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Emerging Drug Profile: Cyclin-Dependent Kinase Inhibitors

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Abstract

As the rational application of targeted therapies in cancer supplants traditional cytotoxic chemotherapy, there is an ever-greater need for a thorough understanding of the complex machinery of the cell and an application of this knowledge to the development of novel therapeutics and combinations of agents. Here, we review the current state of knowledge of the class of targeted agents known as cyclin-dependent kinase (CDK) inhibitors, with a focus on chronic lymphocytic leukemia (CLL). Flavopiridol (alvocidib) is the best studied of the CDK inhibitors, producing a dramatic cytotoxic effect *in vitro* and *in vivo*, with the principal limiting factor of acute tumor lysis. Unfortunately, flavopiridol has a narrow therapeutic window and is relatively non-selective with several off-target (i.e. non-CDK) effects, which prompted development of the second-generation CDK inhibitor dinaciclib. Dinaciclib appears to be both more potent and selective than flavopiridol, with at least an order of magnitude greater therapeutic index, and is currently in phase III clinical trials. In additional to flavopiridol and dinaciclib, we also review the current state of other members of this class, and provide commentary as to the future direction of combination therapy including CDK inhibitors.

Keywords

Pharmacotherapeutics; Chemotherapeutic approaches; Cell cycle and apoptosis changes; Lymphoid Leukemia; Lymphoma and Hodgkin disease

Introduction

It has been recognized for more than twenty years that cyclins are important regulators of cell cycle in both normal and transformed eukaryotic cells [1,2]. In some hematologic malignancies, dysregulation of cyclin (for example, the constitutive expression of cyclin D1 under the control of the immunoglobulin heavy chain promoter in mantle cell lymphoma) is believed to be a primary oncogenic driver, while in other diseases dysfunctional cyclin may be a result of an upstream process. Cyclins in turn regulate so-called cyclin-dependent kinases, which have a variety of function within the cell including and in addition to their eponymous role in the regulation of cell cycle. Because control of cell cycle is an attractive target in anti-cancer therapy, inhibitors of cyclin-dependent kinase (CDK) have been

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explored as a novel therapeutic class. Although control of cell-cycle was a primary motivator for the development of CDK inhibitors, recent investigations into the mechanisms of CDK inhibitor-mediated cytotoxicity have highlighted roles of the cyclins and CDKs unrelated to cell cycle but important in the maintenance of the cancer cell.

CDK inhibitors have been studied in a variety of diseases, but their efficacy has been most appreciated in chronic lymphocytic leukemia (CLL). For this reason the rest of this review will focus primarily on the CDK inhibitors in CLL, with reference made to other leukemias and lymphomas as appropriate.

Cyclins fluctuate with cell cycle and regulate CDKs

The eukaryotic cell cycle results from a complex interplay of extrinsic circulating growth factors, neighborhood anti-growth stimuli, signaling networks, and internal positive and negative regulators of DNA duplication and cell division. Independence of growth factors and insensitivity to anti-growth signals, described by Hanahan and Weinberg as two of the hallmarks of cancer [3], imply unchecked progression through cell cycle checkpoints and consequent loss of control of cell cycle.

The discovery of cyclins, a novel class of proteins whose expression fluctuates regularly inside the cell, provided the first clues about the biochemical workings of the cell cycle [2]. In the normal, non-quiescent eukaryotic cell, the regular rise-and-fall of cyclins is integral to the orderly progression through the cell cycle. Cyclins do not act alone, however, and require the presence of a CDK to do their regulatory work. Typically, each cyclin has one or two CDKs with which it associates, and each CDK has one or two cyclins with which it associates. Cyclins (the regulatory unit) in combination with their partner CDK (the catalytic effector unit) are responsible for phosphorylating other cell cycle regulatory proteins [4]. One of the best-characterized interactions is that of CDK4/Cyclin D with members of the retinoblastoma (Rb) family: Rb proteins sequester E2F family transcription factors, but phosphorylated-Rb dissociates from E2F family transcription factors, thereby permitting transcription [1,5,6]. In this way, CDK4/Cyclin D leads to transcription of Cyclin E, which further represses Rb and advances the cell through mitosis [2]. A partial list of CDKs and their associated cyclins can be found in table 1.

CDKs have a variety of functions

The CDKs are a family of serine/threonine protein kinases that were first discovered to play a key part in the regulation of procession through the cell cycle via phosphorylation and inactivation of Rb, the retinoblastoma tumor suppressor gene product [2,6,7]. It was subsequently discovered that a large variety of cyclins in complex with CDKs were pivotal in all transition points in the cell cycle, including the G_2/M checkpoint. For cancers whose expansion is driven by rapid, out of control proliferation, inhibition of those CDKs that permit progression through the cell cycle is a natural and attractive target, bolstered by *in vitro* evidence that loss of CDK1 and CDK2 activity demonstrably arrests the cell cycle and induces apoptosis in normal and malignant human cells [8–10].

However, not all CDKs control progression throughout the cell cycle: other members of the CDK family take on other roles, and the title *cyclin dependent kinase* then alludes to the cell *cycle* somewhat misleadingly in that these proteins perform in roles seemingly unrelated to cell cycle. For example, CDK5 has a role in neuronal function and tau phosphorylation [11], while CDK7, CDK8, and CDK9 function in regulation of transcription [12,13]. The highly conserved CDKs 7, 8, and 9 play key roles in RNA transcript production through initiation and sustenance of RNA polymerase II (RNAP II) mediated transcription via phosphorylation in the carboxy-terminal domain of RNAP II. While CDK7-effected phosphorylation of

RNAP II permits initiation of transcription, CDK9 and its cyclin partners (T1, T2a, T2b and K) act together to positively promote elongation of existing transcripts [14]. Thus, one strategy in indolent cancers (wherein tumor mass is a result of enhanced survival more so than unchecked proliferation) could be to shut off messenger RNA transcription by inhibition of CDK7 and CDK9. So long as transcription could be repressed, the level of short-lived proteins should then decline. Indeed, anti-apoptotic proteins of the Bcl2 family (Bcl2, Bax, Mcl-1, Bcl-XL, XIAP, and others) are overexpressed in CLL cells, are a putative cause of these cells' extended survival [15,16], and therefore represent a potential target [17–20].

