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ORIGINAL ARTICLE

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Analysis of age, estimated creatinine clearance and pretreatment hematologic parameters as predictors of fludarabine toxicity in patients treated for chronic lymphocytic leukemia: a CALGB (9011) coordinated intergroup study

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Abstract *Purpose:* Fludarabine is a renally excreted agent that is an effective treatment for chronic lymphocytic leukemia (CLL), a disease predominantly of the elderly. We sought to determine whether age, renal function or pretreatment hematologic status predicted toxicity of fludarabine treatment for CLL. *Methods:* We evaluated 192 patients with previously untreated B-cell CLL who were entered onto the fludarabine treatment arm (25 mg/m^2 daily for 5 days every 28 days) of CALGB study 9011, an intergroup study with participation from SWOG, CTG/NCI-C and ECOG. Patients were required to have serum creatinine within 1.5 times normal. Hematologic indices and infections were recorded during the first 28-day cycle of treatment. A time-to-toxicity endpoint was evaluated over the entire course of fludarabine treatment. Creatinine clearance ($CrCl_{est}$) was estimated using serum creatinine, age and body mass index. *Results:* The median age was 64 years (range 37–87 years) and median $CrCl_{est}$ was 62 ml/min (range 27–162 ml/min, interquartile range 52–79 ml/min). We found no association between age and incidence of hematologic toxicity or infection during the first cycle of treatment. There was a strong association between $CrCl_{est}$ and the time-to-toxicity endpoint. Patients with $CrCl_{est}$ below 80 ml/min had increased incidence of toxicity during their treatment course ($P < 0.0001$). Pretreatment anemia, thrombocytopenia and Rai stage were highly associated with the incidence of neutrophil toxicity and grade III/IV hematologic toxicities during the first cycle of treatment ($P < 0.0001$).

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Conclusions: Patient age was not an independent risk factor for fludarabine-related toxicity, but $CrCl_{est}$ was associated with time to toxicity.

Keywords Chronic lymphocytic leukemia · Fludarabine · Aging · Kidney function tests · Drug toxicity

Introduction

Chronic lymphocytic leukemia (CLL) and other hematologic malignancies increase in incidence with advancing patient age. It has been demonstrated that older patients are often not entered onto clinical trials and outside trials they are often under-treated [8, 55, 59]. This may be partially due to concerns of increased toxicity in this group. Only a few studies have focused on the pharmacokinetics of cancer chemotherapy in older subjects [3, 26, 32]. One area of concern is renal function, which is known to decline with age [6, 18, 35, 42]. Several studies have found that in older patients, toxicity of renally cleared chemotherapeutic agents is related to renal function [9, 17, 19]. The issue of renally cleared drugs in older patients is further complicated by the observation that serum creatinine is a poor reflection of renal function because of decreased creatinine production with age. An estimation of renal function using the Cockcroft-Gault equation may be a more accurate reflection of renal function than serum creatinine alone, and was evaluated in this study [11].

Fludarabine is a purine analog that has clinical activity in a variety of hematologic malignancies, including CLL, non-Hodgkin's lymphoma (NHL) and acute myeloid leukemia [2, 10, 12, 14, 24, 30, 39, 47, 48, 50]. Several prospective randomized clinical trials have validated fludarabine as second-line or even first-line therapy for CLL [28, 45]. Fludarabine as a single agent has been shown to result in a superior response rate and progression-free survival than chlorambucil in the treatment of CLL, but toxicity is more frequently observed [41, 45]. Variations in renal function have been correlated with the pharmacokinetic disposition of fludarabine [27, 33, 38].

Fludarabine is rapidly dephosphorylated from the parent drug, 2F-ara-AMP, to the active metabolite, 2-F-ara-A, following intravenous administration. The total body clearance of 2-F-ara-A has been found to be correlated with creatinine clearance, and the mean 24-h urinary excretion of 2-F-ara-A is approximately 60%. Furthermore, the area under the concentration vs time curve (AUC) for 2-F-ara-A is correlated with neutropenia. Since renal function declines with age, we were concerned that older patients or patients with poor renal function would experience a disproportionately high rate of toxicity. Finally, it has been proposed that diminished bone marrow reserve, for example in advanced stages of a hematologic malignancy, may result in susceptibility to treatment toxicities. In the present evaluation, we assessed the impact of age, renal function and pretreatment hematologic parameters on toxicity following initiation of fludarabine treatment for CLL.

