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Risk Factors for Tumor Lysis Syndrome in patients with Chronic Lymphocytic Leukemia Treated with the Cyclin Dependent Kinase Inhibitor, Flavopiridol

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Abstract

Tumor lysis syndrome (TLS) has been described in over 40% of patients with chronic lymphocytic leukemia (CLL) treated with the cyclin dependent kinase inhibitor, flavopiridol. We conducted a retrospective analysis to determine predictive factors for TLS. In 116 patients, the incidence of TLS was 46% (95% CI: 36%-55%). In univariable analysis, female gender, greater

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Authorship

Contribution: K.A. Blum designed and performed the study, supervised patients enrolled on the described trials, analyzed and interpreted the data, and authored the manuscript. A.S. Ruppert analyzed and interpreted the data, performed statistical analysis, assisted with manuscript preparation, and reviewed and edited the draft manuscript. D. Wilkins led the patient care efforts on these trials; collected data on patient response, toxicity, and outcomes; and reviewed the draft manuscript. J.A. Woyach collected and provided the data and reviewed the draft manuscript. J.A. Jones, L. Andritsos, and J.M. Flynn supervised and enrolled patients on these studies and reviewed the draft manuscript. B. Rovin assisted with the treatment of patients on these studies who developed TLS, providing emergent nephrology support and consultation for the management of these patients, and reviewed the draft manuscript. J. Jia provided the pharmacokinetic data, assisted with the analysis, and reviewed and edited the draft manuscript. M. Phelps validated the pharmacokinetic assay, performed the pharmacokinetic sample analysis and modeling, provided the pharmacokinetic data, and reviewed and edited the draft manuscript. A.J. Johnson supervised and performed laboratory correlative work associated with these two trials and reviewed the manuscript. M. Villalona-Calero is the principal investigator on NCI NO1 CM62207 that supported this trial and reviewed and edited the draft manuscript. M.R. Grever is the principal investigator on NCI U01 CA076576 that supported this trial, supervised patients enrolled on these studies, assisted with study design and analysis, and reviewed and edited the draft manuscript. J.C. Byrd served as principal investigator on the described trials, supervised patients enrolled on these studies, assisted with study design and analysis, and reviewed and edited the draft manuscript.

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number of prior therapies, Rai stages III-IV, adenopathy ≥ 10 cm, splenomegaly, del(11q), decreased albumin, and increased absolute lymphocyte count, white blood cell count (WBC), β2-microglobulin, and lactate dehydrogenase (LDH) were associated ($p<0.05$) with TLS. In multivariable analysis, female gender, adenopathy ≥ 10 cm, elevated WBC, increased β2-microglobulin, and decreased albumin were associated with TLS ($p<0.05$). With respect to patient outcomes, 49% and 44% of patients with and without TLS, respectively, responded to flavopiridol ($p=0.71$). In a multivariable analysis controlling for number of prior therapies, cytogenetics, Rai stage, age, and gender, progression-free survival (PFS) was inferior in patients with TLS ($p=0.01$). Female patients and patients with elevated β2-microglobulin, increased WBC, adenopathy ≥ 10 cm, and decreased albumin were at highest risk and should be monitored for TLS with flavopiridol. TLS does not appear to be predictive of response or improved PFS in patients receiving flavopiridol.

Keywords

chronic lymphocytic leukemia; flavopiridol; tumor lysis syndrome

INTRODUCTION

Flavopiridol is a cyclin-dependent kinase inhibitor that induces apoptosis of CLL cell lines through down-regulation of Mcl-1 and the X-linked inactivator of apoptosis (XIAP) via a p53-independent mechanism.(1, 2) Despite robust apoptosis *in vitro*, initial clinical trials utilizing 24 or 72-hour flavopiridol infusion schedules demonstrated limited activity in patients with hematologic malignancies.(3-5) These initial schedules were modeled *in vitro* using media containing fetal bovine serum (FBS). Later studies demonstrated significant protein binding of flavopiridol in human serum with a higher LC_{50} of flavopiridol against CLL cells in human serum compared to FBS.(6) Therefore, the lack of *in vivo* efficacy with the 24-72 hour infusion schedules was postulated to be secondary to human protein binding that limited drug availability to malignant cells. Subsequent phase I and II studies utilizing a pharmacologically derived schedule of flavopiridol with a 30-minute bolus followed by a 4 hour continuous intravenous (IV) infusion (CIVI) designed to increase peak flavopiridol concentrations and overcome human protein binding eventually corroborated the significant activity with flavopiridol previously observed *in vitro* in CLL.(5-7) Specifically in these trials, 40-47% of patients with previously treated CLL responded to flavopiridol, including patients with del(17p13.1). Median progression-free survival (PFS) reported with flavopiridol therapy in patients with relapsed or refractory CLL after a median of 4 prior therapies (range, 1-14) was 10-12 months.

