Flavopiridol Protects Against Inflammation by Attenuating Leukocyte-Endothelial Interaction via Inhibition of Cyclin-Dependent Kinase 9

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Objective—The cyclin-dependent kinase (CDK) inhibitor flavopiridol is currently being tested in clinical trials as anticancer drug. Beyond its cell death–inducing action, we hypothesized that flavopiridol affects inflammatory processes. Therefore, we elucidated the action of flavopiridol on leukocyte–endothelial cell interaction and endothelial activation in vivo and in vitro and studied the underlying molecular mechanisms.

Methods and Results—Flavopiridol suppressed concanavalin A–induced hepatitis and neutrophil infiltration into liver tissue. Flavopiridol also inhibited tumor necrosis factor-α–induced leukocyte–endothelial cell interaction in the mouse cremaster muscle. Endothelial cells were found to be the major target of flavopiridol, which blocked the expression of endothelial cell adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin), as well as NF-κB-dependent transcription. Flavopiridol did not affect inhibitor of κB (IκB) kinase, the degradation and phosphorylation of IκBα, nuclear translocation of p65, or nuclear factor-κB (NF-κB) DNA-binding activity. By performing a cellular kinome array and a kinase activity panel, we found LIM domain kinase-1 (LIMK1), casein kinase 2, c-Jun N-terminal kinase (JNK), protein kinase Cθ (PKCθ), CDK4, CDK6, CDK8, and CDK9 to be influenced by flavopiridol. Using specific inhibitors, as well as RNA interference (RNAi), we revealed that only CDK9 is responsible for the action of flavopiridol.


Key Words: adhesion molecules • endothelium • leukocytes • pharmacology • signal transduction • cyclin-dependent kinase • inflammation • leukocyte extravasation • leukocyte-endothelial cell interaction

Flavopiridol (alvocidib) is a synthetic flavone structurally related to an alkaloid purified from Dysoxylum binectariferum, a plant used in Indian folk medicine.1 Flavopiridol was found to exert cytotoxic effects, which were ascribed to the inhibition of cyclin-dependent kinases (CDKs), ie, the blockade of cell cycle progression.1 CDKs represent crucial regulators of the cell cycle. An overactivity of cell cycle CDKs can be observed in tumor cells, leading to their growth advantage. Consequently, CDK inhibition has been proposed as novel anticancer strategy. Flavopiridol was reported to possess potent antiproliferative action on 60 human cancer cell lines in the US National Cancer Institute screen panel1 and was the first CDK inhibitor to undergo clinical trials. Currently, flavopiridol is evaluated in numerous studies for treating hematologic and solid cancers.

Based on the cell growth-inhibiting feature of flavopiridol, the compound was tested for its effect on the overgrowth of synovial fibroblasts in collagen-induced murine arthritis and was found to suppress synovial hyperplasia.2 Moreover, CDK inhibitors were suggested to induce immune cell death and, thus, to enhance the resolution of inflammation.3 Surprisingly, beyond the antiproliferative and cell death–inducing way of action, no studies have as yet focused on the action of flavopiridol, and CDK inhibitors in general, on an early and crucial step in inflammation, the interaction of leukocytes with endothelial cells (ECs). An excessive leukocyte extravasation from the blood into the tissue—a process that is tightly regulated by the endothelium—is a hallmark of inflammation and contributes to the pathogenesis and progression of many severe inflammation-associated patholo-

Received on: July 29, 2010; final version accepted on: November 8, 2010.

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Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org DOI: 10.1161/ATVBAHA.110.213934
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Neutrophils were primed with 1:3,4-b]diindol-5-IMchloride) was from Sigma-Aldrich (Taufkirchen, Germany). The c-Jun N-terminal kinase inhibitor SP600125 (1,9-pyrazoloanthrone) was from Enzo Life Sciences (Lörrach, Germany), and the casein kinase 2 (CK2) inhibitor 4,5,6,7-tetrabromo-2-azabenzoimidazole was from Tocris (Bristol, UK). The CK2 inhibitor quinalizarin (1,2,5,8-tetrahydroxyanthracene-9,10-dione) was a kind gift of Prof Lorenzo Pinna (University of Padua, Padua, Italy). The myristoylated PKC pseudosubstrate inhibitor (Myr-LHQRQRGAIKQ AKHVVKC-NH3) was from Calbiochem (Darmstadt, Germany).