Specific agents

Although a number of CDK inhibitors have entered into clinical trials, by far the most thoroughly studied is the synthetic flavone flavopiridol; consequently the majority of studies reviewed here involve flavopiridol. More recently, the newer CDK inhibitor dinaciclib appears promising and has entered phase II and III clinical trials. Other compounds are in clinical development, and some of these are surveyed briefly. A partial listing of CDK inhibitors under investigation is included in table 2.

Flavopiridol

Flavopiridol is a semi-synthetic flavone derived from rohitukine alkaloid extracts of *Dysoxylum binectariferum*, a tree native to India and closely related to the Ayurvedic plant *D. malabaricum*, used for the treatment of rheumatoid arthritis [21]. First described as a potential anti-neoplastic agent in 1992, it was initially thought to be cytostatic only, inducing cell-cycle arrest in dividing cells through competitive inhibition of CDK2, but later experiments confirmed the ability to induce apoptosis in quiescent cells [5,22–24]. Subsequent investigations expanded the known spectrum of flavopiridol's activity: CDK1, CDK2, and CDK4 were all confirmed to be inhibited by competitive binding at the ATP-binding pocket [5,22,25]. Flavopiridol also appears to inhibit CDK9, and consequently transcription, through similar ATP-competitive inhibition [26–28].

In preclinical studies, it was quickly recognized that flavopiridol had activity in human tumor cell-lines, and B-cells in particular [29–32]; early phase clinical trials in humans soon followed. Among the CDK inhibitors, flavopiridol is heretofore the most comprehensively studied, and of the diseases in which it has been fielded the most promising work has been in the area of CLL [33], likely due to a few particular biologic effects of CDK inhibition which are relevant to the oncogenic underpinnings of the CLL cell.

Early work with flavopiridol in human tumor cell lines demonstrated drug-mediated cell-cycle arrest and apoptosis [34], but even more remarkably flavopiridol also seemed to induce apoptosis in non-cycling (i.e. G_{0-1} phase) cancer cells [23,24]. In distinction to other leukemias in which the primary problem is rapid, out-of-control proliferation, in CLL a large fraction of the numerous lymphocytes are not actively cycling; the principal driver of the increase in tumor mass is instead believed to be cells' evasion of apoptosis. Flavopiridol, demonstrably capable of inducing apoptosis in non-cycling tumor cells thus seemed an ideal agent to try in CLL. Early studies by several groups demonstrated that despite the non-proliferating nature of CLL cells, flavopiridol mediated robust early apoptosis *in vitro* whereas the effect of this agent against normal T-cells was minimal [29–32,35,36]. Additionally, flavopiridol favorably down-modulated several anti-apoptotic Bcl-2 family proteins and promoted cell death independent of p53. The induction of p53-independent apoptosis strengthened flavopiridol's importance in CLL, a disease in which the complete lack of p53 (the *del(17p)* cytogenetic abnormality) portends particularly poorly [37,38]. Subsequent studies by Chen and Plunkett [39] demonstrated that flavopiridol also inhibits

CDK9 in CLL cells, in turn decreasing phosphorylation of RNA polymerase II, ultimately depleting several short half-life proteins such as Mcl-1 that protect CLL cells from apoptosis (figure 2). Additionally, recent studies with flavopiridol by our group have demonstrated this agent promotes reduction of mitochondrial oxygen consumption and autophagy [40]. Detailed interrogation of autophagy in a subsequent study demonstrated this process to actually be a protective mechanism against flavopiridol-mediated death [41]. Notably in this study we were able to demonstrate that flavopiridol also promoted robust endoplasmic reticulum (ER) stress with downstream activation of ASK1 and caspase 4; inhibition of caspase 4 as well as siRNA knock down of ASK1 prevented apoptosis mediated by flavopiridol. Documentation of activation of ER stress *in vivo* with flavopiridol in this paper further supported this as a very relevant new mechanism of action of flavopiridol mediated cell death in CLL. Further study of this novel mechanism of action is underway at this time with both flavopiridol and other second-generation inhibitors.

Flavopiridol clinical trials

In the first phase I clinical trial to be published, Senderowicz and colleagues reported on flavopiridol given as a continuous intravenous infusion (CIVI) for 72 hours every 2 weeks in seventy-six patients with advanced refractory neoplasms [42]. The initial dose-limiting toxicity was diarrhea, and the maximum tolerated dose (MTD) without antidiarrheal prophylaxis was 50 mg/m^2/d × 3 days. However, when cholestyramine and loperamide were used as antidiarrheal prophylaxis an MTD of 78 mg/m^2/d × 3 days was attainable. Because of the relative frequency of diarrhea in early studies, and because a mean flavopiridol concentration 271 nmol/L (within the range of efficacy in earlier *in vitro* studies [29]) was achieved in the 50 mg/m^2 group, this was put forward as the recommended phase 2 dose. Unfortunately, several subsequent phase II studies using this dose and schedule failed to demonstrate clinical benefit in patients with a variety of relapsed or refractory cancers, including mantle cell lymphoma [43–47]. Similarly, in CLL, no clinical efficacy was found in phase II clinical trials of either a 24-hour (80 mg/m^2) or 72-hour CIVI of flavopiridol [48,49]. However, a second arm in the CALGB 19805 study used a 1-hour bolus of 50 mg/m^2 daily for 3 days based upon leukemia xenograft studies showing benefit to this new schedule [31], and was able to show modest benefit: 11% partial response and 53% stable disease, compared to zero and 27%, respectively, in the 72-hour CIVI arm of the same trial [49].

In light of these disappointments, and in the context of earlier experiments showing markedly differential binding of free drug to human plasma proteins compared to the fetal calf serum typically used in *in vitro* studies [50], estimates of the 1-hour and 24-hour LC_{50} for flavopiridol were revised markedly higher: 3510 nmol/L and 470 nmol/L, respectively– levels that were definitely not achieved with current dosing strategies. The earlier failures were thus attributed to failure to achieve a therapeutic drug concentration with the 72-hour CIVI strategy.