Therapy has been complicated by acute tumor lysis syndrome (TLS) occurring within 4.5 to 24 hours of initiation of flavopiridol. Life-threatening hyperkalemia and hyperphosphatemia requiring therapy with kayexalate, insulin and glucose, sodium bicarbonate, calcium, oral phosphate binders, and occasionally emergent dialysis has been described.(5-7) In the phase I trial, TLS was dose limiting and occurred in 44-55% of patients.(6, 7) As a result of this toxicity, enrollment was restricted to patients with a WBC < 200×10^9/L and aggressive TLS prophylaxis with hydration, rasburicase, and hourly potassium monitoring and

treatment was implemented. Flavopiridol dosing was also reduced to 30 mg/m^2 bolus followed by 30 m/gm^2 CIVI with dose escalation to 30 mg/m^2 bolus and 50 mg/m^2 CIVI only after at least one successful treatment with flavopiridol at the lower dose level without significant TLS. This intra-patient dose escalation, exclusion of patients with white blood cell (WBC) counts > 200 × 10^9/L, and implementation of aggressive TLS prophylaxis greatly improved the tolerability of this agent. However, in the subsequent phase II trial, TLS still occurred in 44% of patients.(5) Some of these patients required dialysis and could not be dose-escalated despite pre-treatment WBC < 200 × 10^9/L and the use of aggressive TLS prophylaxis, monitoring, and treatment, highlighting the unpredictable nature of this toxicity. Therefore, we conducted a retrospective analysis of 116 patients with relapsed or refractory CLL treated with single agent flavopiridol to determine predictive factors for the occurrence of acute TLS.

MATERIALS AND METHODS

Patients

Patients with relapsed or refractory CLL treated with single agent flavopiridol on National Cancer Institute sponsored phase I (NCI-5746, OSU 0055)(6, 7) and phase II trials (NCI-7000, OSU 0491)(5) were evaluated for TLS. These Ohio State University (OSU) trials were approved by the Cancer Therapy Evaluation Program of the NCI and the OSU institutional review board. All patients provided written informed consent in accordance with the Declaration of Helsinki. Fifty-two patients with CLL were treated on the phase I trial between May 2003 and February 2006 and 64 patients received flavopiridol on the phase II trial from February 2006 until June 2008.

Patients at least 18 years of age with CLL requiring treatment according to NCI 1996 criteria(8) who had received at least one prior chemotherapy were enrolled. Additional eligibility criteria for these two trials included Eastern Cooperative Oncology Group performance status of 0-2, creatinine ≤ 2 mg/dL, bilirubin ≤ 1.5 × the upper limit of normal (ULN), and aspartate transaminase ≤ 2 × the ULN.

Treatment plan and Response Assessment

In the phase I and II trials, flavopiridol was administered intravenously over 30 minutes followed by a 4-hour CIVI weekly for 4 consecutive weeks followed by 2 weeks without therapy (6 weeks defined a cycle) for a maximum of 6 cycles. In the phase I trial, flavopiridol was dose escalated from 30-50 mg/m^2 according to Table 1. Ten of the 52 patients in the phase I trial were re-treated with flavopiridol on study at the time of disease progression (2 patients in cohort 3 and 8 patients in cohort 4); however, for the purposes of this analysis these patients were only included at the time of first exposure to flavopiridol. In the phase II trial, flavopiridol was administered at a dose of 30 mg/m^2 over 30 minutes followed by 30 mg/m^2 4-hour CIVI week 1 (Table 1). On week 2 and for all subsequent treatments, flavopiridol was increased to a 30 mg/m^2 bolus with a 50 mg/m^2 CIVI. After 32 patients were treated, the phase II trial was amended to reduce the cycle length from 6 to 4 weeks, decreasing flavopiridol administration to 3 doses with each cycle (days 1, 8, and 15).

Dexamethasone premedication to limit cytokine release symptoms and prophylactic pegfilgrastim day 16 were also added to each cycle.

Response was determined by NCI-WG CLL criteria(8) and toxicity assessed using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Progression-free survival (PFS) was calculated from the date on study until disease progression or death. In patients without documented progression, PFS was censored at the time of last follow-up, next treatment, or stem cell transplantation. Overall survival (OS) was calculated from the date on study until death, censoring patients alive at last follow-up.

Definition, prophylaxis, and management of TLS

TLS was defined as one or more of the following within 24 hours of flavopiridol administration: uric acid > 6.6 mg/dl (the upper limit of normal (ULN)), potassium > 5.1 mmol/L (the ULN), phosphate > 4.5 mg/dL (the ULN), and/or an increase in lactate dehydrogenase (LDH) at least 2 × baseline. All patients received prophylaxis for TLS with allopurinol, rasburicase, intravenous hydration with sodium bicarbonate containing fluid, and oral phosphate binders. Serum potassium levels were monitored hourly and patients with hyperkalemia or hyperphosphatemia were treated with kayexalate, furosemide, albuterol, insulin and glucose, calcium, oral phosphate binders, or emergent dialysis.

Statistical methods

In this retrospective, observational study of patients treated with flavopiridol, the primary aim was to identify potential risk factors associated with the development of TLS. Associations between baseline characteristics and severity of TLS (defined as requiring or not requiring dialysis) were described using Fisher's exact or Kruskal-Wallis tests. Univariable analysis by logistic regression and multivariable analysis with limited backwards selection identified variables significantly associated with the development of TLS. In these analyses, patients were grouped according to whether not they had TLS, due to the small numbers of patients requiring dialysis. Two models were considered: one that included creatinine clearance, which is a function of gender, age, and creatinine levels, and another that did not consider creatinine clearance but did consider gender, age, and creatinine levels as separate variables in the modeling process. The two models were compared using Akaike information criterion (AIC), and the model with the better fit to the observed data was chosen to present herein. The multivariable analysis was repeated in the subset of patients (n=92) with Rai stages III-IV, as only 3 of 24 patients with Rai stages I-II developed TLS. Odds ratios with 95% confidence intervals were included from the models. Variables with p values less than 0.05 were considered significant.

