

## Targeting the Cell Cycle: A New Approach to Cancer Therapy

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### A B S T R A C T

The cell cycle represents a series of tightly integrated events that allow the cell to grow and proliferate. Critical parts of the cell cycle machinery are the cyclin-dependent kinases (CDKs), which, when activated, provide a means for the cell to move from one phase of the cell cycle to the next. The CDKs are regulated positively by cyclins and regulated negatively by naturally occurring CDK inhibitors (CKIs). Cancer represents a dysregulation of the cell cycle such that cells that overexpress cyclins or do not express the CKIs continue to undergo unregulated cell growth. The cell cycle also serves to protect the cell from DNA damage. Thus, cell cycle arrest, in fact, represents a survival mechanism that provides the tumor cell the opportunity to repair its own damaged DNA. Thus, abrogation of cell cycle checkpoints, before DNA repair is complete, can activate the apoptotic cascade, leading to cell death. Now in clinical trials are a series of targeted agents that directly inhibit the CDKs, inhibit unrestricted cell growth, and induce growth arrest. Recent attention has also focused on these drugs as inhibitors of transcription. In addition, there are now agents that abrogate the cell cycle checkpoints at critical time points that make the tumor cell susceptible to apoptosis. An understanding of the cell cycle is critical to understanding how best to clinically develop these agents, both as single agents and in combination with chemotherapy.

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### INTRODUCTION

With advancements in our understanding of the basic mechanisms of oncogenesis and the induction of apoptosis, we have gained a greater appreciation for the critical role that cell cycle regulation plays in malignant transformation and in the development of resistance to chemotherapy. Perturbations in the cell cycle are described commonly in carcinogenesis. Furthermore, with our improved understanding of the effects of chemotherapy on healthy and cancerous cells, it is increasingly apparent that the cell cycle also plays a critical role in the development of resistance to chemotherapy. These observations have led to the development of a new class of anticancer therapeutics in clinical development today: specifically, those drugs that target the motors of the cell cycle, the cyclin-dependent kinases (CDKs).

The development of CDK inhibitors has undergone a gradual evolution. This class of drugs was, at first, primarily applied

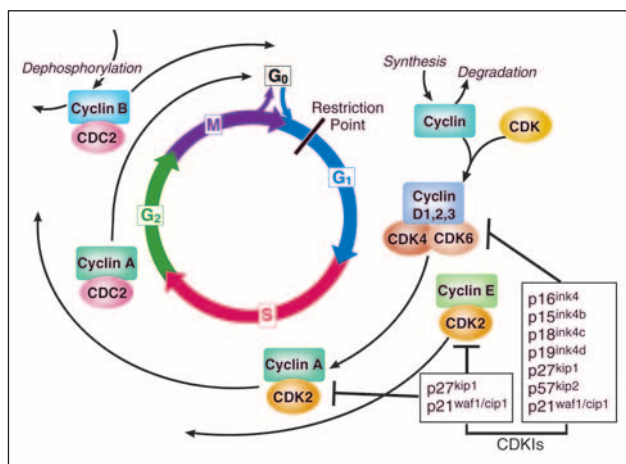
in the treatment of malignancy as single agents, in efforts to target the errors of cell cycle regulation that are already prevalent in malignant cells to achieve tumor specific cytotoxicity. Presently, these agents are used increasingly in combination with traditional cytotoxic drugs to overcome cell cycle-mediated drug resistance and to improve cytotoxic efficacy. Along with this shift in development has come an improved understanding of the role the cell cycle plays in drug resistance.

### THE CELL CYCLE AND ITS REGULATION

The cell cycle is a critical regulator of the processes of cell proliferation and growth as well as of cell division after DNA damage. It governs the transition from quiescence (G<sub>0</sub>) to cell proliferation, and through its checkpoints, ensures the fidelity of the genetic transcript. It is the mechanism by which cells reproduce, and is typically divided into

four phases. The periods associated with DNA synthesis (S phase) and mitosis (M phase) are separated by gaps of varying length called G1 and G2 (Fig 1). Progression of a cell through the cell cycle is promoted by a number of CDKs which, when complexed with specific regulatory proteins called cyclins, drive the cell forward through the cell cycle. There exist corresponding cell cycle inhibitory proteins (CDK inhibitors [CDKIs]) that serve as negative regulators of the cell cycle and stop the cell from proceeding to the next phase of the cell cycle (Fig 1). The INK4 (for inhibitor of cdk4) class of CDKIs, notably p16<sup>Ink4a</sup>, p15<sup>Ink4b</sup>, p18<sup>Ink4c</sup>, and p19<sup>Ink4d</sup>, bind and inhibit cyclin D-associated kinases (CDK2, -4, and -6). The kinase inhibitor protein (KIP) group of CDK inhibitors, p21<sup>waf1</sup>, p27<sup>kip1</sup>, and p57<sup>kip2</sup>, negatively regulate cyclin E/CDK2 and cyclin A/CDK2 complexes.<sup>1</sup>

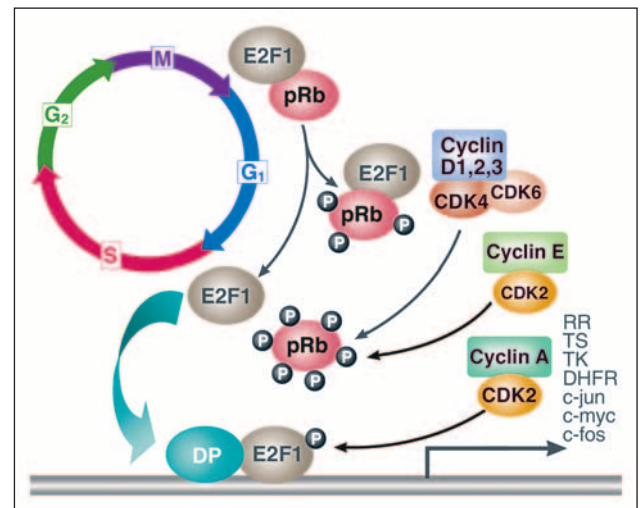
The pattern of cyclin expression varies with a cell's progression through the cell cycle, and this specific cyclin expression pattern defines the relative position of the cell within the cell cycle.<sup>2,3</sup> At least nine structurally related CDKs (CDK1- CDK9) have been identified, though not all have clearly defined cell cycle regulatory roles. A considerable number of cyclins have been identified to date (cyclin A–cyclin T). CDK/cyclin complexes themselves become activated by phosphorylation at specific sites on the CDK by cdk7/cyclin H, also referred to as CDK-activating kinase (CAK).<sup>4</sup> Cyclin D isoforms (cyclin D1-D3) interact with CDK2, -4, and -6 and drive a cell's progression through G1. The association of cyclin E with CDK2 is active at the G1/S transition and directs entry into S phase. S phase progression is directed by the cyclin A/CDK2 complex, and the complex of cyclin A with CDK1 (also known as cdc2) is important in G2. CDK1/cyclin B is necessary for mitosis to occur.



**Fig 1.** The cell cycle. The cell cycle is divided into four phases (G1, S, G2, and M). Progression through the cell cycle is promoted by cyclin-dependent kinases (CDKs), which are regulated positively by cyclins and negatively by CDK inhibitors (CDKIs). The restriction point is the point at which cells progress through the cell cycle independent of external stimuli.