With the foregoing in mind and with a goal to achieve biologically relevant drug concentration *in vivo*, our group used pharmacokinetic (PK) modeling to design a dosing schedule wherein a 30-minute loading dose was followed by a 4 hour infusion of flavopiridol every week for 4 of 6 weeks, with a target plasma concentration of > 1.5 μmol/L [36]. Fifty-two patients (43 with relapsed CLL and 9 with relapsed small lymphocytic lymphoma, SLL) in four cohorts were enrolled on a phase I study using this strategy with tolerability and PK data as endpoints [36,51]. In cohorts three and four, intrapatient dose escalation was permitted on cycle 2 day 1 and cycle 1 day 8, respectively. Retreatment of previous responders was permitted; six with a response to initial study therapy were retreated after progression of disease. Overall, the pretreatment characteristics portended

poorly: at enrollment, forty-two (81%) of patients were Rai stage III or IV, the median number of prior therapies was 4, fifty-one (98%) had been exposed to fludarabine with forty-three (83%) of them being refractory to their latest fludarabine, thirty-eight (73%) had bulky lymphadenopathy, and forty patients (77%) had high risk cytogenetic features.

Eclipsing the previously described diarrhea as the most common grade 3 toxicity was acute tumor lysis syndrome (TLS), which was a dose limiting toxicity in the second dose level resulting in death of a patient due to hyperkalemia prior to the time dialysis could be initiated. This led to temporary study suspension and subsequent introduction of aggressive early monitoring as well as assurance of ability to initiate dialysis within 60 minutes of appreciation of hyper-acute tumor lysis. Through the remaining portion of the study, TLS was observed to some degree in 30 patients (57%). With application of stepped up dosing, aggressive prophylaxis, monitoring, and exclusion of patients with white blood cell count (WBC) greater than $200\times10^3/\mu L$, the drug could then safely be administered with close monitoring. Other common toxicities included diarrhea, transient transaminitis, and an interesting constellation of toxicities including fatigue/malaise, fever, and local tumor pain that was subsequently characterized as a cytokine release syndrome (CRS) [52].

PK analysis of 51 patients was performed in this study with a primary endpoint to reach and sustain a plasma concentration of greater than or equal to 1.5 μmol/L for the duration of the 4-hour infusion. Forty-five patients achieved or exceeded the target peak concentration, but only 20 maintained this concentration for ≥ 4 hours. Across response groups (PR vs. SD vs. PD), only area under the curve (AUC) was statistically significant at the $p \leq 0.05$ level, although other PK parameters (C_{max}, CL) showed a trend toward significance.

Impressively, of 52 patients receiving therapy, 21 (40%) achieved a PR with an overall median progression free survival (PFS) of 12 months. Two additional patients with WBC > $200\times10^3/\mu L$ had 89 and 98% drops in white count, but were excluded from the responders due to having only received one cycle of therapy. Although all 21 patients eventually relapsed, 6 were re-treated, with an encouraging 5/6 achieving another PR which was itself relatively durable: the median PFS after re-treatment was another 10 months (range 6.5–19.1).

Encouraged by these findings, our group conducted a phase II trial of flavopiridol in patients with relapsed or refractory CLL [53]. The previously successful bolus plus infusion strategy was used at an initial dose of 30 mg/m^2 IV bolus + 30 mg/m^2 CIVI × 4 hours for the first dose, with escalation of the infusional dose to 50 mg/m^2 CIVI × 4 hours for the second and subsequent doses if the patient did not experience severe TLS on cycle 1 day 1. Sixty-four patients enrolled and the overall patient characteristics were similar to the phase I trial with respect to number of prior therapies, exposure to purine nucleoside analogues, and bulky lymphadenopathy. With vigilance for TLS and the enrollment criterion that WBC be fewer than $200\times10^3/\mu L$, only 3 patients needed hemodialysis. As with prior studies, cytokine release syndrome was a common feature until 20 mg of IV dexamethasone prior to flavopiridol was instituted as a preventative measure. With this intervention, the rate of CRS decreased from 56 to 22%. In addition to dexamethasone prophylaxis, the study was amended to increase tolerability by shortening the cycle length from six weeks to four, and decreasing the number of weekly treatments from four to three. Pegfilgrastim was added in the third week. Overall response rate in this study compared favorably with the previous phase I trial: 34 patients (53%), with 30 (47%) PRs, 3 (5%) nodular PRs, and 1 CR. Seven of the 34 responders (21%) were able to proceed to allogeneic stem cell transplant due to a reduction of bulky lymphadenopathy. Median PFS for all patients was 8.6 months, for responders 12 months, and 10 months for those responders who did not go on to receive transplant. Historically poor-risk predictors, including del(17p13.1), del(11q22.3), complex

karyotype, elevated β_2-microglobulin, and bulky lymphadenopathy were not predictive in this study: responses (the primary endpoint) as well as the secondary endpoint PFS were similar irrespective of high-risk features. Indeed, a majority of the high-risk patients experienced a response. This flavopiridol trial demonstrated not only efficacy, but also that with appropriate monitoring and prophylaxis, TLS can be prevented or managed, and that with dexamethasone premedication, the CRS symptoms can be ameliorated.

Because CLL is a disease of older adults (with a median age of 72 at diagnosis), any new therapy would ideally be well tolerated in the elderly, yet these patients remain poorly represented in cancer clinical trials. We sought to know how older patients with CLL fared in the previous two trials [54]. Retrospective review of data from these two trials demonstrated that among patients seventy and older (21% of a total of 116 patients) there was no difference in response rate or PFS when compared to patients under seventy years. Overall survival was slightly worse at 2.1 years versus 2.4 years, but this difference disappeared when adjusted for other factors (for example, older patients were more likely to have a complex karyotype). Unlike fludarabine [55], flavopiridol appears safe and effective in older adults.

