**Advances in Brief**

**Flavopiridol-related Proinflammatory Syndrome Is Associated with Induction of Interleukin-6**

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**Abstract**

**Background:** Flavopiridol is a flavonoid with antiproliferative effects mediated, in part, by inhibition of cyclin-dependent kinases. Clinical manifestations in a previous Phase I trial in patients with refractory malignancies treated with a 72-h flavopiridol infusion included a proinflammatory syndrome consisting of fever, fatigue, and “local” tumor pain with concomitant alterations in plasma acute-phase reactant proteins.

**Purpose:** The aim of this study was to determine whether the proinflammatory syndrome observed in this trial was associated with modulation of plasma cytokines.

**Methods:** Patients receiving flavopiridol (n = 76) had serial plasma samples drawn preinfusion and during the infusion for evaluation of interleukin (IL)-6, IL-10, IL-12, granulocyte macrophage colony-stimulating factor, transforming growth factor-β, and tumor necrosis factor-α levels by standard ELISA assays. The Wilcoxon signed rank test was used to test the significance of the difference between the baseline (time 0) plasma cytokine levels compared with the values of each subsequent data collection time points (8, 24, 48, and 72 h).

**Results:** There was a significant and sustained increase in plasma IL-6 levels at all time points when compared with baseline values. Paired values were used in the statistical analysis. Median plasma (interquartile range) values of IL-6 were elevated from 15.5 (9–52) pg/ml at baseline to 23 (4–48) pg/ml at baseline to 46 (21–105) pg/ml (P < 0.001) at 24 h; from 16 (9–52) pg/ml at baseline to 61 (32–170) pg/ml (P < 0.001) at 48 h; and from 15.5 (6–48) pg/ml to 68 (40–200) pg/ml (P < 0.001) at 72 h. Significance was maintained when adjusted for multiple comparisons. The relative increase in IL-6 concentration was dose-dependent. Moreover, IL-6 elevation had a direct correlation with flavopiridol peak plasma concentration, flavopiridol area under the curve, and plasma C-Reactive protein levels. A significant decrease in plasma granulocyte macrophage colony-stimulating factor occurred at the 8-h sampling point: 50 pg/ml (interquartile range 10–205 pg/ml, P < 0.01) when compared with baseline plasma levels and 71 pg/ml (interquartile range 5–152 pg/ml, P < 0.01). No changes in the other pro or anti-inflammatory cytokines were observed. Immunohistochemistry studies in bone marrow aspirates from a prospective group of patients in this trial demonstrated a 4-fold induction of IL-6 (compared with baseline), mostly in non-T cells.

**Conclusion:** Biochemical analysis of plasma in patients undergoing infusional flavopiridol found a significant dose-dependent induction of IL-6. IL-6 elevation could be a marker for the process leading to the appearance of the proinflammatory syndrome observed in patients treated with infusional flavopiridol. The mechanism(s) underlying IL-6 induction and its significance are still unknown but may influence strategies to modulate flavopiridol’s clinical effects.

**Introduction**

Flavopiridol (HMR1275) is a flavonoid with antiproliferative effects attributable to the capacity to inhibit most cdk2 (1–3). Loss of cdk activity may explain cell cycle arrest and induction of apoptosis after exposure to this compound (4–6). Moreover, flavopiridol is able to modulate transcriptional processes as determined by down-regulation of cyclin D1 and vascular endothelial growth factor, among others (7–9). Modulation of transcription by flavopiridol could be explained, at least in part, by the potent inhibition of positive-transcription elongation factor B, also known as cdk9/cyclin T, a kinase crucial in the elongation phase of transcription (10). On the basis of the novel mechanism of action and preclinical antitumor effects attributable to the capacity to inhibit most cdk2 (1–3). Loss of cdk activity may explain cell cycle arrest and induction of apoptosis after exposure to this compound (4–6). Moreover, flavopiridol is able to modulate transcriptional processes as determined by down-regulation of cyclin D1 and vascular endothelial growth factor, among others (7–9). Modulation of transcription by flavopiridol could be explained, at least in part, by the potent inhibition of positive-transcription elongation factor B, also known as cdk9/cyclin T, a kinase crucial in the elongation phase of transcription (10). On the basis of the novel mechanism of action and preclinical antitumor...
effects of flavopiridol, several clinical trials are under way or have been completed recently (2, 11, 12).