Estimated probabilities of PFS and OS were calculated using the Kaplan-Meier method and differences among survival distributions were evaluated with the log-rank test. Logistic regression and proportional hazards models were used to determine the impact of TLS on response and survival, respectively, controlling for study, number of prior therapies, cytogenetic risk group (using Dohner's classification(9): del(17p), del(11q), other), Rai stage, patient age, and gender. In these analyses, where TLS might be associated with

clinical outcome, a 3-level variable of TLS taking into consideration the severity of TLS was initially utilized.

Lastly, a subset analysis in 83 patients, using logistic regression determined the association of peak flavopiridol levels and its glucoronide metabolite (flavo-G) during cycle 1 with TLS. In logistic regression models predicting TLS, all pharmacokinetic measures were log transformed.

RESULTS

Patient Characteristics

One hundred and sixteen patients enrolled and treated with one or more doses of single agent flavopiridol on OSU 0055 (n=52) or OSU 0491 (n=64) were included in this analysis. In 116 patients, the median age was 60 (range, 31-84), 69% of patients were male, the median number of prior therapies was 4 (range, 1-14), and 79% of patients had Rai stage III-IV disease at the time of study enrollment. All but one patient had received a previous nucleoside analogue (fludarabine or pentostatin) and 76% were purine analogue-refractory. No patient had previously received flavopiridol or a cyclin dependent kinase inhibitor. Prior to flavopiridol treatment, 53% of patients had at least one lymph node mass ≥ 10 cm, 52% had splenomegaly, and 66% had adverse cytogenetics with del(17p) or del(11q). Median pre-treatment laboratory characteristics included β2-microglobulin of 4.4 mg/L, absolute lymphocyte count (ALC) of 8.86×10^9/L, WBC of 13.95×10^9/L, and LDH of 199 U/L. Additional patient characteristics including baseline potassium, uric acid, and serum creatinine are provided in Table 2. Pre-treatment phosphate levels were not routinely obtained in all patients and were not included in the analysis.

Incidence of TLS

In 116 patients, the incidence of TLS was 46% (95% CI: 36-55%), with 14 of 53 patients (26%) with TLS requiring dialysis for management of hyperkalemia or hyperphosphatemia not responsive to medical therapy. TLS occurred with the first dose of flavopiridol or at the time of dose escalation. Those patients who required dialysis were typically removed from protocol therapy and completed fewer cycles of flavopiridol (median 1.3, range 0.25-5.0 cycles) compared to patients with TLS who did not require dialysis (median 3.5, range 0.25-6.0 cycles) or patients without TLS (median 3.75, range 0.25-6.0 cycles). Twenty-five (48%) and 28 (44%) patients had TLS on the phase I and phase II trials, respectively. The proportion of patients who required dialysis on the phase II trial was 6%, compared to 19% in the phase I study.

Risk factors associated with TLS

Baseline characteristics are described in Table 3 for patients who did not develop TLS, patients with TLS that did not require dialysis, and patients with TLS requiring dialysis. In univariable analysis using logistic regression (Table 3), patient characteristics highly associated with the occurrence of TLS were female gender ($p<0.001$), increased number of prior therapies ($p=0.001$), Rai stages III-IV ($p<0.001$), lymphadenopathy ≥ 10 cm ($p=0.002$), splenomegaly ($p=0.04$), del(11q) ($p=0.03$), decreased albumin ($p=0.04$), reduced creatinine

clearance (p<0.001), and increased pre-treatment WBC (p<0.001), ALC (p<0.001), β2-microglobulin (p<0.001) and LDH (p=0.003). Specifically, 72% of females compared to 34% of males developed TLS. Fourteen percent of patients treated with 2 or fewer therapies compared to 53% of patients treated with 3 or more prior therapies developed TLS. In addition, TLS was observed in 54% of patients with Rai stages III-IV, but only 12% of patients with Rai stages I-II and in 59% of patients with adenopathy ≥ 10 cm as opposed to 31% patients with adenopathy < 10 cm. Median pre-treatment WBC, β2-microglobulin, and LDH were all higher in patients with TLS. For example, TLS occurred in 100%, 75%, and 38% of patients with a WBC > 150 (n=8), 100-150 (n=12), and < 100×10^9/L (n=96), respectively. Rates of TLS were 74% and 19% in patients with β2-microglobulin levels above and below the median value of 4.4 mg/L, respectively.

In a multivariable analysis using limited backwards selection (Table 4), the following 5 variables remained significantly associated with the development of TLS: female gender (p<0.001), bulky adenopathy ≥ 10 cm (p=0.03), elevated WBC (p=0.01), increased β2-microglobulin (p<0.001), and decreased albumin (p=0.04). This model had excellent discrimination between patients with and without TLS with an area under the ROC curve=0.897. As only 3 of 24 patients with Rai stages I-II developed TLS, the small numbers of these patients precluded use of this variable in the multivariable analysis. When the multivariable analysis was restricted to patients with Rai stages III-IV (n=92), the same 5 variables remained significantly associated with TLS (data not shown). A second model incorporating creatinine clearance failed to provide as good of a fit to the observed data as the previous model (data not shown).