On the basis of this positive single-center experience, a large international multi-center study of flavopiridol in relapsed and refractory CLL was initiated and accrued 165 patients. A preplanned interim analysis of the data was presented at the American Society of Hematology (ASH) 2010 annual meeting [56] with 68 patients (41% of planned accruals) having completed at least two cycles. Using the rationally designed “bolus plus infusion” strategy given weekly for four weeks followed by a two week break, investigators were able to show as much as 31% objective response rate, though all responses were PR. Only nine percent of patients progressed. Among the responders, the responses were again durable: median PFS was about one year (12.2 months). Sixteen and twenty evaluable patients had del(17p13.1) and del(11q22.3), respectively, and objective responses were as high as 25% and 30% in these two groups. Patients with bulky lymphadenopathy (about two thirds of evaluable patients) also benefitted, with 32–39% (depending on criteria) of patients responding. Toxicities were similar to previous trials, and CRS and TLS were effectively ameliorated with corticosteroids and prompt hemodialysis. The response noted in this study matches that observed by OSU investigators using this non-optimized schedule of administration (i.e., four weekly doses in a six week cycle). Further efforts to develop flavopiridol as a monotherapy in CLL should utilize the three-week schedule with steroid prophylaxis identified in our phase II study.

Flavopiridol in combination

Based upon the observation that flavopiridol downregulates Mcl-1 [35], an antiapoptotic protein responsible in part for fludarabine and rituximab resistance, Lin et al. in a phase I trial combined flavopiridol with fludarabine and rituximab for patients with mantle cell lymphoma (MCL) and indolent B-cell malignancies [57]. Fludarabine and Rituximab were given in the standard fashion (fludarabine 25 mg/m^2 daily for five days and rituximab 375 mg/m^2 on day 1, on a 28-day cycle) with the addition of flavopiridol in one of four schedules, two of which were of the bolus plus CIVI type as described above. Of a total of 38 patients (10 with MCL, 9 with follicular lymphoma, 4 with marginal zone lymphoma, 1 with lymphoplasmacytic lymphoma, 3 with SLL, and 11 with CLL), 31 (82%) had a response, while 21 (55%) had a complete response; responses were independent of prior treatment. Median progression free survival was relatively durable at 25.6 months.

A shown in preclinical studies, flavopiridol exerts its lymphocyte killing effect in a manner independent of p53 status. Because alterations in *TP53* play an important role in the

outcomes of B-cell leukemias and lymphomas [37,58], the combination of flavopiridol with other agents that act independently of p53 has theoretical therapeutic potential. The immunomodulator lenalidomide, though incompletely understood, may enhance T-cell mediated killing of malignant B lymphocytes and has shown promise in heavily pretreated CLL patients with adverse prognostic features [59]. To test its synergy with flavopiridol, we conducted a phase I study of flavopiridol and lenalidomide in patients with relapsed CLL [59]. Weekly flavopiridol bolus plus infusion was combined with escalating doses of daily lenalidomide each for three weeks with a two-week break (cycle length 35 days). Data were last reported at the 2011 ASH annual meeting. At that time, 23 patients had completed one or more cycles of therapy and were evaluable. A PR was observed in 13 patients (57%), among them 7 with del(17p13.1) and 6 with del(11q22.3), 9 with complex cytogenetics, 5 who were fludarabine-refractory, and 6 with bulky lymphadenopathy. Six patients were able to proceed to transplant, and four of them remain in remission. Median PFS was 7 months (with a range of up to 24 months) and median OS was 23 months. Toxicities were manageable, with only two patients requiring hemodialysis and there was minimal tumor flare with steroid prophylaxis. Flavopiridol and lenalidomide appear to be a safe and effective combination that should be explored in larger studies.

Bortezomib, a proteasome inhibitor, is another novel therapy that has shown activity in a variety of hematologic cancers, presumably through inhibition of over-expressed and constitutively activated cell cycle regulators and transcription factors, including cyclins, Bcl-2 family proteins, and NF-κB. The combination of bortezomib with CDK inhibitor has been studied and has shown *in vitro* activity in both lymphoid and myeloid cell lines with (among other effects) diminished expression of Bcl-2 family proteins and decreased Akt and STAT family activity [60,61]. Early results in humans have been equally as promising: results of a phase I trial of bortezomib in combination with flavopiridol in patients with Non-Hodgkin Lymphoma (NHL; 9 patients) and plasma cell dyscrasias (7 patients) were notable for a number of complete responses (2/16; 12%) as well as PR (5/16, 31%), all at the MTD [62]. As this was a phase I dose-finding study, future study is warranted.

Flavopiridol pharmacokinetics

Average peak serum concentration of 271 nmol/L was achieved in early studies of a 50 mg/m2 given as a 72-hour continuous infusion. Although this was above the predicted LC50 for flavopiridol, lack of clinical response prompted further investigation which revealed that differential drug binding to fetal calf serum versus human serum meant that actual LC50 for flavopiridol was much higher. With a target of 1500 nmol/L (1.5 μmol/L) sustained for four hours, a bolus plus infusion strategy was designed. With this new strategy, serum drug concentrations easily exceeded this target (AUC range 4.51 to 31.4 μM/h), even at the lower dose levels [36].

Flavopiridol resistance

Although traditional risk factors do not predict response to flavopiridol, and although initial response is often brisk, patients do relapse. As a class of drugs, mechanisms of resistance to CDK inhibitors have so-far been poorly described, although in 2012 our group described autophagy as a novel mechanism of resistance for flavopiridol [41]. Future studies are warranted, especially in the typically very refractory population of flavopiridol patients.

Flavopiridol looking forward

Flavopiridol showed early promise as a single agent, but the paucity of complete responses prompted a search for combinations to increase its effectiveness. Combination both with traditional cytotoxic chemoimmunotherapy as well as with other novel agents has

demonstrated the ability to produce CR in patients even with very advanced disease, with the caveat that there seems to be a fine line between ineffectiveness and life-threatening tumor lysis. Flavopiridol's strengths and limitations are informing the design of future drugs and therapeutic combinations.