CELL CYCLE REGULATION: FROM G0 TO M

Healthy cells need to decide when to divide (ie, enter the cell cycle) and when to stay in G0. Although often termed a quiescent phase, G0 is in fact quite an active phase in which, cellular functions and cellular growth occur. It is necessarily tightly regulated because the alternative (ie, uncontrolled cell division without cell growth) would lead to smaller cells with each division. The entry into the cell cycle (G1) is historically governed by the restriction point—a transition point beyond which cell progression through the cell cycle is independent of external stimuli such as exposure to nutrients or mitogen activation.<sup>5</sup> This point of determination is thought to divide the early and late G1 phase of the cell cycle. Mitogenic signaling of a variety of growth signals is mediated by the RAS/RAF/MAPK pathway, whose end point is the stimulation of D-type cyclin production. The retinoblastoma tumor suppressor gene product (Rb) governs the G1/S transition (Fig 2). In its active state, Rb is hypophosphorylated and forms an inhibitory complex with a group of transcription factors known as E2F-DP (E2F-1, -2, and -3), thus controlling the G1/S transition. The activity of Rb is modulated by the sequential phosphorylation by CDK4/6-Cyclin D and CDK2/Cyclin E (reviewed by Malumbres and Barbacid<sup>6</sup>). When Rb is partially phosphorylated by CDK4/6-CDKs, Rb remains bound to E2F-DP, but this transcription factor is still able to transcribe some genes such as *cyclin E*. Cyclin E then binds to CDK2 and this active complex then completely hyperphosphorylates Rb, thus releasing the E2F-DP complex and fully activating the E2F transcription factors, resulting in transcriptional



**Fig 2.** The G1/S transition and retinoblastoma protein (Rb). Hypophosphorylated Rb complexes with the transcription factor E2F1. CDK2, CDK4, and CDK6 phosphorylate Rb. E2F1 is released, binds to DNA with DP, and induces transcription of ribonucleotide reductase (RR), thymidylate synthase (TS), thymidine kinase (TK), dihydrofolate reductase (DHFR). E2F1 phosphorylation by cyclin A-CDK2 results in its degradation.

activation of numerous S-phase proteins, such as thymidylate synthase (TS) and dihydrofolate reductase (DHFR). In addition to Rb, CDK2 phosphorylates other substrates involved in DNA replication.<sup>7</sup>

Early in S phase, cyclins D and E are targeted by ubiquitination for proteasome degradation.<sup>8</sup> The production of cyclin A and its complexing with CDK2 enables S-phase progression, with the production of other enzymes and proteins involved in DNA synthesis, including histones and proliferating cell nuclear antigen (PCNA; reviewed by Ford and Pardee<sup>9</sup>). However, orderly S-phase progression requires the timely inactivation of E2F, in part accomplished by cyclin A-dependent kinase activity. Cyclin A-CDK2 stably associates with E2F-1, and directs phosphorylation of the E2F/DP heterodimer, neutralizing its DNA binding capacity.<sup>10-13</sup>

During late S and throughout G<sub>2</sub>, cells prepare for mitosis by increasing levels of cyclins A and B. As the level of cyclin B rises, it forms a complex with cdc2 (CDK1) in the cytoplasm, where it remains until mitosis, at which point it shuttles into the nucleus. Recently, an S-phase checkpoint, termed the replication checkpoint, has been described.<sup>14,15</sup> This checkpoint monitors progression through S phase and slows the rate of ongoing DNA synthesis. The S-phase checkpoint is thought to involve activations of ATM and ATR kinases with subsequent activation of Chk1 and Chk2.<sup>14-17</sup> These pathways ultimately control the ability of a cell to enter mitosis, which is dependent on the completion of S phase. Entry into mitosis is determined by the activity of the cyclin B/cdc2 complex, which is tightly regulated by its phosphorylation status, both by an activating phosphorylation at Thr161 by CAK and inhibitory phosphorylations at Thr14 and Thr15. At the completion of S phase, wee1 kinase is degraded by proteolysis in a cdc34 dependent fashion, and the phosphatase, cdc25c, is activated by a regulatory phosphorylation, which leads to activation of the cyclin B/CDK1 complex (also called cyclin B/cdc2). This complex then rapidly is relocated into the nucleus and mitosis begins.<sup>9,18</sup> On DNA damage, however, ATM and ATR are activated leading to a Chk1 and Chk2 phosphorylation, and an inhibitory phosphorylation of cdc25c which prevents the activation of cyclin B/cdc2 and halts further S-phase and G<sub>2</sub> progression and the entry into mitosis.<sup>15,17</sup>

Progression through mitosis is dependent on the anaphase-promoting complex (APC)/cyclosome and the degradation of cyclin B.<sup>18</sup> During mitosis, the assembly of a bipolar spindle by the centrosome is vital to the preservation of genetic fidelity between daughter cells, and is monitored by a checkpoint that senses microtubule defects<sup>19</sup> or aberrant kinetochore attachment.<sup>20,21</sup> Centrosome abnormalities are often observed in malignancy, responsible for chromosome mis-segregation and resultant genomic instability.<sup>22</sup> Centrosome maturation is critical for cell division to occur and is regulated by several kinases including *polo*

kinase and Aurora kinase. Centrosome maturation begins with centriole duplication, which occurs in G<sub>1</sub> and is triggered by CDK2/cyclin E and CDK2/cyclin D activity. Elongation of the centriole occurs throughout S phase so that by prophase, the cell has two pairs of centrioles within the pericentriolar material (reviewed by Blagden and Glover<sup>23</sup>). Polo kinase is involved in recruiting  $\gamma$ -tubulin and in activating the Asp protein, abnormal spindles gene product.<sup>23</sup> Aurora kinase is also involved in centrosome maturation. This protein kinase appears to be required for correct spindle pole structure and bipolarity of the spindle,<sup>24</sup> and appears to be essential in the duplication/separation stage of the centriole cycle.<sup>25</sup> Survivin has been implicated in the regulation of the mitotic spindle and in the preservation of cell viability, due in large part to its expression during cell division in a cell cycle-dependent manner and localization to the mitotic apparatus.<sup>26,27</sup> Cyclin B1/cdc2 activity during mitosis plays a critical role in survivin expression and function in cell viability.<sup>28</sup>