Association of TLS with patient response and survival

In 116 patients, the overall response rate (ORR) was 47%, with 1 complete response. Responses were observed in 44% of patients without TLS and 49% of patients with TLS (p=0.71). Within the TLS group, the ORR was 59% in patients who did not require dialysis and 21% in patients who underwent dialysis (p=0.03). Eighty-six patients have died, with 80 deaths due to disease progression and 6 deaths due to toxicity including infection (n=4), gastrointestinal hemorrhage (n=1) and TLS (n=1). Median PFS was 0.9 years (95% CI: 0.7-1.1) in patients without TLS, 0.8 years (95% CI: 0.4-0.9) in patients with TLS but without dialysis, and 0.4 years (95% CI: 0.1-1.4) in patients with TLS requiring dialysis (p=0.03, Figure 1). OS medians were 2.7 (95% CI: 2.1-3.2), 2.1 (95% CI: 1.1-3.6) and 0.9 years (95% CI: 0.1-1.4) in patients without TLS, with TLS but not requiring dialysis, and with TLS and dialysis, respectively (p<0.001, Figure 2).

In a multivariable model controlling for the number of prior treatments, cytogenetic risk group, Rai stage, age, and gender, response rates were not significantly different (p=0.38) among patients without TLS, with TLS not requiring dialysis, and with TLS requiring dialysis. OS was inferior in patients with TLS when controlling for other variables (p=0.03); however, this was secondary to increased deaths in the patients that required dialysis. When patients with TLS not requiring dialysis were evaluated, there was no significant difference in OS compared to patients without TLS (p=0.88, HR=1.0, 95% CI: 0.6-1.9). PFS was also significantly different among TLS groups when controlling for other variables (p=0.04).

However, risk of progression was not solely due to the higher rate of death in the patients requiring dialysis. The risk of progression for patients with TLS with and without dialysis, respectively, was 2.3 and 2.0 times higher than patients without TLS. Collectively, patients with TLS had a 2-fold higher rate of progression than patients without TLS (p=0.01, 95% CI: 1.2-3.6).

Flavopiridol pharmacokinetics and TLS

In a subset analysis of 83 patients with available pharmacokinetic data, we examined if peak levels (C_{max}) of flavopiridol and its glucoronide metabolite, flavo-G, were correlated with TLS. Peak levels of flavopiridol were not significantly associated with TLS (p=0.66). However, peak levels of flavo-G did correlate with TLS (p<0.001). This association with TLS was independent of patient gender, a potentially confounding variable. Even though higher levels of flavo-G were observed in females (n=29, median Cmax=3.2 μM, range: 0.9-23.8) compared to males (n=54, median Cmax=2.5 μM, range:0.2-9.2), higher levels of flavo-G were associated with a higher incidence of TLS within both sexes. Specifically when peak flavo-G levels were distributed into quartiles, the proportions of males with TLS in quartiles 1-4 were 27% (4/15), 27% (4/15), 27% (4/15), and 78% (7/9), respectively. Among women, the proportions of patients with TLS in quartiles 1-4 were 50% (3/6), 80% (4/5), 50% (3/6), and 100% (12/12), respectively. Therefore, the increased risk of TLS in women was still greater than men with similar flavopiridol pharmacokinetic profiles.

DISCUSSION

Traditional consensus recommendations for the management and prophylaxis for TLS call for identification of patients with high risk features based upon pre-treatment uric acid or LDH levels, renal impairment, bulky disease, and the disease histology; aggressive pre-treatment hydration with allopurinol in low risk patients and rasburicase in high risk patients; and monitoring of potassium, phosphate, calcium, uric acid, LDH, and creatinine every 6-12 hours.(10-12) However, these recommendations do not adequately address TLS induced by flavopiridol in patients with CLL, traditionally considered low risk for TLS, which begins as early as 4.5 hours and peaks 12 hours from the start of the flavopiridol infusion.(11, 12) Despite the significant efficacy of flavopiridol in patients with relapsed or refractory CLL, the unpredictable occurrence of TLS with administration of flavopiridol, the aggressive hourly potassium monitoring, and the requirement for emergently available dialysis has limited the use of this agent outside of clinical trials and academic institutions. Therefore, we conducted a retrospective analysis to evaluate pre-treatment patient characteristics associated with the development of TLS in patients receiving single agent flavopiridol that should assist with the future development of this agent.

In two recently completed trials with flavopiridol for patients with previously treated CLL, the incidence of TLS was 46% (95% CI: 36-55%). Risk factors significantly associated with TLS by multivariable analysis were female gender, increased WBC, bulky adenopathy ≥ 10 cm, elevated β2-microglobulin, and decreased albumin. The association of TLS with WBC, β2-microglobulin, and adenopathy ≥ 10 cm is likely reflective of increased disease burden, while the association with albumin may be related to the protein binding of flavopiridol.

Greater protein-binding in the setting of higher albumin likely decreases available flavopiridol to malignant tissues, reducing the risk of TLS. The association with female gender was surprising and could not be solely explained by reduced creatinine clearance or elevated levels of flavopiridol or its metabolite, flavo-G, in women compared to men.

Unexpectedly, the occurrence of TLS and its severity did not correlate with higher response rates (p=0.38) in a multivariable model controlling for other variables. TLS was associated with inferior OS (p=0.03) and PFS (p=0.04). With respect to OS, the inferior outcome was largely due to poor outcomes in patients requiring dialysis. In contrast, risk of progression was approximately 2-fold higher in patients with TLS, regardless of severity, compared to patients without TLS. Therefore, although presumably the occurrence of TLS results from rapid tumor break-down and exquisite sensitivity of CLL cells to flavopiridol, patients without TLS have meaningful, durable responses with flavopiridol.