The first Phase I clinical study of flavopiridol determined that the dose-limiting toxicity of a 72-h continuous i.v. infusion was secretory diarrhea, which was easily managed with loperamide and cholestyramine (13). Subsequent flavopiridol dose escalation in the presence of anti diarrheal prophylaxis defined hypotension as a second dose-limiting toxicity. In addition, at these higher dose levels, most patients developed a proinflammatory syndrome characterized by anorexia, fever, malaise, arthralgia, and pain, particularly related to tumor sites. Laboratory analysis of patients during this “inflammatory phase” showed a transient increase in plasma concentration of the “positive” APRs, CRP, and fibrinogen with a related decline in “negative” APRs, including prealbumin and albumin levels. The nature of this syndrome and the basis for APR modulation is still unknown (13). A second Phase I trial of 72-h continuous infusion of flavopiridol confirmed these findings (14).

The APR proteins (CRP, fibrinogen, haptoglobin, ceruloplasmin, and transferrin) are produced by hepatocytes in response to host injury and in association with a variety of illnesses, including cancer, rheumatoid arthritis, and infections (15–17). The proinflammatory cytokines IL-1 and IL-6 appear to be major stimuli for the hepatic synthesis of these APRs (15–18). On the other hand, cytokines are produced by a variety of cells and mediate many immunological and inflammatory activities (19, 20). Certain cytokines can be characterized as proinflammatory (TNF-α, IL-1β, IL-2, IL-6, and IFN-γ) or anti-inflammatory (TGF-β1, IL-4, IL-10, and IL-13) with plasma levels that vary in a complex and interrelated manner (19–23).

In this study, we describe our efforts to elucidate the basis for the inflammatory syndrome observed during clinical trials with infusional flavopiridol by measuring the plasma concentration of several pro and anti-inflammatory cytokines from baseline (preinfusion) and intratreatment (8, 24, 48, and 72 h)–banked plasma samples. We report here a dose-dependent induction of plasma IL-6 levels in patients that received infusional flavopiridol. Moreover, this induction correlates with induction of CRP levels, and it was temporally related to the proinflammatory syndrome observed. Bone marrow aspirates from a prospectively selected group of patients that received infusional flavopiridol demonstrated that most patients have induction of IL-6 in non-T bone marrow cells. Whether the induction of proinflammatory cytokines by flavopiridol occurs with other schedules of administration or if the induction of cytokines has a beneficial or toxic effect is still an unresolved issue that may be clarified in future trials with this agent.

Materials and Methods

Plasma Cytokine Measurements. A Phase I trial of flavopiridol administered as a 72-h continuous infusion was conducted at the National Cancer Institute (Bethesda, MD) between December 1994 and March 1997 (13). Adult patients with advanced and refractory malignancies, who met standard eligibility criteria, underwent placement of a triple lumen central venous catheter to facilitate drug administration and plasma sampling. Flavopiridol administration and blood drawing occurred via different ports on the catheter. Serial 10-ml blood samples were collected through the catheter and deposited into heparinized tubes at baseline (i.e., before infusion) and at 8, 24, 48, and 72-h intervals after the commencement of the flavopiridol infusion. Plasma was separated subsequently from whole blood by cold centrifugation and stored at −70°C in polyethylene tubes. Stored plasma samples were analyzed to determine the concentration of cytokines IL-6, IL-10, IL-12, GM-CSF, and TNF-α using Beckman Coulter enzyme assay kit (Fullerton, CA) and basic-fibroblast growth factor and TGF-β using R&D Systems, Inc. (Minneapolis, MN) kit. All cytokine assays used in this study, including IL-6, are universally accepted commercial kit assays. To measure each cytokine, we strictly followed manufacturer’s recommendations. Of note, all cytokine determinations were done at the same time after the trial was completed, minimizing lot-to-lot variations. Moreover, the assays were run by only one individual, and this individual was blinded to the clinical results. The limits of detection of the IL-6 assay, as described by the manufacturer, is ≥3.4 pg/ml.

