Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia

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Despite promising preclinical studies with the cyclin-dependent kinase inhibitor flavopiridol in chronic lymphocytic leukemia (CLL) and other diseases, previous clinical trials with this agent have been disappointing. The discovery of differential protein binding of flavopiridol in human and bovine serum contributed to an effective pharmacokinetic-derived schedule of administration of this agent. On the basis of pharmacokinetic modeling using our in vitro results and data from a previous trial, we initiated a phase 1 study using a 30-minute loading dose followed by 4 hours of infusion administered weekly for 4 of 6 weeks in patients with refractory CLL. A group of 42 patients were enrolled on 3 cohorts (cohort 1, 30 mg/m^2 loading dose followed by 30 mg/m^2 4-hour infusion; cohort 2, 40 mg/m^2 loading dose followed by 40 mg/m^2 4-hour infusion; and cohort 3, cohort 1 dose for treatments 1 to 4, then a 30 mg/m^2 loading dose followed by a 50 mg/m^2 4-hour infusion). The dose-limiting toxicity using this novel schedule was hyperacute tumor lysis syndrome. Aggressive prophylaxis and exclusion of patients with leukocyte counts greater than 200 × 10^9/L have made this drug safe to administer at the cohort 3 dose. Of the 42 patients treated, 19 (45%) achieved a partial response with a median response duration that exceeds 12 months. Responses were noted in patients with genetically high-risk disease, including 5 (42%) of 12 patients with del(17p13.1) and 13 (72%) of 18 patients with del(11q22.3). Flavopiridol administered using this novel schedule has significant clinical activity in refractory CLL. Patients with bulky disease and high-risk genetic features have achieved durable responses, thereby justifying further study of flavopiridol in CLL and other diseases. (Blood. 2007;109:399-404)

Introduction

Significant advances have been made in the treatment of chronic lymphocytic leukemia (CLL).[1] However, these treatments are not curative, and virtually all patients eventually relapse and become fludarabine resistant. In addition, dramatic antitumor responses associated with acute tumor lysis syndrome (TLS) have only rarely been observed in CLL.

Studies examining the genetic features of fludarabine-refractory CLL identified a high (42%-50%) frequency of deletion and/or mutation of the *p53* gene relative to that in untreated patients (7%-10%).[2,3] Mutations or deletions of *p53* are predictive of treatment failure with alkylating agents,[4] fludarabine,[5] and rituximab-based therapies.[6] Only alemtuzumab (Campath-1H; Genzyme, Cambridge, MA)[7,8] or high-dose methylprednisolone[9] have been shown to have efficacy in this highly drug-resistant subset of CLL. Unfortunately, these treatments are strongly immunosuppressive and can result in life-threatening complications. Developing new therapies for patients with CLL, particularly those with high-risk disease or *p53* mutations and/or deletions, remains a priority.

Flavopiridol is a broad cyclin-dependent kinase inhibitor[10-12] that effectively induces apoptosis in leukemic cell lines and in human CLL cells in vitro at clinically achievable concentrations.[13-16] Importantly, this antitumor activity is p53 independent.[13,17] Flavopiridol also decreases expression of Mcl-1 and XIAP, proteins that mediate resistance to apoptosis in CLL cells in vitro.[18] These studies provided justification for pursuing clinical studies of flavopiridol in CLL.

Despite promising preclinical results, clinical activity observed with flavopiridol has been disappointing in CLL and a variety of other types of cancers.[19-26] In CLL, no activity was seen when using 72-hour or 24-hour continuous intravenous infusion schedules,[27,28] and only an 11% response rate was achieved with a 1-hour bolus in recurring CLL.[27] These schedules were modeled after in vitro studies using media containing fetal bovine serum (FBS).[13] In our in vitro studies, we have noted significantly higher protein binding of flavopiridol in human serum than in FBS. Furthermore, the LC_{50} of flavopiridol against CLL cells in human serum was substantially higher than that in FBS and likely had not been attained with either

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the 1-hour,[29] 24-hour,[28] or 72-hour[30] schedule of flavopiridol administration previously investigated in this disease. We therefore used our in vitro findings, together with pharmacokinetic data from a previous study,[28] to model a schedule of flavopiridol to attain an exposure to the drug pharmacologically predicted to be active.