Concanavalin A–Induced Murine Hepatitis

Male mice (C57BL/6, 6 to 8 weeks; Charles River, Sulzfeld, Germany) were performed in accordance with the local animal protection legislation (Government of Upper Bavaria). Surgical preparation of cremaster muscles and intravital microscopy was performed as described previously.4

Materials

Neutrophil Adhesion Assay

Neutrophils were primed with 1 μmol/L dihydrorhodamine-123 (Invitrogen, Karlsruhe, Germany) for 10 minutes, treated as indicated, and analyzed by flow cytometry (FACS Calibur, Becton Dickinson). HUVECs were treated as indicated, trypsinized, formalin fixed, incubated with the respective antibodies, and analyzed by flow cytometry (FACS Calibur).

Cell Culture

Primary human umbilical vein endothelial cells (HUVECs) were isolated and cultured as described previously.6 The human microvascular EC line CDC/EU.HMEC-1 was kindly provided by the Centers for Disease Control and Prevention (Atlanta, Ga).7 Human neutrophil granulocytes were separated from heparinized peripheral blood of healthy volunteers by using CD15 MicroBeads (Miltenyi, Bergisch Gladbach, Germany).

Intravital Microscopy and Cremaster Muscle Preparation

Experiments with male mice (C57BL/6, 6 to 8 weeks; Charles River, Sulzfeld, Germany) were performed in accordance with the local animal protection legislation (Government of Upper Bavaria). Surgical preparation of cremaster muscles and intravital microscopy was performed as described previously.4

Flow Cytometric Analysis

Neutrophils were primed with 1 μmol/L dihydrorhodamine-123 (Invitrogen, Karlsruhe, Germany) for 10 minutes, treated as indicated, and analyzed by flow cytometry (FACS Calibur, Becton Dickinson). HUVECs were treated as indicated, trypsinized, formalin fixed, incubated with the respective antibodies, and analyzed by flow cytometry (FACS Calibur).

Quantification of Apoptosis and Cell Viability

Quantification of apoptosis in HUVECs was carried out as described by Nicoletti et al.6 HUVEC viability was measured by the CellTiter-Blue assay (Promega, Mannheim, Germany).

Western Blot Analysis

Western blot analysis was performed as described previously.9 Densitometric analysis on normalization (loading control) was performed with ImageJ, version 1.43u (National Institutes of Health).

In Vitro IKKβ Kinase Activity Assay

The effect of flavopiridol on purified IKK β (IKKβ) activity was determined using the HTScan IKK β Kinase Assay Kit (Cell Signaling).

NF-κB p65 Translocation and NF-κB DNA-Binding Activity

Immunocytochemistry and electrophoretic mobility shift assay were performed as described previously.5 Densitometric analysis was performed with ImageJ, version 1.43u (National Institutes of Health).

Quantitative Reverse Transcription–Polymerase Chain Reaction

Total mRNA from HUVECs, liver tissue, and cremaster muscle tissue was isolated (RNeasy Mini or Fibrous Tissue Kit; Qiagen, Hilden, Germany). Quantitative reverse transcription–polymerase chain reaction was performed as described previously.6

Gene Silencing

Transfection of HUVECs was performed with the Amaxa HUVEC Nucleofactor kit (Lonza). On target plus short interfering RNA (siRNA) from Dharmacon (Lafayette, Colo) was used. In addition, HUVECs treated with infectious adenoviruses encoding short hairpin RNA (shRNA; nontargeting shRNA or CDK9 shRNA) were purchased from Sirion (Martinsried, Germany).

Kinome Chip Analysis (PepChip)

The PepChip kinome array was performed by Pepscan Presto BV (Lelystad, the Netherlands) as described previously.10 Briefly, HUVECs were treated either with tumor necrosis factor-α (TNF-α; 10 ng/mL) for 15 minutes or with flavopiridol (100 nmol/L) for 30 minutes before TNF-α. Native protein lysates were generated by the M-PER buffer (Pierce, Rockford, Ill). Aliquots of the lysates were mixed with activation solution containing 2 μCi of [γ-32P]ATP. Supernatants of this mixture were loaded on the chip and incubated for 2 hours at 37°C. On the chip, 1152 different peptides with specific phosphorylation motifs for the respective kinase are spotted in triplicate. Phosphor-storage screens were exposed to the chip.