Bone Marrow Aspiration and Bone Marrow IHC. After signing the informed consent, bone marrow aspirates from a total of 6 prospective patients were obtained by standard procedures before and ~48 h after flavopiridol initiation (first cycle only). Bone marrow mononuclear cells were isolated by Ficoll gradient from Amersham (Uppsala, Sweden), following manufacturer’s instructions. Human bone marrow cells were cyto- spun onto silanized slides from Digene (Bethsville, MD). Slides were stored at −80°C until used for immunohistochemical staining. Slides were air dried at room temperature; fixed in 3.7% PBS-buffered formalin for 5 min; delipidized in 50% aqueous acetone (2 min), absolute acetone (2 min), and 50% aqueous acetone (2 min); rinsed in PBS (5 min); and blocked in PBS/0.5% BSA (30 min). Antihuman IL-6 (1 μg/ml; R&D), antihuman TNF-α (0.2 μg/ml), and antihuman IFN-γ (1 μg/ml), both from BioSource (Camarillo, CA), were diluted in PBS/0.5% BSA/0.3% Triton X-100 solution. Then, bone marrow cytopsins were incubated overnight at 4°C in a humidified chamber. Samples were rinsed (2 × 5 min each) at room temperature with PBS and incubated with biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) diluted in PBS/0.5% BSA/0.1% Triton X-100 solution for 1 h at room temperature. Cytospins were then rinsed in PBS as above, incubated in avidin-biotin complex solution from Vector Laboratories, and diluted in PBS for 45 min. Cells were rinsed with Tris-buffered saline (pH 7.5) and incubated in 0.03% diaminobenzidine in Tris-buffered saline with 0.003% hydrogen peroxide. Slides were incubated for 5 min and rinsed in deionized water for 5 min. Cytospins were then blocked in 3% hydrogen peroxide in PBS for 30 min to quench any residual peroxidase. Slides were rinsed 3 × 10 min each in PBS and incubated in antihuman CD3 (DAKO, Carpinteria, CA; dilution: 1:5000) overnight at 4°C as described earlier. Sections were then processed as for the first labeling and rinsed with PBS before incubation in True Blue solution (KPL Laboratories, Gaithersburg, MD), instead of diaminobenzidine, for ≤15 min. Sections were then briefly rinsed in deionized water and dehydrated through an ascending series of ethanol, air dried, and covered-slipped with Permount (Fisher Scientific, Fairlawn, NJ). Cytospins were examined using a Zeiss Axiount S100 inverted
microscope (Carl Zeiss, Jena, Germany), and digitized images were taken using a SPOT II (Diagnostic Instruments, Sterling Heights, MI) digital camera and software system. A minimum of 50 marrow cells per field was counted. The average of cytokine-positive cells for all of the slides of each patient (pre and post-treatment) was determined and used to calculate the fold increase in post-treatment samples relative to pretreatment samples. The scoring was performed in a blinded manner by two independent investigators.

