Flavopiridol (alvocidib) in chronic lymphocytic leukemia

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A B S T R A C T

Nearly half of patients with relapsed chronic lymphocytic leukemia (CLL) develop loss of 17p13, resulting in loss of the p53 tumor suppressor gene and resistance to most standard therapies. Thus, new therapies for relapsed CLL patients with del(17p13) and other high-risk cytogenetic abnormalities are needed. The synthetic flavone flavopiridol down-regulates the anti-apoptotic protein Mcl-1 and induces apoptosis of CLL cells *in vitro*. However, initial clinical trials using 24-72-hour continuous intravenous infusion (CIVI) dosing schedules observed no clinical activity, due to extensive drug binding to human plasma proteins and inadequate free flavopiridol concentrations. Pharmacokinetic modeling indicated that a 30-minute IV bolus followed by a 4-hour CIVI would achieve serum concentrations which induced *in vitro* apoptosis. A phase I study of this novel dosing regimen in 58 patients demonstrated that flavopiridol is highly active in patients with relapsed, genetically high-risk CLL. The dose limiting toxicity was acute tumor lysis syndrome (TLS) resulting in fatal hyperkalemia in one patient. Increased monitoring of serum potassium levels, aggressive intervention for hyperkalemia, and limitation of treatment to patients with white blood cell counts less than 200×10^9/L allowed flavopiridol to be administered safely. The response rate was 47%, with a median progression free survival of 11 months in responders, and flavopiridol was active in patients with high-risk features such as del(11q22) and del(17p13) or bulky nodal disease. Mean area under the curve (AUC) of drug exposure correlated with response to therapy, and mean AUC of the glucoronide metabolite correlated with severe TLS. A recently completed phase II study in 63 patients confirmed these results. Thus, flavopiridol is highly active in relapsed, high-risk CLL. An international, multi-center registration study is ongoing.

Introduction

Modern chemoimmunotherapy regimens have achieved overall response (OR) and complete response (CR) rates of over 90% and 70%, respectively, in patients with previously untreated chronic lymphocytic leukemia (CLL), but therapy is not curative and patients invariably relapse. As their disease progresses with successive cycles of remission and relapse, many patients acquire high-risk cytogenetic abnormalities such as del(17p13) and del(11q22), which result in loss of p53 and ATM, respectively. While del(17p13) is initially observed in only 5% of patients with untreated CLL, nearly half of patients eventually develop loss of 17p13. Unfortunately, del(17p13) and other high-risk cytogenetic abnormalities

induce resistance to most current therapies. Treatment options for such high-risk patients are limited, as agents such as alkylators, fludarabine and rituximab are ineffective against relapsed, p53-deficient CLL. Alemtuzumab is effective but is associated with immunosuppression and infectious complications. Thus, a major focus of CLL research is the identification of novel agents with biological and clinical activity in this high-risk subset of CLL patients.

Del(17p13) and del(11q22) confer resistance to standard therapies

Studies have identified several cellular factors which modulate resistance of CLL cells to fludarabine, although resistance factors are less well understood for other agents. Loss of the p53 tumor suppressor gene is highly predictive of resistance to fludarabine and other agents and confers a markedly inferior survival. Patients with del(17p13) constitute the poorest cytogenetic prognostic group in CLL, and fludarabine based regimens fail to overcome this poor prognosis. Fludarabine induces apoptosis of CLL cells by a p53-dependent mechanism and, not surprisingly, is ineffective in p53-deficient patients. While del(17p13) is observed in 5% of CLL patients at diagnosis, the p53 gene is lost in nearly 50% of fludarabine refractory patients and results in resistance to standard therapies. Secondary dysfunction of the p53 pathway can also occur through loss of 11q22. Patients with del(11q22) have a poor prognosis, and it is hypothesized that del(11q22) results in loss of the ATM (ataxia telangiectasia mutated) tumor suppressor gene. ATM phosphorylates p53 and is required for proper functioning of the p53 tumor suppressor pathway in normal cells. Loss of ATM results in secondary dysfunction of p53 and confers an inferior survival in CLL, and primary CLL cells from patients with p53 or ATM mutations failed to undergo apoptosis following *ex vivo* irradiation. Thus, CLL cells with primary or secondary p53 dysfunction may be predisposed to further chromosomal damage, resulting in increasing chemo-resistance.