Materials and methods

The present study was based on a subset of patients enrolled on a phase III intergroup study coordinated by the Cancer and Leukemia Group B (CALGB 9011), with participation by the Southwest Oncology Group (SWOG), Clinical Trials Group of the National Cancer Institute of Canada (CTG/NCI-C) and the Eastern Cooperative Oncology Group (ECOG). The title of this study was: A phase III comparison of fludarabine phosphate (NSC #312887) vs. chlorambucil vs. fludarabine phosphate + chlorambucil in previously untreated B-cell chronic lymphocytic leukemia [45]. Between October 1990 and December 1994, 544 patients with previously untreated CLL were entered on the study, and 192 were randomized to receive single-agent fludarabine. For the present analysis, only patients randomized to the single-agent fludarabine arm were evaluated.

Patients with intermediate-risk (Rai stages I-II) and high-risk (Rai stages III-IV) CLL were included. Diagnosis of CLL was based on persistent lymphocytosis $>5000/\mu l$ with appropriate immunophenotype ($CD5^+$ along with surface immunoglobulin$^+$, $CD19^+$, $CD20^+$, or $CD24^+$; presence of either kappa or lambda light chain, and low expression of surface immunoglobulin) and bone marrow biopsy showing >30% lymphocytes. The Rai stages were defined according to the following criteria: I – enlarged lymph nodes; II – splenomegaly or hepatomegaly; III – anemia, hemoglobin less than 11 g/dl; and IV – thrombocytopenia, platelets less than 100,000/μl. Other eligibility criteria included CALGB performance status of 0-2, negative direct Coombs test, bilirubin ≤ 2.0 mg/dl, SGOT/SGPT not more than 1.5 times the upper limit of normal, creatinine not more than 1.5 times the upper limit of normal, and BUN not more than 1.5 times the upper limit of normal. The normal range for these laboratory tests was defined independently by each institution, and usually represents two standard deviations from the mean for a test population from that institution.

Patients on the fludarabine arm of this study received fludarabine ($25\ mg/m^2$) as an intravenous injection daily for 5 days every 28 days. The institutional review boards of the respective institutions approved the protocol, and all patients were required to provide written informed consent.

For the present analysis, the first cycle of treatment, which included the 5 days of fludarabine administration followed by the remainder of the 28-day cycle, was thoroughly evaluated. Blood was drawn for a complete blood count on day 14 of the cycle and when otherwise clinically indicated. For hematologic toxicities, the nadir value during the 28-day cycle was used to determine the grade of toxicity. Platelet and hemoglobin toxicities were determined based on the percentage change from the on-study value: grade 1, 11–24% decrease; grade 2, 25–49% decrease; grade 3, 50–74% decrease; and grade 4, ≥75% decrease. Neutrophil toxicity was based on the actual neutrophil count: grade 1, 1500–2000/μl; grade 2, 1000–1500/μl; grade 3, 500–1000/μl, and grade 4, $<500/\mu l$. Dose modification in the second cycle of treatment was based on treatment-related toxicity during the first cycle of treatment. Patients with grade 1, 2, 3 or 4 toxicity would receive 75%, 50%, 25% or 0% of full dose, respectively. If the toxicity category for the nadir neutrophil count was equal to or less than that for the on-study value, then no treatment-related neutrophil toxicity was recorded in the present analysis. Case reports and data flow sheets for each patient were reviewed to determine the presence or absence of infectious complications, including bacterial, fungal, viral, parasitic and undefined infections during the first 28-day cycle of fludarabine treatment.

Treatment-related toxicity was evaluated beyond the first cycle by determining a time-to-toxicity endpoint. The time-to-toxicity endpoint was reached when a patient had a dose reduction,

experienced a toxicity-related dose delay, or went off fludarabine treatment for a toxicity-related reason. For patients experiencing such an event, the value recorded for time to toxicity was the first cycle number of fludarabine that was reduced, delayed or missed during the study due to fludarabine-related toxicity. For patients not experiencing one of these events, time to toxicity was recorded as the last cycle number the patient received fludarabine.

Creatinine clearance was estimated ($CrCl_{est}$) using the Cockcroft-Gault equation $CrCl_{est} = (140 - \text{age in years}) \times LBW_{kg} / (72 \times Cr_{mg/dl})$ [11], where LBW is lean body weight and Cr is serum creatinine. A correction factor of 0.85 was used for females. LBW for males was 50 kg + 2.3 kg for each inch over 5 feet in height, and for females was 45.5 kg + 2.3 kg for each inch over 5 feet in height. Urine creatinine was not a variable in this extrapolation.

