Flavopiridol, the First Cyclin-Dependent Kinase Inhibitor: Recent Advances in Combination Chemotherapy

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Abstract: The cell cycle is the series of events necessary for the division and duplication of a cell. The dysregulation of the cell cycle can promote the development of cancer. A group of proteins, cyclin-dependent kinases (CDKs), that control the cell cycle, provide new targets for treating cancer. As a result, cyclin-dependent kinase inhibitors (CDKIs) represent a novel class of chemotherapeutic agents. Of these, flavopiridol, a semisynthetic flavonoidal alkaloid, emerged as the first CDKI to enter clinical trials. Preclinical data indicate that flavopiridol could block the proliferation of neoplastic cells and induce programmed cell death as a single agent. Furthermore, recent emerging data revealed that flavopiridol can potentiate, generally in a dose- and sequence-dependent manner, the anti-tumor effects of many established chemotherapeutic agents. This review is primarily focused on the role of flavopiridol in combination with various therapeutic agents that are in or near clinical development.

Keywords: Anticancer, cancer, CDKIs, combination therapy, flavopiridol, flavonoid.

1. INTRODUCTION

Flavonoid is one of the most representative classes of plant secondary metabolites, occurring widely throughout the plant kingdom [1]. Flavonoid can inhibit, and sometimes induce, a large number of mammalian enzyme systems, that sometimes are involved in important pathways that regulate cell division and proliferation [1]. Flavopiridol is a semisynthetic flavonoid, which was originally isolated from the stem bark of the Indian plant *Dysoxylum binectariferum* (also called rohitukine) (Fig. (**1**)) [2]. The anticancer effect of flavopiridol has been identified as the result of drug screening developed by the National Cancer Institute (NCI) on 60 human cancer cell lines [3]. When administered alone, flavopiridol can induce cell cycle arrest at G1 or G2/M phase, associated with direct inhibition of CDK-1, -2, -4, -6 and -7 by competing with ATP at the nucleotide- binding site on CDKs [4]. Research in the last few years has indicated that besides inhibiting CDK activity, flavopiridol can also suppress the expression of some anti-apoptotic proteins, such as Bcl-2 [5], Mcl-1 [6-8], cyclin D1 [9, 10], and vascular endothelial growth factor (VEGF) [11]. Flavopiridol has also been demonstrated to induce apoptosis, inhibit angiogenesis and potentiate the effect of chemotherapeutic agents in a variety of *in vitro* and *in vivo* models [11-14]. In view of the antitumor effect of flavopiridol, many researchers have tried to elucidate possible structure-activity relationships that might lead to more efficient drug discovery.

During pre-clinical and clinical studies, flavopiridol induced apoptosis significantly both as a single agent and in combination with other anti-cancer agents [15]. In clinical trials as a single agent, flavopiridol showed dose-limiting toxicities (DLT) of severe diarrhea and vascular thrombotic events, such as myocardial infarction, transient neurological ischemic attacks, deep vein thrombosis and pulmonary embolism [16]. Further clinical development of flavopiridol will likely depend on the ability to successfully combine it with other antineoplastic agents. On one hand, recent data indicated that flavopiridol can kill noncycling cells *in vitro*, raising the possibility that flavopiridol might be profitably combined with agents that inhibit cell cycle progression. On the other hand, the observation that flavopiridol, like other CDKIs, could itself induce cell cycle arrest in G1 and G2 phase raises the possibility that flavopiridol might inhibit the effects of proliferation-dependent agents [13]. Based on these possibilities, flavopiridol has been reported to enhance the cytotoxic effects of various chemotherapeutics. In several preclinical studies, sequence-dependent synergistic cytotoxic effects were observed when flavopiridol was administered after a variety of chemotherapeutic drugs. These agents included paclitaxel [12], gemcitabine [17], docetaxel [18, 19], cisplatin [13, 17, 20], virinostat [21], TNF-2 [22], HA14-1 [23] and so on. Current studies have shown that the synergistic effect of flavopiridol with other chemotherapeutic agents has more ascendancy. Thus the aim of this review is to report in a critical manner all the various combinations so that the reader may be able to design further clinical studies for cancer eradication.

2. STRUCTURE ACTIVITY RELATIONSHIP (SAR) STUDIES OF FLAVOPIRIDOL

Flavopiridol is derived from rohitukine($C_{16}H_{19}O_5N$), a chromane alkaloid, which was first reported from *Amoora rohituka* (Roxb.) and then from *Dysoxylum binectariferum*, both from the family Meliaceae (Fig. (**1**)) [24]. Although there have been effort toward the total synthesis of flavopiri-

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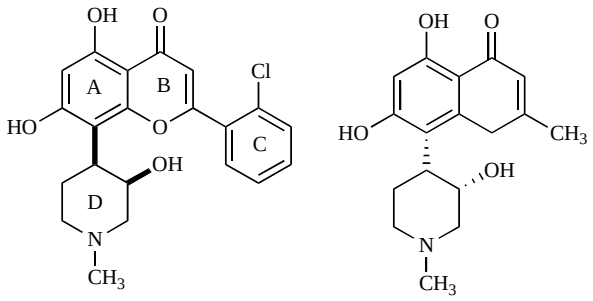
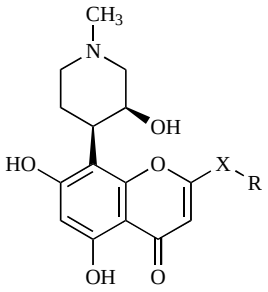


Fig. (1). Structures of flavopiridol and rohitukine.

dol, none has been successful, owing to the difficulty in the installation of the *cis*-alcohol functional group in the piperidine ring (namely D-ring) [24]. Thus flavopiridol is semi-synthetically derived from rohitukine [24]. SAR studies of flavopiridol analogues have increased in recent years (Fig. (**2**)). Many studies have been conducted to examine the structural requisites involved in the anticancer activity of flavopiridol, given that this knowledge might lead to new drug discovery. Our discussion will concentrate on recent progress of the molecular framework, i.e. thio- and oxoflavopiridols, D-, C- and B-rings.

2.1. Thio- and Oxoflavopiridols

Kim *et al.* [25] reported that Flavopiridol analogues, thio- and oxoflavopiridols which contain a sulfur (**2**) or oxygen (**3**) atom linker between a chromone ring and the hydrophobic side chain (Fig. (**3**)), are selective CDK1 inhibitors with an IC_{50} of 110 and 130 nM. Thio- and oxoflavopiridols **2** and **3** are not only selective within the CDK family but also discriminated between unrelated serine/threonine and tyrosine protein kinases. CDK1 selective thio- and oxoflavopiridol analogues inhibit the colony-forming ability of multiple human tumor cell lines and possess a unique anti-proliferative profile in comparison to flavopiridol (Table **1**).



2: X=S, R=2-chlorophenyl (-)

3: X=O, R=2-chlorophenyl (-)

Fig. (3). Structures of thio- and oxoflavopiridols.

2.2. D-Ring and its Substitutions

Two naturally occurring poly-hydroxylated flavonoids quercetin and genistein are potent tyrosine kinase inhibitors but have poor CDK inhibitory activity (Fig. (**4**)). Structurally both quercitin and genistein lack the 3-hydroxy-1-methylpiperidinyl ring (D-ring) present in flavopiridol [26]. Therefore the importance of D-ring of flavopiridol in determining the key structural requirements for CDK inhibitory activity has been acknowledged for some years now (Fig. (**5**)).

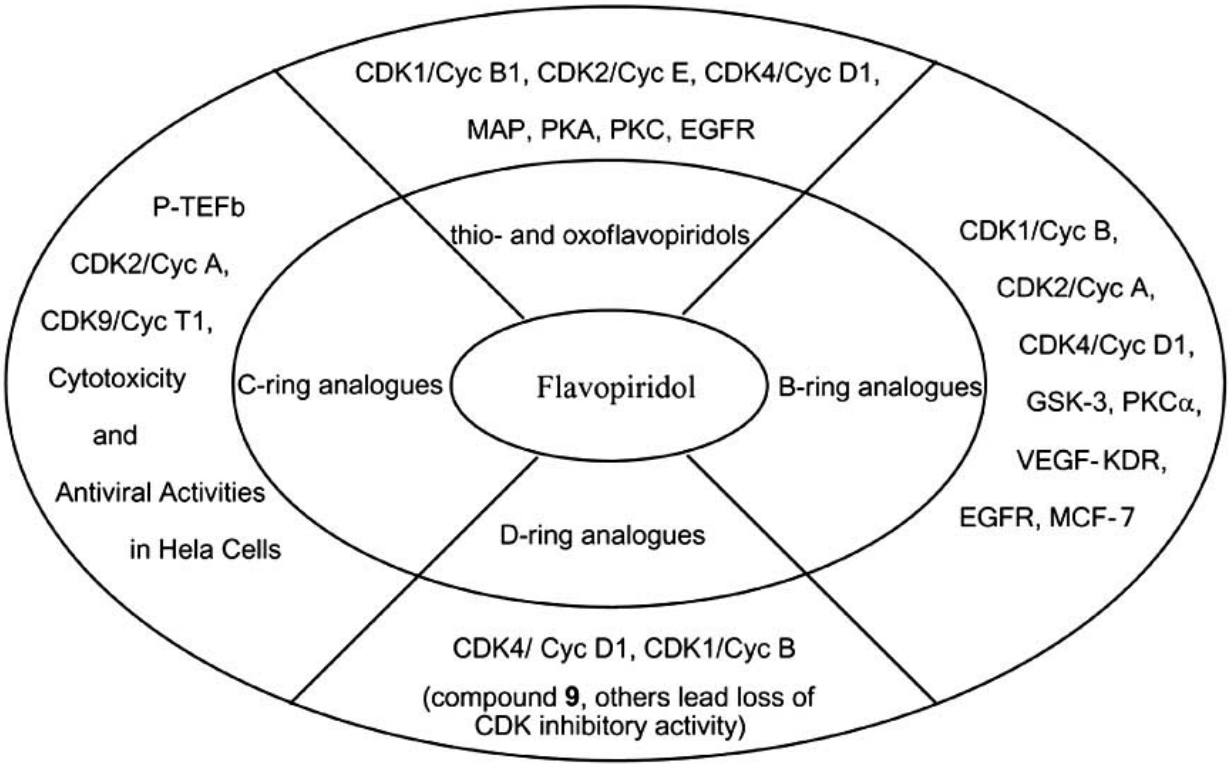


Fig. (2). Activity of flavopiridol analogues.

Table 1. Activity Data of CDKIs Against Other Different Kinases [25]

Compounds	IC_{50} (μM)						
	CDK1/CycB1	CDK2/CycE	CDK4/CycD1	MAP	PKA	PKC	EGFR
flavopiridol	0.03	0.17	0.10	19.0	>50	14.0	22.0
2	0.11	2.10	16.2	>25	>50	16.1	>50
3	0.13	2.11	6.15	>25	>50	>50	>50

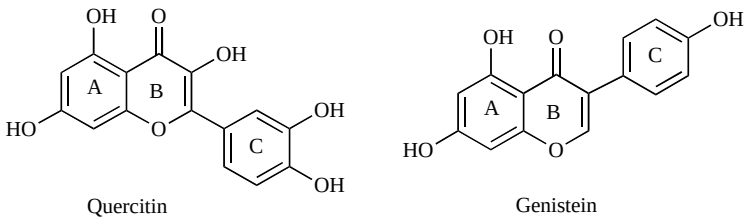


Fig. (4). Structures of quercitin and genistein.