Relapsed CLL patients with loss or mutation of p53 are resistant to standard therapies such as alkylators, fludarabine, and rituximab. Alemtuzumab and high dose methylprednisolone are active in relapsed CLL patients with del(17p13) but are associated with immunosuppression and increased susceptibility to severe infections such as cytomegalovirus (CMV) disease. In addition, single agent alemtuzumab has limited clinical activity in relapsed CLL patients with bulky lymph nodes > 5 cm. Thus, therapeutic options for relapsed CLL patients with high-risk chromosomal abnormalities are limited, and new treatments for these poor-risk patients are needed.

Flavopiridol: preclinical background

Flavopiridol (NSC 649890) is an N-methylpiperidinyl, chlorophenyl flavone which was initially developed as a tyrosine kinase inhibitor by the National Cancer Institute (NCI) and was subsequently noted to be a broad cyclin dependent kinase (CDK) inhibitor. Flavopiridol inhibited CDK1 and CDK2 by alteration of tyrosine phosphorylation and competitive inhibition with ATP. In addition, flavopiridol inhibited CDK4-cyclin D1 *in vitro* and induced apoptosis in HL-60 leukemia and SUDHL-4 lymphoma cell lines. Subsequent studies demonstrated that flavopiridol affects other intracellular pathways and induces apoptosis by down-modulating anti-apoptotic proteins such as Mcl-1 and X-linked inactivator of apoptosis (XIAP), which mediate resistance to fludarabine and other therapeutic agents in CLL. Down-modu-

lation of Mcl-1 may be critical for flavopiridol's activity, as over-expression of Mcl-1 correlates with resistance to therapies such as fludarabine and rituximab in CLL.

Flavopiridol inhibited RNA polymerase II (RNAP II). Inactivation of positive transcription elongation factor b (P-TEFb) resulted in decreased phosphorylation and transcriptional activity of RNAP II, decreased gene transcription, down-modulation of Mcl-1, and induction of apoptosis. In preclinical studies, flavopiridol induced apoptosis of CLL cell lines and primary CLL cells at concentrations which are attainable clinically. Furthermore, flavopiridol induced apoptosis by activating caspase 3, which exerts its pro-apoptotic effects distally to p53. Animal studies using lymphocytes derived from p53 knockout mice confirmed that flavopiridol induces apoptosis of B-cell lymphoid malignancy cells in a p53-independent manner. Thus, these studies provided strong preclinical data that flavopiridol can induce apoptosis of p53-deficient CLL cells.

Flavopiridol: initial studies of 24-72 hour continuous IV schedules

In vitro preclinical studies demonstrated that flavopiridol inhibited tumor cell growth after 72-hours' exposure, and a 72-hour continuous intravenous infusion (CIVI) administration schedule was chosen by the NCI for the initial phase I study of flavopiridol in humans. Diarrhea was the dose limiting toxicity (DLT), and fatigue, malaise, fever, hypoalbuminemia, anorexia, lightheadedness, nausea, vomiting, hypotension, and pericardial and pleural effusions were also observed. Subsequent phase II studies in relapsed solid and hematologic cancers administered flavopiridol at 50 mg/m^2/day by 72-hour CIVI, but no clinical activity was observed.

Initial studies in relapsed CLL were similarly disappointing. A phase I/II study administered flavopiridol 80-140 mg/m^2 by 24-hour CIVI, every 2 weeks for up to 12 doses, to 26 patients with fludarabine refractory CLL. Toxicity was acceptable, but no clinical responses were observed. Fifteen patients received flavopiridol 50 mg/m^2/day by 72-hour CIVI every 2 weeks in the CALGB 19805A study. No clinical responses were observed, and 73% of patients experienced progression. Animal studies suggested that IV bolus dosing might be more clinically effective than the CIVI schedule, and the amended CALGB 19805B study gave flavopiridol 50 mg/m^2 by 60-minute IV bolus daily for 3 consecutive days every 3 weeks. Four of 36 patients (11%) attained a partial response (PR), and 53% of patients had stable disease. Tumor lysis syndrome (TLS) was observed in 2 patients, and the peripheral lymphocyte count decreased in all non-responders. Thus, IV bolus dosing showed greater clinical promise than CIVI dosing, although clinical responses remained modest.