#### THE CELL CYCLE AS A TARGET FOR CANCER THERAPEUTICS

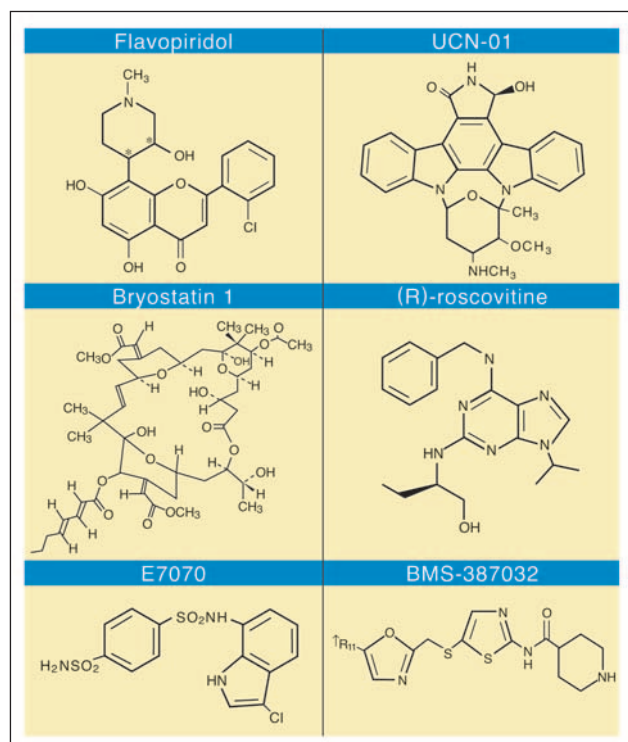
The rationale for targeting the cell cycle and, in particular, the CDKs in anticancer therapy has been based on the frequency of their perturbations in human malignancy and the observation that cell cycle arrest by CDK inhibition could induce apoptosis. Most tumor-suppressor genes and oncogenes are components of signal transduction pathways that control several cellular functions including cell cycle entry and exit.<sup>29,30</sup> In contrast to healthy cells, tumor cells are unable to stop at predetermined points of the cell cycle because of loss of checkpoint integrity. This can be due to inactivation of critical CDKs, or to overexpression of cyclins. For example, *p16* is an INK4 gene that is particularly sensitive to epigenetic silencing by hypermethylation of its promoter region, which results in inhibition of transcription and loss of gene expression. When this occurs uncontrolled proliferation can result. Accordingly, loss of *p16* function has been associated with a multitude of malignancies including melanoma, lung, breast, and colorectal tumors.<sup>31</sup> Similarly, overexpression of cyclin D1 has been associated with the development and progression of breast cancer.<sup>32,33</sup> Thus, targeting CDKs would recapitulate cell cycle checkpoints that would necessarily limit a tumor cell's ability to cycle, and this may then facilitate the induction of apoptosis.<sup>34</sup>

This rationale led to the development of CDKIs as novel antitumor agents. These compounds can inhibit CDKs by direct effects that target the catalytic CDK subunit, or by indirect means that target regulatory pathways that govern CDK activity.<sup>35</sup> Recently, attention has shifted to these drugs as inhibitors of transcription. Transcription is carried out by three different RNA polymerases, including

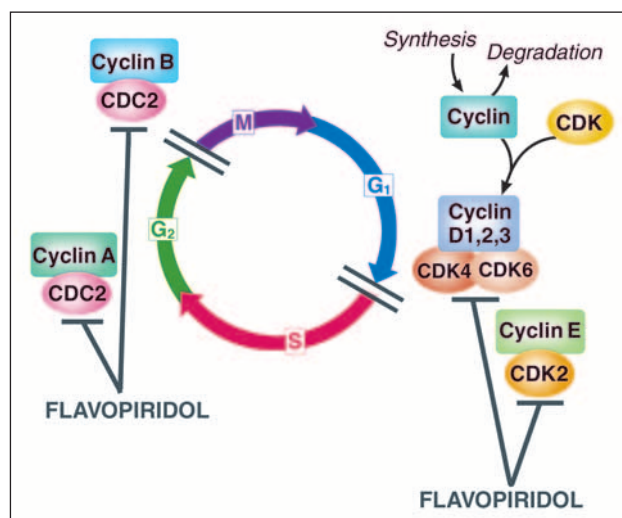
RNA polymerase II (RNA pol II). Phosphorylation of RNA pol II at its C-terminal domain (CTD) affects its function in transcription.<sup>36</sup> The protein kinases responsible for this phosphorylation include CDKs -1, -7, -8, and -9.<sup>36,37</sup> In particular, cyclin T-CDK9 (*p*-TEFb) phosphorylates the CTD of RNA pol II to control efficient transcriptional elongation. Cyclin B-CDK1 and cyclin C-CDK8 also phosphorylate the CTD of RNA pol II, resulting in suppression of mRNA production during mitosis and inhibition of protein factors required to initiate transcription. CDK2 also phosphorylates RNA pol II and this function appears to be important for the transcription of the viral genome in cells infected with HIV-1.<sup>38</sup> Tumor cells appear especially sensitive to RNA pol II inhibition.<sup>39,40</sup> Thus, the inhibition of RNA pol II CTD phosphorylation by CDKIs may contribute to the pro-apoptotic effect of this class of drugs.<sup>36-40</sup> Several CDKIs currently in clinical development are described in the following paragraphs.

### Flavopiridol

Flavopiridol is a novel antineoplastic agent that originally was noted for its ability to inhibit the activity of a number of protein kinases. Flavopiridol (Fig 3) is now best classified as a CDKI because of its considerable affinity for CDKs and its ability to induce cell cycle arrest in a number of cell lines.<sup>41,42</sup> It has been shown to bind to and directly inhibit CDK1 (cyclin B1-cdc2 kinase), CDK2, CDK4, and CDK6 (Fig 4).

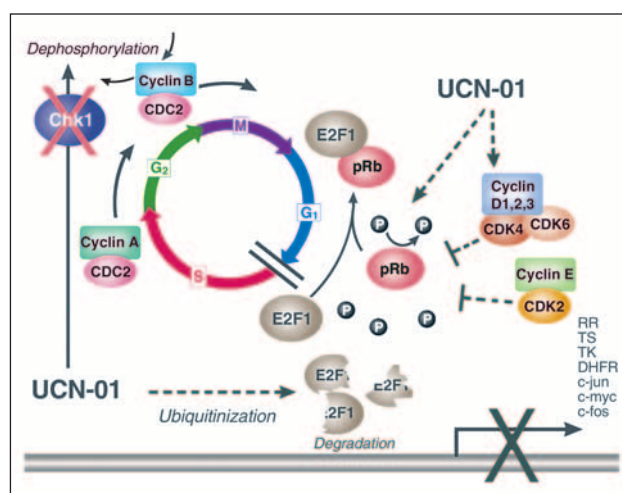


**Fig 3.** Selected group of cyclin-dependent kinase inhibitors currently under clinical development.



**Fig 4.** Flavopiridol cell cycle effects. Flavopiridol is a pan-cyclin-dependent kinase (CDK) inhibitor of CDK2, CDK4, and CDK6 at nanomolar concentrations, resulting in cell cycle arrest at both the G<sub>1</sub>/S transition and the G<sub>2</sub>/M transition.

