Mascarenhas M, Kamble S, Lal B, Sharma S. In vitro antitumor properties of a novel cyclin-dependent kinase inhibitor, P276-00. *Mol Cancer Ther* 2007;6:918–925.
95. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, Albassam M, Zheng X, Leopold WR, Pryer NK, Toogood PL. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol Cancer Ther* 2004;3:1427–1438.
96. Brasca MG, Amboldi N, Ballinari D, Cameron A, Casale E, Cervi G, Colombo M, Colotta F, Croci V, D'Alessio R, Fiorentini F, Isacchi A, Mercurio C, Moretti W, Panzeri A, Pastori W, Pevarello P, Quartieri F, Roletto F, Traquandi G, Vianello P, Vulpetti A, Ciomei M. Identification of N,1,4,4-tetramethyl-8-{{[4-(4-methylpiperazin-1-yl)phenyl]amino}-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (PHA-848125), a potent, orally available cyclin dependent kinase inhibitor. *J Med Chem* 2009;52:5152–5163.
97. Meijer L, Borgne A, Mulner O, Chong JP, Blow JJ, Inagaki N, Inagaki M, Delcros JG, Moulinoux JP. Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. *Eur J Biochem* 1997;243:527–536.
98. Kapasi AJ, Spector DH. Inhibition of the cyclin-dependent kinases at the beginning of human cytomegalovirus infection specifically alters the levels and localization of the RNA polymerase II carboxyl-terminal domain kinases cdk9 and cdk7 at the viral transcriptosome. *J Virol* 2008;82:394–407.
99. Parry D, Guzi T, Shanahan F, Davis N, Prabhavalkar D, Wiswell D, Seghezzi W, Paruch K, Dwyer MP, Doll R, Nomeir A, Windsor W, Fischmann T, Wang Y, Oft M, Chen T, Kirschmeier P, Lees EM. Dinaciclib (SCH 727965), a novel and potent cyclin-dependent kinase inhibitor. *Mol Cancer Ther* 2010;9:2344–2353.
100. Chen R, Wierda WG, Chubb S, Hawtin RE, Fox JA, Keating MJ, Gandhi V, Plunkett W. Mechanism of action of SNS-032, a novel cyclin-dependent kinase inhibitor, in chronic lymphocytic leukemia. *Blood* 2009;113:4637–4645.
101. DePinto W, Chu XJ, Yin X, Smith M, Packman K, Goelzer P, Lovey A, Chen Y, Qian H, Hamid R, Xiang Q, Tovar C, Blain R, Nevins T, Higgins B, Luistro L, Kolinsky K, Felix B, Hussain S, Heimbrook D. In vitro and in vivo activity of R547: A potent and selective cyclin-dependent kinase inhibitor currently in phase I clinical trials. *Mol Cancer Ther* 2006;5:2644–2658.
102. Goh KC, Novotny-Diermayr V, Hart S, Ong LC, Loh YK, Cheong A, Tan YC, Hu C, Jayaraman R, William AD, Sun ET, Dymock BW, Ong KH, Ethirajulu K, Burrows F, Wood JM. TG02, a novel oral multi-kinase inhibitor of CDKs, JAK2 and FLT3 with potent anti-leukemic properties. *Leukemia* 2012;26:236–243.

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***SUPPORTING INFORMATION***

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

**Table S1.** Clinical combination of CDK inhibitors with current chemotherapeutics (source: clinicaltrials.gov)