Patients, materials, and methods

Patients

Patients were enrolled on this National Cancer Institute (NCI)–sponsored and Ohio State University institutional review board–approved study following written informed consent. Enrollment requirements included: age older than 17 years; symptomatic CLL or small lymphocytic lymphoma by the NCI criteria[31]; platelet count greater than 49×10^9/L (cohorts 1 and 2); Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less; no active infection or inflammatory bowel disease; and serum creatinine and total bilirubin levels lower than 2 times the normal value. Cohort 3 of this trial allowed enrollment of patients with any baseline platelet count.

Design, treatment, and dose modifications

This study used a modified phase 1 design, enrolling 3 to 6 patients per level until 2 of the first 6 patients experienced dose-limiting toxicity. The dose level below this was then expanded to a minimum of 6 patients to ensure safety for phase 2 investigation. The schedule in cohorts 1 and 2 included receiving 50% of the flavopiridol dose as a 30-minute bolus infusion and the remaining 50% dose as a 4-hour infusion every week for 4 consecutive weeks, followed by 2 weeks off. The total dose administered in cohort 1 was 60 mg/m^2 (ie, 30 mg/m^2 as a 30-minute bolus followed by a 30 mg/m^2 4-hour continuous infusion). In cohort 2, the dose was 80 mg/m^2 (ie, 40 mg/m^2 as a 30-minute bolus and a 40 mg/m^2 4-hour continuous infusion). In cohort 3, the schedule was modified to administer flavopiridol at the cohort 1 dose for 4 weekly doses. Subsequently, if evidence of a partial response was not present as defined by NCI 96 criteria, a 30 mg/m^2 30-minute bolus followed by a 50 mg/m^2 4-hour continuous infusion (total dose, 80 mg/m^2) was administered. This modification was based on pharmacokinetic data derived from cohort 1, which suggested that an increased continuous infusion dose was required to maintain the targeted concentration throughout the infusion period. All patients received allopurinol at a dose of 300 mg/day beginning one day prior to initiation of treatment and continuing during therapy. All patients received prophylactic antiviral and *Pneumocystis carinii* therapy as described previously[3] during and for 6 months following completion of therapy. Following an episode of dose-limiting acute TLS in cohort 2, the remaining patients in cohorts 1 and 3 received inpatient pretreatment hydration and urine alkalization, prophylactic phosphate-binder treatment, and hourly potassium monitoring during and for 4 hours after treatment. Aggressive management of hyperkalemia, including kayexalate therapy, insulin and glucose therapy, and calcium therapy, was used according to an established protocol. The ability to perform emergent dialysis within 1 hour was assured. Prophylactic rasburicase (0.15 mg/kg) was used for the first dose in all patients and for those whose dose was increased with the fifth dose. Following 5 doses with flavopiridol, treatment was transitioned to outpatient status, with 2 hours of hydration before and during therapy with abbreviated laboratory monitoring. Patients discontinued therapy in cases of stable disease without improvement after 2 cycles (8 doses). Otherwise, patients continued therapy in the absence of progression or toxicity that prohibited further treatment for a maximum of 6 cycles (24 doses). Patients were evaluated for response after 2, 4, and 6 cycles of therapy. A bone marrow biopsy was performed at baseline and at 1 month after therapy to assess response. Baseline interphase cytogenetic studies were performed as previously published.[7] Following completion of therapy, patients were seen 1 and 2 months after therapy and then every 3 months until progression as defined by the NCI[31] was noted. Following this, patients were followed for survival only.

Dose-limiting toxicity

Dose-limiting toxicity was defined as nonhematologic toxicity of grade 3 or greater severity (excluding transient liver function abnormalities, transient non–life-threatening electrolyte abnormalities, fatigue, or diarrhea that resolved within 4 days), or in some cases grade 2 toxicity (ie, irreversible renal, chronic pulmonary, neurologic, or cardiac toxicity). Hematologic toxicity was dose limiting only if grade 4 thrombocytopenia or neutropenia occurred in the setting of an infection that persisted for 7 days or more. The NCI Common Toxicity Criteria were used to grade nonhematologic toxicities, and the NCI 96 CLL criteria were used for hematologic toxicities.[31] Hyperacute TLS was defined as tumor lysis requiring dialysis within 6 hours of initiating therapy.