Statistical methods

Six dichotomous outcomes were selected for analysis. The outcomes were measured in the first cycle only, since dose reductions could have occurred during subsequent cycles, confounding the association of baseline covariates with these outcomes. The outcomes included: (1) presence of infection of any grade, (2) presence of hemoglobin toxicity of any grade, (3) presence of platelet toxicity of any grade, (4) presence of neutrophil toxicity of any grade, (5) presence of any hematologic toxicity of any grade, and (6) presence of hematologic toxicity of grade 3 or greater. The associations of these variables with baseline age, $CrCl_{est}$, hematologic parameters and Rai stage were evaluated with the likelihood ratio test of logistic regression models. Age and $CrCl_{est}$ were tested both as continuous variables and as dichotomized variables. Age was dichotomized a priori at age 70 years or greater versus age less than 70 years. $CrCl_{est}$ was dichotomized a priori at 50 ml/min or greater versus less than 50 ml/min, and at 70 ml/min or greater versus less than 70 ml/min. These cut-points were chosen since pharmacokinetic studies have demonstrated that fludarabine clearance is significantly reduced in patients with a creatinine clearance less than 70 ml/min [38]. A creatinine clearance of less than 50 ml/min has been used by others as a factor in making dosing decisions for renally excreted agents [5]. Age was not controlled for in the analyses involving $CrCl_{est}$ since an age correction was included in the calculation of $CrCl_{est}$.

Analysis of the time-to-toxicity endpoint required the assumption that all non-toxicity-related reasons for going off fludarabine occurred independently of the toxicity-related reasons and thus did not pose a competing risk. The likelihood ratio test of the proportional hazards model was used to test the association of time to toxicity with $CrCl_{est}$, age, and Rai stage. The method of Kaplan-Meier was used to plot time to toxicity curves by quartile of $CrCl_{est}$ [29].

All tests were performed under a two-sided alpha level of 0.05 and are presented as "statistically significant" if their P values are < 0.05. However, it should be kept in mind that the problem of multiplicities inherent in these analyses has created a true alpha level that is indeterminate but obviously greater than 0.05.

Results

Patient characteristics

Table 1 shows the baseline characteristics of the entire cohort of 192 patients from the CALGB 9011 study who were randomized to receive single-agent fludarabine treatment for CLL. The median age of this population was 64 years (range 37–87 years), and 50 patients (26%) were at least 70 years old. Patients presented with a range of $CrCl_{est}$ from 27 to 162 ml/min, with the 25th percentile at 52 ml/min and the 75th percentile at 79 ml/min. We verified that the distribution of $CrCl_{est}$ calculated from this population showed an inverse correlation with age (–0.63, $P < 0.0001$). No association was found between age and Rai stage ($P = 0.37$). There was, however, an association between age and performance status, with older patients having poorer performance status ($P = 0.006$). The mean age of patients with a performance status of 0 was 60 years, while the mean age of patients with a performance status of greater than 0 was 65 years.

Table 1. Baseline patient characteristics ($n = 192$)

Age (years)	
Median	64
25th percentile	54
75th percentile	70
Range	37–87
Women (%)	30
Rai stage I or II (%)	61
Rai stage III or IV (%)	39
Serum creatinine (mg/dl)	
Median	1.1
25th percentile	0.9
75th percentile	1.3
Range	0.6–2.2
$CrCl_{est}$ (ml/min)	
Median	62
25th percentile	52
75th percentile	79
Range	27–162
Hemoglobin, median (g/dl)	12.2
Platelets, median (/μl)	154,000
Neutrophils, median (/μl)	4,400
Lymphocytes (/μl)	68,200

Analysis of age as a predictor of fludarabine-related toxicity

Older patients were similar to younger patients with respect to pretreatment hematologic parameters and disease stage. We found no association of age with hemoglobin, platelet, or neutrophil toxicity in cycle 1 when age was dichotomized at 70 years (Table 2). In fact, an inverse relationship between age and incidence of any hematologic toxicity was observed when age was used as a continuous variable ($P = 0.04$). CLL patients undergoing fludarabine therapy frequently experienced infectious complications. However, we found no correlation between age and number of infectious complications ($P = 0.51$). Finally, there was no association between age and the time-to-toxicity endpoint ($P = 0.19$); the hazard ratio for a 10-year increase in age was 1.12 (95% confidence interval 0.94–1.33).