Pharmacokinetic modeling achieves a clinical active dosing schedule

Pharmacokinetic (PK) data showed that CIVI dosing achieved plasma drug concentrations (200-400 nM) that had induced apoptosis *in vitro* in preclinical studies, but activity was not observed *in vivo*. The discrepancy between preclinical activity and clinical outcome resulted from increased binding of flavopiridol to human plasma proteins. Initial preclinical studies of flavopiridol used fetal calf serum (FCS) in culture media. However, substitution of human plasma for FCS *in vitro* decreased free drug from 63-100% of total drug concentration to only 5-8%, with an increase in 1-hour and 24-hour LC50 values from 670 nM and 120 nM to 3510 nM and 470 nM, respec-

tively. This increase in LC50 may be critical, as the 24 hr LC50 of 470 nM was not achieved *in vivo* with CIVI dosing. Thus, binding to human plasma proteins resulted in failure to achieve a pharmacologically effective *in vivo* plasma flavopiridol concentration when the drug was given by CIVI. Therefore, no clinical activity was observed.

PK modeling indicated that administering flavopiridol by a 30-minute IV bolus followed by a 4-hour IV infusion could achieve and maintain a plasma drug concentration which induced apoptosis in preclinical studies. We conducted an NCI-approved phase I dose escalation study using this PK-derived schedule. Fifty-eight patients with relapsed CLL or small lymphocytic lymphoma (SLL) received flavopiridol by 30-minute IV bolus followed by 4-hour CIVI weekly for 4 doses every 6 weeks, for up to 6 cycles. Toxicity and response data on the first 42 patients were published previously and are presented here (Table 1). Median age was 60 years (range, 39-84). Patients had received a median of 4 prior therapies (range, 1-13), 41 patients (98%) had failed fludarabine based therapy, and 35 patients (83%) were refractory to or intolerant of their last fludarabine therapy. Thirty-two patients were Rai stage III/IV (76%), and 31 patients (74%) had bulky lymphadenopathy with at least one node >5 cm. Six dose levels were planned, with 30 mg/m^2 IVB + 30 mg/m^2 CIVI in the first dose level increasing by increments of 10 mg/m^2 IVB + 10 mg/m^2 CIVI to a maximum of 80 mg/m^2 IVB + 80 mg/m^2 CIVI in dose level 6.

Table 1. Demographic and disease characteristics of patients (n=42).

Median age (range)	60(39-84)
Rai stage III/IV	32(76%)
Bulky lymph nodes > 5 cm	31(74%)
Median prior therapies (range)	4(1-13)
Prior fludarabine therapy	41(98%)
Fludarabine refractory	35(83%)
Del(11q22)	18(43%)
Del(17p13)	12(29%)
Complex karyotype	23(55%)
Any high-risk cytogenetics	33(78%)

Dose limiting toxicity: acute tumor lysis and hyperkalemia

Six patients were treated at dose level 1. The maximum tolerated dose (MTD) was exceeded at dose level 2; acute TLS and hyperkalemia were observed as the DLT in 2 of 3 patients in cohort 2. One patient in cohort 2 was hospitalized for TLS after the first dose of flavopiridol, did not require hemodialysis, and safely completed cycle 1 without further significant TLS. Patient 2 in cohort 2 developed rapidly progressive, overwhelming acute TLS which resulted in uncontrollable hyperkalemia, cardiac arrhythmia, asystole and death. Postmortem examination showed extensive necrosis of bulky abdominal lymph nodes. Patient 3 in cohort 2 experienced no significant TLS to four treatments at 40 mg/m^2 IVB + 40 mg/m^2 CIVI, but required transient hemodialysis after developing acute TLS and hyperkalemia after reduction of the dose to 30 mg/m^2 IVB + 30 mg/m^2 CIVI with cycle 2. The study was temporarily suspended, and we instituted a rigorous NCI-approved inpatient management plan using IV hydration, allopurinol, rasburicase and aggressive intervention for hyperkalemia to prevent life threatening episodes of acute TLS.