In Vitro Kinase Panel

A radiometric protein kinase assay (33PanQinase Activity Assay, ProQinase, Freiburg, Germany) was used for measuring the kinase activity of 255 protein kinases as described previously.11 Briefly, the kinase assays were performed in 96-well FlashPlates (BioLytic, Biberach, Germany). The reaction cocktail contained nonradioactive and radiometric kinase substrates. The reaction was started by addition of kinase (33PanQ inase Activity Assay, ProQinase, Freiburg, Germany) and the reaction was terminated by addition of 100 mmol/L EDTA. The radioactivity was measured in a liquid scintillation counter (Perkin-Elmer, Waltham, Mass).
ATP, the test sample (in 10% dimethyl sulfoxide), and a kinase/substrate mixture. Flavopiridol was tested at 9 concentrations in the range of $10^{-11}$ to $10^{-9}$ mol/L at a final dimethyl sulfoxide concentration of 1%.

### Statistical Analysis

GraphPad Prism, version 3.03, was used. An unpaired t-test was performed to compare 2 groups. For 3 or more groups, 1-way analysis of variance (ANOVA) followed by the Newman-Keuls post hoc test was used. Data from the cremaster model were analyzed with the Mann-Whitney rank sum test. Data are expressed as mean±SEM.

### Results

#### Flavopiridol Reduces Inflammation in ConA-Induced Liver Injury

In mice, hepatitis was induced by intravenous application of ConA (15 mg/kg). The liver injury was greatly suppressed by pretreatment (15 minutes) with flavopiridol (44 ng, IV), as proven by a large decrease in the serum levels of alanine transaminase and aspartate transaminase (Figure 1A). This protective effect of flavopiridol was further confirmed by microscopic examinations of liver sections (hematoxylin and eosin staining) showing that flavopiridol lowered ConA-triggered necrosis (Figure 1B). Neutrophils are the key initiators of the subsequent lymphocyte recruitment and liver injury caused by ConA. Flavopiridol strongly inhibited neutrophil infiltration of the liver, as shown in the histological images in Figure 1C. A quantification of the neutrophil count is given in Figure 1D. Moreover, the activity of MPO, a marker for neutrophil infiltration, was dramatically decreased by flavopiridol in liver tissue (Figure 1E). In addition, we detected a significant decrease of the endothelium-specific adhesion molecule E-selectin (Figure 1F, left) and of the intercellular adhesion molecule-1 (ICAM-1) mRNA expression in flavopiridol-treated mice (Figure 1F, right). These data show that the antiinflammatory action of flavopiridol is associated with the reduction of leukocyte infiltration and cell adhesion molecule expression.

#### Flavopiridol Abrogates Leukocyte Transmigration and Adhesion in TNF-α-Activated Venules of the Mouse Cremaster Muscle

Intravital microscopy of the mouse cremaster muscle showed that the number of leukocytes that migrated through the

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Flavopiridol protects against ConA-induced liver injury. Mice were treated IV with 15 mg/kg ConA. Flavopiridol (FP, 44 ng, IV) was administered 15 minutes before ConA. Liver tissues were removed 8 hours after ConA administration. n=6 per group. A, Plasma levels of aspartate transaminase (AST) and alanine transaminase (ALT) were assessed. B, Representative hematoxylin/eosin–stained sections of livers. Scale bar represents 50 μm. Arrows indicate necrotic area. Bar graph: The percentage of liver parenchyma with necrotic injury was assessed per high power field (HPF). C, Representative image of liver sections stained for naphthol-AS-D-chloroacetate esterase. Arrows indicate neutrophils. Bar represents 100 μm. D, Neutrophil count per HPF. E, MPO activity in the liver tissue. F, E-selectin and ICAM-1 mRNA expression in the liver tissue. Data are expressed as ICAM-1/GAPDH and E-selectin/GAPDH ratios. *P<0.05 versus ConA.
endothelium (transmigration) on intrascrotal injection of TNF-α (300 ng) was significantly decreased in the group concurrently treated with flavopiridol (11 ng, IV) (Figure 2A, left). Leukocyte adherence to the endothelium was also reduced (Figure 2A, right). Flavopiridol did not affect hemodynamic parameters, such as the vessel diameter, centerline blood flow velocity, and wall shear rate (Supplemental Table I, available online at http://atvb.ahajournals.org). Also, the systemic white blood cell count was not altered by flavopiridol, suggesting that no pronounced cytotoxic effect (eg, apoptosis induction) occurred on leukocytes. Furthermore, we assessed the motility of interstitially migrating leukocytes in detail (Supplemental Table II), indicating that flavopiridol did also not affect this important parameter of leukocyte activation. We conclude that leukocytes might not be the primary target of flavopiridol. In analogy to the liver injury model, we also analyzed ICAM-1 and observed a significant decrease of ICAM-1 mRNA expression in flavopiridol-treated cremaster muscle tissue (Figure 2B).