Statistical Analysis. Baseline plasma samples were drawn from each subject before the administration of flavopiridol. The infusion was then started, and plasma samples were drawn at 8, 24, 48, and 72 h. Each set of paired data consisted of plasma samples from those patients that had both a baseline sample drawn as well as a sample for the time point being compared (i.e., baseline sample versus 8-h sample, baseline sample versus 24-h sample, etc.). Only these paired data were included in the statistical analysis. Of the 76 subjects evaluated, a subset of 39 subjects had both baseline and 8-h plasma samples collected and subsequently analyzed for IL-6 concentrations, whereas 50 patients had the baseline and 24-h samples analyzed. Similarly, 47 patients had a baseline and a 48-h sample, and 49 had both a baseline and 72-h sample. Moreover, only 27 patients have “complete sampling”: baseline, 8-, 24-, 48-, and 72-h collection. Thus, the fact that these are relatively small fractions of total subjects at each time point may require cautious interpretation of the comparisons. Histograms revealed a nonparametric distribution for all cytokine concentrations that were measured. Paired data were, therefore, analyzed by nonparametric methods. Specifically, the Wilcoxon signed-rank test was used to evaluate the significance of the difference between the baseline values compared with the values from each collection time point (8, 24, 48, and 72 h). Because multiple comparisons were performed, Ps were adjusted by the Bonferroni method. Similarly, correlation coefficients were obtained using Spearman’s rank correlation procedure for evaluation of data that were not normally distributed. The measured plasma concentration for each pharmacologic parameter was ranked according to magnitude of change from baseline/preinfusion levels to substantially achieved levels.

Results

Specific Elevation of Plasma IL-6 in Patients Receiving Flavopiridol. Patients that received infusional flavopiridol demonstrated clear evidence of a proinflammatory syndrome (~24 h after drug administration that lasted ~3–5 days postinfusion (13). This syndrome was accompanied by increased positive APRs, such as fibrinogen, and a decline in negative APRs, such as albumin (13). To explore a possible role for cytokine elaboration in this proinflammatory syndrome, we measured a battery of pro and anti-inflammatory cytokines by standard ELISA assays before and during flavopiridol administration. There was a significant and sustained increase in plasma IL-6 levels at all time points when compared with baseline values. Paired values were used in the statistical analysis. Median plasma (interquartile range) values of IL-6 were elevated from 15.5 (9–52) pg/ml at baseline to 23 (4–48) pg/ml (P < 0.01) at 8 h, from 15 (2–48) pg/ml at baseline to 46 (21–105) pg/ml (P < 0.001) at 24 h, from 16 (9–52) pg/ml at baseline to 61 (32–170) pg/ml (P < 0.001) at 48 h, and from 15.5 (6–48) pg/ml to 68 (40–200) pg/ml (P < 0.001) at 72 h. Significance was maintained even when adjusted for multiple comparisons as described in “Materials and Methods.” As shown in Fig. 1B, a subgroup analysis of patients who received complete sampling (n = 27) also demonstrated significant induction of IL-6 for each time point (P < 0.05). The median (pg/ml, interquartile range) for each group (baseline, 8-, 24-, 48-, and 72-h collection) was 15 (6.5–53.5), 25 (15–79.5), 46 (28–121), 63 (31.5–153.5), and 68 (40–187.5), respectively. The increase in plasma IL-6 level was already detectable at the 8-h time point of the infusion and precedes the onset of the inflammatory clinical symptoms and the modulation of APRs observed in our Phase I clinical trial (13). An analysis of individual data points (baseline and highest difference IL-6 values from baseline-highest ∆) from the subgroup shown in panel B (patients with complete sampling) revealed that most (except 1) patients have increased plasma IL-6 when compared with baseline. This information clearly reinforces the notion that the plasma IL-6 elevations observed in this trial occur in the majority of patients (Fig. 1C). Additional findings include a significant but transient decrease in plasma concentration of GM-CSF with baseline of 71 pg/ml versus 8-h median 50 pg/ml (n = 42, P < 0.01; data not shown).

The plasma IL-6 cytokine level returned to baseline before the beginning of the second course of flavopiridol in those individuals that were retreated (data not shown). GM-CSF levels returned to baseline within 16 h after nadir levels were achieved. Changes in GM-CSF at >8 h, as well as time points for IL-10, IL-12, basic-fibroblast growth factor, TGF-β, and TNF-α, were not statistically significantly different from baseline (data not shown). Thus, the only cytokine with detectable changes in this battery was IL-6.