With other therapies typically utilized in CLL including fludarabine, fludarabine and cyclophosphamide, rituximab, pentostatin, or 2-chlorodeoxyadenosine,(13-17) the incidence of TLS is less than 5%. TLS typically occurs several days after the administration of nucleoside analogues or within 12 hours following rituximab. In the largest analysis of TLS in patients with CLL, TLS occurred in 20 (0.33%) of 6,137 patients receiving single agent fludarabine, within 7 days (range 5-14 days) after administration.(15) This incidence is strikingly low compared to the 46% incidence observed with flavopiridol and contrasts with the rapid onset of TLS with flavopiridol. In the analysis of 6,137 patients, risk factors for TLS after fludarabine included fewer prior therapies (p=0.004) and hepatosplenomegaly (p=0.05).(15) The median WBC pre-treatment was 108.5×10^9/L in patients with TLS compared to 83.6×10^9/L in patients without TLS, although this was not statistically significant. Similar to our data, the occurrence of TLS was associated with significant morbidity with 40% of patients with TLS dying during the first cycle of fludarabine.(15)

Our retrospective analysis was limited by the lack of a standardized definition and grading system for TLS. Historically, TLS has been defined as “a group of metabolic abnormalities that result from the rapid release of intracellular metabolites such as nucleic acids, proteins, phosphorus, and potassium from lysed malignant cells.”(11) A more specific definition includes the separation of TLS into laboratory TLS defined as “abnormalities of two or more of the following serum values: uric acid, potassium, phosphorus, or calcium” or clinical TLS defined as “the presence of laboratory TLS and one of the following: renal failure, cardiac arrhythmia, or seizure.”(10, 11) For the purposes of this analysis we utilized conservative criteria for TLS, defined as a rise above the ULN of uric acid, potassium, phosphate, and/or doubling of the lactate dehydrogenase (LDH) within 24 hours of flavopiridol administration, in order to capture all patients experiencing TLS. Aggressive pre-treatment prophylaxis incorporated into the phase I and II flavopiridol trials with kayexalate, oral phosphate binders, and rasburicase, frequently mitigated rises in potassium, phosphate, or uric acid confounding the diagnosis of TLS. This study utilized NCI CTCAE version 3.0 to assess the severity of TLS. According to NCI CTCAE version 3.0, TLS is grade 3 if it is present and grade 5 if associated with death, with no recognition of grades 1, 2, or 4 TLS. Therefore, in this retrospective study, separation of patients according to the severity of TLS was challenging. While some patients primarily had elevations in LDH, other patients

experienced life-threatening hyperkalemia or hyperphosphatemia. Therefore, in order to characterize the severity of TLS, TLS was classified into TLS requiring dialysis and TLS without dialysis, as this was clinically relevant. NCI CTCAE v. 4.0 does recognize grade 3, 4, and 5 TLS with grade 4 defined as TLS with life-threatening consequences which should make future evaluation of TLS more accurate and reproducible among institutions at least with respect to the grade 4 category. Grade 3 TLS still remains poorly defined. In the future, strict definitions and grading of TLS should be utilized in prospective evaluations of TLS with flavopiridol in order to standardize diagnosis and management of this complication, which will ultimately improve tolerability and facilitate generalized use of this agent.

Fortunately, the acute occurrence of TLS within 24 hours of initial flavopiridol administration not only allows for close monitoring for this toxicity but also permits development of strategies to minimize TLS with flavopiridol. Such strategies include use of flavopiridol as initial therapy when patients may have a limited disease burden, lower β2-microglobulin, or normal albumin. In addition, flavopiridol after initial debulking chemotherapy or as maintenance therapy may minimize the risk of TLS and these strategies are currently under evaluation at OSU. The development and validation of predictive models for TLS that incorporate pre-treatment clinical risk factors, *in vivo* sensitivity testing of patient derived-CLL cells, or pharmacokinetic modeling may help identify patients at greatest risk for TLS and are also ongoing. Patients at risk for TLS by clinical, *in vivo*, or pharmacokinetic modeling may benefit from either reduced flavopiridol doses or stepped-up administration schedules. While these flavopiridol dosing strategies are under development, we recommend that further studies of flavopiridol be limited to patients with CLL with pre-treatment WBC < 100×10^9/L and adenopathy < 10 cm. In addition, close monitoring of female patients, patients with an elevated β2-microglobulin, and patients with a low serum albumin is recommended. Such recommendations should also be incorporated into studies of other emerging novel cyclin dependent kinase inhibitors(18-20) in patients with CLL in order to minimize the risks of acute TLS until prospective, therapy specific data are available.

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REFERENCES

1. Kitada S, Zapata JM, Andreeff M, Reed JC. Protein kinase inhibitors flavopiridol and 7-hydroxy-staurosporine down-regulate antiapoptosis proteins in B-cell chronic lymphocytic leukemia. Blood. Jul 15; 2000 96(2):393–397. [PubMed: 10887097]
2. Byrd JC, Shinn C, Waselenko JK, Fuchs EJ, Lehman TA, Nguyen PL, et al. Flavopiridol induces apoptosis in chronic lymphocytic leukemia cells via activation of caspase-3 without evidence of bcl-2 modulation or dependence on functional p53. Blood. Nov 15; 1998 92(10):3804–3816. [PubMed: 9808574]
3. Flinn IW, Byrd JC, Bartlett N, Kipps T, Gribben J, Thomas D, et al. Flavopiridol administered as a 24-hour continuous infusion in chronic lymphocytic leukemia lacks clinical activity. Leuk Res. Nov; 2005 29(11):1253–1257. [PubMed: 15916806]
4. Byrd JC, Peterson BL, Gabrilove J, Odenike OM, Grever MR, Rai K, et al. Treatment of relapsed chronic lymphocytic leukemia by 72-hour continuous infusion or 1-hour bolus infusion of

flavopiridol: results from Cancer and Leukemia Group B study 19805. Clin Cancer Res. Jun 1; 2005 11(11):4176–4181. [PubMed: 15930354]