Recently, Murthi *et al.* [26] reported modifications of D-ring of flavopiridol and their effect on CDK inhibitory activity. Oxidation of the alcohol to the ketone analogue **4** resulted in a significant loss of activity. The cyclohexyl D-ring analogue **5** is more than an order of magnitude less active than flavopiridol **1**, indicating that the presence of the nitrogen atom on the D-ring is very important for CDK inhibitory activity. The *cis*- and *trans*- hydroxyl isomers **6a** and **6b** that are derived from 1-methyl-3-piperidinone are completely inactive against CDK4/cyclin D, suggesting that the positioning of the nitrogen atom on the D-ring is also very important for CDK inhibitory activity. In addition, Gross *et al.* [27] outlined a stereocontrolled approach to useful, substituted piperidones and piperidines (compound **7** and **8**), which were further elaborated to flavopiridol D-ring analogs. But the effect on CDK inhibitory activity has not been tested.

The olefin analogue **9**, which lacks the hydroxyl group results only in a 4- to 5-fold loss in activity against both the CDKs. Presumably the lack of activity of compounds **6a**, **6b**, and **5** is due to the loss of the piperidine nitrogen-Asp 145 interaction. The modest loss in activity with the olefin **9** suggests that the hydroxyl group-Lys 33 interaction may not be

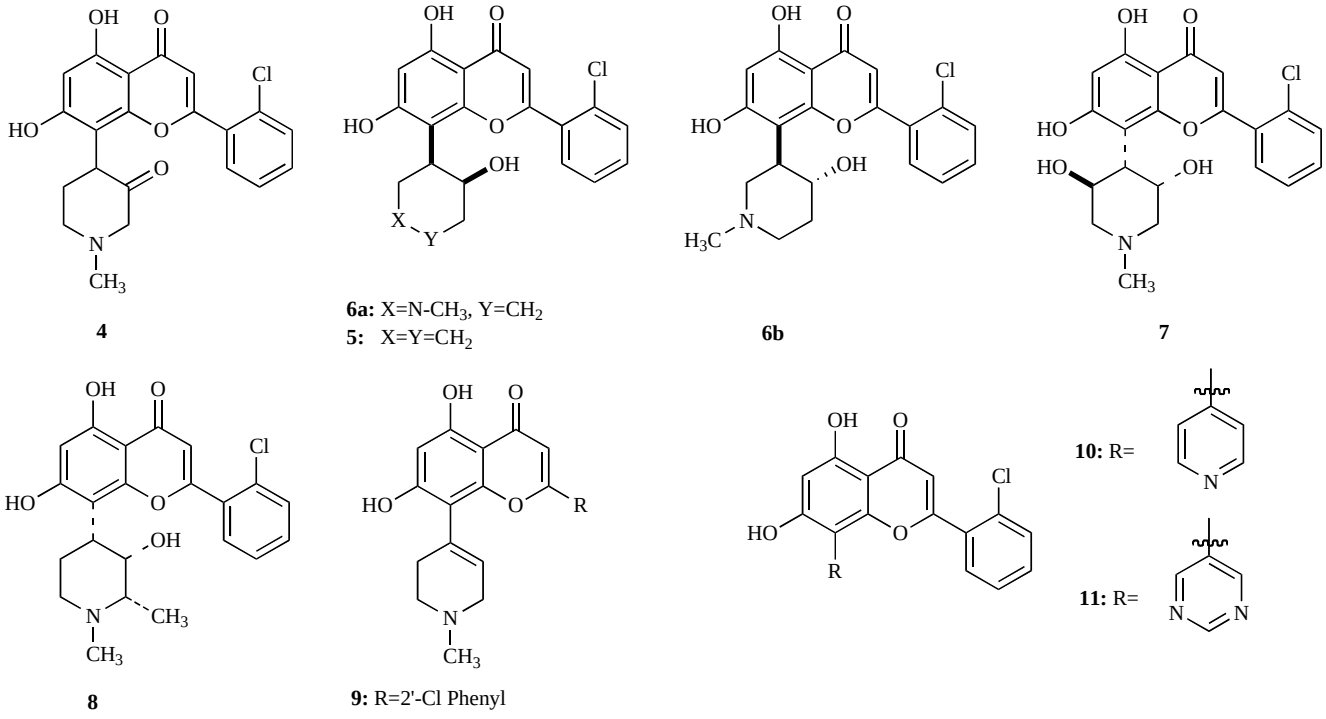


Fig. (5). Structures of flvopiridol D-ring analogues.

very critical for CDK inhibitory activity as long as the nitrogen-Asp 145 interaction is maintained. The major loss in activity of ketone **4** could be due to a loss in one or both hydrogen bonding interactions because of conformational changes of the hydroxy piperidine ring. Replacement of the D-ring with aryl rings such as pyridyl (compound **10**) and pyrimidyl (compound **11**) also results in a major loss of CDK inhibitory activity [26].

2.4. C-Ring and its Substitutions (Fig. (6))

Ali *et al.* [28] prepared a series of chiral flavopiridol analogues with variations in the C-ring (**12a-12p**) and tested the *in vitro* CDK2/cyclin A or P-TEFb kinase activities of these compounds. The deschloroflavopiridol (**12a**), 2-fluorophenyl (**12d**), and 5-methylisoxazole (**12n**) analogues all potently inhibit HIV-1 viral replication in cellular assays (EC_{50} values <10 nm) with respective selectivity indexes two-, three-, and 14-fold higher than that of flavopiridol.

Since the tetrahydropyridyl analogues (**9**) did not result in a major loss of CDK inhibitory activity, Murthi *et al.* pursued additional SAR studies of the series involving changes in the flavone nucleus and the C-ring. Based on the above statement, modication of the D-ring seems to attenuate the binding interactions to some degree between the compounds and CDK enzymes. While modification of the C-ring results in at least a 10-fold loss in activity, the tetrahydropyridine series seems quite tolerant of changes in the C-ring (**13-15, 17-21, 23**) [26]. Ali *et al.* [28] also identified the D-ring olefin analogues (**16, 17, 22**). The 4-fluorophenyl compound, 16a, is a more potent inhibitor of CDK2/cyclin A than the 2-chlorophenyl analogue; it is almost equipotent to flavopiridol. On the other hand, SAR study of the flavopiridol C-ring analogues against CDK2/cyclin A closely resembles the SAR of olefin analogues against CDK4/cyclin D1 [26].

In addition, Kim *et al.* [25] reported that insertion of a nitrogen atom linker (**24**) resulted in a severe reduction in potency against all CDKs tested. And the removal of the nitrogen atom (**25**) led a less reduction.

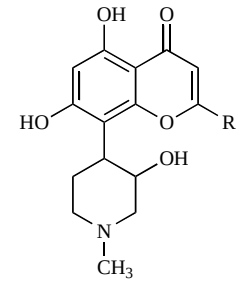
2.4. B-Ring and its Substitutions (Fig. (7))

Schoephfer *et al*. [29] designed and synthesized the 2-benzylidene-benzofuran-3-ones as flavopiridol mimics. Among the mimics, compound **26** is an exact analogue of B-ring modification of flavopiridol. Compared to the *des*-chloro analogue of flavopiridol, introduction of an ortho chloro substituent (compound **26**) did not lead to the beneficial effect on CDK inhibitory activity. On the contrary, some potency against the three CDK enzymes (cyclin A, B, and D1) was lost.

Synthesis of the quinolone and isocoumarin analogues **27** and **28** has also been reported [26]. The alteration of the flavone nucleus to these two compounds resulted in reduced CDK inhibitory activity, suggesting ineffective interactions in the adenine binding pocket.

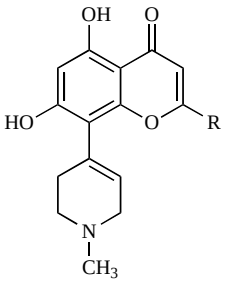
3. MECHANISMS OF ACTION OF FLAVOPIRIDOL

The cell cycle is the mechanism through which cells divide, and is an orderly and tightly regulated phenomenon involving four phases (G1, S, G2 and M) [30, 31]. G1 phase separates M and S phases. In this period, cells commit to enter the cell cycle and prepare to duplicate their DNA. After G1 phase, cells enter S phase, the period of DNA synthesis (genome duplication). After S phase, cells enter G2 phase, the period in which cells can repair errors that might occur during DNA duplication and thus prevent passing these errors to daughter cells. During G2 phase, cells prepare to enter M phase, the period in which chromatids and then daughter cells separate. After M phase, cells can enter G1 phase again or enter G0 phase, a replicatively quiescent phase. In G0 phase, the cells usually have a diploid amount of DNA, which represents the differentiated functioning cell not committed to the cell cycle [37]. The progression through these phases is controlled by a number of CDKs which are

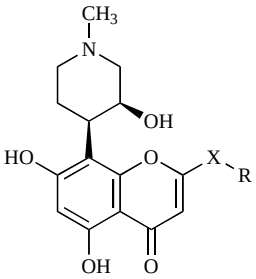


12a: R=phenyl
12b: R=3-chlorophenyl
12c: R=4-chlorophenyl
12d: R=2-fluorophenyl
12e: R=4-fluorophenyl
12f: R=4-bromophenyl
12g: R=4-tert-butylphenyl
12h: R=4-trifluoromethylphenyl

12i: R=4-hydroxyphenyl
12j: R=2-pyridyl
12k: R=3-pyridyl
12l: R=4-pyridyl
12m: R=4-chloro-3-pyridyl
12n: R=5-methylisoxazole
12o: R=3-vinylphenyl
12p: R=4-vinylphenyl



13: R=3'-Cl Phenyl
14: R=4'-Cl Phenyl
15: R=2'-F Phenyl
16: R=4'-F Phenyl
17: R=2'-Br Phenyl
18: R=2'-I Phenyl
19: R=Phenyl
20: R=2',4'-di Cl Phenyl
21: R=4-Pyridyl
22: R=3-Pyridyl
23: R=Cyclohexyl



24: X=NH, R=phenyl
25: X=none, R=N-piperidyl

Fig. (6). Structures of flvopiridol C-ring analogues.