Fourteen additional patients (cohort 1a, Table 2) were treated at dose level 1, for a total of 20 patients at this dose level. With increased monitoring and aggressive intervention for TLS, toxicity was acceptable with only 1 of 14 patients in cohort 1a requiring dialysis. Nine of 23 patients in cohorts 1 and 2 achieved a PR, indicating that our novel dosing schedule was clinically active. Plasma concentrations at 0.5 hours (C0.5hr) and 4.5 hours (C4.5hrs) were

Table 2. Dosing schedule and number of patients treated by cohort.

Cohort 1:	30 mg/m^2 IVB + 30 mg/m^2 4-hr CIVI	(n=6)
Cohort 2:	40 mg/m^2 IVB + 40 mg/m^2 4-hr CIVI	(n=3)
Cohort 1a:	30 mg/m^2 IVB + 30 mg/m^2 4-hr CIVI	(n=14)
Cohort 3:	30 mg/m^2 IVB + 30 mg/m^2 4-hr CIVI to 30 mg/m^2 IVB + 50 mg/m^2 CIVI with dose 5	(n=19)
Cohort 4:	30 mg/m^2 IVB + 30 mg/m^2 4-hr CIVI to 30 mg/m^2 IVB + 50 mg/m^2 CIVI with dose 5	(n=16)

Table 3. Clinical activity by risk group.

	All Patients	*Del(17p13)*	*Del(11q22)*	*Bulky lymph nodes >5 cm*
No. patients	42	12	18	31
Partial response	19	5	13	16
Response rate	45%	42%	72%	51%

1.56 µM and 0.93 µM, respectively. Thus, 30 mg/m^2 IVB attained the PK target of 1.5 µM, but 30 mg/m^2 4-hour CIVI was unable to maintain this target for the duration of the drug infusion. PK analysis suggested that increasing the 4-hour CIVI dose to 50 mg/m^2 would sustain the PK target of 1.5 µM for the entire infusion.

Pharmacokinetics: increased anti-tumor activity results from higher $C_{4.5hrs}$

The protocol was amended to study intra-patient dose escalation in the next group of patients (cohort 3). Fourteen of 19 patients in cohort 3 underwent dose escalation to 30 mg/m^2 IVB + 50 mg/m^2 CIVI beginning with the second cycle. Five patients were not dose escalated due to severe TLS in cycle 1. $C_{0.5hr}$ and $C_{4.5hrs}$ with dose 1 of cycle 1 (30 mg/m^2 IVB + 30 mg/m^2 CIVI) were 2.21 µM and 1.03 µM, respectively. Increasing the dose to 30 mg/m^2 IVB + 50 mg/m^2 CIVI attained C0.5hr and $C_{4.5hrs}$ of 1.95 µM and 1.54 µM, respectively. Thus, the higher 4-hour CIVI dose maintained the PK target of 1.5 µM for the duration of the 4.5-hour treatment period. Furthermore, this increase in C4.5hrs correlated with increased anti-tumor activity. The median increase in serum lactate dehydrogenase (LDH) was 665 IU after dose 1 of cycle 1, compared to 1058 IU after dose escalation of the 4-hour CIVI dose at the start of cycle 2. Ten of 19 patients (52%) in cohort 3 experienced a clinical response.

A markedly elevated white blood count (WBC) >200×10^9/L was identified as the major laboratory and clinical risk factor for developing severe TLS and hyperkalemia requiring hemodialysis. Five of 8 patients (63%) with WBC > 200×10^9/L required transient hemodialysis for severe TLS, compared to only 1 of 34 patients (3%) with WBC < 200 ×10^9/L. The study was therefore amended to exclude patients with WBC >200×10^9/L, to minimize the risk of severe TLS and the need for dialysis. Furthermore, the study was amended to dose escalate patients at dose 2 of cycle 1, to determine whether earlier dose escalation is possible in patients who do not develop severe TLS with dose 1. Cohort 4 enrolled 16 patients with WBC <200×10^9/L (Table 2), and dose escalation was safely performed at dose 2. These data have been submitted for publication.

