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A phase 1 clinical trial of flavopiridol consolidation in chronic lymphocytic leukemia patients following chemoimmunotherapy

Farrukh T. Awan¹, Jeffrey A. Jones¹, Kami Maddocks¹, Ming Poi¹, Michael R. Grever¹, Amy Johnson¹, John C. Byrd¹, and Leslie A. Andritsos^{1,2}

¹Division of Hematology, Department of Internal Medicine, The Ohio State University Comprehensive Cancer Center, 320 W. 10th Avenue, Columbus, Ohio 43210, USA

²Starling Loving Hall, 320 W. 10th Avenue, Columbus, Ohio 43210, USA

Abstract

Patients with chronic lymphocytic leukemia (CLL) who receive chemoimmunotherapy and do not achieve complete remission experience significantly shortened progression-free interval (PFS). Additionally, the majority of patients treated for relapsed disease demonstrate evidence of measurable disease. Eradication of minimal residual disease (MRD) results in improved PFS and overall survival. Maintenance therapy might result in eradication of MRD and improve response duration but might be associated with an increase in incidence of infectious complications. Flavopiridol is a broad cyclin-dependent kinase (CDK) inhibitor with established safety and efficacy in patients with relapsed CLL, particularly patients with high-risk cytogenetic features. A pharmacologically derived schedule was utilized as consolidation therapy in this phase I study to assess the safety and feasibility of outpatient therapy with flavopiridol in patients with low tumor burden. Flavopiridol was administered as a 30-min loading dose of 30 mg/m² followed by a 4-h infusion of 30 mg/m² once weekly for 3 weeks every 5 weeks (1 cycle) for planned 2 cycles in ten patients. Therapy was extremely well tolerated and no patient developed acute tumor lysis syndrome. The most common toxicities were gastrointestinal. Of the patients, 22 % improved their response from a PR to CR. Eighty-eight percent experienced a reduction in tumor burden as measured by extent of bone marrow involvement including patients with del17p and complex karyotype. The study establishes the safety and efficacy of flavopiridol as consolidation therapy after chemoimmunotherapy for patients with CLL. Further evaluation is required in larger trials for the utility of CDK inhibitors as consolidation or maintenance strategies.

Registration number at [ClinicalTrials.gov](https://clinicaltrials.gov): NCT00377104.

Keywords

Flavopiridol; Chronic lymphocytic leukemia

[©]Farrukh T. Awan, Farrukh.awan@osumc.edu, Leslie A. Andritsos, leslie.andritsos@osumc.edu.

Compliance with ethical standards

Conflict of interest The authors declare no relevant conflicts of interest except for research funding from Lymphoma Research Foundation, Leukemia and Lymphoma Society, and D Warren Brown Foundation.

Introduction

The majority of patients with chronic lymphocytic leukemia (CLL) who receive chemoimmunotherapy will achieve a complete remission [1]. However, for those patients who do not achieve a complete remission, progression-free interval is significantly shortened [1–3]. In addition, the majority of patients with relapsed CLL who receive treatment have evidence of measurable marrow or lymph node disease [2, 3]. Eradication of this minimal residual disease (MRD) has been associated with improved progression-free and overall survival and serves as an important endpoint to determine early treatment efficacy [4]. A number of agents have undergone clinical evaluation as consolidation or maintenance options to improve the depth of response after standard chemotherapy. Notably, the use of alemtuzumab in the consolidation setting after combination chemoimmunotherapy was demonstrated to improve progression-free survival [5]. However, its use was associated with significant infectious complications that resulted in early discontinuation of multiple trials utilizing this approach. Other agents that have been utilized in this manner include rituximab, ofatumumab, and lenalidomide, with demonstration of improved responses and progression-free interval, albeit at a higher risk of infectious complications especially with rituximab [6–8].

Flavopiridol is an *N*-methylpiperidiny, chlorophenyl flavone with pleiotropic effects on CLL cells, including broad cyclin-dependent kinase (CDK) inhibition, inhibition of RNA polymerase II, and mitochondrial depolarization. Early clinical trials have demonstrated the effectiveness of flavopiridol for the treatment of relapsed CLL, particularly in patients with high-risk cytogenetic features [9–11]. For patients with significant tumor burden, treatment is initiated in the inpatient setting because of the risks of tumor lysis syndrome and cytokine release syndrome. If treatment is tolerated, patients are transitioned to the outpatient setting. We hypothesized that for patients with non-bulky disease, flavopiridol may be safely initiated in the outpatient setting. We performed a phase I dose-escalation study of flavopiridol administered in the outpatient setting in patients who had received prior cytoreduction with chemotherapy. The purpose of this study was to assess the safety and feasibility of outpatient therapy with flavopiridol in patients with low tumor burden disease, with a secondary endpoint of assessing eradication of minimal residual disease.