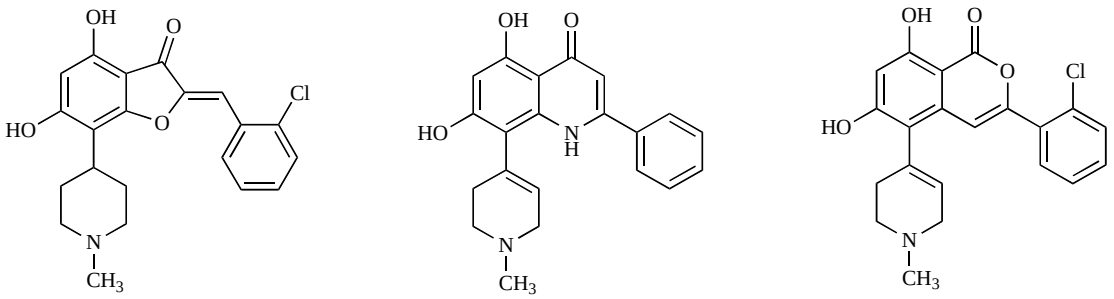


Fig. (7). Structures of flvopiridol B-ring analogues.

heterodimeric complexes composed of a catalytic kinase subunit and a regulatory cyclin subunit [30, 31]. Through cell cycle progressions, cyclin D/CDK4 and cyclin D/CDK6 are the first complexes to become active, appearing during mid-to-late G1 phase, followed by cyclin E/CDK2 complexes in late G1 phase. Cyclin E/CDK2 complexes are present in the cell cycle progressions until the cell has completed the G1/S transition. Active cyclin A/CDK2 complexes drive S phase progression, and after the cell has entered G2 phase, cyclin A trades its partner, CDK2, for CDK1. The G2/M phase transition heralds the appearance of cyclin B-CDK1 [32-34]. Dysregulation of the cell cycle is a hallmark of malignancy. Benson C *et al.* [35] have summarized the association between deregulated cyclins, CDKs, endogenous CDKIs and tumours completely. The activity of CDKs is negatively regulated by direct interactions with proteins referred to as CDKIs. CDKIs are divided into two major families: the INK4 (inhibitor of CDK4) family, including $p16^{ink4a}$, $p15^{ink4b}$, $p18^{ink4c}$ and $p19^{ink4d}$, which specifically inhibit cyclin D-associated kinases (CDK 4 and CDK 6); and the Cip/Kip (kinase inhibitor protein) family, consisting of $p21^{cip1/waf1}$, $p27^{kip1}$ and $p57^{kip2}$, which inhibit most CDKs [36]. Numerous CDKIs are currently undergoing preclinical and clinical evaluation, and the search for new CDKIs continues. Flavopiridol, the first CDK inhibitor to undergo clinical evaluation in humans, is one of the pan-CDK inhibitors which are non-selective.

Initially, flavopiridol was reported as a potent *in vitro* epidermal growth factor receptor (EGFR) tyrosine kinase and protein kinase A (PKA) inhibitor (IC_{50}=21 and 122 μM, respectively) [37, 38]. However, when flavopiridol was tested in the NCI Development Therapeutics Program panel of 60 human tumor cell lines, it was shown to inhibit cell proliferation at more physiologically relevant concentrations (IC_{50}=66nM)[39]. Moreover, the antiproliferative effect did not correlate with the presence of the EGFR [38, 39].

Flavopiridol can inhibit cell cycle progression by a variety of mechanisms related to CDK inactivation. Studies using purified CDKs showed that flavopiridol inhibits CDK1, CDK2, CDK4, CDK6 (all IC_{50} ~41 nM) and CDK7 (IC_{50} = 300 nM) by competing with ATP [38, 40-44]. Inhibition of CDK2, 4, and 6 led to cell cycle arrest at G1 phase. This inhibition also resulted in inhibition of phosphorylation of pRb, p107 and p130, and the activation of E2F. Inhibition of CDK1 led to inhibition of activation of topoisomerase activators, of lamin proteoglycans histone 1 protein and chromatin condensation and retardation in cell cycle progression through S-phase and accumulation in G2 phase. In addition, inhibition of CDK7 resulted in reduced activation of other CDK's and RNA polymerase [45].

In addition to directly inhibiting CDKs, flavopiridol causes a decrease in the level of cyclin D1, an oncogene that is overexpressed in many human neoplasias. When MCF-7 human breast carcinoma cell lines were incubated with flavopiridol, cyclin D1 protein levels decreased within 3 hours. This effect was followed by a decline in the levels of cyclin D3 with no alteration in the levels of cyclin D2 and cyclin E, the remaining G1 cyclins, and then 2 hours later, loss of CDK4 activity occurred [10]. Cyclin D1 depletion was a consequence of downregulation of cyclin D1 mRNA and was associated with specific decline in cyclin D1 promoter measured by luciferase reporter assay [10]. Another study showed a G1/S cell-cycle arrest after exposure of nonmalignant breast epithelial cell line MCF10A to flavopiridol, which was accompanied by a loss in CDK6 activity as measured by reduced Rb phosrylation [43]. The loss in CDK6 activity was preceded by decline in cyclin D1. Therefore, cyclin D1 transcriptional repression is likely to be related to the inhibition of positive transcription elongation factor b (P-TEFb) by flavopiridol [43, 46]. P-TEFb is a complex of CDK9 and T-type cyclins, and it phosphorylates the carboxyl-terminal domain of the large subunit of RNA polymerase Ⅱ, thereby facilitating transcription elongation [47, 48]. P-TEFb is also required for activation of transcription of the HIV-1 genome by the viral transactivator Tat [48]. Thus, flavopiridol is likely to have promising potential for AIDS therapy. In summary, flavopiridol can induce cell cycle arrest by at least three mechanisms: 1) by direct inhibition of CDK activities by binding to the ATP-binding site; 2) by prevention of the phosphorylation of CDKs at threonine-160/161 by inhibition of CDK7/cyclin H; and 3) for G1-phase arrest, by a decrease in the amount of cyclin D1, an important cofactor for CDK4 and CDK6 activation [37].

Flavopiridol has also been shown to have an antiangiogenic effect by down-regulating VEGF mRNA and limiting blood vessel formation in mouse matrigel model [49]. Brusselbach *et al.* [50] incubated human umbilical vein endothelial cells (HUVECs) with flavopiridol and reported apoptotic cell death even in cells that were not cycling. In another report, Kerr *et al.* [51] tested flavopiridol in an *in vivo* angio-

genesis model and observed that flavopiridol decreased blood vessel formation in the mouse Matrigel models of angiogenesis. Melillo *et al.* [11] demonstrated that, at low nanomolar concentrations, flavopiridol blunted the induction of VEGF by hypoxia in human monocytes. This effect was caused by a decreased stability of VEGF mRNA, which paralleled the decline in VEGF protein. Thus, the antitumor activity of flavopiridol may be supplemented by antiangiogenic effects [37].

Another significant feature of flavopiridol's activation is the induction of apoptosis. Apoptosis plays a vital role in drug- and radiation-induced cytotoxicity. Although various hematopoietic cell lines were exquisitely sensitive to flavopiridol-induced apoptosis [38], the mechanisms by which flavopiridol induced apoptosis have not yet been fully explained. By inhibition of CDKs, flavopiridol may reduce or block function of antiapoptotic, cell cycle promoting proteins, enabling their antagonists to inhibit cell proliferation, to induce differentiation and/or to induce apoptosis [45]. In oral cancer cells, flavopiridol induced apoptosis by regulating Bcl-x, accompanied by a larger percentage of cells with a sub-G1 DNA content [32]. In the context of B-cell chronic lymphocytic leukaemia cells, flavopiridol has been shown to induce cytotoxic effects independent of p53 status and circumvent the Bcl-2 family mediated inhibition of apoptosis [52, 53]. This effect is mediated through up-regulation of E2F1 and repression of Mcl-1 [54]. The exact mechanisms of flavopiridol-induced apoptosis are being undertaken by several groups. Transcriptional inhibition of genes that encode antiapoptotic regulators has been postulated to play a central role in the cytotoxicity of flavopiridol [36, 53]. In addition, flavopiridol was shown to induce mucinous differentiation in lung carcinoma cells accompanied by loss in CDK2 activity [55].

4. PRECLINICAL ACTIONS IN COMBINATION THERAPY

4.1. Combinations with Taxanes

The taxanes act by stabilizing microtubules, thereby causing a G2/M arrest followed by apoptosis. The two primary taxanes in clinical use today are paclitaxel (Paclitaxel) and docetaxel (Taxotere).

Docetaxel, a semisynthetic derivative of paclitaxel originally derived from the yew tree, is a promising anticancer drug shown to inhibit a wide variety of tumor cells, including prostate cancer cells, by diverse mechanisms that include cell cycle arrest, induction of apoptosis, stabilization of microtubules, and inhibition of angiogenesis [56]. Preclinical studies in human gastric and breast cancer cell lines have shown that the greatest increase in apoptosis occurs when docetaxel is followed by flavopiridol [18]. The hypothesis of this regimen is that flavopiridol treatment after the docetaxel-mediated mitotic block results in the inhibition of cyclin B1-CDK activity, a decrease in phosphorylated survivin, a more rapid exit from mitosis, and an increase in apoptosis. Gomez LA *et al.* [56] reported that in androgen-dependent and androgen-independent LNCaP prostate cancer cells, sequential treatment with flavopiridol followed by docetaxel (FD) produced the greatest enhancement of apoptotic cell death. The data indicated that substantial decreases in X-linked inhibitor-of-apoptosis protein (XIAP, member of the IAP family) and AKT (prosurvival factor) proteins are important mediators for increased apoptosis in FD-treated LNCaP cells. A later study showed that the sequences of flavopiridol followed by docetaxel (FD) or docetaxel followed by flavopiridol (DF) produced greater apoptosis and inhibited angiogenesis better than either drug alone, leading to a significant inhibition of primary and metastatic prostate tumors [57]. The results suggested that the highest levels of cleaved (activated) caspase-3 in the FD group could be correlated with a reduction in metastases and a decrease in the anti-apoptosis proteins XIAP and the Bcl-xL in the DF group correlated with smaller primary prostate tumors. Furthermore, the combination of docetaxel plus flavopiridol may enhance the effect of radiation therapy *in vitro* and *in vivo* by enhancing apoptosis and changing cell cycle [58].

The combinations of flavopiridol with paclitaxel demonstrate the concept of cell-cycle-mediated drug resistance. Synergism between flavopiridol and paclitaxel has been observed in A549 non-small cell lung cancer cells [13]. The study indicated that the combination of paclitaxel and flavopiridol was highly sequence dependent, such that paclitaxel should precede flavopiridol to achieve the maximal effect. Cell-cycle-mediated resistance was demonstrated when flavopiridol exposure was followed by paclitaxel. Flavopiridol's multiple cell-cycle arrest effects (including the inhibition of CDK4, CDK6, and CDK2 at G1, and the inhibition of cyclin B1-cdc2 kinase activity at G2 [12]), created 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 pacitaxel sensitivity in culture [12]. The reverse sequence of paclitaxel followed by flavopiridol is associated with an increased induction of apoptosis [12, 13], as evidenced by caspase-3 activation and poly-(ADP-ribose)-polymerase (PARP) degradation [12].

4.2. Combinations with Histone Deacetylase Inhibitors (HDACIs)

Histone deacetylase enzymes (HDAC) reciprocally regulated histone acetylation, which play a significant role in the control of transcription in multiple cell types, with the acetylation of histone permitting chromatin to assume a relaxed conformation [59-61]. HDACIs are a group of agents that block histone deacetylation, thus altering the rates of transcription of many different genes, such as the genes that regulating cell survival, the cell cycle, and cellular differentiation [21]. At low concentrations *in vitro*, HDACIs have been shown to induce cellular differentiation, such as sodium butyrate and suberoylanilide hydroxamic acid (SAHA; vorinostat; Zolinza) [62, 63].