Flavopiridol administration has been associated with the selective induction of apoptotic cell death, particularly in hematopoietic cell lines.<sup>43,44</sup> This induction of apoptosis may be mediated by an early activation of the mitogen-activated protein kinase (MAPK) protein kinase family of proteins (MEK, p38, and JNK), leading to activation of caspases.<sup>45</sup> It has also been shown to inhibit antiapoptotic molecules including bcl-2,<sup>43</sup> XIAP,<sup>46</sup> p21,<sup>46</sup> mcl-1,<sup>47,48</sup> cyclin D1,<sup>49</sup> and phospho-survivin.<sup>50</sup> Flavopiridol has been shown to be a potent inhibitor of Cyclin T-CDK9, resulting in the reduced efficiency of transcriptional elongation. This



**Fig 5.** UCN-01 cell cycle effects. At G<sub>1</sub>/S, UCN-01 inhibits cyclin-dependent kinase (CDK) phosphorylation of retinoblastoma protein (Rb) and targets E2F1 for proteasome degradation, inducing G<sub>1</sub> cell cycle arrest and reduction in transcription of S-phase genes. At G<sub>2</sub>/M, following DNA damage, UCN-01 inhibits Chk1, increasing Cyclin B/CDC2 activity and abrogating the G<sub>2</sub> checkpoint.

leads to the cellular depletion of mRNAs with short half lives, including genes that promote cellular proliferation (cyclin D1) and inhibit apoptosis (mcl-1 and XIAP).<sup>51,52</sup> This collective effect by flavopiridol on transcriptional suppression is then thought to promote apoptosis or to inhibit cell proliferation. Flavopiridol has also been shown to inhibit transcription of p21, as well as drg1, as a means of overcoming resistance to irinotecan therapy.<sup>53,54</sup>

Cell cycle arrest and the induction of apoptosis by flavopiridol have been demonstrated in squamous head and neck cell lines as well as other preclinical models.<sup>55,56</sup> The initial schedule of administration, based on the preclinical data, was a 72-hour continuous infusion schedule administered every 2 weeks, resulting in peak nanomolar drug concentrations.<sup>57-62</sup> However, recent data, generated by investigators at Ohio State University (Columbus, OH),<sup>63</sup> indicate that the proapoptotic effect of flavopiridol may be dependent on dose and schedule. In particular, flavopiridol is highly protein bound (> 90%) to human plasma proteins. The activity of this drug depends on obtaining free drug levels of 250 to 300 nmol/L. Pharmacokinetic models now indicate that this is possible by administering flavopiridol as a 30-minute bolus infusion followed by a 4-hour infusion. This not only achieves high peak levels of the drug, but also allows them to be sustained for up to 4 hours. Using this approach, the investigators at Ohio State have reported an approximately 50% response rate with prolonged survival in fludarabine refractory chronic lymphocytic leukemia (CLL). In fact, this response rate has been associated with significant tumor lysis syndrome.<sup>63</sup>

### UCN-01

UCN-01 (7-hydroxystaurosporine) is a staurosporine analog isolated from the culture broth of *Streptomyces* species, and is a selective inhibitor of protein kinase C.<sup>64</sup> UCN-01 is associated with a G1/S cell cycle arrest,<sup>65</sup> associated with the induction of p21CIP/Waf1, dephosphorylation of CDK2,<sup>66</sup> and with the resultant dephosphorylation of the retinoblastoma gene product (pRb).<sup>65,66</sup> Hypophosphorylated pRb remains tightly bound to E2F-1, thereby preventing cell cycle progression into S phase.<sup>67</sup> Additionally, UCN-01 causes the degradation of E2F-1 by targeting the transcription factor for proteasome degradation by ubiquitination (Fig 5).<sup>68</sup> UCN-01 also abrogates the G2 checkpoint by inhibiting Chk1 kinase, which is involved in the regulation of the phosphatase cdc25C and the protein 14-3-3.<sup>69-71</sup> If this exit out of G2 into M phase occurs in the setting of coexistent DNA damage, the tumor cells undergo apoptosis, micronucleation, and induction of a form of cell death called "mitotic catastrophe."<sup>72,73</sup>

The first clinical trial of UCN-01 as a single agent was completed by the National Cancer Institute (NCI; Bethesda, MD).<sup>74</sup> In vitro protein binding experiments dem-

onstrated that UCN-01 is significantly bound to human alpha1-acid glycoprotein (AGP) with high affinity.<sup>74-76</sup> However, hyperglycemia remains a difficult problem for patients receiving UCN-01. Using the recommended phase II dose of UCN-01 in a phase I study with escalating doses of flurouracil, Kortmansky et al have reported that one patient with hyperglycemia required hospitalization for continuous insulin infusion. Furthermore, after amending the eligibility criteria to exclude patients with diabetes mellitus, four additional episodes of grade 3 hyperglycemia were reported.<sup>77</sup> The etiology of the hyperglycemia is unclear. In their phase I study, Sausville et al<sup>74</sup> noted hyperglycemia in association with hyperinsulinemia. It has been shown recently that at clinically relevant concentrations, UCN-01 inhibits glucose transport in the presence of increasing concentrations of insulin.<sup>78</sup> These investigators also show that UCN-01 inhibits insulin-induced phosphorylation of AKT at Thr308 but not Ser473. The data suggest that UCN-01 induces clinical insulin resistance by blocking AKT activation and subsequent glucose transport in response to insulin.

### Bryostatins

The bryostatins are macrocyclic lactones with a unique polyacetate backbone (Fig 3). Bryostatins were first isolated from the marine invertebrate *Bugula neritina* and first characterized after showing high activity against the murine P388 lymphocytic leukemia.<sup>79</sup> Currently there are 20 known natural bryostatins, distinguished by their substituents at C7 and C20.<sup>80</sup> As a modulator of the cell cycle, bryostatins produce transient induction of p21 and subsequent dephosphorylation, inactivation of CDK2, and inhibition of tumor cell growth.<sup>81</sup> However, bryostatins also interfere with the upregulation of p21 produced by phorbol esters.<sup>82</sup> In U937 cells, pretreatment with deoxycytidine blocked bryostatin-mediated p21 induction, and enhanced apoptosis.<sup>83</sup> Bryostatin also decreased cyclin B expression in tumor xenografts, resulting in the prevention of paclitaxel-mediated cdc2 kinase activation.<sup>84</sup> The net effect is an arrest of cells in G2. Phase I studies of bryostatin have been explored with various infusion rates and dosing schedules.<sup>85,86</sup> The dose-limiting toxicity (DLT) has been myalgia.<sup>87,88</sup> Limited single-agent activity was noted with bryostatin in patients with melanoma, ovarian cancer, and non-Hodgkin's lymphoma in a phase I trial.<sup>87</sup>

### Other CDKs in Clinical Development

The agent CYC202 (*R*-roscovitine; Cyclacel Ltd, Dundee, United Kingdom; Fig 3) is a potent inhibitor of CDK2 with an inhibitory concentration of 50% of 100 nmol/L.<sup>89,90</sup> Similar to flavopiridol, CYC202 has been shown to suppress transcription of genes that inhibit apoptosis, which may significantly contribute to its antitumor effect.<sup>91-94</sup> It is one of a growing list of CDK inhibitors currently under clinical development that are orally bioavailable. CYC202 has been shown to have single-agent in

in vitro activity against a broad range of tumor cell types.<sup>95</sup> In vivo activity has also been reported against human colon and uterine cancer xenografts.<sup>95</sup> Preclinical studies with this agent in combination with chemotherapy have not been reported.