Flavopiridol Reduces TNF-α-Induced Adhesion of Neutrophils to HUVECs
To proceed with mechanistic analyses, we first confirmed that leukocyte-EC interaction is also impaired in vitro. In fact, the adhesion of formyl-methionyl-leucyl-phenylalanine-activated neutrophils onto a TNF-α-activated EC monolayer was reduced when (only) ECs were treated with flavopiridol (Figure 2C). The number of adhered leukocytes did not change when neutrophils were also treated with flavopiridol. Furthermore, we checked the influence of flavopiridol on the respiratory burst, an important marker of neutrophil activation, and found that the formyl-methionyl-leucyl-phenylalanine-induced production of reactive oxygen species was not altered in the presence of flavopiridol, even at high concentrations (Figure 2D). These data indicate that neutrophils are not the major target of flavopiridol.

Flavopiridol Strongly Reduces the Expression of Endothelial CAMs
Treatment of ECs with flavopiridol concentration-dependently inhibited the TNF-α-evoked total (Figure 3A), as well as cell surface levels of the EC adhesion molecules ICAM-1 (IC50: 27 nmol/L), vascular cell adhesion molecule-1 (VCAM-1; IC50, 74 nmol/L), and E-selectin (IC50: 118 nmol/L) (Figure 3B). As shown in Figure 3C, flavopiridol effectively inhibited the TNF-α-induced ICAM-1 mRNA expression, suggesting that flavopiridol might interfere with transcriptional processes. Under basal conditions, flavopiridol (alone) did not affect ICAM-1
mRNA expression (Figure 3C). To exclude the possibility that the observed effects on ECs are simply due to cytotoxicity, we measured the apoptotic cell rate, as well as the metabolic cell viability and observed that flavopiridol had no significant effect on both parameters up to a concentration of 100 nmol/L. (Figure 3D). Moreover, to exclude effects due to EC heterogeneity, the results regarding the influence of flavopiridol on cell adhesion molecule expression, apoptosis, and cell viability obtained in macrovascular HUVECs were confirmed in human microvascular endothelial cells as shown in Supplemental Figure I.

**Flavopiridol Inhibits NF-κB Consensus Promoter Activity but Does Not Influence the Activation Cascade of NF-κB**

As shown in Figure 4A, flavopiridol concentration-dependently suppressed NF-κB consensus promoter activity, as assessed by a dual luciferase reporter gene assay. We analyzed the activation cascade of NF-κB and observed no effect of flavopiridol on recombinant human IKK β kinase (Figure 4B) or on the phosphorylation of IKKα/β in ECs (Figure 4C). Flavopiridol did not prevent the degradation of the NF-κB inhibitor IκB-α (Figure 4D) and did not affect the TNF-α-induced phosphorylation of the NF-κB p65 subunit (Figure 4E). Neither the TNF-α-triggered nuclear translocation of p65 (as assessed microscopically; Figure 4F and Supplemental Figure II) nor the NF-κB DNA-binding activity (Figure 4G) was altered by flavopiridol. These findings suggest that flavopiridol is able to impair NF-κB consensus promoter activity but does not interfere with the canonical activation cascade of this proinflammatory transcription factor.