Assessment of response

The revised NCI-sponsored Working Group Guidelines[31] were used as criteria for response and progression. Fludarabine refractory was defined by criteria previously reported.[32]

Pharmacokinetics

Plasma samples were obtained prior to dosing and at 30 minutes, 4.5 hours, 6 hours, 8 hours, 12 hours, 18 hours, 24 hours, and 32 hours after the start of the bolus infusion. Following review of data from the first 13 patients, the 18- and 32-hour specimen collection times were deleted and a 48-hour specimen collection time was added to better characterize the terminal elimination phase. Plasma proteins were precipitated with acetonitrile containing internal standard, dried, and reconstituted in mobile phase. Reconstituted analytes were separated using a reversed-phase gradient of methanol and water containing ammonium acetate (pH 4.15). Flavopiridol (402 $[M+H]^+$) was detected using a validated liquid chromatography tandem mass spectrometry (LC/MS/MS) electrospray ionization method in the positive ion mode. The limit of quantitation for this assay was 5 nM, with a linear range up to 1 μM. This assay measures total flavopiridol plasma concentration.

Statistics

Data were analyzed using the Wilcoxon signed-rank test for nonparametric comparisons and the student *t* test for parametric comparisons. Progression-free survival was estimated using the Kaplan-Meier method. Nonlinear least squares regression with a 2-compartment model was used for refining the dosing schedule (WinNon version 4.1; Pharsight, Mountain View, CA). The area under the plasma drug concentration–versus–time profile (AUC), volume of distribution (V_{ss}), clearance (CL), and half-life ($t_{1/2}$) were calculated by fitting the concentration-time data using noncompartmental analysis.

Results

Patient characteristics

A group of 42 patients were treated on cohorts 1 (n = 20), 2 (n = 3), and 3 (n = 19) (Table 1). The median number of cycles administered was 2 (range, 1-6 cycles) for cohort 1, 1 (range, 1-3 cycles) for cohort 2, and 3 (range, 1-6 cycles) for cohort 3. Patients had a high frequency of high-risk cytogenetic abnormalities and were heavily pretreated, and most patients were fludarabine refractory as defined previously.

Hyperacute TLS is observed with flavopiridol

Six patients were treated at the cohort 1 dose, with one patient exhibiting dose-limiting toxicity (neutropenic fever). Patient 2 on cohort 2 experienced TLS requiring a 2-day hospitalization, but not dialysis, and attained a durable (longer than 13 months) partial response. Patient 3 on cohort 2 developed hyperacute TLS with

Table 1. Pretreatment patient characteristics

	Cohorts 1 and 2	Cohort 3
No. patients	23	19
Median age, y (range)	61 (44-84)	55 (39-69)
Stage, %		
Rai intermediate risk	39	11
Rai high risk	61	89
Female, %	35	16
Median no. therapies (range)	4 (2-13)	4 (1-9)
Prior fludarabine treatment, no. (%)	22 (96)	19 (100)
Fludarabine refractory, no. (%)	17 (74)	18 (95)
Del(11q22.3), %	39	47
Del(17p13.1), %	35	21
Complex karyotype, %	35	53
Complex, del(11q22.3), or del(17p13.1), %	70	79
Median leukocyte count, $\times 10^9$/L (range)	8.3 (3.1-200)	44.5 (1.9-253)
Median hemoglobin, mM (range)	10.1 (6.5-15.4)	9.5 (5.8-13.7)
Median platelets, $\times 10^9$/L (range)	117 (53-254)	64 (16-213)
Patients with splenomegaly, %	39	74
Patients with adenopathy, %	100	100
Patients with bulky adenopathy above 5 cm, %	87	63
Patients with bulky adenopathy above 10 cm, %	26	37

uncontrollable hyperkalemia and subsequent fatal asystole on day 1 before dialysis could be initiated. On autopsy, extensive apoptosis/necrosis of diffuse intra-abdominal lymphadenopathy was found. No additional patients were treated at this dose, but patient 1 of cohort 2, who previously had not experienced TLS at the 80 mg/m^2 dose, developed hyperacute TLS requiring emergent dialysis on day 1 of cycle 2 while receiving a 60-mg/m^2 dose. The protocol was temporarily suspended, and an aggressive TLS monitoring procedure was initiated. An additional 14 patients were treated at the cohort dose. While biochemical evidence of TLS was present in most of these patients, only one developed hyperacute TLS requiring dialysis. Rise in lactate dehydrogenase (LDH), phosphate, and potassium levels was generally noted by completion of the 4.5 hours of treatment, peaked 24 to 48 hours after therapy, and normalized by the second treatment dose. In patients requiring dialysis for TLS, complete normalization of LDH sometimes did not occur until 14 days after therapy. In patients developing TLS, cytokine release symptoms as previously reported with flavopiridol[33] were generally observed.