CYC202 is now completing phase I clinical trials in Europe.<sup>96</sup> Preliminary results have been reported with an oral starting dose of 100 mg twice daily for 7 days on an every-21-days cycle. No toxicities were reported.<sup>96</sup> Additional schedules using 5, 7, or 10 days have been reported. DLT was reached at 1,600 mg daily and 800 mg twice daily. DLTs consisted of nausea and vomiting, hypokalemia, elevated creatinine, and skin rash, which were all rapidly reversible. One partial response in a patient with hepatocellular carcinoma and 10 stable diseases lasting for more than 4 months have been reported. This included two patients with non-small-cell lung cancer, both of whom remained stable for more than 1 year.<sup>97,98</sup>

Another CDKI under clinical investigation is BMS-387032 (Fig 3), an *N*-acyl-2-aminothiazole analog.<sup>99,100</sup> This agent is reported to be a selective inhibitor of CDK2/cyclin E with an inhibitory concentration of 50% of 48 nmol/L. It induces cell cycle arrest, and apoptosis in a large panel of tumor cell lines. In combination with chemotherapy it provides additive or synergistic effects. Preliminary clinical results have been reported from two single-agent phase I clinical trials in which BMS-387032 was administered as either a 1-hour or a 24-hour infusion every 3 weeks. In both trials, fatigue was the most common adverse effect. Other toxicities included nausea, vomiting, diarrhea, and constipation.<sup>99,100</sup>

E7070 (Fig 3) is a synthetic sulfonamide that targets the G1 phase of the cell cycle by depleting cyclin E, inducing p53 and p21, and inhibiting cdc2 phosphorylation.<sup>101,102</sup> A phase II trial administering E7070 as a 1-hour infusion every 3 weeks in patients with advanced head and neck cancers was associated with limited clinical activity.<sup>103</sup> However, post-treatment biopsies from selected patients on the clinical trial indicated inhibition of Rb phosphorylation, suggesting that more frequent administration of drug may be required to sustain this effect.

The imidazopyridines are also a novel class of CDKIs with particular specificity for CDK2.<sup>104</sup> One such compound (AstraZeneca-Compound 1; AstraZeneca, Wilmington, DE) has been shown to enhance both gemcitabine- and cisplatin-induced apoptosis.<sup>105</sup> This effect was sequence (chemotherapy followed by the CDK inhibitor) and E2F-1 dependent. Phase I clinical trials with this class of oral agent, in which different drug schedules are being tested, are ongoing.

A pyrido[2,3-*d*]pyrimidine-7-ones (PD 0332991) has also been reported by Pfizer Global Research (New York, NY)<sup>106</sup> to have a relative selectivity for CDK4. This agent inhibits a broad panel of Rb-positive solid tumor cell lines in low micromolar concentrations. In MDA-MB-453 human breast cancer cells, PD 0332991 inhibited Rb phosphorylation by 50% at Ser<sup>780</sup> and Ser<sup>795</sup> with

concentrations of 0.066 and 0.063  $\mu\text{mol/L}$ , respectively. There was a G1 cell cycle arrest with PD 0332991 at a concentration as low as 0.04  $\mu\text{mol/L}$ . Similar results were obtained in vitro with the human colon cancer cell line Colo-205. Daily oral administration of PD 0332991 to animals bearing Colo-205 xenografts resulted in significant tumor regressions. With 14 days of therapy at the highest dose tested (150 mg/kg), there was a tumor growth delay of approximately 50 days and more than one log of tumor-cell kill. Despite complete regressions, tumors did grow back after these short treatment periods. In order to test whether the tumors that re-emerged were still sensitive to drug, the Colo-205 tumors were harvested and then reimplanted into treatment-naïve mice. After the tumors regrew to 100 to 150 mg, the tumor bearing mice were re-treated with PD 0332991. The tumors responded with equal sensitivity to the drug, indicating that no resistance had developed with the initial therapy.<sup>107,108</sup> A phase I clinical trial with PD 0332991 in patients with Rb-positive tumors is now under way in advanced solid tumors using a once-a-day dosing schedule.

#### CDKIS IN COMBINATION WITH CHEMOTHERAPY

Cell cycle-mediated drug resistance is best described as a relative insensitivity to a chemotherapeutic agent due to the position of the cell in the cell cycle, or more precisely, due to the activation of cell cycle checkpoints, that interrupt cell cycle progression to allow time for repair.<sup>109</sup> This is a recently recognized mechanism of resistance to cytotoxic chemotherapy. In combination chemotherapy, for example, one chemotherapeutic agent can impact the cell cycle such that the next chemotherapeutic agent administered immediately in sequence becomes less effective.<sup>109</sup> Cell cycle-mediated drug resistance limits the efficacy of standard cytotoxic drugs and can be overcome by the appropriately scheduled administration of novel CDKIs. Different cytotoxic agents are more effective in certain points during the cell cycle, and may elicit different cell cycle checkpoint responses. In the following sections, we present the preclinical and clinical data that support the use of CDK inhibitors to modulate their cytotoxicity in an effort to overcome cell cycle mediated drug resistance.

#### COMBINATIONS OF TAXANES WITH FLAVOPIRIDOL AND BRYOSTATIN: LABORATORY EVALUATION

The combination of taxanes with either flavopiridol or bryostatin-1 demonstrates the concept of cell cycle-mediated drug resistance. Paclitaxel was examined in combination with flavopiridol in various sequences in the MKN-74

human gastric cancer cell line as well as the human breast cancer cell line MCF-7, which are both wild type for p53.<sup>110</sup> Cell cycle-mediated resistance was demonstrated when flavopiridol exposure was followed by paclitaxel. Flavopiridol's multiple cell cycle effects (including the inhibition of CDK4, CDK6, and CDK2 at G1, and the inhibition of cyclin B1-cdc2 kinase activity at G2<sup>110</sup>) creates a cell cycle arrest. This prevents cells from entering M-phase, the phase during which paclitaxel is most active, and leads to a significant reduction in paclitaxel sensitivity in culture.<sup>110</sup> Similar results were reproduced in vitro and in vivo with flavopiridol in combination with docetaxel.<sup>111</sup> Cotreatment or pretreatment of MKN-74 gastric cancer cells with flavopiridol prevented docetaxel-induced activation of cyclin B1/cdc-2 kinase. Thus, when compared with docetaxel alone when 62% of the docetaxel treated cells were arrested in M phase, pretreatment with flavopiridol prevented these cells from entering the G2 and M phase of the cell cycle. These results were reproduced in vivo. With sequential docetaxel followed by flavopiridol, the reductions in tumor volumes of MKN-74 gastric cancer xenografts were statistically greater than with docetaxel alone. Furthermore, when flavopiridol was administered concomitantly with docetaxel or was administered before docetaxel, the effect was no greater than docetaxel alone.<sup>111</sup> Koutcher et al<sup>84</sup> demonstrated similar effects in vitro with bryostatin-1 followed by paclitaxel in the treatment of human MKN-74 gastric cancer cells and in vivo with a mouse mammary tumor xenograft system.