**The Inhibition of the Activity of Several TNF-α-Induced Kinases Is Not Responsible for the Actions of Flavopiridol**

We performed a cellular kinome array (PepChip) to get insights into the kinases that are (directly or indirectly) influenced by flavopiridol. ECs were treated either with TNF-α alone or with both flavopiridol and TNF-α. The kinases with the highest rate of inhibition by flavopiridol are stated in the supplemental materials (Table 3). As a next step, we sought to answer the question whether the inhibition of these kinases is the cause for the observed effects of flavopiridol. The reduction of ICAM-1 expression served as readout parameter. The silencing of LIMK1 via siRNA did not affect the ability of TNF-α to induce ICAM-1 (Figure 5A), nor did the inhibitor of c-Jun N-terminal kinase (Figure 5B), the inhibitors of CK2 (Figure 5C), or the PKCθ blocker (Figure 5D). Thus, we conclude that the inhibition of these kinases by flavopiridol is not linked to the ICAM-1 reduction.
Inhibition of CDK9 by Flavopiridol Is Responsible for Its Effects

Because the kinome array did not entirely cover CDKs, we additionally determined the IC50 profile of flavopiridol in a 33PanQinase in vitro kinase activity assay (comprising 255 kinases) and revealed that flavopiridol in concentrations lower than 100 nmol/L exclusively targets CDK4, CDK6, CDK8, CDK9, and LIMK1 (Supplemental Table IV). Consequently, we used a CDK4/6 inhibitor and silenced CDK8 and CDK9 to figure out their role in ICAM-1 reduction. We found that CDK4 and CDK6 are not involved in the effect of flavopiridol (Figure 6A) and that CDK8-silenced ECs show a greatly increased ICAM-1 expression in response to TNF-α (Figure 6B). Most importantly, in the absence of CDK9, the expressions of ICAM-1 (Figure 6C), VCAM-1 (Figure 6D), and E-selectin (Figure 6E) were significantly inhibited, suggesting that CDK9 is crucially involved in the effect of flavopiridol.

Discussion

Besides their role as promising antitumor therapeutics, CDK-inhibiting drugs have been proposed as novel antiinflammatory agents by their properties to increase apoptosis of neutrophil granulocytes and to inhibit lymphocyte proliferation.12,13 Nevertheless, investigations regarding an antiinflammatory potential of flavopiridol are rare. In the context of
hepatic acute phase response, 1 study showed that the interleukin (IL)-6/STAT3 signaling was disrupted by flavopiridol in hepatoblastoma cells.\textsuperscript{14} Moreover, flavopiridol was found to attenuate the proliferation of synovial fibroblasts in vitro and to inhibit synovial hyperplasia in a murine model of arthritis without suppressing lymphocyte function.\textsuperscript{2}

In our present work, we provide for the first time evidence that flavopiridol works as an antiinflammatory agent independent of affecting proliferative and cell death–inducing actions. It effectively blocked leukocyte infiltration and leukocyte-EC interaction by hampering endothelial activation, a crucial event in the onset and maintaining of inflammation.

In the model of ConA-induced murine hepatitis, flavopiridol effectively blocked neutrophil infiltration of the liver and reduced ICAM-1 and E-selectin expression. It was recently demonstrated that neutrophil recruitment in the liver sinusoids does not primarily depend on these classic adhesion molecules but on the interaction of CD44 and hyaluronan.\textsuperscript{15} A novel set of experiments is required to analyze whether flavopiridol affects the CD44-hyaluronan system; however, it can be speculated that flavopiridol might influence both the CD44-hyaluronan and the ICAM-1/E-selectin pathway of leukocyte recruitment.

An important finding of this work is the fact that flavopiridol effectively blocks NF-κB-dependent gene expression (luciferase reporter gene, ICAM-1 expression), but not by affecting the canonical NF-κB activation cascade (IKK activation, IkB degradation, nuclear p65 translocation, NF-κB DNA-binding). Supporting our data, an inhibitory effect of flavopiridol on NF-κB-dependent reporter gene and ICAM-1 expression was also described in studies evaluating the action of flavopiridol on TNF-α-induced genes in A293 (human kidney) and HL60 (human myeloid leukemia) cells, respectively.\textsuperscript{16,17} However, in contrast to our study, flavopiridol was reported to suppress NF-κB signaling by inhibition of IKK activation, IkB degradation, p65 phosphorylation and translocation, as well as NF-κB DNA-binding activity in different cancer cell lines.\textsuperscript{16} This striking discrepancy cannot be easily explained. We assume that it might be based on a strong cell type–dependent action of flavopiridol.