The Elevation of Plasma IL-6 in Patients Receiving Infusional Flavopiridol Is Dose-dependent. To determine whether the plasma IL-6 induction observed in this trial is dose-dependent, we correlated pharmacokinetic parameters and IL-6 levels. In Fig. 2A–D, changes in plasma levels of IL-6 were ranked and separated into low and high groups (above and below the median ranked plasma IL-6 level, respectively) and displayed along the ordinate axis. These groups were then compared with ranked change in plasma CRP, dose level, AUC, and Cmax as displayed on the abscissa. When we compared high and low plasma IL-6 groups with plasma CRP (panel A), dose level (panel B), AUC (panel C), and Cmax (panel D), we found significant differences (one-sided t test, P < 0.05). Increasing plasma levels of IL-6 were weak to moderately well correlated with change in plasma CRP levels (n = 15, r = 0.52, and P < 0.05), dose level (n = 30, r = 0.5, and P < 0.05), AUC (n = 30, r = 0.38, and P < 0.05), and Cmax (n = 30, r = 0.41, and P < 0.05). A poor correlation was noted between changes in plasma fibrinogen, albumin, and prealbumin with IL-6 levels during flavopiridol infusion (data not shown).

Induction of Plasma IL-6 in Patients Is Attributable to Increased Expression of IL-6 in Non-T Bone Marrow Cells. To determine the source of plasma IL-6 observed in these patients, we prospectively obtained bone marrow aspirates from
6 patients before and ~48 h during the first cycle of flavopiridol infusion as described in “Materials and Methods.” IHC studies from cytospins of these bone marrow cells revealed that IL-6 was elevated (~4-fold induction), whereas TNF-α or IFN-γ was mostly unaltered (Fig. 3, A and B). The IL-6-producing cells were almost exclusively non-T cells. Unfortunately, we were unable to further identify the exact lineage of these IL-6-producing cells, because the macrophage-specific antibodies did not properly stain these samples. Thus, the elevation observed in plasma IL-6 levels may reflect in part the induction of IL-6 in non-T bone marrow cells during infusional flavopiridol.

Discussion

In this study, we describe, for the first time, the association between the proinflammatory syndrome observed in patients with infusional flavopiridol and the dose-dependent induction of the proinflammatory cytokine IL-6. The source of plasma IL-6 appears to involve, at least in part, non-T bone marrow cells.

Flavopiridol is a novel cdk inhibitor in early clinical trials with the capacity to arrest cell cycle progression, induce apoptosis, and modulate gene transcription with clear evidence of in vivo preclinical antitumor effects (2, 4–8, 10, 24–32). Most cellular effects have to this point been relatable to inhibition of one or more cdks (2). Flavopiridol was evaluated recently in a Phase I setting administered as a 72-h continuous infusion (13). In this trial, flavopiridol infusion has been found to cause a proinflammatory “flu-like” clinical syndrome with modulation in plasma levels of APR proteins (13). In this study, we retrospectively evaluated the plasma concentration of a variety of pro and anti-inflammatory cytokines in samples taken during that flavopiridol Phase I clinical trial to determine whether these inflammatory effects might be associated with modulation of plasma cytokine levels. Our results showed that, although most cytokines were not affected in a statistically significant way, plasma IL-6 concentration was induced as earlier as 8 h during the infusion. A moderate, but statistically significant, correlation...
was further determined when comparing the change in plasma IL-6 concentration with plasma concentration of CRP and the following pharmacological parameters: (a) dose level; (b) AUC; and (c) $C_{\text{max}}$. Although our original 72-h continuous infusion flavopiridol study (13) showed an increase in plasma APR proteins, this study extends these observations by providing evidence that IL-6 plasma levels were increased throughout the infusion. This leads to the hypothesis that the modulation of the APR proteins may be in part the result of increased IL-6 concentrations. This speculation will require further investigation in model systems. Elevated levels of IL-6 during flavopiridol chemotherapy could potentially modulate the cytotoxicity of flavopiridol in normal and tumor tissues. Moreover, plasma IL-6 levels may also be a useful marker of biological activity of flavopiridol.