Dinaciclib

Studies with flavopiridol have been encouraging, but limited due in part to the narrow therapeutic window. Efforts to overcome this limitation and develop a CDK inhibitor with a broader therapeutic index have seen early success in dinaciclib (SCH-727965, later MK-7965), a compound developed using a traditional drug-screening program with permutations on a basic scaffold assessed for tumor response in an *in vivo* model with simultaneous calculation of the therapeutic index, taking flavopiridol as a benchmark [63]. The result of these efforts is a newer potent and selective inhibitor of CDKs with a therapeutic index in initial mouse studies more than ten times that of flavopiridol [63]. Although dinaciclib is specific for CDKs (whereas flavopiridol affects a broader range of serine/threonine and tyrosine kinases), there is considerable structural homology in the CDK family and for that reason dinaciclib, like other current-generation CDK inhibitors, exerts its effects through a specific combination (likely different from drug-to-drug) upon various CDKs; in this case, inhibition of CDKs 1, 2, 5, and 9 with IC_{50} of 3, 1, 1, and 4 nmol/L, respectively [64].

Dinaciclib, like flavopiridol, induces apoptosis in CLL cells irrespective of traditionally negative prognostic factors such as prior fludarabine exposure, unmutated IGHV, or del(17p13.1) status [65]. This is tremendously advantageous, as discussed above under flavopiridol. Dinaciclib-mediated cytotoxicity is not inhibited by cytokines which otherwise prevent spontaneous apoptosis in CLL cells: CD40 ligand, BAFF, TNF-alpha, and IL-4. Overcoming these survival signals seems to be through down-regulation of the respective receptors. Dinaciclib is ineffective, however, at overcoming the protective effect of direct contact of CLL cells with stromal cells. Notably, the PI3K inhibitor PIK-75 (an alpha isoform specific inhibitor) is able to abrogate the protective effect of the stromal environment in dinaciclib-treated CLL cells, suggesting a potential rational combination of targeted therapies [65].

Dosing and administration

Two alternative schedules have been described with dinaciclib. First, a 2-hour I.V. infusion on days 1, 8, and 15 of a 28-day cycle was explored in phase I studies with expansion into a phase II study of patients with indolent and large cell lymphomas [66]; the recommended phase II dose in this case was 12 mg/m^2, although two patients (12.5%) required de-escalation to 10 mg/m^2 because of toxicity [66]. The schedule has been used successfully in CLL patients, although in this case the maximally tolerated dose was 14 mg/m^2 [67]. Second, a much larger dose (up to 50 mg/m^2) given by 2-hour I.V. infusion once every 21 days has been used in acute leukemia. This 50 mg/m^2 dose and 21-day schedule is based on safety and tolerability data from a trial of dinaciclib in 81 patients with advanced malignancies, including solid tumors [68]. Interestingly, this study also explored dose-limiting toxicities in extended 8-hour and 24-hour infusions, finding acceptable safety profile at 7.4 and 10.4 mg/m^2 for 8- and 24-hour infusions, respectively, which may have relevance in light of data regarding transience of Mcl-1 inhibition, described below.

Clinical trials in CLL

Although dinaciclib has entered into phase II clinical trials for CLL, these data have yet to be published. An update on the phase I study of dinaciclib in patients with relapsed or

refractory CLL made at the 2011 ASCO annual meeting reported on a total of 33 patients in five dose cohorts, 16 of whom were enrolled as an expansion cohort at the maximally tolerated dose of 14 mg/m^2 [69]. Partial response by international working-group criteria [70] was observed in 15 of 33 patients (45%), fourteen of whom had prior fludarabine exposure. Fifteen patients had del(17p13.1), and seven (47%) achieved PR. Of all those who did respond, responses were observed at all dose levels and both early (after 2 cycles) as well as late (after 6 cycles) in treatment. When considering the sixteen patients who received the maximally tolerated dose (the recommended phase 2 dose) of 14 mg/m^2, the PR rate increased to 62.5%. Among all thirty-three patients, twenty-four remained on study at the time of analysis with a follow up period ranging from 3 to 73 weeks. The median PFS was not yet reached. In addition, the majority of those with active, proliferative disease at the time they began therapy experienced stability of disease while on treatment. As expected, 25 of 27 patients with correlatives performed in this study had a sharp decline in the level of the antiapoptotic protein Mcl-1. The half-life ($t_{1/2}$) of the drug was calculated as 2.1 to 3.8 hours. Toxicities echoed strongly those of flavopiridol, including diarrhea and hematologic toxicity manifested in all cell lines (in one case perhaps contributing to a DLT of bacterial pneumonia); most notably five patients experienced TLS, two of whom required dialysis (one each at the MTD of 14 mg/m^2 and the maximum administered dose of 17 mg/m^2). Overall, this study provided a good rationale for future clinical trials of dinaciclib in CLL, especially in patients with prior fludarabine exposure or del(17p13.1), as neither of those traditionally poor-risk factors seemed to influence response.

Clinical trials in lymphoma

A phase II clinical trial of dinaciclib in sixteen patients with indolent and large cell lymphoma utilized the 12 mg/m^2 dose and days 1, 8, and 15 every 28 days schedule established in phase I trials of solid tumor patients [66]. Four patients (25%) have had measurable activity: one patient with diffuse large B-cell lymphoma experienced a partial response good enough to proceed to autologous hematopoietic cell transplant, while three patients experienced activity not meeting criteria for partial response by decrease in size of nodes. The commonest severe toxicities were hematologic.

On the basis of the foregoing positive results, dinaciclib as a single agent is now in phase III clinical trials in patients with refractory CLL and SLL and will be compared in a randomized fashion with ofatumumab. Earlier phase studies of dinaciclib in combination therapy are ongoing.

Other agents

A partial list of cyclin-dependent kinase inhibitors is given in table 2, along with their structures and current status. We will survey briefly some of these drugs, bearing in mind that at this time few agents apart from flavopiridol and dinaciclib have undergone much scrutiny in human trials. Information about the current status of clinical trials was gathered from http://clinicaltrials.gov and https://www.clinicaltrialsregister.eu/.