We expanded eligibility to patients with platelet counts greater than 50×10^9/L to gain safety data on the frequency of hyperacute TLS in patients with more advanced CLL. Cohort 3 also allowed for dose escalation of patients who did not attain a partial response after the fourth dose of therapy to a total dose of 80 mg/m^2/week (30 mg/m^2 bolus followed by a 50 mg/m^2 4-hour infusion). This was based on pharmacokinetic analyses from patients treated on cohort 1 demonstrating that a 1.5-μM or greater flavopiridol concentration, previously demonstrated to be required for in vitro CLL cell cytotoxicity, was not being maintained for the entire treatment. A group of 19 patients were enrolled into this new cohort, with 4 developing hyperacute TLS requiring dialysis with the first cycle. Two of these patients had complicated courses. The first patient developed fatal Gram-negative sepsis related to a central venous catheter. The second patient exhibited recurrence of pulmonary hemorrhage previously seen during participation on a prior institutional review board–approved rituximab and etanercept clinical trial at Ohio State University. A group of 14 patients treated in this cohort were dose escalated, and 3 required short-term temporary dialysis (1-2 days) with no long-term consequences. No patient developed tumor lysis requiring hemodialysis beyond the fifth dose of flavopiridol therapy. Grades 3 and 4 toxicities observed are presented in Table 2 as the number of toxicities that occurred among patients treated in this trial. Toxicity observed with dose escalation during course 5 was not notably different except for biochemical tumor lysis. Grades 3 and 4 neutropenia were frequent with flavopiridol. Neutropenia often occurred within 24 hours of therapy in a manner different from that in standard chemotherapy and was resolved by the second dose of therapy. With successive weekly doses of flavopiridol neutropenia was noted to be more prolonged with patients recovering to baseline in time for course 2 of therapy.

Elevated leukocyte count is a major risk factor for hyperacute TLS

Most (5 [63%]) of the 8 patients with a pretreatment leukocyte count greater than 200×10^9/L developed hyperacute TLS requiring dialysis with the first dose, whereas only 1 (3%) of 34 patients with pretreatment counts less than 200×10^9/L developed hyperacute TLS requiring dialysis ($P < .001$). In contrast, lymph nodes

Table 2. Toxicity observed with flavopiridol

	No. patients with toxicities (%)	
	Grade 3	Grade 4
Hematologic		
ANC	0 (0)	30 (77)
Hgb	6 (15)	1 (3)
Platelets	8 (21)	7 (18)
Hemorrhage	3 (8)	0 (0)
Thrombosis	1 (3)	0 (0)
Cardiac		
Hypotension	1 (3)	3 (8)
Tachycardia or SVT	2 (6)	0 (0)
Atrioventricular heart block	1 (3)	0 (0)
Chest pain	1 (3)	0 (0)
Respiratory		
Dyspnea	3 (8)	0 (0)
Hypoxia	4 (10)	2 (5)
Apnea	1 (3)	0 (0)
Respiratory failure	0 (0)	1 (3)
GI/Hepatic		
Diarrhea	16 (41)	0 (0)
Abdominal pain/cramping	2 (5)	0 (0)
AST or ALT	11 (28)	2 (5)
Bilirubin	1 (3)	0 (0)
Renal*		
Renal failure	2 (5)	1 (3)
CNS		
Seizure	0 (0)	1 (3)
CNS cerebrovascular inschemia	0 (0)	1 (3)
Neuropathic pain	1 (3)	0 (0)
Possible infection		
Fever without neutropenia	3 (8)	0 (0)
Febrile neutropenia	4 (10)	0 (0)
Infection with neutropenia	6 (15)	1 (3)
Infection without neutropenia	1 (3)	0 (0)
Other		
Edema	1 (3)	1 (3)
Fatigue	7 (18)	1 (3)
Anorexia	3 (8)	0 (0)
Biochemical tumor lysis	23 (55)	0 (0)

ANC indicates absolute neutrophil count; Hgb, hemoglobin; SVT, supraventricular tachycardia; GI, gastrointestinal; AST, aspartate aminotransferase; ALT, alanine aminotransferase; and CNS, central nervous system.