5. Lin TS, Ruppert AS, Johnson AJ, Fischer B, Heerema NA, Andritsos LA, et al. Phase II Study of Flavopiridol in Relapsed Chronic Lymphocytic Leukemia Demonstrating High Response Rates in Genetically High-Risk Disease. J Clin Oncol. Dec 10; 2009 27(35):6012–6018. 2009. [PubMed: 19826119]
6. Byrd JC, Lin TS, Dalton JT, Wu D, Phelps MA, Fischer B, et al. Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. Blood. Jan 15; 2007 109(2):399–404. 2007. [PubMed: 17003373]
7. Phelps MA, Lin TS, Johnson AJ, Hurh E, Rozewski DM, Farley KL, et al. Clinical response and pharmacokinetics from a phase 1 study of an active dosing schedule of flavopiridol in relapsed chronic lymphocytic leukemia. Blood. Mar 19; 2009 113(12):2637–2645. 2009. [PubMed: 18981292]
8. Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood. Jun 15; 1996 87(12):4990–4997. [PubMed: 8652811]
9. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. Dec 28; 2000 343(26):1910–1916. [PubMed: 11136261]
10. Tosi P, Barosi G, Lazzaro C, Liso V, Marchetti M, Morra E, et al. Consensus conference on the management of tumor lysis syndrome. Haematologica. Dec 1; 2008 93(12):1877–1885. 2008. [PubMed: 18838473]
11. Cairo MS, Coiffier B, Reiter A, Younes A. Recommendations for the evaluation of risk and prophylaxis of tumour lysis syndrome (TLS) in adults and children with malignant diseases: an expert TLS panel consensus. Br J Haematol. May; 149(4):578–586. [PubMed: 20331465]
12. Coiffier B, Altman A, Pui C-H, Younes A, Cairo MS. Guidelines for the Management of Pediatric and Adult Tumor Lysis Syndrome: An Evidence-Based Review. J Clin Oncol. Jun 1; 2008 26(16): 2767–2778. 2008. [PubMed: 18509186]
13. Byrd JC, Waselenko JK, Maneatis TJ, Murphy T, Ward FT, Monahan BP, et al. Rituximab Therapy in Hematologic Malignancy Patients With Circulating Blood Tumor Cells: Association With Increased Infusion-Related Side Effects and Rapid Blood Tumor Clearance. Journal of Clinical Oncology. Mar 1.1999 17(3):791. 1999. [PubMed: 10071268]
14. Dann EJ, Gillis S, Polliack A, Okon E, Rund D, Rachmilewitz EA. Tumor Lysis Syndrome Following Treatment with 2-Chlorodeoxyadenosine for Refractory Chronic Lymphocytic Leukemia. New England Journal of Medicine. 1993; 329(21):1547–1548. [PubMed: 8105383]
15. Cheson BD, Frame JN, Vena D, Quashu N, Sorensen JM. Tumor lysis syndrome: an uncommon complication of fludarabine therapy of chronic lymphocytic leukemia. J Clin Oncol. Jul 1; 1998 16(7):2313–2320. 1998. [PubMed: 9667245]
16. O'Brien S, Moore JO, Boyd TE, Larratt LM, Skotnicki A, Koziner B, et al. Randomized Phase III Trial of Fludarabine Plus Cyclophosphamide With or Without Oblimersen Sodium (Bcl-2 antisense) in Patients With Relapsed or Refractory Chronic Lymphocytic Leukemia. J Clin Oncol. Mar 20; 2007 25(9):1114–1120. 2007. [PubMed: 17296974]
17. Marotta G, Bigazzi C, Lenoci M, Tozzi M, Bocchia M, Lauria F. Low-dose fludarabine and cyclophosphamide in elderly patients with B-cell chronic lymphocytic leukemia refractory to conventional therapy. Haematologica. Dec 1; 2000 85(12):1268–1270. 2000. [PubMed: 11114133]
18. Tong W-G, Chen R, Plunkett W, Siegel D, Sinha R, Harvey RD, et al. Phase I and Pharmacologic Study of SNS-032, a Potentand Selective Cdk2, 7, and 9 Inhibitor, in Patients With Advanced Chronic Lymphocytic Leukemia andMultiple Myeloma. J Clin Oncol. May 17.2010 JCO. 2009.2026.1347.
19. Flynn, JM.; Johnson, AJ.; Andritsos, L.; Blum, KA.; Jones, JA.; Wiley, EA., et al. The Cyclin Dependent Kinase Inhibitor SCH 727965 Demonstrates Promising Pre-Clinical and Early Clinical Activity in Chronic Lymphocytic Leukemia; ASH Annual Meeting Abstracts; 2009 November 20; 2009. p. 886abstr

20. Lock, V.; Cooke, L.; Yule, M.; Thompson, NT.; Croce, KD.; Lyons, JF., et al. AT7519, a Potent Multi-Targeted CDK Inhibitor, Is Active in CLL Patient Samples Independent of Stage; ASH Annual Meeting Abstracts; 2008 November 16; 2008. p. 3161abstr