BAY-1000394 is an oral pan-CDK inhibitor which has shown activity against human tumor xenografts in mice, and has now entered early phase clinical trials [71].

P276-00, like flavopiridol, is a synthetic flavone which inhibits CDKs, but with less off-target inhibitor effect on non-CDK kinases, and with particular selectivity for the CDK4/Cyclin D1, CDK1/Cyclin B, and CDK9/Cyclin T1 complexes [20,72–74]. Preclinical studies of P276-00 have demonstrated decrease in phosphorylated Rb (pRb), seemingly confirming CDK4/Cyclin D1 inhibition, and these observations have formed the basis for 10 clinical trials in Cyclin D1-driven or expressing solid tumors and hematologic cancers, five

of which remain active. An early report suggests safety and tolerability are good, achieving concentrations adequate for target inhibition [75].

R547 is a selective inhibitor of CDK1, CDK2, and CDK4 at physiologically relevant doses, and results from a single phase Ia clinical trial in patients with advanced cancers was reported at the 2007 American Society of Clinical Oncology (ASCO) annual meeting [76–78]. This study demonstrated drug concentrations predictive of efficacy in preclinical models, with mostly mild, non-hematologic toxicities. Enthusiasm for the compound has been limited, however, and no additional studies have been registered or reported.

Seliciclib (CYC-202, R-roscovitine) is an oral purine analogue which inhibits CDK2 strongly (mediated by ATP-competitive binding), as well as a number of other CDKs with less potency, including CDK1, CDK5, and CDK7 [79–82]. Roscovitine has shown activity *in vitro* against a number of human tumor cell lines with evidence of death by apoptosis [83,84]. As CLL cells are dependent upon constitutive expression of anti-apoptotic proteins, this provides an attractive target. In a preclinical study of roscovitine, Hahntow and colleagues demonstrated caspase-mediated apoptosis in 21 of 28 CLL samples [85]. As a potential explanation for this apoptosis, the anti-apoptotic proteins Mcl-1 and X-linked Inhibitor of Apoptosis (XIAP), but not Bcl-2, were down regulated. Relevant clinically, apoptosis was induced irrespective of Zap-70 status, and at concentrations that did not kill normal B-lymphocytes.

Seliciclib (the R-isomer of roscovitine) was further tested against a panel of 26 CLL samples (11 with mutations in either *ATM* or *TP53*) with similar results [86]. Within 24 hours, all 26 samples exposed to CYC-202 had experienced apoptosis, independent of p53. Down regulation of Mcl-1, but not Bcl-2 was observed in line with previous studies and probably due to the latter's longer half-life. They further demonstrated that the decrease in the level of transcripts was due to decrease in activation of RNAP II, providing indirect evidence that seliciclib inhibits the CDK9/Cyclin T complex.

Two phase I clinical trials of seliciclib in advanced malignancy have been published [87,88]. Although in one study, dose-limiting toxicities (DLTs) were seen at 800 mg b.i.d., in the other the use of alternative dosing schedules permitted doses as high as 1600 mg twice daily for 3 days every two weeks. Interestingly, the high-grade toxicities were similar, consisting of hyponatremia and hypokalemia, and in each study there was a single case of reversible acute kidney injury. Of 77 total patients, there was one partial response (hepatocellular carcinoma) and 14 patients experienced stabilization of disease. Despite the early promising results in clinical and preclinical studies, however, two phase II trials of seliciclib in solid tumors have been terminated and future development remains uncertain.

SNS-032 (formerly BMS-387032) selectively inhibits CDK2, CDK7, and CDK9, with far less activity against CDK1 and CDK4, as well as a panel of other kinases [89,90]. As with other inhibitors of CDK7 and CDK9, the mechanism of action appears to be down-regulation of short-lived members of the Bcl-2 family including Mcl-1 and XIAP, which is of particular interest in CLL as it has been shown that CLL cells are dependent on consistent expression of antiapoptotic signals to prevent cell death [91–93]. SNS-032 has completed phase I trials in solid tumors and hematologic malignancies [94]. In a trial with 37 patients (19 with CLL), there was limited evidence of efficacy, although this may have been limited by infusion duration or a schedule that was modeled after tumor cell lines as opposed to primary CLL cells. As with flavopiridol, tumor lysis syndrome was the dose-limiting toxicity (DLT) in CLL patients, and other grade 3/4 toxicities were almost entirely hematologic.

TG02 (SB1317) is an orally bioavailable multi-kinase inhibitor which inhibits CDK1, 2, 7, and 9 as well as FLT3, JAK2 in an equipotent fashion [95,96]. TG02 also inhibits other kinases, including ERK5, with lesser potency. It is hoped that this multi-kinase activity will provide benefit beyond less broad inhibitors (e.g. sunitinib), and preclinical studies have shown excellent activity in acute myeloid leukemia blasts [96,97]. It is currently in phase I trials for ALL, AML, CLL, myeloma, and myelodysplastic syndrome [98].

Concluding remarks and future directions

The cyclin dependent kinase inhibitors flavopiridol and dinaciclib have shown remarkable promise *in vitro* as well as in early phase human trials, both alone and in combination with other agents. As a class, CDK inhibitors all seem to induce apoptosis of B lymphocytes, but misleadingly not necessarily in a way related to the cell-cycle from which they derive their name. As we have described here, the differential efficacy and toxicity profile of the agents in this class likely stems from each agent's unique combination of inhibition of protein kinases, and CDKs in particular.