*Excludes transient electrolyte abnormalities.

larger than 5 or 10 cm as determined by physical exam or computed tomography (CT) scan did not predict TLS. These 5 patients all required significant supportive care, and only one received further treatment with flavopiridol. Thus, patients with 6 prior therapies received 3 cycles of flavopiridol and had a remission of 10 months. On the basis of the high risk of life-threatening TLS in patients with leukocyte counts greater than 200×10^9/L that often precluded further therapy, these patients are now excluded in ongoing phase 2 CLL studies of flavopiridol. In contrast to this finding among patients with high lymphocyte counts, the 3 patients requiring dialysis during course 2 of therapy with the dose escalation did not have leukocyte counts greater than 200×10^9/L, and all were able to be retreated.

Response to treatment and characteristics associated with response

All response data are reported using an intent-to-treat basis. Less than half (19 [45%]) of the patients attained a partial response as defined by the NCI 96 criteria. Response by cohort was 8 (40%) of 20 in cohort 1, 1 (33%) of 3 in cohort 2, and 10 (52%) of 19 in cohort 3. There were no nodular partial responses or complete responses in which patients simply failed to recover from cytopenias. Four patients had complete resolution of lymph node and spleen enlargement. The median follow-up time since enrollment on the study was 28 months (range, 14-39 months). Progression-free survival for responding patients was a median of 13 months (95% CI of 7.7-18.8 months), with all but 3 patients having relapsed at this time. Of the original patients enrolled on this trial, 19 have died. Although most responses were noted in the first 2 cycles, continued reduction of disease was noted throughout therapy.

We next sought to determine whether flavopiridol was effective in patients with features predicting resistance to other available therapies. Of the 31 patients who had lymph nodes larger than 5 cm, 16 (51%) responded to flavopiridol therapy. Similarly, responses were noted in 5 (42%) of 12 patients with del(17p13.1) and 13 (72%) of 18 of patients with del(11q22.3) features associated with adverse treatment outcome in CLL.

Pharamacokinetic studies

Table 3 summarizes pharmacokinetic data for patients enrolled in cohorts 1 and 3. The plasma concentrations declined in a biexponential manner after the end of the 4-hour infusion, with a terminal $t_{1/2}$ of 1.1 to 42.5 hours (14.0 ± 10.2 hours; mean ± SD). Significant interpatient variability was observed, with C_{max} varying 2- to 3-fold at each dose level. The mean CL of flavopiridol was 14.5 ± 6.5 L/h/m^2, similar to that reported in prior studies. There was no clear relationship of any pharmacokinetic parameter to response. Table 3 demonstrates the 0.5- and 4.5-hour C_{max} in patients enrolled in cohort 3 among those patients receiving dose escalation. By increasing the 4-hour infusion dose to 50 mg/m^2 during the second cycle of therapy, we observed a significant increase in the mean (± SD) flavopiridol concentration at 4.5 hours from 1.03 (0.47 at the 30 mg/m^2 dose) to 1.54 (0.48 at the 50 mg/m^2 dose [$P = .001$]). This was accompanied by both biochemical and clinical evidence of rapid response. The average increase in LDH with the first dose of therapy was 665 IU on day 1 of cycle 1 ($P < .001$), and 1058 IU on day 1 of cycle 2 ($P < .001$) when dose escalation occurred.