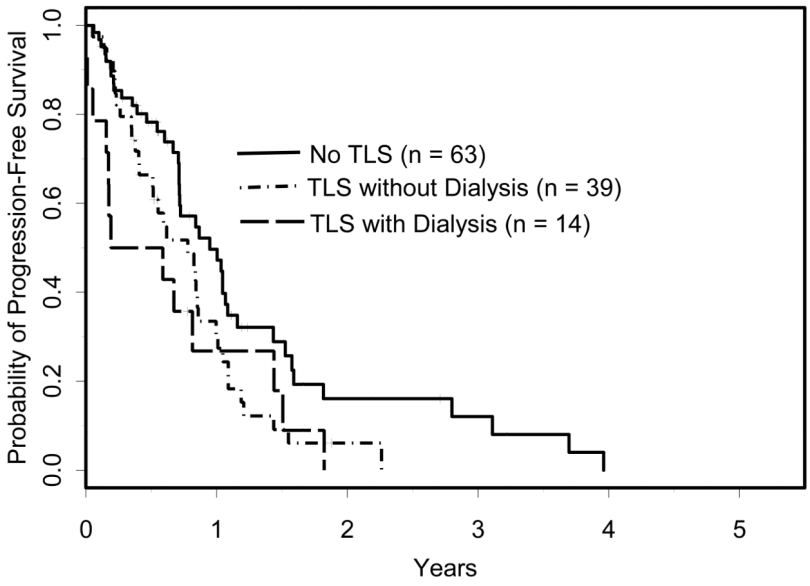

Figure 1.

PFS of CLL patients with respect to occurrence of TLS after treatment with flavopiridol. (Solid line: patients without TLS; dotted line: patients with TLS who did not require dialysis; dashed line: patients with TLS who required dialysis).

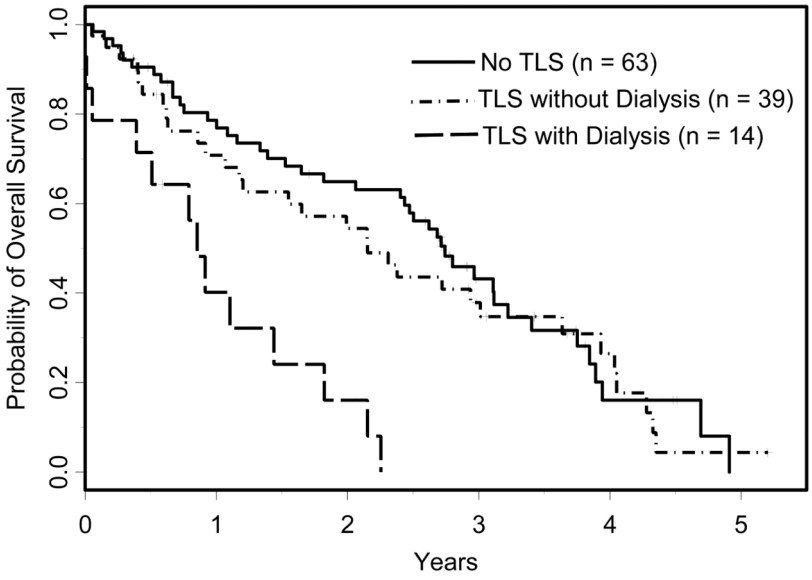

Figure 2.

OS of CLL patients with respect to occurrence of TLS after treatment with flavopiridol. (Solid line: patients without TLS; dotted line: patients with TLS who did not require dialysis; dashed line: patients with TLS who required dialysis).

Table 1

Dose Escalation of flavopiridol on OSU 0055 and OSU 0491

Study	Cohort	IVB (mg/m^2)	CIVI (mg/m^2)	Weeks	Number of patients (n=116)
OSU 0055					
	1	30	30	Weeks 1-4, cycles 1-6	20
	2	40	40	Weeks 1-4, cycles 1-6	3
	3	30 30	30 50	Weeks 1-4, cycle 1 Weeks 1-4, cycles 2-6	17
	4	30 30	30 50	Week 1, cycle 1 Weeks 2-4, cycle 1 and Weeks 1-4, cycles 2-6	12
OSU 0491					
	1	30 30	30 50	Week 1, cycle 1 Weeks 2-4, cycle 1 and Weeks 1-4, cycles 2-6	32*
	2 (amended)	30 30	30 50	Week 1, cycle 1 Weeks 2-3, cycle 1 and Weeks 1-3, cycles 2-6	32

Abbreviations: IVB: intravenous bolus over 30 minutes, CIVI: continuous IV infusion over 4 hours.

*Cohort 1 on OSU 0491 includes 5 patients who transitioned to the amended dose level (cohort 2) after the first cycle of flavopiridol.(5)