As single agents, CDK inhibitors are unlikely to produce prolonged remissions, but their side effect of rapid tumor de-bulking may find utility in bridging otherwise refractory patients to stem-cell transplantation or other therapy. Ultimately, a better understanding of mechanisms of action on a cellular basis will provide a framework for rational combination of targeted therapies in future clinical trials. As with trials of other agents, both patients and science will benefit when eligibility for trials is determined according to the molecular mechanisms of each individual's tumor, rather than a broad disease grouping. Further, future clinical trials have the opportunity to explore alternative dose plans (for example, stepped-up dosing to ameliorate the effects of tumor lysis) and infusion schedules (in the case of dinaciclib, the anti Mcl-1 effect has been shown to disappear by 24 hours in malignant lymphoid blasts [99]). Alternatively, different delivery vehicles have the potential to prolong the exposure of tumor cells to the active agent: a liposomal formulation of flavopiridol has been developed with the goal of increased half-life, AUC, and consequently efficacy [100]. In addition to combinations of CDK inhibitors with traditional cytotoxic agents, future trials should explore rational combinations of therapy suggested by our ever-better understanding of cell signaling, mRNA transcription, and the specific inhibition patterns of individual agents. Finally, because even as recently as 2012 an entirely new mechanism of action for flavopiridol has been discovered [41], we must remain attuned to the possibility that we will question existing mechanisms or discover new ones in this remarkable and promising class.

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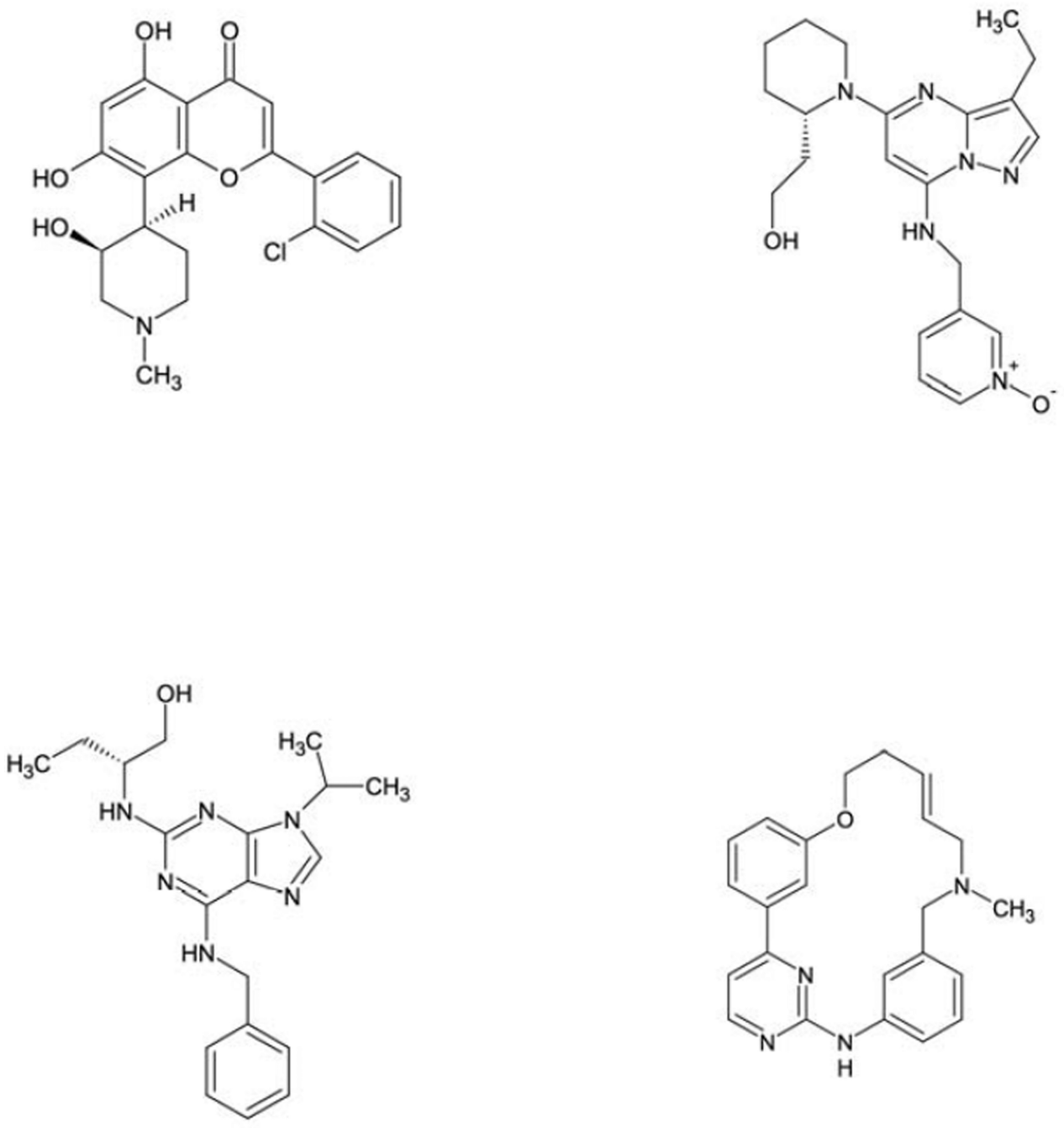

Figure 1.
Molecular structures of representative cyclin-dependent kinase inhibitors alvocidib (flavopiridol; A), dinaciclib (B), seliciclib (C), and TG02 (D).