Table 3. Flavopiridol pharmacokinetic parameters for patients enrolled in cohort 1

		Cohort 3	
	Cohort 1	Cycle 1, d 1	Cycle 2, d 1
No. patients	20	19	14
AUC, μM per h	10.9 ± 6.3	11.3 ± 5.2	12.7 ± 3.7
CL, L/h/m^2	14.5 ± 6.5	13.6 ± 6.2	15.2 ± 5.4
V_d, L/m^2	267 ± 199	270 ± 255	294 ± 161
$T_{1/2}$, h	14.0 ± 10.2	12.6 ± 6.8	12.8 ± 3.8
$C_{0.5\ h}$, μM	1.56 ± 0.92	2.21 ± 1.06	1.95 ± 0.78
$C_{4.5\ h}$, μM	0.93 ± 0.54	1.03 ± 0.07	1.54 ± 0.48

Cohort 1 consisted of a 30 mg/m^2 bolus plus a 30 mg/m^2 4-hour infusion; n = 20. AUC indicates area under the plasma concentration time curve from time 0 to infinity; CL, total systemic clearance; V_d, volume of distribution, $T_{1/2}$, terminal elimination half-life; $C_{0.5\ h}$, flavopiridol plasma concentration obtained 0.5 h after starting the bolus infusion (ie, end of the 0.5-h bolus infusion); and $C_{4.5\ h}$, flavopiridol plasma concentration obtained 4.5 h after starting the bolus infusion (ie, end of the 4-h continuous infusion). Values are presented as means ± standard deviation.

Discussion

This report describes dramatic clinical activity of the cyclin-dependent kinase inhibitor flavopiridol in genetically high-risk, fludarabine-refractory CLL. Remarkably, the dose-limiting toxicity in this trial was hyperacute TLS, a toxicity only rarely observed in CLL.[34-38] With experience derived from this trial, we were able to identify that patients at highest risk for this toxicity have elevated leukocyte counts greater than 200×10^9/L. Exclusion of patients with counts greater than 200×10^9/L has provided for safe administration relative to hyperacute TLS in the next 17 patients treated using this same stepped-up dosing on days 1 and 8 of therapy.[39] The frequency of responses in patients with genetically high-risk and fludarabine-refractory CLL indicates substantial potential for this agent for both this patient group as well as for the initial treatment of CLL as part of combination therapies and as a single agent. In addition, it suggests that flavopiridol may have similar activity in other B-cell malignancies such as low-grade lymphoma and mantle cell lymphoma, in which response profiles similar to those in CLL are often observed with other agents.

The development of flavopiridol in CLL exemplifies the importance of continued translational investigation throughout the clinical development of an agent and the adaptation of models that better approximate the in vivo human setting. Pharmacokinetic data from the 72-hour[30] and 24-hour[25] continuous infusion studies and 1-hour bolus[29] studies demonstrated the achievement of plasma drug concentrations that should have been sufficient to induce apoptosis. Despite this, only minimal activity was observed in these trials. One explanation for the lack of concordance between the promising in vitro data and disappointing clinical trial results may be the significant flavopiridol protein binding in human serum. Our experiments demonstrated that substitution of human serum for FBS, as is typically used in laboratory studies, resulted in a decrease in free drug levels from 63% to 100% to 5% to 8%. This was accompanied by an increase in the 1-hour and 24-hour LC_{50} required to induce apoptosis in CLL cells in vitro, from 670 nM and 120 nM, respectively (using FBS), to 3510 nM and 470 nM, respectively (using human serum). This observation may be critical, as the 24-hour LC_{50} of 470 nM was not achieved in vivo.[28,40] In addition, the 1-hour LC_{50} concentration for CLL cells in human serum was generally not obtained in the solid-tumor phase 1 trial using this schedule.[29]

Given the novel mechanism of action of flavopiridol, as well as its ability to induce p53-independent apoptosis, we pursued a study using pharmacokinetic modeling from our in vitro data as well as the 24-hour continuous infusion study.[28] This modeling suggested that a 30-minute bolus followed by a 4-hour continuous infusion schedule could attain a flavopiridol concentration of 1.5 μM for at least 4.5 hours, a concentration sufficient to induce apoptosis in CLL cells in vitro using media with human serum. While this concentration of flavopiridol was often obtained following the initial 30-minute infusion in our study, a significant decline was usually observed during the 4-hour infusion. The reason for discordance between our modeling and these results is likely due to the overestimation of the $t_{1/2}$ of flavopiridol resulting from shortcomings in previous assays. A new LC/MS/MS assay was developed in our laboratory, and the dose escalation to 50 mg/m^2 in cohort 3 was based on pharmacokinetic data derived from the first 23 patients treated on this study using this improved assay. Pharmacokinetic and pharmacodynamic modeling efforts are underway to better understand the relationship between flavopiridol exposure, toxicity, and efficacy. In addition, we are developing an assay to measure free flavopiridol concentrations and attempting to identify the protein that binds to flavopiridol, which may be relevant to predicting patient response.