A major caveat in regarding IL-6 as the sole mediator of flavopiridol-induced proinflammatory effects is that plasma assays may miss locally elaborated or acting cytokines, e.g., in tumor or vascular endothelial compartments. Thus, IL-6 may be one of the many influences that could govern the appearance of the syndrome. On the other hand, the elucidation of a dose- and time-related increase in IL-6 after flavopiridol infusion offers an additional context by which the drug desired and nondesired effects might be considered.

IL-6 possesses pleiotropic activities, playing a central role in host defense (33, 34). Included among these activities is the induction of APR proteins by hepatic cells. In cultured fibroblasts, lipopolysaccharide, viruses, IL-1, TNF, and platelet-derived growth factor enhance IL-6 production (33, 34). Mononuclear phagocytes, vascular endothelial cells, activated T cells, and other cells produce IL-6 in response to IL-1 and to a lesser extent TNF-$\alpha$ (17). In vivo, cells do not normally produce IL-6 unless appropriately stimulated, such as in a clinical setting of sepsis or shock (17).

Phase I/II clinical trials evaluating the administration of recombinant IL-6 demonstrated clear evidence of adverse effects, such as fever, fatigue, anemia, increased CRP levels, and a decrease in plasma albumin (35, 36). Of note, this clinical profile was similar to the effects seen during our continuous infusion flavopiridol trial (13). Thus, the effects observed in this patient population could be explained in part by the elevation of IL-6. An additional factor is a small but significant decrease in plasma GM-CSF, 8 h into the flavopiridol infusion. These effects were transient in that the plasma levels of GM-CSF

![Fig. 2](image-url) Serum levels of CRP, dose level, AUC, and $C_{\text{max}}$ in patients receiving flavopiridol when segregated according to low and high plasma IL-6 levels. Ranked change in serum CRP (A), dose level (B), AUC (C), and $C_{\text{max}}$ (D) segregated into high and low ranked change in plasma IL-6 groups (X axis) during flavopiridol infusion, where “x” denotes median values for each box plot displayed. See “Results” for full details.
returned to baseline by the next sampling time point of 24 h. Although statistically significant, the mechanism and/or meaning of the GM-CSF decline are unknown. The remaining cytokines measured in plasma did not show obvious changes.

To the best of our knowledge, this is the first study of a cdk inhibitor to modulate cytokine expression in patients. Another protein kinase modulator, bryostatin 1, demonstrated immunomodulatory properties, including the ability to induce the proinflammatory cytokines IL-6 and TNF-α (37, 38) One study of bryostatin 1 (37) showed that the proinflammatory syndrome observed in this trial was associated with in vivo evidence of cytokine modulation with an early increase in plasma TNF-α followed by a
rise in IL-6 documented at 24 h (38). The exact mechanism by which bryostatin induces cytokines is unknown, because it can acutely increase protein kinase C activity or cause protein kinase C down-regulation after chronic exposure (31, 39).

Why plasma IL-6 concentrations increased, whereas other proinflammatory cytokines, such as TNF-α, did not, remains yet to be clarified. Flavopiridol administration may target the production of IL-6 specifically, without influencing other cytokines. Perhaps the cytokines that did not exhibit a change turned over more rapidly and thus did not manifest increasing plasma levels. Moreover, some cytokines are labile and difficult to measure. Nevertheless, in the case of TNF-α and IFN-γ, we were not able to detect changes in their production either in T or non-T bone marrow cells (Fig. 3C–F). It is still possible that although IL-6 concentration rose throughout the study, the onset of other proinflammatory cytokines may have occurred after our last plasma sample was drawn (72 h). Alternatively, local elaboration of cytokines in specific compartments, undetectable by conventional plasma ELISA methodology, remains to be completely explored. Although still possible, the elevation of non-IL-6 proinflammatory cytokines, several days after the onset of the clinical signs and symptoms of systemic inflammation, could not explain this proinflammatory syndrome. An additional limitation in our analysis relates to the lack of correlation between severity of symptoms and IL-6 plasma levels.