Some studies have suggested that sequential depsipeptide (one of HDACIs)/flavopiridol treatment enhances BAX:Bcl-2 protein ratios and mediates apoptosis in cultured lung and esophageal cancer cells *via* mitochondrial dependent mechanisms [64]. These observations are consistent with the data reported by Almenara *et al.* [65] demonstrating that flavopiridol inhibits p21 induction, and synergistically enhances mitochondrial damage and apoptosis in human leukemia cells mediated by SAHA; apoptosis after

SAHA/flavopiridol treatment coincides with caspase-dependent cleavage of Bcl-2 and phosphorylated Rb. Rosato *et al.* [66] observes that flavopiridol inhibits p21 induction, and markedly potentiates cytotoxicity mediated by sodium butyrate in leukemia cells; apoptosis coincides with diminished Bcl-2, Mcl-1, and XIAP levels after combination drug treatment. Collectively, these data indicate that depletion of a variety of antiapoptotic proteins and disruption of mitochondrial integrity contributes to flavopiridol-mediated potentiation of cytotoxicity by HDACIs in cancer cells. Nguyen DM *et al.* [67] have found that in addition to inhibiting p21 expression, sequential depsipeptide/flvopiridol treatment decreases expression of a variety of cell signaling and cell cycle-related proteins in malignant pleural mesothelioma cells, such as cyclin B expression. Based on the above findings, the interactions may stem from flavopiridol-mediated transcriptional repression of antiapoptotic genes (e.g., $p21^{CIP1/WAF1}$, Mcl-1, and XIAP). However, it is also known that HDACIs trigger perturbations in NF-κB activity and that such an event may protect cells from apoptosis [68]. In view of the evidence that flavopiridol acts as an IκBα kinase (IKK) inhibitor [2], Gao N *et al*. [68] conducted a study to testify the possibility that flavopiridol-mediated NF-κB inhibition might contribute to synergistic interactions with HDACIs (such as NaB and SAHA). The results indicated that flavopiridol dramatically blocked HDACI-mediated NF-κB activation in human leukemia cells and that this event played a significant, albeit limited, role in antileukemic synergism. Nevertheless, these findings also suggested that interactions between flavopiridol and HDACIs were multifactorial and that, more directly, NF-κB-independent flavopiridol actions (e.g., downregulation of $p21^{CIP1/WAF1}$, Mcl-1, and XIAP) in all likelihood contributed substantially to this phenomenon.

4.3. Combinations with Radiation

Flavopiridol has been investigated for its potential to enhance the effect of radiotherapy in several models, including human colon, esophageal, gastric, and lung xenografts and murine ovarian, breast and lymphoma syngeneic tumors, and anti-tumor activity was potentiated when flavopiridol was administered following radiation [15, 16, 58, 69]. Kim *et al.* investigated the time- and sequence-related effects of a combination therapy involving docetaxel, flavopiridol and irradiation in H460 human lung cancer cells [58]. The interaction between radiation and flavopiridol was also found to be sequence-dependent, with the cell death reaching maximal levels when the radiation treatment preceded the flavopiridol treatment by 2 hours [70]. Jung *et al.* [15] reported an increased radiosensitivity of gastric and colon cancer cell lines as a result of flavopiridol treatment, and also determined it to be sequence- and time-dependent. A possible mechanism for flavopiridol's radiosensitizing effects involves the reduction of p21 expression, and the augmentation of apoptotic rates. When combined with radiation, flavopiridol proves to significantly enhance the radioresponse of the GL261 glioma tumors [71]. Another possible mechanism relies on flavopiridol's ability to inhibit angiogenesis. Because flavopiridol is able to decrease the level of a HIF-receptor gene, accompanied by its ability to inhibit mRNA synthesis. Raju *et al.* [72] have investigated the *in vitro* radiosensitizing effect of flavopiridol in the murine ovarian cancer cell line OCA-I and reported a radiosensitizing effect. The mechanism underlying this phenomenon is thought to involve cell cycle redistribution, and the blocking of sublethal DNA damage repair processes. In contrast, Chien *et al.* [73] based on their studies conclude that flavopiridol had no effect on the radiosensitivity of human bladder carcinoma cell lines. Given the conflicting results obtained from murine and human tumor cells, Camphausen K *et al.* [74] have investigated the effects of flavopiridol on the radiosensitivity of two human prostate carcinoma cell lines. The results indicate that flavopiridol at concentrations ranging from 60 to 90 nM significantly enhances the radiosensitivity of the two human prostate carcinoma cell lines. This enhancement occurs in the absence of significant CDK inhibition, reduced P-TEFb activity, or abrogation of G2 arrest. However, based on the prolonged expression of gH2AX after irradiation, it appears that the flavopiridol-induced radiosensitization involves the altered metabolism of DNA double strand breaks (DSBs).

4.4. Combinations with Proteasome Inhibitors

Dai Yun's group has reported that flavopiridol interactes synergistically with proteasome inhibitors (e.g., MG-132 and lactacystin) to induce mitochondrial dysfunction and apoptosis in human myeloid and lymphoid leukemia cells *in vitro* [75]. Bcr/Abl^+ cells are relatively resistant to apoptosis induced by conventional agents and depend upon unique Bcr/Abl-dependent signaling pathways for their survival. Preclinical studies indicate that flavopiridol enhances the activity of targeted agents such as imatinib mesylate in Bcr/Abl^+ leukemia cells, including those displaying imatinib mesylate resistance [76]. Based on this, a report suggests that combined administration of flavopiridol and proteasome inhibitor, bortezomib effectively triggers apoptosis in Bcr/Abl^+ cells that are both sensitive and resistant to imatinib mesylate through a mechanism that involves inactivation of the NF-κB and STAT3/STAT5 axes. Furthermore, this regimen is active against chronic myeloid leukemia cells displaying a recently described Bcr/Abl-independent form of imatinib mesylate resistance [77].

4.5. Combinations with Monoclonal Antibodies

Trastuzumab (Herceptin) is a humanized monoclonal antibody directed against the extracellular domain of the Neu receptor that reduces its activation and the activation of the Ras- MAPK pathway. Wu K *et al.* [9] have reported the combined use of trastuzumab and flavopiridol in two Neu-overexpressing human breast cancer cell lines. Their study suggests that the combination of trastuzumab and flavopiridol synergistically inhibits the Ras-MAPK and/or PI3K/Akt pathway, which is associated with reduced abundance of cyclin D1 and/or CDKs and decreased S-phase progression. In another study, cyclin D1 levels are unaltered when cells are treated with flavopiridol and trastuzumab [78], so the synergistic interaction between these two drugs appears to be independent of CDK4/6 [79]. Instead, EGFR is an essential target of the flavopiridol-trastuzumab combination. The observations suggest an alternative strategy to interrupt EGFR and erbB2 interactions by inhibiting EGFR/erbB2 expression levels, and not simply by inhibiting kinase activity. It would

be important to clarify how trastuzumab-flavopiridol downregulates the EGFR and erbB2 so that future therapies might use multiple converging inhibitors of these two key contributors to breast cancer mortality [79]. In addition, the significantly improved therapeutic efficacy that results from combining flavopiridol with humanized anti-Tac antibody (HAT), which recognizes CD25, provides motivation for a clinical trial in the treatment of Adult T-cell leukemia [80].

4.6. Combinations with Gemcitabine

Gemcitabine is a broad-spectrum anticancer drug that is commonly used in the treatment of patients with a variety of non-hematological malignancies that include breast cancer. The cellular effects of gemcitabine are maximally exerted on cells in the S-phase of the cell cycle by virtue of its DNA synthesis inhibitory effects. It is conceivable that potential additivity or synergy may exist with drugs that also target the regulatory elements of the cell cycle [81]. Flavopiridol is a drug that targets several of the key proteins in the regulation of the cell cycle [4]. Preclinical studies have shown at least additive effect of flavopiridol when combined with conventional cytotoxic drugs [82] including gemcitabine [83]. In a study testing flavopiridol and gemcitabine in gastrointestinal cancer cell lines using higher concertrations of both drugs, flavopiridol induced a transcriptional downregulation of the M-2 subunit of the ribonucleotide reductase enzyme in gemcitabine treated cells [83]. In another study, sequential treatment of breast cancer cells by sub-cytotoxic concentrations of gemcitabine followed by flavopiridol significantly sensitized these cells to undergo apoptosis. The mechanisms of such interaction include the sensitization of cells by gemcitabine in the S-phase to apoptosis induced by flavopiridol, mediated by the upregulation of $p21^{WAF1}$ and p16 cell cycle inhibitory proteins [81]. This result also suggests a role of gemcitabine followed by flavopiridol in inducing apoptotic cell death in breast cancer cells irrespective of ER and p53 status. In addition, the upregulation of $p21^{WAF1}$ is contrast to the downregulation of $p21^{WAF1}$ HCT-116 cells that are sensitized to SN-38-induced apoptosis by flavopiridol [84-86].

4.7. Combinations with other Chemotherapeutic Agents

In recent years, many studies have focused on the synergy between flavopiridol and various other antineoplastic agents, such as UCN-01, TNF, doxorubicin, TRAIL, minocycline, colchicine, bryostatin, HA14-1, and so on [7, 22, 23, 87-100]. Synergistic effects are demonstrated when flavopiridol follows treatment of A549 lung carcinoma cell lines with cytarabine, topotecan, doxorubicin, or etoposide. In contrast, 5-fluorouracil requires flavopiridol to be present for 24 hours previous to be synergistic. Synergistic effects with cisplatin are not schedule-dependent [13]. Flavopiridol has also been shown to synergistically increase the percentage of mitomycin-C-induced apoptosis in MKN-74 gastric and MDA-MB-468 cells [99]. Treatment with either mitomycin-C or flavopiridol alone induced apoptosis in less than 18% of either of the cell lines. Sequential treatment with mitomycin-C followed by flavopiridol was significantly better at inducing apoptosis (63% to 76% of cells) than the other order or simultaneous exposure [99]. In addition, in a recent study in a rabbit VX2 liver tumour model, the combination of cisplatin-eluting gelatin microspheres (GMSs) and flvopiridol significantly enhanced antitumor effects [100]. In all, the mechanisms of these combinations remain to be unclosed.

5. CLINICAL TRIALS IN COMBINATION THERAPY

The potency of flavopiridol in preclinical *in vitro* and *in vivo* models has not been reflected in the clinical setting to date [4]. Monotherapy trials have not been designed to distinguish prolonged disease stabilization from indolent tumor growth [4]. Based on encouraging preclinical data of flavopiridol in combination with other chemotherapies in xenograft models and *in vivo* [82, 84], many combination clinical trials have been initiated. Several phase I and phase II studies of flavopiridol have been reported in a variety of solid tumors and hematologic malignancies.