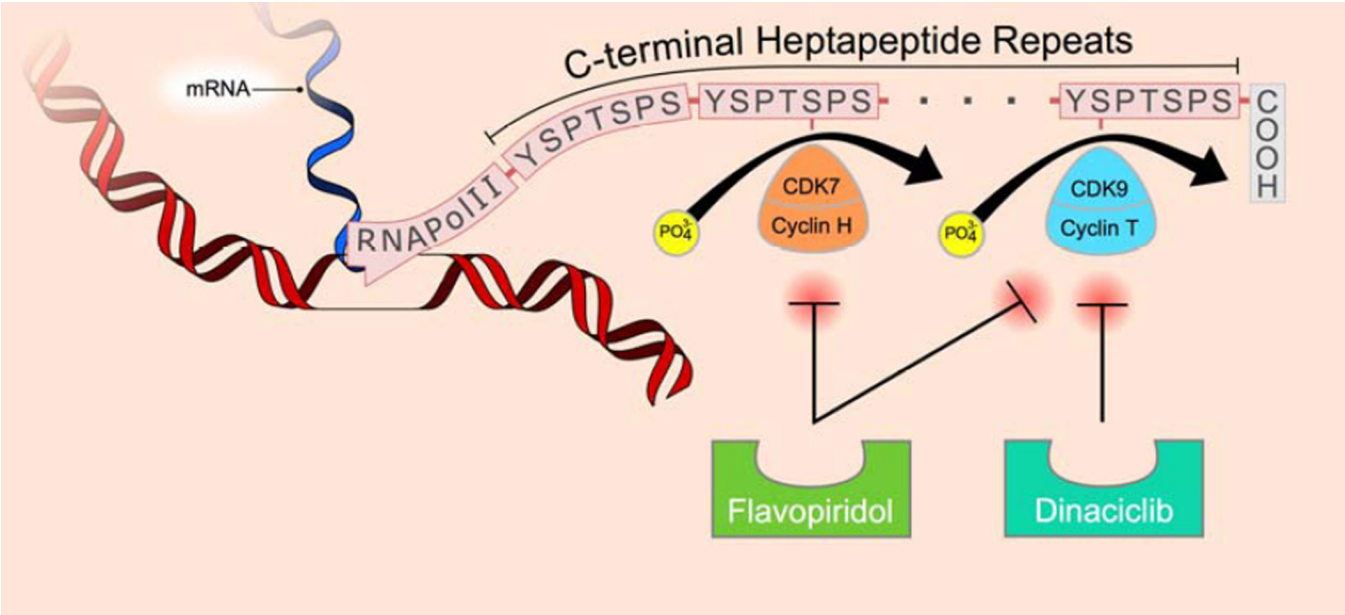

Figure 2.

Pro-survival (anti-apoptotic) mediators such as Mcl-1 have a short half-life and are actively transcribed in some cancer cells by RNA polymerase II, which requires phosphorylation at serine 2 and serine 5 within the heptapeptide repeats that comprise its C-terminal domain (CTD). Cyclin-dependent kinases 7 and 9 phosphorylate these serines, but can be inhibited by cyclin-dependent kinase inhibitors, ultimately leading to decreased levels of anti-apoptotic proteins and therefore increased cell death.

Table 1

Selected CDK/cyclin targets.

CDK	Cyclin Partner(s)	Function
CDK1	Cyclin A, B	G_2 phase; M phase
CDK2	Cyclin A, E	S phase
CDK4	Cyclin D*	G_1 phase
CDK5	Cyclin D*, E, G	Senescence
CDK6	Cyclin D*	G_1 phase
CDK7	Cyclin H	Transcription (RNAP II) initiation
CDK8	Cyclin C	Mediator complex; Beta-catenin modulation
CDK9	Cyclin T**, K	Transcription (RNAP II) elongation

*human cyclin D takes three isoforms: D1, D2, D3

**human cyclin T isoforms T1, T2a, and T2b

Table 2

Cyclin dependent kinase inhibitors in clinical development.

Agent (alternative names)	Targets	Status*
Alvocidib (flavopiridol, HMR-1275, L86-8275, NSC-649890)	CDKs 1,2,4,6,7,9	Phase II
BAY-1000394	CDKs 1 ?,7,9	Phase I
Dinaciclib (MK-7965, SCH-727965)	CDKs 1,2,5,9	Phase III
P276-00	CDKs 1,4,9	Phase II
R547	CDKs 1,2,4	Phase I completed
Seliciclib (R-roscovitine, CYC-202)	CDKs 1,2,5,7	Phase II terminated
SNS-032 (formerly BMS-387032)	CDKs 2,7,9	Phase I completed
TG02	CDKs 1,2,7,9 FLT3, JAK2	Phase I

*based on trials registered at http://clinicaltrials.gov and https://www.clinicaltrialsregister.eu/

Table 3

Quick Profile: Alvocidib (Flavopiridol)

Drug name	**Alvocidib**
Company	Sanofi-Aventis, Paris, France
Other names	Flavopiridol, HMR-1275, L86-8275, NSC-649890
MoA	Inhibition of CDKs 1, 2, 4, 6, 7, and 9
MoR	Autophagy
MTD	80 mg/m^2 when given by bolus plus infusion 78 mg/m^2/day when given as 72-hour infusion
DLT	Acute tumor lysis, cytokine release syndrome, and diarrhea
Schedule	Alvocidib days 1, 8, 15 on 28-day cycle Cycle 1: 30 mg/m^2 by 30-minute IV bolus, followed by 30 mg/m^2 by 4-hour CIVI If no TLS, escalate to Cycle 2: 30 mg/m^2 by 30-minute IV bolus, followed by 50 mg/m^2 by 4-hour CIVI
Plasma concentration	Target > 1.5 μmol/L
Plasma half-life	16 +/− 13.1 h for 60 mg/m^2 dose 13.2 +/− 8.8 h for 80 mg/m^2 dose
Cost	Not yet available outside of trial; this medication is available through CTEP in the United States
Other targets	Also inhibits GSK3β at higher nanomolar concentration

Table 4

Quick Profile: Dinaciclib

Drug name	**Dinaciclib**
Company	Merck, Whitehouse Station, NJ
Other names	MK-7965, SCH-727965
MoA	Inhibition of CDKs 1, 2, 5, and 9
MoR	Unknown
MTD	12–14 mg/m^2 by 2-hour infusion weekly 50 mg/m^2 by 2-hour infusion every 21 days 7.4, 10.4 mg/m^2 by 8- or 24-hour infusion, respectively
DLT	Acute tumor lysis, cytokine release syndrome, and diarrhea
Schedule	NHL and CLL: 12–14 mg/m^2 by 2-hour infusion on days 1, 8, 15 of 28-day cycle
Plasma concentration	1210 ng/mL after a 29.6 mg/m^2 dose
Plasma half-life	3 hours after a 29.6 mg/m^2 dose
Cost	Not yet available outside of trial; this medication is available through CTEP in the United States
Other	Has now entered phase III trials

Abbreviations: CTEP, Cancer Therapy Evaluation Program; MoA, Mechanism of action; MoR, Mechanism of resistance; MTD, Maximum tolerated dose; DLT, Dose limiting toxicity.