Cell cycle-mediated drug resistance may be overcome by appropriate sequencing of the drug combination. The reverse sequence of paclitaxel or docetaxel followed by flavopiridol is associated with an increased induction of apoptosis.<sup>110,112</sup> This sequence is associated with an accelerated exit of cells from mitosis, an event that may be critical for the sequence dependent enhancement of paclitaxel induced apoptosis by flavopiridol. In the case of paclitaxel followed by bryostatin-1, there is decreased tumor metabolism and blood flow,<sup>84</sup> which may impact on tumor growth. The increased sensitivity to paclitaxel when followed by bryostatin-1 may be explained in part by Bcl-2:Bax, the heterodimer pair that is closely associated with mitochondrial dysfunction and the initiation of apoptosis. Administration of bryostatin-1 after paclitaxel can overcome paclitaxel resistance in U937 cells ectopically expressing Bcl-x<sub>L</sub>,<sup>113</sup> and is associated with an increase in the proapoptotic factor Bax with resultant increased sequence dependent apoptosis.<sup>114</sup>

#### TAXANE COMBINATIONS WITH FLAVOPIRIDOL AND BRYOSTATIN: CLINICAL EVALUATION

The sequential combination of 175 mg/m<sup>2</sup> of paclitaxel followed by escalating doses flavopiridol administered every 3 weeks has been evaluated in a phase I study.<sup>57</sup> At a

flavopiridol dose of 94 mg/m<sup>2</sup> dose-limiting neutropenia and pulmonary toxicity were observed. Peak mean flavopiridol levels of 400 to 600 nmol/L were observed in this study. The clinical results were remarkable for major responses in patients with chemotherapy refractory malignancies (ie, prostate and esophagus), including patients who have received prior paclitaxel therapy. A phase II study of this combination in patients with paclitaxel refractory esophagus cancer was deemed inactive.<sup>115</sup>

More recently, there have been attempts to change the flavopiridol schedule with taxanes so as to achieve higher peak doses. This approach takes into account that flavopiridol is highly protein bound and that higher peak doses will result in greater free levels of the drug. On the basis of the preclinical data, this was tested in the phase I clinical trial of sequential docetaxel and flavopiridol. In this study, patients were treated with weekly docetaxel at a fixed dose of 35 mg/m<sup>2</sup> followed 4 hours later by escalating doses of flavopiridol administered as a 1 hour infusion, in order to achieve higher peak flavopiridol levels.<sup>116</sup> Flavopiridol could be escalated to 80 mg/m<sup>2</sup>/wk (3 of 4 weeks) without DLT. Pharmacokinetic studies now demonstrated peak flavopiridol levels in the micromolar range (1.3 ± 0.6 μmol/L with 20 mg/m<sup>2</sup> of flavopiridol to 4.1 ± 0.02 μmol/L with 60 mg/m<sup>2</sup> of flavopiridol).<sup>57</sup> In this study, five partial responses (PRs) were observed in patients within pancreatic,<sup>2</sup> breast,<sup>2</sup> and ovarian<sup>1</sup> cancers. In contrast, results from a phase I study in metastatic breast cancer patients were disappointing in that the two agents were difficult to combine because of dose-limiting neutropenia and hypotension.<sup>117</sup> However, in this study, the docetaxel was followed by flavopiridol in 24 hours, and administered as either a 72-hour infusion or by 1-hour bolus infusion, administered daily for 3 consecutive days. Thus, the toxicity of flavopiridol in combination with taxanes appear to be highly schedule dependent.<sup>117</sup>

In a separate trial combining paclitaxel and bryostatin, patients were treated with a weekly dose of paclitaxel 80 mg/m<sup>2</sup> followed 24 hours later with increasing doses of bryostatin-1. The recommended phase II dose was 80 mg/m<sup>2</sup> of paclitaxel followed in 24 hours by 50 μg/m<sup>2</sup> of bryostatin-1.<sup>118</sup> A phase II study of this drug combination was initiated in patients with newly diagnosed metastatic esophageal cancer. In the first nine patients enrolled on the protocol, four patients with partial responses and one with stable disease were noted in first seven assessable patients.<sup>118</sup> However, in the first five patients treated at the recommended dose, there was an unexpected increase in grade 3 and 4 myalgias (in five of five patients) and grade 3 fatigue (in two of five patients) requiring dose reductions in both bryostatin-1 and paclitaxel. Unfortunately, even with dose reductions, progressive worsening myalgias were observed with cumulative dosing, which limited the number of cycles of therapy that could be delivered. A second phase

II trial combining bryostatin-1 and paclitaxel for non-small-cell lung cancer also required dose reductions for myalgia.<sup>119</sup> Thus, myalgias remain a major issue with bryostatin-1 in combination with paclitaxel and this may limit the clinical development of this drug combination. Analogs of bryostatin (“bryologs”) are currently in preclinical development and may have future clinical utility if deemed not to cause significant myalgias.<sup>120</sup>

#### COMBINATIONS OF IRINOTECAN AND FLAVOPIRIDOL: PRECLINICAL AND CLINICAL DEVELOPMENT

Cell cycle-mediated drug resistance has been demonstrated in the human colon cancer cell line HCT-116 (with an intact p53-p21 axis), both with irinotecan alone and with the combination of flavopiridol and irinotecan. The *p21* gene is transcriptionally activated by p53 and is responsible for the p53-dependent checkpoint that results in a G1 and G2 arrest after DNA damage.<sup>46</sup> Treatment of human colon cancer HCT-116 cells with SN-38 alone (the active metabolite of irinotecan) is associated with an induction of p21, and a concomitant G2 arrest, thereby rendering these cells resistant to SN-38 (and therefore to irinotecan), which is 1,000 times more effective in S phase.<sup>121</sup> This cell cycle-mediated drug resistance was demonstrated in culture by quantitative fluorescence microscopy (QFM) analysis, in which SN-38 exposure to HCT-116 cells resulted in only 1% ± 1% cell death.<sup>46</sup>

As a CDKI, flavopiridol itself induces a G1 and G2 cell cycle arrest. Therefore, when flavopiridol precedes irinotecan, cell cycle-mediated drug resistance should again be demonstrated. When HCT-116 cells were exposed to the drug sequence of flavopiridol followed by SN-38, QFM analysis demonstrated only 15% ± 2% cell death. However, this cell cycle-mediated resistance was again overcome by appropriate drug sequencing: SN-38 → flavopiridol resulted in significantly increased HCT-116 cell death at 44% ± 2% ( $P < .001$ ).<sup>46</sup> The augmentation of irinotecan’s antitumor effect in this sequence dependent fashion was confirmed in vivo with HCT-116 xenografts.<sup>46</sup>

The combination of sequential weekly irinotecan followed by flavopiridol has been examined in a phase I clinical trial. On the basis of the best preclinical models, the investigators selected a 7-hour interval between irinotecan and flavopiridol, administered 4 of 6 weeks, with flavopiridol administered as a 1-hour infusion.<sup>122</sup> Fifty-one patients with advanced solid tumors were enrolled onto this protocol. Peak flavopiridol concentrations > 2 μmol/L were observed starting at flavopiridol doses of 50 mg/m<sup>2</sup> on this weekly schedule. PRs were noted in patients with gastric, esophagus, and colorectal cancers and prolonged stable disease (ie, > 6 months) in 36% of all enrolled patients including patients with adrenocortical cancer and hepatocellular cancer, and 52% of patients

with previously treated colorectal cancer.<sup>122</sup> Clinical benefit appeared to be most pronounced in patients whose tumors were wild type for p53. The importance of p53 status to response with this combination therapy has been reviewed recently.<sup>123</sup> The combination of flavopiridol with irinotecan and cisplatin is also being evaluated in a phase I clinical trial.<sup>124</sup>