Clinical activity in high-risk CLL

NCI Working Group criteria noted clinical responses in 19 of 42 patients (45%); all responders achieved a PR. Responses were durable, with a median progression free survival (PFS) of 12 months. Furthermore, flavopiridol exhibited clinical activity in poor-risk CLL populations for whom treatment options are limited. PR was observed in 5 of 12 patients (42%) with del(17p13) and 13 of 18 patients (72%) with del(11q22). Sixteen of 31 patients (51%) with bulky lymph nodes > 5 cm achieved PR (51%). These findings were con-

firmed by the additional 16 patients in cohort 4. Thus, flavopiridol demonstrated remarkable clinical activity in heavily pretreated patients with relapsed CLL; more importantly, the drug was highly active in patients with poor risk cytogenetic features and bulky nodal disease, who otherwise had limited treatment options.

Phase II study: confirmation of clinical activity and increased tolerability

To confirm these phase I findings, we enrolled 63 patients with relapsed CLL with WBC <200×10⁹/L in a phase II study. Median age was 61 years (range, 31-82), 40 patients were male (65%), and 11 patients were ≥70 years. Median number of prior therapies was 4 (range, 1-11). All patients had failed purine analog therapy. Rai stage was I/II (n=14) or III/IV (n=48). Patients received flavopiridol 30 mg/m² IVB + 30 mg/m² CIVI for dose 1, with escalation to 30 mg/m² IVB + 50 mg/m² CIVI beginning at dose 2. Therapy was given weekly for 4 doses every 6 weeks for up to 6 cycles. The study was amended to change the schedule to 3 doses every 4 weeks to see if this schedule change would improve tolerance.

Three patients did not undergo dose escalation due to grade 4-5 tumor lysis with dose 1, and 2 patients required hemodialysis. Cytokine release syndrome (CRS) was common and was associated with IL-6 release, but was averted by use of pre-treatment steroids. Patients received a median of 4 cycles (range, 0.25-6), and 19 pts completed all 6 planned cycles. Thirty-two patients responded (51%), including 28 PR, 3 CR, and 1 patient who had insufficient counts but otherwise achieved a CR (CRi). Furthermore, the phase II study confirmed the activity of flavopiridol in high-risk patients, with response rates of 63% in patients with del(17p13), 46% in patients with del(11q22), 50% in patients with a complex karyotype, and 51% in patients with lymph nodes ≥5 cm. Of 26 pre-amendment patients, 2 completed therapy, while 16 of 37 post-amendment patients completed 6 cycles. Furthermore, this improvement in treatment delivery resulted in an improved response rate. Thus, reducing the number of doses per cycle from four to three, reducing the cycle length from 42 to 28 days, and using prophylactic steroids eliminated IL-6 release and CRS, allowed more patients to complete therapy, and resulted in a higher response rate.

Summary

Flavopiridol is a novel agent which induces apoptosis of CLL cells by a p53-independent mechanism. High plasma protein binding resulted in a lack of clinical activity in studies using 24-72-CIVI schedules. A pharmacokinetically derived schedule administering the drug by 30-minute IVB followed by 4-hour CIVI demonstrated significant clinical activity in heavily pretreated CLL patients, with responses in approximately half of patients. More impressively, flavopiridol was highly active in relapsed CLL patients with high-risk genetic features such as del(17p13) and del(11q22). Ongoing studies are focusing on optimization of the dosing schedule to reduce toxicity, most notably side effects related to cytokine release, and improve tolerability and feasibility of this drug in relapsed CLL patients. Furthermore, ongoing pharmacokinetic and pharmacodynamic studies are focused upon identifying PK parameters or metabolites which may identify patients at greatest risk for severe TLS and other toxicities, with the goal of improving safety of this dosing schedule. Flavopiridol is under study as consolidation therapy to eliminate minimal residual disease (MRD) after cytoreductive therapy and in combination regiments with

other agents. Additionally, the drug is under active investigation in a variety of hematologic and solid malignancies.

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