5.1. Phase I Clinical Trials

With the absence of activity as a single agent and the encouraging preclinical models, combinations of flavopiridol with cytotoxic chemotherapeutic drugs were designed [101]. A subsequent phase I trial of paclitaxel followed by a 24-h continuous venous infusion of flavopiridol reports neutropenia and pulmonary toxicity as the DLT [102]. The maximal tolerated doses (MTD) for paclitaxel and flavopiridol are 175, and 70 mg/m2day, respectively. Accordingly, a phase I study of flavopiridol in combination with paclitaxel/carboplatin was conducted in patients with advanced non-small-cell lung cancer. The results indicate that the combination is feasible with accepted toxicities, such as asthenia, pain, nausea, diarrhea, vomiting, leucopenia, myalgia, and alopecia [103]. Based on these findings, a phase I study of docetaxel followed by a 72-hour infusion of flavopiridol every 3 weeks was conducted [104]. Furthermore, another phase I trial evaluating docetaxel and flavopiridol as a 24-h infusion was also designed. 10 patients were treated with flavopiridol and docetaxel given once every 21 days. The DLTs were neutropenia and infection. The MTD was docetaxel 60mg/m^2 followed 24hours later by flavopiridol 50mg/m^2 over 24 hours [101]. The 24-h infusion of flavopiridol is, however, cumbersome for the patients because it is associated with clinical toxicities. Prior data show that weekly flavopiridol is feasible in combination with irinotecan [105]. Based on these promising clinical obsevations, Fornier M.N. *et al.* [19] conducted a phase I trial of weekly, sequential docetaxel followed by flavopiridol in patients with advanced solid tumors such that docetaxel was administered 4 h before flavopiridol. The combination was well tolerated, with one DLT occurring at flavopiridol 70 mg/m^2 (grade 3 mucositis) and one DLT at 80 mg/m^2 (grade 4 neutropenia). These promising results lead us to keep an eye on ongoing phase II study of weekly sequential docetaxel and flavopiridol at a dose of 80 mg/m^2 for patients with metastatic, refractory pancreatic carcinoma.

The serine/threonine kinase inhibitor flavopiridol targets multiple cyclin-dependent kinases, induces checkpoint arrest, and interrupts transcriptional elongation. Based on this, Judith E. Karp *et al.* [106] designed a phase I clinical trial in which flavopiridol, given for the dual purpose of initial cytoreduction and enhancing the cell cycle progression of the remaining leukemic cell cohort, was followed by ara-C and

mitoxantrone. DLT occurred at 60 mg/m^2/d with profound neutropenia >40 days duration, and MTD was 50 mg/m^2/d. These findings warrant continuing development of flavopiridol at 50 mg/m^2/d × 3 days in combination with cytotoxic and biological agents for acute leukemias.

Dana Rathkopf *et al.* [107] have conducted a phase I trial of flavopiridol+FOLFOX (folinic acid, 5-FU, and oxaliplatin) for advanced solid tumors. Flvopiridol was administered every 2 weeks with oxaliplatin before 5-FU, based on sequence-dependent growth inhibition. With dose escalation of oxaliplatin (85 mg/m^2) and 5-FU (2,400 mg/m^2), DLT included hyponatremia, thrombocytopenia, and neutropenia. 5-FU was subsequently reduced to allow for dose escalation of flavopiridol. DLT with escalation of flavopiridol was nausea, vomiting, and neutropenia. The MTD was 70 mg/m^2 flavopiridol, 85 mg/m^2 oxaliplatin, and 1,800 mg/m^2 5-FU continuous infusion over 48 hours. The results indicate that flavopiridol with FOLFOX is a safe and tolerable regimen.

Flavopiridol downmodulates antiapoptotic proteins associated with resistance to fludarabine and rituximab are effective against p53-mutated chronic lymphocytic leukemia (CLL). Thomas S. Lin *et al.* [108] conducted a phase I study of flavopiridol, fludarabine, and rituximab (FFR) in patients with mantle-cell lymphoma (MCL), indolent B-cell non-Hodgkin's lymphomas (B-NHL), and CLL to determine the activity of FFR. Cytopenias and fatigue were the most common reasons for early discontinuation and main DLT were seizures and renal insufficiency. FFR was active in MCL, indolent B-NHL, and CLL and should be studied for older patients with MCL who are not candidates for aggressive chemotherapy.

In addition, Keith C. Bible *et al.* [20] have conducted a phase I trial of flavopiridol combined with either cisplatin or carboplatin in the treatment of patients with advanced solid tumors. The MTD was 60mg/m^2 cisplatin and 100 mg/m^2 flavopiridol over 24 hours. Carboplatin area under (the plasma concentration time) curve (AUC) 2 with 100mg/m^2 flavopiridol over 24 hours was deemed intolerable because of significant toxicity, including fatigue, nausea, diarrhea, and myelosuppression. The best response was stable disease.

5.2. Phase II Clinical Trials

Flavopiridol followed in a timed sequential manner by the cell cycle-dependent antileukemia drugs cytosine arabinoside (ara-C) and mitoxantrone (FLAM) has been studied [106, 109, 110].In a phase II trial of FLAM, 15 patients had newly diagnosed, poor-risk acute myelogenous leukemia (AML) with multiple poor-risk features including older age (100% > 50 years), secondary AML (100%), and adverse genetic features (53%) [109]. Twelve (75%) achieved complete remission (CR), with a 2-year disease-free survival (DFS) of 50%. To establish a more accurate estimate of efficacy in inducing durable CRs, Judith E. Karp *et al.* [111] conducted a recent Phase II trial of FLAM. In the trial, thirty patients (67%) achieved CR, with a 12.5–31 month DFS of 67%. The regimen (FLAM) exhibited meaningful and reproducible clinical activity in AML with multiple poor-risk biologic features.

In another phase II study of flavopiridol in combination with docetaxel in refractory, metastatic pancreatic cancer [112], 3 patients (33%) achieved transient stable disease, with one of these patients achieving a 20% reduction in tumor size. Median survival was 4.2 months and adverse events were significant. The results of this study suggested that the combination of flavopiridol and docetaxel has minimal activity and significant toxicity, reflecting the challenges of treating patients with pancreatic cancer.

Several important clinical questions remain unanswered: How is flavopiridol best combined with other chemotherapeutic agents? What are the best pharmacodynamic endpoints to be followed? How can the “stable disease phenomenon” in the phase II setting be captured scientifically?

CONCLUSIONS

Based on the above discussion, the inhibiting activity of flavopiridol is strongest for CDK. And this activity is highly associated with the specific structure of flavopiridol. Single-agent evaluation of flavopiridol has demonstrated limited clinical activity, especially with respect to serious adverse effects. Collectively, the combination of flavopiridol with other cytotoxic agents is an emerging, alternative approach to anticancer therapy. There are presently several clinical investigations underway examining the combination of flavopiridol with other cytotoxic agents, with particular attention being paid to sequence and schedule. The phase II and phase III studies will definitely provide useful care for patients with cancer.

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ABBREVIATIONS

AML	=	acute myelogenous leukemia
AUC	=	area under (the plasma concentration time) curve
CDK	=	cyclin-dependent kinase
CDKIs	=	Cyclin-dependent kinase inhibitors
CR	=	complete remission
DFS	=	disease-free survival
DLT	=	dose-limiting toxicities
DSBs	=	DNA double strand breaks
EGFR	=	epidermal growth factor receptor
ER	=	estrogen-receptor
gH2AX	=	phosphorylated histone H2AX
GMSs	=	cisplatin-eluting gelatin microspheres
HAT	=	humanized anti-Tac antibody
HDACIs	=	Histone deacetylase inhibitors
HIF	=	Hypoxia-inducible factor

HUVECs = human umbilical vein endothelial cells

IKK = IκBα kinase

MAPK = mitogen-activated protein kinase

MTD = maximal tolerated dose

NCI = National Cancer Institute

PARP = poly-(ADP-ribose)-polymerase

PI3K = Phosphoinositide 3-kinase

PKA = protein kinase A

P-TEFb = positive transcription elongation factor b

SAHA = suberoylanilide hydroxamic acid

SAR = structure-activity relationship

STAT = signal transducer and activator of transcription

TNF = tumor necrosis factor

TRAIL = TNF -related apoptsis-inducing ligand

VEGF = vascular endothelial growth factor

XIAP = X-linked inhibitor-of-apoptosis protein.

REFRENCES

[1] López-Lázaro, M. Flavonoids as anticancer agents: structure-activity relationship study. *Curr. Med. Chem. Anticancer Agents.*, **2002**, *2*, 691-714.

[2] Takada, Y.; Aggarwal, B.B. Flavopiridol inhibits NF-κB activation induced by various carcinogens and inflammatory agents through inhibition of IκBα kinase and p65 phosphorylation: abrogation of cyclin D1, cyclooxygenase-2, and matrix metalloprotease-9. *J. Biol. Chem.*, **2004**, *279*, 4750-4759.

[3] Kelland, L.R. Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinic: current status. *Expert. Opin. Investig. Drugs.*, **2000**, *9*, 2903-2911.

[4] Shapiro, G.I. Preclinical and clinical development of the cyclin-dependent kinase inhibitor flavopiridol. *Clin. Cancer Res.*, **2004**, *10*, 4270s-4275s.

[5] König, A.; Schwartz, G.K.; Mohammad, R.M.; Al-Katib, A.; Gabrilove, J.L. The novel cyclin-dependent kinase inhibitor flavopiridol downregulates Bcl-2 and induces growth arrest and apoptosis in chronic B-cell leukemia lines. *Blood*, **1997**, *90*, 4307-4312.

[6] Gojo, I.; Zhang, B.; Fenton, R.G. The cyclin-dependent kinase inhibitor flavopiridol induces apoptosis in multiple myeloma cells through transcriptional repression and down-regulation of Mcl-1. *Clin. Cancer Res.*, **2002**, *8*, 3527-3538.

[7] Kitada, S.; Zapata, J.M.; Andreeff, M.; Reed, J.C. Protein kinase inhibitors flavopiridol and 7-hydroxy-staurosporine down-regulate antiapoptosis proteins in B-cell chronic lymphocytic leukemia. *Blood*, **2000**, *96*, 393-397.

[8] Wittmann, S.; Bali, P.; Donapaty, S.; Nimmanapalli, R.; Guo, F.; Yamaguchi, H.; Huang, M.; Jove, R.; Wang, H.G.; Bhalla, K. Flavopiridol down-regulates antiapoptotic proteins and sensitizes human breast cancer cells to epothilone B-induced apoptosis. *Cancer Res.*, **2003**, *63*, 93-99.

[9] Wu, K.; Wang, C.; D'Amico, M.; Lee, R.J.; Albanese, C.; Pestell, R.G.; Mani, S. Flavopiridol and trastuzumab synergistically inhibit proliferation of breast cancer cells: association with selective cooperative inhibition of cyclin D1-dependent kinase and Akt signaling pathways. *Mol. Cancer Ther.*, **2002**, *1*, 695-706.

[10] Carlson, B.; Lahusen, T.; Singh, S.; Loaiza-Perez, A.; Worland, P.J.; Pestell, R.; Albanese, C.; Sausville, E.A.; Senderowicz, A.M. Down-regulation of cyclin D1 by transcriptional repression in MCF-7 human breast carcinoma cells induced by flavopiridol. *Cancer Res.*, **1999**, *59*, 4634-4641.

[11] Melillo, G.; Sausville, E.A.; Cloud, K.; Lahusen, T.; Varesio, L.; Senderowicz, A.M. Flavopiridol, a protein kinase inhibitor, down-regulates hypoxic induction of vascular endothelial growth factor expression in human monocytes. *Cancer Res.*, **1999**, *59*, 5433-5437.