Methods

Study design

From October of 2006 to February of 2008, patients with a diagnosis of CLL or small lymphocytic lymphoma (SLL) were enrolled on this phase I clinical study of flavopiridol following approval by the Institutional Review Board at The Ohio State University. All patients provided written informed consent. At the time of enrollment, all patients had received at least one but not more than three prior chemotherapy regimens, had attained at least a partial response or greater to the most recent therapy without evidence of progressive disease as defined by National Cancer Institute (NCI)-96 criteria, and were >2 but <12 months from most recent therapy. All patients were >18 years old, had an ECOG performance status of 0–2, creatinine <2.0 mg/dL, bilirubin <1.5 times the upper limit of normal (ULN), and transaminases <2 times ULN. Patients were required to have a platelet

count of $50 \times 10^9/L$, an absolute neutrophil count of $1.0 \times 10^9/L$, and a white blood count of $200 \times 10^9/L$. Pregnant and breastfeeding women were excluded.

Treatment

Flavopiridol was administered as a 30-min loading dose of 30 mg/m², followed by a 4-h infusion of 30 mg/m² once weekly for 3 weeks every 5 weeks (1 cycle) for planned 2 cycles. Intra-patient dose escalation to dose level 2 of 30-mg/m² loading dose followed by a 4-h infusion of 50 mg/m² was ensued in patients who did not have tumor lysis requiring hemodialysis during the first dose of flavopiridol. The study was initially designed to allow for dose escalation based on the result of concurrent phase I trials of flavopiridol but did not require dose escalation since the current dose was determined to be the maximally tolerated dose. The modified design allowed for treatment in a standard 3 + 3 design for toxicity assessment without dose escalation, and the study was essentially performed as an extension cohort of a phase I trial. De-escalation rules were based on dose-limiting toxicity assessments.

Supportive care and prophylactic measures

All patients received allopurinol 300 mg daily for prevention of hyperuricemia and 20 mg dexamethasone IV on days 1, 8, and 15 of flavopiridol treatment for the prevention of cytokine release syndrome [10–12]. An additional dose of 4 mg dexamethasone orally on days 2, 9, and 15 was utilized as needed to diminish symptoms of cytokine release. Of valacyclovir, 1000 mg daily (or equivalent) was administered to all patients for prophylaxis of *Varicella zoster* virus, and *Pneumocystis jirovecii* pneumonia prophylaxis was administered at the discretion of the treating physician. All patients received prophylactic 500 mg ciprofloxacin orally twice daily. Of pegfilgrastim, 6 mg subcutaneously was administered on day 16 of each treatment cycle.

Toxicity and response assessment and dose-limiting toxicity

Dose-limiting toxicity was defined as non-hematologic toxicity of grade 3 severity excluding transient liver function abnormalities, transient electrolyte abnormalities that were not life threatening, fatigue, or diarrhea that resolve within 4 days. Hematologic toxicity was evaluated by the modified NCI-96 criteria and considered dose-limiting for (1) grade 4 thrombocytopenia for 7 days, (2) grade 3 or 4 neutropenia that did not resolve to grade 2 or less by week 5 of therapy, and (3) occurrence of febrile neutropenia. The National Cancer Institute Common Toxicity Criteria (CTCAE) version 3.0 was used to characterize toxicity. Dose-limiting toxicity was defined during the first cycle. Responses were assessed following cycle 2 using the NCI-96 Working Group response criteria. All patients underwent bone marrow assessment 2 months after completion of therapy, and minimal residual disease was assessed by a validated four-color flow cytometry assay.

Pharmacokinetics, pharmacodynamics, and cytokine studies

Whole blood samples were collected for pharmacokinetic (PK) analysis prior to dosing ($t = 0$) and at 30 min (0.5 h), 4.5, 6, 8, 24, and 48 h after the start of the bolus infusion. Similar PK samples were collected at similar time points during week 2 if dose escalation occurred.