#### COMBINATIONS OF GEMCITABINE WITH FLAVOPIRIDOL AND UCN-01

The NCI is now sponsoring a phase I clinical trial combining gemcitabine followed by flavopiridol. Flavopiridol was found to potentiate gemcitabine-induced apoptosis in a sequence dependent manner in several epithelial gastrointestinal cell lines.<sup>125</sup> Cells were treated with gemcitabine, flavopiridol, and their combination in varying sequences and schedules. In pancreas, human gastric, and colon cancer cell lines, maximal antitumor effect was observed with the combination of gemcitabine followed by flavopiridol.<sup>125</sup> The reverse sequence of flavopiridol followed by gemcitabine demonstrated no additional antitumor efficacy over that of flavopiridol or gemcitabine alone. Similar results have been reported in the breast cancer cell line MCF-7.<sup>126</sup> This sequence antagonism for flavopiridol followed by gemcitabine is believed to be due to a G1/S arrest induced by flavopiridol before gemcitabine therapy, as demonstrated by a significant reduction of incorporation of [<sup>3</sup>H]Thd into DNA.<sup>125</sup> The synergy for gemcitabine followed by flavopiridol was associated with a reduction in the RNA and protein levels of the M2 subunit of the early S-phase protein, ribonucleotide reductase. Jung et al<sup>125</sup> demonstrated that the addition of flavopiridol to gemcitabine-treated cells is associated with a reduction in the M2 subunit of ribonucleotide reductase, thereby potentiating gemcitabine’s antitumor activity.

The S-phase delay induced by gemcitabine may also play an important role in flavopiridol’s ability to potentiate gemcitabine’s activity.<sup>127,128</sup> Flow cytometry studies demonstrate that the cells at risk for programmed cell death are those in the S phase population, after a G1/S arrest. Deregulated and persistent E2F transcriptional activity in S phase selectively sensitizes many transformed cells to apoptosis. In view of this, inhibition of cyclin A-cdk2 kinase activity during S phase can result in inappropriately persistent E2F-1 activity, which constitutes an apoptotic trigger, especially in transformed cells.<sup>34</sup> It has been demonstrated recently that flavopiridol treatment during S-phase traversal results in persistent expression of E2F-1 and that phosphorylation of E2F-1 is markedly diminished. The response to flavopiridol during S phase is blunted in cells expressing a nonphosphorylatable E2F-1 mutant incapable of binding cyclin A, suggesting that the modulation of E2F-1 activity produced by flavopiridol-mediated CDK inhibition is critical for the apoptotic response of S-phase cells.<sup>129</sup>

Cell cycle arrest plays a critical role in resistance to nucleoside analogs such as cytarabine, fludarabine, and gemcitabine. For example, when primary leukemic cells in the marrow are exposed to cytarabine, a substantial portion of the cells undergo a cell cycle arrest, with resultant termination of DNA synthesis, and accumulation in S phase. Decreasing DNA synthesis results in less cytotoxicity of the nucleoside analog, and resultant protection from cell death.<sup>130,131</sup> *In vitro*, UCN-01 has demonstrated an enhancement of the antitumor efficacy of these analogs, perhaps through G2 cell cycle checkpoint abrogation.<sup>132</sup> A study by Shi et al<sup>131</sup> indicates that gemcitabine increases the percentage of cells arrested in S phase with a significant reduction in DNA synthesis. Subsequent exposure to UCN-01 demonstrated no reactivation of DNA synthesis, but rather an increase in nucleosomal DNA fragmentation, activation of caspase-3, and induction of apoptosis. Therefore, the augmentation of apoptosis by UCN-01 may not require cells to proceed through the S-phase arrest induced by nucleoside analogs, such as gemcitabine. The combination of gemcitabine and UCN-01 is now being tested in a phase I clinical trial for patients with advanced solid tumors (Mayo Clinic, Rochester, MN).

#### NEW TARGETS IN MITOSIS

Additional targets involving the mitotic apparatus are being evaluated presently. Polo-kinase 1 and Aurora kinases are attractive novel targets attacking the mitotic machinery and intimately related to the G2-M transition checkpoint.<sup>133</sup> These proteins are overexpressed in a variety of malignancies, including colon cancer,<sup>134</sup> pancreatic cancer, and non-Hodgkin's lymphoma,<sup>135</sup> and may provide additional options for cancer patients in the future. For proper segregation of DNA and for the activation of the APC, there must be proper binding of kinetochores to tubulin fibers. The chromosome passenger complex (CPC) is found within the kinetochore.<sup>136,137</sup> This complex consists of the inner centromere protein (INCENP) Aurora kinase B and survivin. Through phosphorylation, these proteins are activated enabling the attachment of tubulin fibers to the kinetochores. Inhibition of Aurora kinase B within this complex will impair chromosomal alignment, resulting in abnormal chromosomal segregation, polyploidy and eventual apoptosis. Several inhibitors of Aurora kinases, including those with relative specificity for Aurora B kinase, have been reported and clinical trials with this class of agents are planned.<sup>138,139</sup>

Mammalian cells contain at least four members of the polo-like kinase (Plk) family, termed Plk1, Plk2 (also known as Snk), Plk3 (also known as Prk/Fnk), and Plk4 (also known as Sak).<sup>140</sup> Members of this protein kinase family are characterized by the presence of a conserved C-terminal domain, termed the POLO box, in addition to

the kinase domain. Of these, Plk1 has been the most extensively studied member of the family and is important for numerous aspects of mitotic progression, including centrosome maturation, proper assembly of mitotic spindle, and activation of the APC.<sup>141,142</sup> ONO1910, a small-molecule inhibitor of Plk,1 has been shown to induce mitotic arrest in a wide range of tumor cells, characterized by spindle abnormalities leading to apoptotic death.<sup>143</sup> This compound, which was well tolerated *in vivo*, was found to be a potent inhibitor of tumor growth of human liver (Bel-7402), breast (MDF-7), and pancreatic (MIA PaCa) cancer xenografts. In addition, it enhanced the effect of several chemotherapeutic agents including doxorubicin, oxaliplatin, and gemcitabine. Phase I clinical trials with this agent are now underway.

Mitosis is a dynamic process which involves the function of at least 11 kinesins. These molecular motors are required throughout mitosis. Several members of the kinesin family of microtubule motor proteins are potential targets for the discovery of novel antimitotic cancer therapies. Kinesis spindle motor protein (KSP), also known as Eg5 or kinesin-5, is a kinesin that plays an essential role in the formation of a bipolar mitotic spindle and is required for cell cycle progression through mitosis.<sup>144,145</sup> Inhibition of KSP produces a monopolar spindle with a rosette of condensed mitotic chromosomes attached to a radial array of microtubules. Recently, a potent inhibitor of KSP, CK0106023, has been identified which causes growth inhibition of human tumor cell lines *in vitro* and the human ovarian carcinoma SKOV3 *in vivo*.<sup>146</sup> CK0106023 exhibited antitumor activity comparable with or exceeding that of paclitaxel and caused the formation of monopolar mitotic figures identical to those produced in cultured cells.<sup>146</sup> KSP inhibitors have now entered clinical trial. SB-743921 (GlaxoSmithKline, Philadelphia, PA) is a potent and selective inhibitor of KSP with a Ki of 100 pM. Phase I results indicate that DLTs are prolonged neutropenia and liver function abnormalities at a dose of 8 mg/m<sup>2</sup> with the drug administered as a 60-minute intravenous infusion every 21 days.<sup>147</sup>

#### FUTURE DIRECTIONS

Presently, CDKIs have demonstrated limited clinical activity as single agents in advanced solid tumors. The reason for this is unclear. Many of these single-agent clinical trials have focused on flavopiridol. However, it has become apparent only recently that this drug is highly protein bound and that this may have a significant impact on the biologic activity of the drug. This theoretical approach has been applied recently to the treatment of patients with refractory CLL. In this study, patients with CLL were treated with flavopiridol for 30 minutes, followed by a 4-hour continuous infusion of the drug. This resulted in profound tumor lysis and a dramatic

anticancer effect.<sup>63</sup> This is believed related to inhibition of the CDK complexes that phosphorylate CTD of RNA pol II. This results in transcription suppression of genes that inhibit apoptosis. Thus, with pharmacokinetic modeling it may be possible to make flavopiridol an active single-agent drug, at least in the treatment of hematologic malignancies. Whether this applies to solid tumors, as well, is currently being tested.