[12] Motwani, M.; Delohery, T.M.; Schwartz, G.K. Sequential dependent enhancement of caspase activation and apoptosis by flavopiridol on paclitaxel-treated human gastric and breast cancer cells. *Clin. Cancer Res.*, **1999**, *5*, 1876-1883.

[13] Bible, K.C.; Kaufmann, S.H. Cytotoxic synergy between flavopiridol (NSC 649890, L86-8275) and various antineoplastic agents: the importance of sequence of administration. *Cancer Res.*, **1997**, *57*, 3375-3380.

[14] Patel, V.; Senderowicz, A.M.; Pinto, D.Jr.; Igishi, T.; Raffeld, M.; Quintanilla-Martinez, L.; Ensley, J.F.; Sausville, E.A.; Gutkind, J.S. Flavopiridol, a novel cyclin-dependent kinase inhibitor, suppresses the growth of head and neck squamous cell carcinomas by inducing apoptosis. *J. Clin. Invest.*, **1998**, *102*, 1674-1681.

[15] Jung, C.; Motwani, M.; Kortmansky, J.; Sirotnak, F.M.; She, Y.; Gonen, M.; Haimovitz-Friedman, A.; Schwartz, G.K. The cyclin-dependent kinase inhibitor flavopiridol potentiates γ-irradiation-induced apoptosis in colon and gastric cancer cells. *Clin. Cancer Res.*, **2003**, *9*, 6052-6061.

[16] Sato, S.; Kajiyama, Y.; Sugano, M.; Iwanuma, Y.; Tsurumaru, M. Flavopiridol as a radio-sensitizer for esophageal cancer cell lines. *Dis. Esophagus.*, **2004**, *17*, 338-344.

[17] Matranga, C.B.; Shapiro, G.I. Selective sensitization of transformed cells to flavopiridol-induced apoptosis following recruitment to S-phase. *Cancer Res.*, **2002**, *62*, 1707-1717.

[18] Motwani, M.; Rizzo, C.; Sirotnak, F.; She, Y.; Schwartz, G.K. Flavopiridol enhances the effect of docetaxel *in vitro* and *in vivo* in human gastric cancer cells. *Mol. Cancer Ther.*, **2003**, *2*, 549-555.

[19] Fornier, M.N.; Rathkopf, D.; Shah, M.; Patil, S.; O'Reilly, E.; Tse, A.N.; Hudis, C.; Lefkowitz, R.; Kelsen, D.P.; Schwartz, G.K. Phase I dose-finding study of weekly docetaxel followed by flavopiridol for patients with advanced solid tumors. *Clin. Cancer Res.*, **2007**, *13*, 5841-5846.

[20] Bible, K.C.; Lensing, J.L.; Nelson, S.A.; Lee, Y.K.; Reid, J.M.; Ames, M.M.; Isham, C.R.; Piens, J.; Rubin, S.L.; Rubin, J.; Kaufmann, S.H.; Atherton, P.J.; Sloan, J.A.; Daiss, M.K.; Adjei, A.A.; Erlichman, C. Phase I trial of flavopiridol combined with cisplatin or carboplatin in patients with advanced malignancies with the assessment of pharmacokinetic and pharmacodynamic end points. *Clin. Cancer Res.*, **2005**, *11*, 5935-5941.

[21] Mitchell, C.; Park, M.A.; Zhang, G.; Yacoub, A.; Curiel, D.T.; Fisher, P.B.; Roberts, J.D.; Grant, S.; Dent, P. Extrinsic pathway- and cathepsin-dependent induction of mitochondrial dysfunction are essential for synergistic flavopiridol and vorinostat lethality in breast cancer cells. *Mol. Cancer Ther.*, **2007**, *6*, 3101-3112.

[22] Kim, D.M.; Koo, S.Y.; Jeon, K.; Kim, M.H.; Lee, J.; Hong, C.Y.; Jeong, S. Rapid induction of apoptosis by combination of flavopiridol and tumor necrosis factor (TNF)-α or TNF-related apoptosis-inducing ligand in human cancer cell lines. *Cancer Res.*, **2003**, *63*, 621-626.

[23] Pei, X.Y.; Dai, Y.; Grant, S. The small-molecule Bcl-2 inhibitor HA14-1 interacts synergistically with flavopiridol to induce mitochondrial injury and apoptosis in human myeloma cells through a free radical-dependent and Jun NH_2-terminal kinase-dependent mechanism. *Mol. Cancer Ther.*, **2004**, *3*, 1513-1524.

[24] Mohanakumara, P.; Sreejayan, N.; Priti, V.; Ramesha, B.T.; Ravikanth, G.; Ganeshaiah, K.N.; Vasudeva, R.; Mohan, J.; Santhoshkumar, T.R.; Mishra, P.D.; Ram, V.; Shaanker, R.U. Dysoxylum binectariferum Hook.f (Meliaceae), a rich source of rohitukine. *Fitoterapia.*, **2010**, *81*, 145-148.

[25] Kim, K.S.; Sack, J.S.; Tokarski, J.S.; Qian, L.; Chao, S.T.; Leith, L.; Kelly, Y.F.; Misra, R.N.; Hunt, J.T.; Kimball, S.D.; Humphreys, W.G.; Wautlet, B.S.; Mulheron, J.G.; Webster, K.R. Thio- and oxoflavopiridols, cyclin-dependent kinase 1-selective inhibitors: synthesis and biological effects. *J. Med. Chem.*, **2000**, *43*, 4126-4134.

[26] Murthi, K.K.; Dubay, M.; McClure, C.; Brizuela, L.; Boisclair, M.D.; Worland, P.J.; Mansuri, M.M.; Pal, K. Structure-activity relationship studies of flavopiridol analogues. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1037-1041.

[27] Gross, A.; Borcherding, D.R.; Friedrich, D.; Sabol, J.S. A stereocontrolled approach to substituted piperidones and piperidines: flavopiridol D-ring analogs. *Tetrahedron. Lett.*, **2001**, *42*, 1631-1633.

[28] Ali, A.; Ghosh, A.; Nathans, R.S.; Sharova, N.; O'Brien, S.; Cao, H.; Stevenson, M.; Rana, T.M. Identification of flavopiridol analogues that selectively inhibit positive transcription elongation factor (P-TEFb) and block HIV-1 replication. *ChemBioChem.*, **2009**, *10*, 2072-2080.

[29] Schoepfer, J.; Fretz, H.; Chaudhuri, B.; Muller, L.; Seeber, E.; Meijer, L.; Lozach, O.; Vangrevelinghe, E.; Furet, P. Structure-based design and synthesis of 2-benzylidene-benzofuran-3-ones as flavopiridol mimics. *J. Med. Chem.*, **2002**, *45*, 1741-1747.

[30] Deep, G.; Agarwal, R. New combination therapies with cell-cycle agents. *Curr. Opin. Investig. Drugs.*, **2008**, *9*, 591-604.

[31] Vermeulen, K.; Van Bockstaele, D.R.; Berneman, Z.N. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell. Prolif.*, **2003**, *36*, 131-149.

[32] Mihara, M.; Shintani, S.; Nakashiro, K.; Hamakawa, H. Flavopiridol, a cyclin dependent kinase (CDK) inhibitor, induces apoptosis by regulating Bcl-x in oral cancer cells. *Oral. Oncol.*, **2003**, *39*, 49-55.

[33] Pines, J. Cyclins and cyclin-dependent kinases: theme and variations. *Adv. Cancer Res.*, **1995**, *66*, 181-212.

[34] Atherton-Fessler, S.; Parker, L.L.; Geahlen, R.L.; Piwnica-Worms, H. Mechanisms of p34cdc2 regulation. *Mol. Cell. Biol.*, **1993**, *13*, 1675-1685.

[35] Benson, C.; Kaye, S.; Workman, P.; Garrett, M.; Walton, M.; de Bono, J. Clinical anticancer drug development: targeting the cyclin-dependent kinases. *Br. J. Cancer*, **2005**, *92*, 7-12.

[36] Dai, Y.; Grant, S. Cyclin-dependent kinase inhibitors. *Curr. Opin. Pharmacol.*, **2003**, *3*, 362-370.

[37] Senderowicz, A.M.; Sausville, E.A. Preclinical and Clinical Development of Cyclin-Dependent Kinase Modulators. *J. Natl. Cancer Inst.*, **2000**, *92*, 376-387.

[38] Senderowicz, A.M. Flavopiridol: the first cyclin-dependent kinase inhibitor in human clinical trials. *Invest. New. Drugs*, **1999**, *17*, 313-320.

[39] Sedlacek, H.H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A.; Senderowicz, A.; Sausville, E. Flavopiridol (L86-8275, NSC-649890), a new kinase inhibitor for tumor therapy. *Int. J. Oncol.*, **1996**, *9*, 1143-1168.

[40] Carlson, B.; Pearlstein, R.; Naik, R.; Sedlacek, H.; Sausville, E.; Worland, P. Inhibition of CDK2, CDK4 and CDK7 by flavopiridol and structural analogs [abstract]. *Proc. Am. Assoc. Cancer Res.*, **1996**, *37*, 424.

[41] Carlson, B.A.; Dubay, M.M.; Sausville, E.A.; Brizuela, L.; Worland, P.J. Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. *Cancer Res.*, **1996**, *56*, 2973-2978.

[42] Losiewicz, M.D.; Carlson, B.A.; Kaur, G.; Sausville, E.A.; Worland, P.J. Potent inhibition of CDC2 kinase activity by the flavonoid L86-8275. *Biochem. Biophys. Res. Commun.*, **1994**, *201*, 589-595.

[43] Singh, S.S.; Sausville, E.A.; Senderowicz, A.M. Cyclin D1 and CDK6 are the targets for flavopiridol-mediated G1 block in MCF10A breast epithelial cell line [abstract]. *Proc. Am. Assoc. Cancer Res.*, **1999**, *40*, 28.

[44] Worland, P.J.; Kaur, G.; Stetler-Stevenson, M.; Sebers, S.; Sartor, O.; Sausville, E.A. Alteration of the phosphorylation state of p34cdc2 kinase by the flavone L86-8275 in breast carcinoma cells. Correlation with decreased H1 kinase activity. *Biochem. Pharmacol.*, **1993**, *46*, 1831-1840.

[45] Sedlacek, H.H. Mechanisms of action of flavopiridol. *Crit. Rev. Oncol. Hematol.*, **2001**, *38*, 139-170.

[46] Senderowicz, A.M. The cell cycle as a target for cancer therapy: basic and clinical findings with the small molecule inhibitors flavopiridol and UCN-01. *Oncologist*, **2002**, *7*(Suppl 3), 12-19.

[47] Chao, S.H.; Fujinaga, K.; Marion, J.E.; Taube, R.; Sausville, E.A.; Senderowicz, A.M.; Peterlin, B.M.; Price, D.H. Flavopiridol inhibits P-TEFb and blocks HIV-1 replication. *J. Biol. Chem.*, **2000**, *275*, 28345-28348.

[48] Price, D.H. P-TEFb, a cyclin-dependent kinase controlling elongation by RNA polymerase II. *Mol. Cell. Biol.*, **2000**, *20*, 2629-2634.

[49] Monga, M.; Sausville, E.A. Developmental therapeutics program at the NCI: molecular target and drug discovery process. *Leukemia*, **2002**, *16*, 520-526.