Blood samples were collected in sodium heparin tubes, and plasma was immediately separated and stored at -20°C for later analysis. Flavopiridol quantification in plasma samples was achieved using a previously validated liquid chromatography-tandem mass spectrometry assay [12]. Plasma flavopiridol concentration–time data were analyzed using standard non-compartmental methods in PhoenixTM WinNonlin[®] 6.3 (Pharsight, Mountain View, CA). Descriptive statistical analyses of PK parameters were performed on all enrolled patients with evaluable PK data.

Statistical considerations

No formal hypothesis testing was planned. Descriptive statistics were provided for the primary endpoints of safety and tolerability. The evaluable population included all patients completing 1 cycle of therapy or discontinuing therapy during the first cycle secondary to toxicity. Median duration of response calculated from the date of best response until the earliest time of either disease progression or death was estimated among responding patients using the Kaplan–Meier method.

Results

Patient characteristics

Ten patients with previously treated CLL and currently with stable disease were enrolled on this phase I study designed to assess the safety and preliminary efficacy of flavopiridol as consolidation therapy. Baseline characteristics are detailed in Table 1. Median age was 59.5 years and 90 % of the patients were males. The majority of patients (80 %) had no palpable lymphadenopathy or splenomegaly at the time of initiating consolidation therapy, but up to 60 % had evidence of disease on CT imaging. Thirty percent had no morphologic evidence of CLL in the bone marrow when starting treatment and 10 % were negative on flow cytometry.

Toxicity

Patients received a median of 1.5 cycles of treatment (range 1–3). No patient developed acute tumor lysis syndrome during outpatient therapy with flavopiridol. Nine patients received the planned 2 cycles of therapy. One patient developed acute diverticulitis following cycle 1 and was removed from the study. The other dose-limiting toxicity was sinus tenderness secondary to an upper respiratory tract infection. Therapy was generally well tolerated and the most common toxicities were gastrointestinal (Table 2).

Response

Nine patients were evaluable for response, and utilizing the NCI-96 criteria, two patients improved their response from a partial response to complete response after flavopiridol. Most patients (88 %) experienced a reduction in their tumor burden as measured by morphologic evaluation of the extent of bone marrow involvement (Fig. 1). Of the patients, 77 % experienced an improvement in the burden of CLL cells in the marrow as assessed by flow cytometry. This included patients with del (17p) and complex karyotype (Table 3). None of the patients however was able to achieve a minimal residual disease negative status by four-color flow cytometry performed on bone marrow aspirate. After a median follow-up

of more than 5 years, median progression-free survival (PFS) was 321 days and median time to next treatment (TTNT) was 1.8 years (689 days; Fig. 2).

Pharmacokinetics

A total of 121 plasma concentrations from all 9 patients were evaluable for PK analysis. Figure 3 represents the mean concentration-time profiles for these patients. All nine patients received flavopiridol dose (30 + 30) mg/m² on C1D1 and (30 + 50) mg/m² on C1D8. A summary of non-compartmental analysis (NCA) parameter estimates is presented in Table 4. A previous study has reported a significant correlation between area under the curve (AUC) and response to flavopiridol treatment in relapsed and refractory non-Hodgkin's lymphoma [13]. In this study, we did not observe a significant correlation between the $C_{\max}$ or AUC of plasma flavopiridol concentration and the depth of response to the treatment (data not shown). All parameter estimates were within the range of previously published data for dose levels (30 + 30)mg/m² and (30 + 50)mg/m² administered as 30- min loading dose followed by a 4-h infusion [13–15].

Discussion

A hybrid-dosing schedule derived from serial pharmacokinetic analyses was utilized in this study to evaluate the safety and preliminary efficacy of flavopiridol in patients with CLL as consolidation therapy in the outpatient setting. Two dose-limiting toxicities (DLTs) were observed but none in the expansion cohort. Flavopiridol was therefore found to be safe, and a 30-min loading dose of 30 mg/m² followed by a 4-h infusion of 30 mg/m² once weekly for 3 weeks, every 5 weeks (1 cycle) for 2 cycles, with dose escalation of the 4-h infusion dose to 50 mg/m² after the first dose is the recommended phase 2 dose for patients with CLL. This dose can be utilized as a short consolidation regimen for patients who achieve at least a partial response after chemoimmunotherapy. The dosing schedule, regimen, and the clinical situation all ensured that patients do not develop hyperacute tumor lysis and cytokine release syndrome that has been a major concern for the clinical utility of this agent and required extensive supportive care [9–11, 13]. Flavopiridol can therefore be administered in the above schedule with pre-medications as indicated as consolidation therapy for patients with CLL.