The importance of the pharmacologic optimization of this schedule is demonstrated in the significantly increased biochemical evidence of TLS at the higher doses, at which the target concentration of 1.5 μM was maintained for the duration of therapy. In addition, at this higher dose level there was also increased evidence of clinical activity relative to the first treatment. The success of this study emphasizes both the importance of considering plasma-protein interactions in preclinical investigations of new agents and the use of detailed pharmacologic studies to direct schedule optimization. Indeed, development of flavopiridol by our group and others would have been much more efficient if comparative protein-binding experiments had been done prior to the clinical trials. This issue should be carefully considered as new therapies move toward the clinic.

Our observation of a high response rate with durable remissions in this heavily pretreated group of patients, including a large proportion of patients with high-risk genetic abnormalities such as del(17p13.1) and del(11q22.3) who are poorly responsive to other therapies, suggests that addition of flavopiridol will likely have a substantial role in the future treatment of CLL and related diseases. Future studies should also examine the association of treatment response with IgV_H mutational status. While this agent can induce durable remissions in patients with bulky disease, strategies to use flavopiridol can improve. Future efforts must focus on ways to optimally administer flavopiridol to avoid hyperacute TLS. Our data using this novel schedule suggest that patients with leukocyte counts greater than 200×10^9/L are at highest risk for developing life-threatening hyperacute TLS requiring dialysis, but even in patients with lower leukocyte counts, administration of flavopiridol still requires significant supportive care and detailed monitoring. Future strategies for patients with previously untreated CLL will include effective cytoreductive therapy followed by flavopiridol to eliminate residual disease. This approach will provide an active agent for resistant disease while reducing the elevated leukocyte count, which, in our opinion, will circumvent the biggest risk of life-threatening TLS. Finally, given the dramatic response observed to date, trials using flavopiridol in patients with lymphoma, acute leukemia, and solid tumors should also be pursued using this novel schedule of administration.

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Authorship

Contribution: J.C.B. and M.R.G. authored and co-investigated the study, identified the initial schedule of administration, directed modifications of the study, and authored the manuscript. T.S.L. was the principal investigator on the clinical trial, supervised patients enrolled on study, and assisted with the manuscript writing. J.T.D. supervised pharmacokinetic analyses and assisted with the manuscript writing. D.W. developed and validated the new pharmacokinetic assay for flavopiridol. M.A.P. performed the pharmacokinetic assay, analyzed data, and assisted with the manuscript writing. B.F. led the patient care effort, assisted in development of TLS management procedures, and assisted with the manuscript writing. M.M. was co-investigator on the study and, with the principal investigator, oversaw patient management and assisted with the manuscript writing. K.A.B. was co-investigator on the study, evaluated patients, and assisted with the manuscript writing. B.R. supervised management of nephrology issues and assisted with the manuscript writing. M.B.-M. was key in patient care and management of TLS and assisted with the manuscript writing. S.B. performed data management for the trial and assisted with the manuscript writing. L.J.S. was director of the patient clinical treatment unit, sample processing laboratory, where he assisted in developing the outpatient algorithm, and assisted with the manuscript writing. A.J.J. and D.M.L. supervised and performed laboratory correlative work and protein-binding studies and assisted with the manuscript writing. N.A.H. directed interphase cytogenetic analysis on all patients and assisted with the manuscript writing. G.L. directed immunophenotype analysis on all patients to confirm eligibility and assisted with the manuscript writing. D.C.Y. performed statistical analyses and assisted with the manuscript writing. J.-R.S. was involved in planning the study and interpreting data to support the schedule. A.D.C. oversaw the NCI flavopiridol program and provided input into study design.

Conflict-of-interest disclosure: J.-R.S. is an employee of Sanofi Aventis. A patent has been filed on this method of administering flavopiridol, but it has not been reviewed. Inventors on this patent are J.C.B., M.R.G., T.S.L., J.T.D., and J.-R.S. It has no financial value at the present time.

J.C.B. and T.S.L. contributed equally to this work.

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