[50] Brüsselbach, S.; Nettelbeck, D.M.; Sedlacek, H.H.; Müller, R. Cell cycle-independent induction of apoptosis by the anti-tumor drug Flavopiridol in endothelial cells. *Int. J. Cancer*, **1998**, *77*, 146-152.

[51] Kerr, J.S.; Wexler, R.S.; Mousa, S.A.; Robinson, C.S.; Wexler, E.J.; Mohamed, S.; Voss, M.E.; Devenny, J.J.; Czerniak, P.M.; Gudzelak, A. Jr.; Slee, A.M. Novel small molecule alpha v integrin antagonists: comparative anti-cancer efficacy with known angiogenesis inhibitors. *Anti Cancer Res.*, **1999**, *19*, 959-968.

[52] Byrd, J.C.; Shinn, C.; Waselenko, J.K.; Fuchs, E.J.; Lehman, T.A.; Nguyen, P.L.; Flinn, I.W.; Diehl, L.F.; Sausville, E.; Grever, M.R. Flavopiridol induces apoptosis in chronic lymphocytic leukemia cells *via* activation of caspase-3 without evidence of bcl-2 modulation or dependence on functional p53. *Blood*, **1998**, *92*, 3804-3816.

[53] Pepper, C.; Thomas, A.; Hoy, T.; Fegan, C.; Bentley, P. Flavopiridol circumvents Bcl-2 family mediated inhibition of apoptosis and drug resistance in B-cell chronic lymphocytic leukaemia. *Br. J. Haematol.*, **2001**, *114*, 70-77.

[54] Ma, Y.; Cress, W.D.; Haura, E.B. Flavopiridol-induced apoptosis is mediated through up-regulation of E2F1 and repression of Mcl-1. *Mol. Cancer Ther.*, **2003**, *2*, 73-81.

[55] Lee, H.R.; Chang, T.H.; Tebalt, M.J. 3rd.; Senderowicz, A.M.; Szabo, E. Induction of differentiation accompanies inhibition of CDK2 in a non-small cell lung cancer cell line. *Int. J. Oncol.*, **1999**, *15*, 161-166.

[56] Gomez, L.A.; de Las Pozas, A.; Perez-Stable, C. Sequential combination of flavopiridol and docetaxel reduces the levels of X-linked inhibitor of apoptosis and AKT proteins and stimulates apoptosis in human LNCaP prostate cancer cells. *Mol. Cancer Ther.*, **2006**, *5*, 1216-1226.

[57] Reiner, T.; de las Pozas, A.; Perez-Stable, C. Sequential combinations of flavopiridol and docetaxel inhibit prostate tumors, induce apoptosis, and decrease angiogenesis in the Gγ/T-15 transgenic mouse model of prostate cancer. *Prostate*, **2006**, *66*, 1487-1497.

[58] Kim, J.C.; Saha, D.; Cao, Q.; Choy, H. Enhancement of radiation effects by combined docetaxel and flavopiridol treatment in lung cancer cells. *Radiother. Oncol.*, **2004**, *71*, 213-221.

[59] Carrozza, M.J.; Utley, R.T.; Workman, J.L.; Côté, J. The diverse functions of histone acetyltransferase complexes. *Trends Genet.*, **2003**, *19*, 321-329.

[60] Peterson, C.L. HDAC's at work: everyone doing their part. *Mol. Cell.*, **2002**, *9*, 921-922.

[61] Gray, S.G.; Teh, B.T. Histone acetylation/deacetylation and cancer: an "open" and "shut" case? *Curr. Mol. Med.*, **2001**, *1*, 401-429.

[62] Rivero, J.A.; Adunyah, S.E. Sodium butyrate stimulates PKC activation and induces differential expression of certain PKC isoforms during erythroid differentiation. *Biochem. Biophys. Res. Commun.*, **1998**, *248*, 664-648.

[63] Kim, D.; Dan, H.C.; Park, S.; Yang, L.; Liu, Q.; Kaneko, S.; Ning, J.; He, L.; Yang, H.; Sun, M.; Nicosia, S.V.; Cheng, J.Q. AKT/PKB signaling mechanisms in cancer and chemoresistance. *Front. Biosci.*, **2005**, *10*, 975-987.

[64] Nguyen, D.M.; Schrump, W.D.; Tsai, W.S.; Chen, A.; Stewart, J.H. 4th; Steiner, F.; Schrump, D.S. Enhancement of depsipeptide-mediated apoptosis of lung or esophageal cancer cells by flavopiridol: activation of the mitochondria-dependent death-signaling pathway. *J. Thorac. Cardiovasc. Surg.*, **2003**, *125*, 1132-1142.

[65] Almenara, J.; Rosato, R.; Grant, S. Synergistic induction of mitochondrial damage and apoptosis in human leukemia cells by flavopiridol and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). *Leukemia*, **2002**, *16*, 1331-1343.

[66] Rosato, R.R.; Almenara, J.A.; Cartee, L.; Betts, V.; Chellappan, S.P.; Grant, S. The cyclin-dependent kinase inhibitor flavopiridol disrupts sodium butyrate-induced $p21^{WAF1/CIP1}$ expression and maturation while reciprocally potentiating apoptosis in human leukemia cells. *Mol. Cancer Ther.*, **2002**, *1*, 253-266.

[67] Nguyen, D.M.; Schrump, W.D.; Chen, G.A.; Tsai, W.; Nguyen, P.; Trepel, J.B.; Schrump, D.S. Abrogation of p21 expression by flavopiridol enhances depsipeptide-mediated apoptosis in malignant pleural mesothelioma cells. *Clin. Cancer Res.*, **2004**, *10*, 1813-1825.

[68] Gao, N.; Dai, Y.; Rahmani, M.; Dent, P.; Grant, S. Contribution of disruption of the nuclear factor-κB pathway to induction of apopto-

sis in human leukemia cells by histone deacetylase inhibitors and flavopiridol. *Mol. Pharmacol.*, **2004**, *66*, 956-963.

[69] Mason, K.A.; Hunter, N.R.; Raju, U.; Ariga, H.; Husain, A.; Valdecanas, D.; Neal, R.; Ang, K.K.; Milas, L. Flavopiridol increases therapeutic ratio of radiotherapy by preferentially enhancing tumor radioresponse. *Int. J. Radiat. Oncol. Biol. Phys.*, **2004**, *59*, 1181-1189.

[70] Kim, S.; Wu, H.G.; Shin, J.H.; Park, H.J.; Kim, I.A.; Kim, I.H. Enhancement of radiation effects by flavopiridol in uterine cervix cancer cells. *Cancer Res. Treat.*, **2005**, *37*, 191-195.

[71] Newcomb, E.W.; Lymberis, S.C.; Lukyanov, Y.; Shao, Y.; Schnee, T.; Devitt, M.; Rosenstein, B.S.; Zagzag, D.; Formenti, S.C. Radiation sensitivity of GL261 murine glioma model and enhanced radiation response by flavopiridol. *Cell Cycle*, **2006**, *5*, 93-99.

[72] Raju, U.; Nakata, E.; Mason, K.A.; Ang, K.K.; Milas, L. Flavopiridol, a cyclin-dependent kinase inhibitor, enhances radiosensitivity of ovarian carcinoma cells. *Cancer Res.*, **2003**, *63*, 3263-3267.

[73] Chien, M.; Astumian, M.; Liebowitz, D.; Rinker-Schaeffer, C.; Stadler, W.M. *In vitro* evaluation of flavopiridol, a novel cell cycle inhibitor, in bladder cancer. *Cancer Chemother. Pharmacol.*, **1999**, *44*, 81-87.

[74] Camphausen, K.; Brady, K.J.; Burgan, W.E.; Cerra, M.A.; Russell, J.S.; Bull, E.E.; Tofilon, P.J. Flavopiridol enhances human tumor cell radiosensitivity and prolongs expression of γH2AX foci. *Mol. Cancer Ther.*, **2004**, *3*, 409-416.

[75] Dai, Y.; Rahmani, M.; Grant, S. Proteasome inhibitors potentiate leukemic cell apoptosis induced by the cyclin-dependent kinase inhibitor flavopiridol through a SAPK/JNK- and NF-κB-dependent process. *Oncogene*, **2003**, *22*, 7108-7122.

[76] Yu, C.; Krystal, G.; Dent, P.; Grant, S. Flavopiridol potentiates STI571-induced mitochondrial damage and apoptosis in BCR-ABL-positive human leukemia cells. *Clin. Cancer Res.*, **2002**, *8*, 2976-2984.

[77] Dai, Y.; Rahmani, M.; Pei, X.Y.; Dent, P.; Grant, S. Bortezomib and flavopiridol interact synergistically to induce apoptosis in chronic myeloid leukemia cells resistant to imatinib mesylate through both Bcr/Abl-dependent and -independent mechanisms. *Blood*, **2004**, *104*, 509-518.

[78] Nahta, R.; Iglehart, J.D.; Kempkes, B.; Schmidt, E.V. Rate-limiting effects of Cyclin D1 in transformation by ErbB2 predicts synergy between herceptin and flavopiridol. *Cancer Res.*, **2002**, *62*, 2267-2271.

[79] Nahta, R.; Trent, S.; Yang, C.; Schmidt, E.V. Epidermal growth factor receptor expression is a candidate target of the synergistic combination of trastuzumab and flavopiridol in breast cancer. *Cancer Res.*, **2003**, *63*, 3626-3631.

[80] Zhang, M.; Zhang, Z.; Goldman, C.K.; Janik, J.; Waldmann, T.A. Combination therapy for adult T-cell leukemia-xenografted mice: flavopiridol and anti-CD25 monoclonal antibody. *Blood*, **2005**, *105*, 1231-1236.

[81] Ali, S.; El-Rayes, B.F.; Aranha, O.; Sarkar, F.H.; Philip, P.A. Sequence dependent potentiation of gemcitabine by flavopiridol in human breast cancer cells. *Breast Cancer Res. Treat.*, **2005**, *90*, 25-31.

[82] Guo, J.; Zhou, A.W.; Fu, Y.C.; Verma, U.N.; Tripathy, D.; Frenkel, E.P.; Becerra, C.R. Efficacy of sequential treatment of HCT116 colon cancer monolayers and xenografts with docetaxel, flavopiridol, and 5-fluorouracil. *Acta Pharmacol. Sin.*, **2006**, *27*, 1375-1381.

[83] Jung, C.P.; Motwani, M.V.; Schwartz, G.K. Flavopiridol increases sensitization to gemcitabine in human gastrointestinal cancer cell lines and correlates with down-regulation of ribonucleotide reductase M2 subunit. *Clin. Cancer Res.*, **2001**, *7*, 2527-2536.

[84] Motwani, M.; Jung, C.; Sirotnak, F.M.; She, Y.; Shah, M.A.; Gonen, M.; Schwartz, G.K. Augmentation of apoptosis and tumor regression by flavopiridol in the presence of CPT-11 in Hct116 colon cancer monolayers and xenografts. *Clin. Cancer Res.*, **2001**, *7*, 4209-4219.