Our study was not designed to evaluate the efficacy of flavopiridol in the consolidation setting, but efficacy analysis revealed that the majority of patients were able to improve the depth of their response. In this heterogeneous population of patients with multiple prior chemotherapies, none of the patients attained a MRD negative state as assessed by bone marrow aspirate flow cytometry. Moreover, PFS was slightly shorter than what was historically observed with bendamustine [16] and fludarabine, cyclophosphamide, and rituximab in the relapsed setting [2, 17], reflecting the heterogeneity and small sample size of the study. However, toxicity was modest and an argument can be made that a longer course of therapy may have the potential to further improve outcomes and extend treatment-free intervals. Our previous work has already established the safety and efficacy of flavopiridol in patients with CLL, and further trials with more specific cyclin-dependent kinase inhibitors are being conducted [13, 18, 19].

The use of maintenance therapy for CLL has been studied, and multiple trials of maintenance therapy with monoclonal antibodies and lenalidomide have been reported [5, 7, 20]. Treatment with rituximab or ofatumumab as maintenance was shown to improve progression-free survival in a subset of patients with genetically low-risk CLL albeit at the cost of a higher incidence of infectious complications and neutropenia [8]. This led to the recent approval of ofatumumab in the maintenance setting for patients with relapsed disease [8]. Similarly, lenalidomide use was also shown to be a feasible option but more than half of the patients experienced grade 3–4 neutropenia [6]. Therefore, flavopiridol could possibly be utilized to enhance the depth of response with kinase inhibitors since complete responses are infrequently seen in patients treated with ibrutinib or idelalisib [21, 22]. However, venetoclax has been shown to affect more CRs and MRD-negative states and appears to be a more effective agent [23].

The small sample size precludes definitive recommendations regarding the role of flavopiridol in the consolidation or maintenance setting but does suggest its feasibility in routine practice. Additionally, the hybrid-dosing schedule has already established efficacy in CLL and non-Hodgkin lymphomas [10, 11, 13, 15, 18]. Conversely, newer agents like dinaciclib (formerly SCH-727965) are more specific in their inhibition of cyclin-dependent kinases and may be potentially more advantageous as compared to flavopiridol. Furthermore, the prolonged 4-h infusion of flavopiridol makes it less attractive in this era of well-tolerated oral agents. The field of CLL therapy has also moved to targeted agents such as ibrutinib, acalabrutinib [24], idelalisib [25], and venetoclax [23] with high response rates and durable remissions, which questions the utility and position of flavopiridol in the current field. However, given that cyclin-dependent kinase inhibitors such as flavopiridol influence pathways independent of these agents, combination strategies using this or other CDK inhibitors with these agents should be considered as part of future clinical trials.

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Fig. 1.

Pre- and post-treatment assessment of morphologic bone marrow involvement by CLL. Mean plasma concentration vs. time profiles for all 9 patients on C1D1 (left) and C1D8 (right). Each data point is mean plasma flavopiridol concentration $\pm$ standard deviation

Fig. 2.
Kaplan–Meier curves of progression-free survival (*solid line*) and treatment-free survival (*dashed line*)

Fig. 3. Plasma flavopiridol concentration versus time. Mean plasma concentration vs. time profiles for all 9 patients on C1D1 (left) and C1D8 (right). Each data point is mean plasma flavopiridol concentration $\pm$ standard deviation