Of note, the analysis of IL-6/APRs in this trial was prospectively obtained only after several dose levels were completed, when it was clinically obvious that this agent induces a proinflammatory syndrome (13). Thus, most patients in our analysis have, at least, moderate (grade 2) proinflammatory syndrome despite a wide range in IL-6 levels. Moreover, no IL-6/APRs data are available from patients with minimal or no symptoms, a situation that occurred at lower dose levels, just before our prospective collection efforts began. Thus, based on the available data, we cannot conclude that there is a clear relationship between plasma IL-6 levels (or IL-6 fold induction) and proinflammatory signs/symptoms in this trial. It is noteworthy to mention, similar to our findings, that there is no relationship between IL-6 plasma levels and proinflammatory signs in Phase I trials with recombinant IL-6 (40–43). Of note, most patients in these trials have moderate/severe signs or symptoms regardless of the schedule of administration/dose administered. Thus, even minor increases in systemic IL-6 by flavopiridol (our study) or IL-6 increases caused by recombinant exogenous IL-6 administration provoked significant proinflammatory symptoms/signs without a clear relationship between IL-6 concentration and severity of symptoms.

The present results raise the question of how flavopiridol, a cdk antagonist described previously, might up-regulate IL-6. The expression of several cytokines involves the transcriptional activation of NF-κB (44, 45). A study by Perkins et al. (46) described that p21<sup>cip1</sup> or, an endogenous cdk inhibitor, transcriptionally activates NF-κB. Of note, flavopiridol also demonstrated clear activation of NF-κB in several human cell lines, such as 293 and HaCaT cells, as measured by reporter and gel shift assays. The exact nature for this transcriptional activation is being investigated in our lab. However, it is also clear that flavopiridol may modulate other transcriptional events as observed in the case for cyclin D1, vascular endothelial growth factor, and HIV transcription (2, 7, 8, 10). Accumulation of proinflammatory cytokines could also occur by the activation of the MAPK family (17, 47–49). Production of IL-1 and TNF-α from stimulated human monocytes is inhibited by a series of pyridinyl-imidazole compounds, and the target of these compounds was identified as p38 MAPK (49). Initial experiments in our laboratory demonstrated that flavopiridol is able to activate the three subfamilies of MAPK (mitogen-activated protein/extracellular signal-regulated kinase kinase, c-Jun-NH<sub>2</sub>-terminal kinase, and p38) in T Jurkat cell lines (50). Activation of MAPKs by flavopiridol is not specific for T cells because flavopiridol can activate MAPKs in other cell lines, such as HaCaT keratinocyte-immortalized cell lines. Although the activation of MAPKs by flavopiridol is intriguing, the relationship between this activation and flavopiridol cellular effects, including the elaboration of cytokines, is unclear.

Although it is still unknown whether IL-6 induction observed in these patients is attributable to flavopiridol’s capacity to inhibit cdks or other signaling pathways, it is clear that the proinflammatory syndrome observed in this population is attributable to, at least in part, induction of IL-6 in non-T cells. Cells from macrophage lineage, stromal, and endothelial cells are all sources of IL-6 production (33). Of note, it has been reported that IL-6 possesses antitumor effects in preclinical models (51, 52), and it can modulate the expression of tumor-associated and major histocompatibility antigens in tumors (53). However, it is also possible that the induction of IL-6 by flavopiridol may promote tumor proliferation/resistance to antitumor effects of other compounds, e.g., IL-6 prevented apoptosis and supported cell growth of multiple myeloma cells (54), and plasma IL-6 was directly related to disease status and progression in multiple myeloma (55). Thus, the role of IL-6 in the antitumor (and/or pro-survival) effects of flavopiridol and the means to modulate IL-6 expression are important questions that need to be addressed in future preclinical and clinical research studies with flavopiridol in solid and hematological malignancies.

**Acknowledgments**

We thank the assistance of Anthony Murgo, Maria Lourdes Villalba, Constanza Quiroz, and Stephen Schweiger in completing this project.

**References**


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