[85] Motwani, M.; Schwartz, G.K. Flavopiridol (Flavo) potentiates the SN-38-induced apopotosis in association with down regulation of cyclin dependent kinase inhibitor p21*waf1/cip1* in HCT-116 cells. *Proc. Am. Assoc. Cancer Res.*, **2000**, *40*, 209.

[86] Ambrosini, G.; Seelman, S.L.; Qin, L.X.; Schwartz, G.K. The cyclin-dependent kinase inhibitor flavopiridol potentiates the effects of topoisomerase I poisons by suppressing Rad51 expression in a p53-dependent manner. *Cancer Res.*, **2008**, *68*, 2312-2320.

[87] Wu, K.; D'Amico, M.; Wang, C.; Albanese, C.; Pestell, R.G.; Mani, S. A study of cytotoxic synergy of UCN-01 and flavopiridol in syngeneic pair of cell lines. *Invest. New. Drugs.*, **2005**, *23*, 299-309.

[88] Li, W.; Fan, J.; Bertino, J.R. Selective sensitization of retinoblastoma protein-deficient sarcoma cells to doxorubicin by flavopiridol-mediated inhibition of cyclin-dependent kinase 2 kinase activity. *Cancer Res.*, **2001**, *61*, 2579-2582.

[89] Budak-Alpdogan, T.; Chen, B.; Warrier, A.; Medina, D.J.; Moore, D.; Bertino, J.R. Retinoblastoma tumor suppressor gene expression determines the response to sequential flavopiridol and doxorubicin treatment in small-cell lung carcinoma. *Clin. Cancer Res.*, **2009**, *15*, 1232-1240.

[90] Palacios, C.; Yerbes, R.; López-Rivas, A. Flavopiridol induces cellular FLICE-inhibitory protein degradation by the proteasome and promotes TRAIL-induced early signaling and apoptosis in breast tumor cells. *Cancer Res.*, **2006**, *66*, 8858-8869.

[91] Rosato, R.R.; Dai, Y.; Almenara, J.A.; Maggio, S.C.; Grant, S. Potent antileukemic interactions between flavopiridol and TRAIL/Apo2L involve flavopiridol-mediated XIAP downregulation. *Leukemia*, **2004**, *18*, 1780-1788.

[92] Iyirhiaro, G.O.; Brust, T.B.; Rashidian, J.; Galehdar, Z.; Osman, A.; Phillips, M.; Slack, R.S.; Macvicar, B.A.; Park, D.S. Delayed combinatorial treatment with flavopiridol and minocycline provides longer term protection for neuronal soma but not dendrites following global ischemia. *J. Neurochem.*, **2008**, *105*, 703-713.

[93] Jorda, E.G.; Verdaguer, E.; Canudas, A.M.; Jiménez, A.; Bruna, A.; Caelles, C.; Bravo, R.; Escubedo, E.; Pubill, D.; Camarasa, J.; Pallàs, M.; Camins, A. Neuroprotective action of flavopiridol, a cyclin-dependent kinase inhibitor, in colchicine-induced apoptosis. *Neuropharmacology*, **2003**, *45*, 672-683.

[94] Cartee, L.; Maggio, S.C.; Smith, R.; Sankala, H.M.; Dent, P.; Grant, S. Protein kinase C-dependent activation of the tumor necrosis factor receptor-mediated extrinsic cell death pathway underlies enhanced apoptosis in human myeloid leukemia cells exposed to bryostatin 1 and flavopiridol. *Mol. Cancer Ther.*, **2003**, *2*, 83-93.

[95] Lee, Y.K.; Isham, C.R.; Kaufman, S.H.; Bible, K.C. Flavopiridol disrupts STAT3/DNA interactions, attenuates STAT3-directed transcription, and combines with the Jak kinase inhibitor AG490 to achieve cytotoxic synergy. *Mol. Cancer Ther.*, **2006**, *5*, 138-148.

[96] Ge, Y.; Byun, J.S.; De Luca, P.; Gueron, G.; Yabe, I.M.; Sadiq-Ali, S.G.; Figg, W.D.; Quintero, J.; Haggerty, C.M.; Li, Q.Q.; De Siervi, A.; Gardner, K. Combinatorial antileukemic disruption of oxidative homeostasis and mitochondrial stability by the redox reactive thalidomide 2-(2,4-difluoro-phenyl)-4,5,6,7-tetrafluoro-1H-isoindole-1,3(2H)-dione (CPS49) and flavopiridol. *Mol. Pharmacol.*, **2008**, *74*, 872-883.

[97] Hooijberg, J.H.; Broxterman, H.J.; Scheffer, G.L.; Vrasdonk, C.; Heijn, M.; de Jong, M.C.; Scheper, R.J.; Lankelma, J.; Pinedo, H.M. Potent interaction of flavopiridol with MRP1. *Br. J. Cancer*, **1999**, *81*, 269-276.

[98] Incles, C.M.; Schultes, C.M.; Kelland, L.R.; Neidle, S. Acquired cellular resistance to flavopiridol in a human colon carcinoma cell line involves up-regulation of the telomerase catalytic subunit and telomere elongation. Sensitivity of resistant cells to combination treatment with a telomerase inhibitor. *Mol. Pharmacol.*, **2003**, *64*, 1101-1108.

[99] Schwartz, G.K.; Farsi, K.; Maslak, P.; Kelsen, D.P.; Spriggs, D. Potentiation of apoptosis by flavopiridol in mitomycin-C-treated gastric and breast cancer cells. *Clin. Cancer Res.*, **1997**, *3*, 1467-1472.

[100] Nitta, N.; Sonoda, A.; Seko, A.; Ohta, S.; Nagatani, Y.; Tsuchiya, K.; Otani, H.; Tanaka, T.; Kanasaki, S.; Takahashi, M.; Murata, K. A combination of cisplatin-eluting gelatin microspheres and flavopiridol enhances antitumour effects in a rabbit VX2 liver tumour model. *Br. J. Radiol.*, **2010**, *83*(989), 428-432.

[101] El-Rayes, B.F.; Gadgeel, S.; Parchment, R.; Lorusso, P.; Philip, P.A. A phase I study of flavopiridol and docetaxel. *Invest. New. Drugs.*, **2006**, *24*, 305-310.

[102] Schwartz, G.K.; O'Reilly, E.; Ilson, D.; Saltz, L.; Sharma, S.; Tong, W.; Maslak, P.; Stoltz, M.; Eden, L.; Perkins, P.; Endres, S.; Barazzoul, J.; Spriggs, D.; Kelsen, D. Phase I study of the cyclin-dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors. *J. Clin. Oncol.*, **2002**, *20*, 2157-2170.

[103] George, S.; Kasimis, B.S.; Cogswell, J.; Schwarzenberger, P.; Shapiro, G.I.; Fidias, P.; Bukowski, R.M. Phase I study of flavopiridol in combination with Paclitaxel and Carboplatin in patients with non-small-cell lung cancer. *Clin. Lung. Cancer*, **2008**, *9*, 160-165.

[104] Tan, A.R.; Yang, X.; Berman, A.; Zhai, S.; Sparreboom, A.; Parr, A.L.; Chow, C.; Brahim, J.S.; Steinberg, S.M.; Figg, W.D.; Swain, S.M. Phase I trial of the cyclin-dependent kinase inhibitor flavopiridol in combination with docetaxel in patients with metastatic breast cancer. *Clin. Cancer Res.*, **2004**, *10*, 5038-5047.

[105] Shah, M.A.; Kortmansky, J.; Motwani, M.; Drobnjak, M.; Gonen, M.; Yi, S.; Weyerbacher, A.; Cordon-Cardo, C.; Lefkowitz, R.; Brenner, B.; O'Reilly, E.; Saltz, L.; Tong, W.; Kelsen, D.P.; Schwartz, G.K. A phase I clinical trial of the sequential combination of irinotecan followed by flavopiridol. *Clin. Cancer Res.*, **2005**, *11*, 3836-3845.

[106] Karp, J.E.; Passaniti, A.; Gojo, I.; Kaufmann, S.; Bible, K.; Garimella, T.S.; Greer, J.; Briel, J.; Smith, B.D.; Gore, S.D.; Tidwell, M.L.; Ross, D.D.; Wright, J.J.; Colevas, A.D.; Bauer, K.S. Phase I and pharmacokinetic study of flavopiridol followed by 1-beta-D-arabinofuranosylcytosine and mitoxantrone in relapsed and refractory adult acute leukemias. *Clin. Cancer Res.*, **2005**, *11*, 8403-8412.

[107] Rathkopf, D.; Dickson, M.A.; Feldman, D.R.; Carvajal, R.D.; Shah, M.A.; Wu, N.; Lefkowitz, R.; Gonen, M.; Cane, L.M.; Dials, H.J.; Winkelmann, J.L.; Bosl, G.J.; Schwartz, G.K. Phase I study of flavopiridol with oxaliplatin and fluorouracil/leucovorin in advanced solid tumors. *Clin. Cancer Res.*, **2009**, *15*, 7405-7411.

[108] Lin, T.S.; Blum, K.A.; Fischer, D.B.; Mitchell, S.M.; Ruppert, A.S.; Porcu, P.; Kraut, E.H.; Baiocchi, R.A.; Moran, M.E.; Johnson, A.J.; Schaaf, L.J.; Grever, M.R.; Byrd, J.C. Flavopiridol, fludarabine, and rituximab in mantle cell lymphoma and indolent B-cell lymphoproliferative disorders. *J. Clin. Oncol.*, **2010**, *28*, 418-423.

[109] Karp, J.E.; Smith, B.D.; Levis, M.J.; Gore, S.D.; Greer, J.; Hattenburg, C.; Briel, J.; Jones, R.J.; Wright, J.J.; Colevas, A.D. Sequential flavopiridol, cytosine arabinoside, and mitoxantrone: a phase II trial in adults with poor-risk acute myelogenous leukemia. *Clin. Cancer Res.*, **2007**, *13*, 4467-4473.

[110] Karp, J.E.; Ross, D.D.; Yang, W.; Tidwell, M.L.; Wei, Y.; Greer, J.; Mann, D.L.; Nakanishi, T.; Wright, J.J.; Colevas, A.D. Timed sequential therapy of acute leukemia with flavopiridol: *in vitro* model for a phase I clinical trial. *Clin. Cancer Res.*, **2003**, *9*, 307-315.

[111] Karp, J.E.; Blackford, A.; Smith, B.D.; Alino, K.; Seung, A.H.; Bolaños-Meade, J.; Greer, J.M.; Carraway, H.E.; Gore, S.D.; Jones, R.J.; Levis, M.J.; McDevitt, M.A.; Doyle, L.A.; Wright, J.J. Clinical activity of sequential flavopiridol, cytosine arabinoside, and mitoxantrone for adults with newly diagnosed, poor-risk acute myelogenous leukemia. *Leuk. Res.*, **2009**, [Epub ahead of print].

[112] Carvajal, R.D.; Tse, A.; Shah, M.A.; Lefkowitz, R.A.; Gonen, M.; Gilman-Rosen, L.; Kortmansky, J.; Kelsen, D.P.; Schwartz, G.K.; O'Reilly, E.M. A phase II study of flavopiridol (Alvocidib) in combination with docetaxel in refractory, metastatic pancreatic cancer. *Pancreatology*, **2009**, *9*, 404-409.

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