Recent data indicate that other CDKIs, such as CYC202, also suppress gene transcription through similar mechanisms.<sup>91-94</sup> This would suggest that drugs we call "CDK inhibitors" may be better termed "inhibitors of transcription." It remains to be determined whether all CDKIs currently in clinical development share this similar property. Nevertheless, this effect of CDKIs on transcription could lead to a rethinking on how best to develop these drugs for cancer therapy.

A series of oral CDKIs have also attracted recent attention. One particular advantage of these oral agents is that may allow sustained daily dosing of the drug. The theoretical advantage of such an approach is that, unless apoptosis can be initiated, sustained growth inhibition by a CDKI appears to require chronic dosing. In fact, drug removal will lead to tumor regrowth. Thus, this next generation of oral drugs may allow continuous oral dosing schedules that are not possible with the current class of intravenous CDKIs. This approach may also require a rethinking of what we consider acceptable clinical trial end points, such that an improvement in time to tumor progression, rather than radiologic response, may be more useful.

This new class of oral drugs is also distinguished by increased specificity for individual CDKs. In view of the specificity, the activity of several of these agents will depend on expression of critical downstream effector molecules that must be present in order to induce cell cycle arrest. In the case of a CDK4 inhibitor, this will require an intact pRb pathway. Thus, patient selection with tumors expressing the appropriate molecular phenotype will be critical in evaluating response to a CDK-specific agent. It remains to be determined whether such specificity offers any advantage over a CDKI, such as flavopiridol, which is a pan-CDKI. In fact, recent studies have indicated that selectively inhibiting CDK2 or CDK4 may not be sufficient to induce cell cycle arrest.<sup>148-150</sup> Rather, growth inhibition may be best achieved when multiple CDKs are inhibited concurrently. In view of the redundancy of these pathways at both the G1/S checkpoint, the concept of less CDK specificity for a CDKI, rather than more specificity, may in fact make the most sense for drug development. Nevertheless, there are certain tumor types in which CDKs are highly amplified such that tumor cell survival appears highly dependent on its expression. For example, microarray studies have indicated that CDK4 is highly amplified dedifferentiated liposarcomas.<sup>151</sup> Thus, in the future, microarray technology may allow us to identify patients with particular

tumor types who are most sensitive to CDK-specific, targeted therapy.

However, it is the combination of CDKIs with standard cytotoxic agents that is emerging as an alternative approach to anticancer therapy. This approach exploits the cell cycle perturbations of malignancy. Preclinical studies demonstrate the concept of cell cycle mediated drug resistance, and suggest that the combination of standard cytotoxic agents with CDKIs will require thoughtful sequencing and scheduling. The importance of drug sequence is not limited to this class of targeted drugs. In fact, many of the "targeted" drugs in clinical trials today have an effect on the cell cycle. For example, 17-AAG, a derivative of geldanamycin, an ansamycin antibiotic,<sup>152</sup> has also been shown suppress Chk1 expression, resulting in an abrogation of S phase and G2 phase checkpoint.<sup>153,154</sup> Thus, similar to UCN-01, 17-AAG will abrogate the G2 cell cycle arrest induced by SN-38, activate cyclin B1/cdc2 kinase, and trigger apoptosis in a sequence-dependent manner (SN-38 followed by 17-AAG).

Activation of the ras/raf pathway activates MAPK, which enhances cell growth by activating cyclinD1/CDK4.<sup>155</sup> Thus, inhibition of any step in the ras/raf/erk/MAPK cascade will induce a cell cycle arrest. This has implications for a drug such as sorafenib (BAY 43-9006), which targets Raf-kinase B and inhibits MAPK.<sup>156</sup> Suberoylanilide hydroxamic acid (SAHA) is part of a class of new targeted drugs that inhibit histone deacetylases (HDACs). SAHA has been shown to upregulate transcriptionally the expression of p21, which induces a cell cycle arrest.<sup>157</sup> Nutlins are a new class of drug that interrupt the binding between mdm2 and p53. In cells with an intact p53 pathway, this will result in stabilization of p53, induction of p21 and cell cycle arrest.<sup>158</sup> Gefitinib (ZD1839) is one of series of new drugs developed to target the epidermal growth factor receptor. However, treatment of tumor cells with this agent induces a G1 cell cycle arrest with down-regulation of the expression and function of CDK2, CDK4, CDK6, cyclin-D1 and cyclin-D3, as well as upregulation of the CDKI p27 (KIP1).<sup>159</sup>

Thus, similar to the drugs clinically developed to, in fact, target the CDKs, each of these new drugs, even though they have different putative targets, have the capabilities of inhibiting the cell cycle and antagonizing the effects of chemotherapy. This assertion has been tested recently with epidermal growth factor receptor inhibitor gefitinib. Since this drug induces a G1 cell cycle arrest, pretreatment with this agent before paclitaxel (an M phase-specific drug) should antagonize the paclitaxel effect. As indicated from the preclinical studies, chronic dosing with low levels of gefitinib resulted in protracted cell cycle arrest, and continuous pretreatment with gefitinib significantly attenuated the effect of paclitaxel in mice bearing breast and lung cancer xenografts.<sup>160</sup> The impact of this on the negative outcome of the randomized phase III trial comparing continuous daily administration of gefitinib with paclitaxel and carboplatin with chemotherapy alone in the treatment of

metastatic lung cancer remains unknown.<sup>161</sup> However, it does raise serious questions that with targeted therapies there may be a need to examine the impact of sequence of drug administration before embarking on large clinical trials with cytotoxic agents.

Therefore, it would appear that an understanding of the cell cycle remains critical for the clinical development of CDKIs, as well as for all targeted agents that indirectly affect the cell cycle, especially when considering combining these agents with chemotherapy. Sequence, dosing and schedule of drug administration must all be taken into account in the

clinical development of these agents. Each of these issues is being addressed in phase I and II clinical trials of CDKIs, both as single agents and in combination with chemotherapy. Although these studies will provide initial evidence of antitumor activity, definitive randomized phase III studies of CDKIs either alone or in combination with chemotherapy will still need to be conducted to determine the impact that this new class of targeted drugs will have in the advancement of cancer therapy.

### Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following author or immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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