Table 1**Baseline patient demographics (*n* = 10)**

Age in years, median (range)	59.5 (50–76)
65 years, <i>n</i> (%)	2 (20)
Male, <i>n</i> (%)	9 (90)
WHO performance status, <i>n</i> (%)	
0	4 (40)
1	6 (60)
Lymphadenopathy and organomegaly, <i>n</i> (%) at the time of initiating maintenance therapy	
Lymphadenopathy on physical examination	2 (20)
Splenomegaly on physical examination	None
Lymphadenopathy on imaging studies	6 (60)
Splenomegaly on imaging studies	4 (40)
Bone marrow involvement by CLL at the time of initiating maintenance therapy (%)	
Morphologic assessment, mean/median (range)	23.2/3.3 (0–79)
Flow cytometry assessment, mean/median (range)	28.3/16 (0–80)
Hematologic parameters, mean/median (range)	
WBC ($\times 10^3/\mu\text{L}$)	5.1/3.5 (2.5–12.6)
Hemoglobin (g/dL)	13.3/13.6 (10.4–14.5)
Platelets ($\times 10^9/\text{L}$)	169.6/155 (82–341)
LDH ($\mu\text{g/mL}$), median (range)	148.7/124 (104–268)
IGHV mutational analysis, <i>n</i> (%)	
Unmutated IGHV	5 (50) ^a
Cytogenetic abnormalities, <i>n</i> (%)	
<i>n</i> (%) with del (13q14.3)	5 (50)
<i>n</i> (%) with trisomy 12	2 (20)
<i>n</i> (%) with del (11q22.3)	1 (10)
<i>n</i> (%) with del (17p13.1)	2 (20)
Complex karyotype	2 (20)
Treatment history	
Prior therapies, mean/median (range)	1.8/1.3 (1–3)
Prior alkylator treatment, <i>n</i> (%)	9 (90)
Prior fludarabine-refractory treatment, <i>n</i> (%)	9 (90)
Prior rituximab treatment, <i>n</i> (%)	10 (100)
Most recent prior therapy, <i>n</i> (%)	
Fludarabine, cyclophosphamide, and rituximab (FCR)	4 (40)
Fludarabine and rituximab (FR)	2 (20)
Cyclophosphamide, vincristine, and rituximab (R-CVP)	2 (20)
Other ^b	2 (20)
Response to most recent therapy as assessed by NCI-96 criteria, <i>n</i> (%)	
Complete response	4 (40)
Partial response	5 (50)

Stable disease	1 (10)
Response to most recent therapy as assessed by IWCLL-96 criteria, <i>n</i> (%)	
Complete response	1 (10)
Partial response	8 (80)
Stable disease	1 (10)

^aNot available on four patients

^bFludarabine plus rituximab; flavopiridol; GRN-163L; and pentostatin, cyclophosphamide, and rituximab (PCR)

Table 2

Incidence of all study-related adverse events by system organ class, by preferred term, and by grade (total cycles of therapy administered = 18)

Grades				
	1	2	3	4
Gastrointestinal				
Nausea	1			
Vomiting			1	
Colonic obstruction			1	
Colitis				1
Infection				
Infection with normal ANC or grade 1 or 2 neutrophils—skin (cellulitis)			1	
Infection with normal ANC or grade 1 or 2 neutrophils—upper airway NOS			1	
Vascular				
Thrombosis/thrombus/embolism				1

Table 3End of study assessments of patient demographics ($n = 9$)

Lymphadenopathy and organomegaly, n (%) after completing maintenance therapy	
Lymphadenopathy on physical examination	1 (11)
Splenomegaly on physical examination	None
Lymphadenopathy on imaging studies	4 (44)
Splenomegaly on imaging studies	0
Bone marrow involvement by CLL after completing maintenance therapy (%)	
Morphologic assessment, mean/median (range)	28./16 (0–80)
Flow cytometry assessment, mean/median (range)	15.5/5 (0–60)
Response to consolidation therapy as assessed by NCI-96 criteria, n (%)	
Complete response	5 (55)
Partial response	2 (22)
Stable disease	1 (11)
Progressive disease	1 (11)
Response to consolidation therapy as assessed by IWCLL-96 criteria, n (%)	
Complete response	0
Partial response	7 (77)
Stable disease	1 (11)
Progressive disease	1 (11)

Table 4
Pharmacokinetic parameters estimated by non-compartmental analysis method using Phoenix WinNonlin

Cycle/day	Dose (LD + ID), mg	$T_{1/2}$, h	C_{max} , μM	$AUC_{(0-\infty)}$, $\text{h} \times \mu\text{M}$	V_z , L/m^2	CL , $\text{L}/\text{h}/\text{m}^2$
C1D1 ($n=9$)	30 + 30	9.3 ± 4.8	1.79 ± 0.73	14.5 ± 7.0	147 ± 67	13.3 ± 7.8
C1D8 ($n=9$)	30 + 50	9.7 ± 4.0	2.56 ± 2.00	18.8 ± 10.6	175 ± 105	12.9 ± 4.8

The table lists mean values $\pm$ standard deviations derived from all nine patients. $AUC_{(0-\infty)}$ observed area under the curve from zero to infinity, C_{max} maximum observed concentration, CL clearance, ID infusion dose, LD loading dose, n number of patients, $T_{1/2}$ terminal phase elimination half-life, V_z apparent volume of distribution based on terminal phase