Table 2

Patient characteristics

Characteristic	All Patients, N=116 (%)
Age, years	
Median (range)	60 (31-84)
Gender	
Male	80 (69)
Female	36 (31)
Race	
White	109 (94)
Black	7 (6)
Prior therapies	
Median (range)	4 (1-14)
Previous nucleoside analogue therapy (fludarabine or pentostatin)	115 (99)
Nucleoside analogue refractory (fludarabine or pentostatin)	88 (76)
Rai Stage	
0	0 (0)
1	19 (16)
2	5 (4)
3	29 (25)
4	63 (54)
β_2-microglobulin, mg/L*	
Median (range)	4.4 (0.8-14.9)
Bulky lymphadenopathy	
≥ 5 cm	87 (75)
≥ 10 cm	61 (53)
Splenomegaly	60 (52)
WBC, × 10^9/L	
Median (range)	13.95 (1.3-314.5)
ALC, × 10^9/L	
Median (range)	8.86 (0.17-266.31)
LDH, U/L*	
Median (range)	199 (102-654)
Albumin, g/dL	
Median (range)	3.4 (2.0-4.7)
Potassium, mmol/L	
Median (range)	3.8 (2.5-4.8)
Creatinine, mg/dL	
Median (range)	1.02 (0.50-1.79)
Creatinine clearance, cc/min Median (range)	74 (31-196)
Uric acid, mg/dL	
Median (range)	5.1 (0.7-10.0)
Cytogenetics (presence of any of the following abnormalities)	
Del (13q)	57 (49)
Normal	15 (13)
Tri (12)	18 (16)
Del(11q)	49 (42)
Del(17p)	40 (34)
Not available	4 (3)
Cytogenetics utilizing Dohner's	11 (9)

Characteristic	All Patients, N=116 (%)
prioritization(9)	
Del (13q)	
Normal	15 (13)
Tri (12)	9 (8)
Del (11q)	37 (32)
Del(17p)	40 (34)
Not available	4 (3)

Abbreviations: WBC: white blood cell count, ALC: absolute lymphocyte count, LDH: lactate dehydrogenase.

* Upper limit of normal β_2-microglobulin is 2.5 mg/L, Upper limit of normal LDH is 190 U/L.

Table 3

Patient characteristics in patients with and without TLS

Characteristic	Patients without TLS (n = 63)	Patients with TLS Without Dialysis (n = 39)	Patients with TLS With Dialysis (n = 14)	P-value**
Study, no. (%) OSU0055 (phase I), n=52 OSU0491 (phase II), n=64	 27 (52) 36 (56)	 15 (29) 24 (38)	 10 (19) 4 (6)	0.64
Gender, no. (%) Male, n=80 Female, n=36	 53 (66) 10 (28)	 17 (21) 22 (61)	 10 (13) 4 (11)	<0.001
Age, years Median (range)	 58 (36-77)	 63 (39-82)	 60 (31-84)	0.15
Number of prior therapies Median (range)	 4 (1-10)	 5 (1-14)	 6 (3-10)	0.001
Rai Stage, no. (%) I/II, n=24 III/IV, n=92	 21 (88) 42 (46)	 2 (8) 37 (40)	 1 (4) 13 (14)	<0.001
Bulky lymphadenopathy ≥ 10 cm, no. (%) No, n=55 Yes, n=61	 38 (69) 25 (41)	 13 (24) 26 (43)	 4 (7) 10 (16)	0.002
Enlarged Spleen, no. (%) No, n=56 Yes, n=60	 36 (64) 27 (45)	 15 (27) 24 (40)	 5 (9) 9 (15)	0.04
del(17p), no. (%) No, n=72 Yes, n=40	 37 (51) 26 (65)	 24 (33) 12 (30)	 11 (15) 2 (5)	0.16
del(11q), no. (%) No, n=63 Yes, n=49	 41 (65) 22 (45)	 14 (22) 22 (45)	 8 (13) 5 (10)	0.03
WBC, × 10^9/L Median (range)	 8.3 (2.5-146.5)	 25.1 (1.3-252.1)	 94.2 (1.9-314.5)	<0.001
ALC, × 10^9/L Median (range)	 4.8 (0.2-145.0)	 20.9 (0.7-248.3)	 89.0 (0.5-266.3)	<0.001
Beta-2 Microglobulin, mg/L Median (range)	 3.1 (1.3-9.0)	 5.0 (0.8-14.9)	 8.3 (2.7-12.6)	<0.001
LDH, U/L* Median (range)	 169 (112-654)	 224 (102-506)	 277 (148-545)	0.003
Albumin, mg/dL Median (range)	 3.4 (2.5-4.3)	 3.1 (2.0-4.7)	 3.5 (2.4-3.7)	0.04
Potassium, mmol/L Median (range)	 3.8 (3.0-4.7)	 3.8 (2.5-4.7)	 3.7 (3.2-4.8)	0.72
Creatinine, mg/dL Median (range)	 0.95 (0.65-1.79)	 1.04 (0.50-1.74)	 0.97 (0.79-1.60)	0.61
Creatinine clearance (cc/min)	 82 (38-196)	 62 (31-117)	 61 (37-166)	<0.001
Uric Acid, mg/dL Median (range)	 4.9 (1.9-9.0)	 5.1 (0.7-10.0)	 6.0 (2.9-7.9)	0.80

Abbreviations: TLS: tumorlysis syndrome, WBC: white blood cell count, ALC: absolute lymphocyte count, LDH: lactate dehydrogenase

* Upper limit of normal β2-microglobulin is 2.5 mg/L, Upper limit of normal LDH is 190 U/L.

** P-value is determined by logistic regression analysis where TLS (Yes vs. No) was the dependent variable.

Table 4

Risk Factors for TLS in multivariable analysis

Risk Factor	Odds Ratio*	95% CI	P value
Female vs. Male	8.6	2.6-27.7	<0.001
Bulky lymphadenopathy ≥ 10 cm	3.5	1.1-10.8	0.03
WBC, 50 unit increase	2.0	1.2-3.5	0.01
β2-microglobulin, 1 unit increase	1.6	1.2-2.0	<0.001
Albumin, 1 unit increase	0.3	0.1-0.9	0.04

*An odds ratio >1 (< 1) indicates a greater (lesser) odds of TLS for either the first category listed for dichotomous variables or higher values of continuous variables