We found that instead of affecting the canonical NF-κB activation cascade, flavopiridol exerted its action on ICAM-1 expression via inhibition of CDK9. Interestingly, although high (μmol/L) concentrations of flavopiridol are able to inhibit a wide variety of cellular kinases,\textsuperscript{18} at concentrations as low as the IC\textsubscript{50} values of adhesion molecule reduction (27 nmol/L for ICAM-1), it exerts an inhibitory function on only 5 kinases. This suggests that the antiinflammatory action of flavopiridol is not based on a broad-spectrum kinase inhibition but on a rather selective interference by targeting CDK9. CDK9 is crucially involved in the control of gene transcription. The positive transcriptional elongation factor b (P-TEFb), which regulates the elongation phase of RNA polymerase II–dependent transcription, is a heterodimer composed of CDK9 and cyclin T1. P-TEFb acts by phosphorylation of negative elongation factors, as well as of RNA polymerase II. From cDNA microarray analyses, it is known that flavopiridol suppresses transcription in a concentration-dependent manner: 60 nmol/L does not affect cellular gene expression, whereas 300 nmol/L decreases mRNA levels.\textsuperscript{19} Applying nuclear run-on assays, Chao and Price revealed that flavopiridol did not influence transcription at 10, 30, and 100
nmol/L. In good accordance with these findings, we could detect neither a cytotoxic action nor a cellular protein content–lowering effect (data not shown) of flavopiridol at 100 nmol/L in ECs. Thus, we can exclude detrimental effects on global transcription.

It has been a matter of debate whether CDK9 governs gene transcription in general or regulates restricted sets of genes. Recently, a selective modulation of gene expression by CDK9 has been reported, because CDK9 inhibition induced not only a downregulation but also an (even more pronounced) upregulation of genes. Moreover, CDK9/P-TEFb has been discovered to play an important role in TNF-α-induced NF-κB activation by forming a protein complex with activated p65 in the nucleus. However, P-TEFb regulates only a subset of NF-κB-dependent genes. The P-TEFb-p65 complex is required for IL-8 and Gro-β but not for IkB-α gene activation. Our data prompted us to suggest that P-TEFb-p65 interaction might also be in charge of ICAM-1 gene activation. Moreover, CDK9/P-TEFb functions as a subunit of P-TEFb, CDK9 was found to interact with the basis of a few studies, CDK9 inhibition was thought to be an approach against inflammation-associated pathologies. On the basis of the first study scrutinizing the action of CDK9 inhibition on leukocyte-EC interactions between leukocytes and the endothelium, primary inflammation-triggering event.

Our work highlights inhibition of CDK9 as an interesting approach against inflammation-associated pathologies. On the basis of a few studies, CDK9 inhibition was thought to be of potential value to fight inflammation. Independent of its function as a subunit of P-TEFb, CDK9 was found to interact with cytoplasmic regions of gp130, the receptor for the proinflammatory cytokine IL-6. An additional study reported that CDK9 inhibitors disrupt the IL-6/STAT3 signaling in the liver. HEXIM1, an endogenous inhibitor of P-TEFb, was shown to associate with NF-κB p65 and to repress NF-κB-dependent gene transcription in smooth muscle cells. However, to the best of our knowledge, this is the first study investigating the action of CDK9 inhibition on leukocyte-EC interaction and, in particular, on endothelial activation, a crucial inflammation-triggering event.

In summary, our study provides evidence that low-dose flavopiridol effectively protects against inflammation-induced interactions between leukocytes and the endothelium, primar-
ibly by blocking endothelial activation. This is achieved by an inhibition of the proinflammatory transcription factor NF-κB. Most importantly, the canonical NF-κB activation cascade is not altered by flavopiridol, whereas NF-κB-induced transcription is decreased via inhibition of CDK9. Thus, our work highlights flavopiridol as a promising antiinflammatory agent and, moreover, discloses inhibition of CDK9 as an interesting approach for the treatment of inflammation-associated diseases.

**Acknowledgments**

Flavopiridol was a kind gift from the US National Cancer Institute/ Sanofi Aventis.

**Sources of Funding**

This study was supported in part by a grant from the European Community (FP6–2002 Life Science & Health, Prokinase Research Project, Project No. LSHB-CT-2004-503467).

**Disclosures**

None.

**References**