Analysis of estimated creatinine clearance as a predictor of fludarabine-related toxicity

Since variations in renal function have been correlated with the pharmacokinetic characteristics of fludarabine,

Table 2. Proportion of patients experiencing selected toxicity during the first treatment cycle, by age group. Data are presented as the number of patients experiencing toxicity/number of evaluable patients in the subgroup. This ratio is also shown as a percentage in parentheses. All grades of toxicity are included unless otherwise specified. Hematologic toxicity includes hemoglobin, neutrophil and platelet toxicity. *P* values are from the likelihood ratio test of a logistic regression model

Toxicity	All patients	Age < 70 years	Age ≥ 70 years	*P* value (age dichotomized)	*P* value (age continuous)
Any hematologic	150/189 (79)	114/139 (82)	36/50 (72)	0.13	0.04[a]
Grade 3/4 hematologic	37/189 (20)	28/139 (20)	9/50 (18)	0.74	0.31
Platelet	105/187 (56)	80/137 (58)	25/50 (50)	0.31	0.13
Hemoglobin	77/186 (41)	56/136 (41)	21/50 (42)	0.92	0.11
Neutrophil	65/176 (37)	49/131 (37)	16/45 (36)	0.82	0.85
Infection	47/185 (25)	35/137 (26)	12/48 (25)	0.94	0.51

[a]Less hematologic toxicity in older patients

we hypothesized that renal function would predict fludarabine toxicity. A surrogate index of creatinine clearance is the serum creatinine level. Serum creatinine ranged from 0.6 to 2.2 mg/dl in the patients entered onto the study. We found no statistically significant relationship between serum creatinine and any of the toxicity endpoints during the first cycle of treatment, except for a modest association with presence of any hemoglobin toxicity ($P = 0.04$); the *P* values for all other endpoints were > 0.30. Serum creatinine was associated with time-to-toxicity endpoint ($P = 0.02$), but no distinct cut-points were detected. Serum creatinine level has limitations as a renal function marker because variables such as muscle mass, level of activity and age can affect serum creatinine independently of renal function.

A more widely accepted surrogate marker for renal function is creatinine clearance estimated from a patient's ideal body mass, age and serum creatinine [11, 37]. A wide distribution of $CrCl_{est}$ values was found over the entire study population, ranging from 27 to 162 ml/min, with a median value of 62 ml/min (25th percentile at 52 ml/min, and 75th percentile at 79 ml/min). Again, no significant associations during the first cycle of treatment between calculated creatinine clearance and platelet toxicity ($P = 0.58$) or neutrophil toxicity ($P = 0.80$) were found when the data were analyzed by logistic regression. $CrCl_{est}$ was significantly associated (inversely) only with hemoglobin toxicity ($P = 0.01$). From the logistic model regressing hemoglobin toxicity on $CrCl_{est}$, the predicted probabilities of suffering a hemoglobin toxicity during the first cycle of treatment for patients with a $CrCl_{est}$ of 50, 70, and 100 ml/min were calculated as 48%, 39%, and 27%, respectively.

In addition to evaluating $CrCl_{est}$ as a continuous variable, we also tested two dichotomies of $CrCl_{est}$ selected prior to data analysis for their possible clinical utility ($CrCl_{est}$ < 50 ml/min vs ≥50 ml/min, and $CrCl_{est}$ < 70 ml/min vs ≥70 ml/min). As shown in Table 3, $CrCl_{est}$ dichotomized at 50 ml/min was significantly associated (inversely) only with hemoglobin toxicity ($P = 0.04$). Dichotomization at $CrCl_{est}$ 70 ml/min resulted in comparable conclusions. Hemoglobin toxicity was unrelated to pretreatment hemoglobin level ($P = 0.10$), and bleeding episodes could not account for the decreased hemoglobin. The occurrence of hemolysis was not specifically addressed in this study, but thorough chart review revealed few reports of documented hemolysis.

When looking beyond the first cycle of treatment, we found that $CrCl_{est}$ was related to the time-to-toxicity endpoint ($P = 0.005$). This association is illustrated in Fig. 1 which shows Kaplan-Meier curves by quartile of $CrCl_{est}$. The three lower quartiles are closely grouped well below the fourth quartile. Only 12% of the patients in the fourth quartile, i.e., those with a $CrCl_{est}$ of 80 ml/min or greater, experienced the toxicity endpoint at cycle 2, while 28% of the remaining patients experienced the toxicity endpoint at cycle 2. This dichotomized variable was highly significant in predicting time to toxicity ($P < 0.0001$).

Analysis of pretreatment hematologic parameters and disease stage as predictors of fludarabine-related toxicity

Indicators of bone marrow reserve as well as the severity of CLL include pretreatment hematologic

Table 3. Proportion of patients experiencing selected toxicities during the first treatment cycle, by $CrCl_{est}$ level. Data are presented as the number of patients experiencing toxicity/number of evaluable patients in each subgroup. This ratio is also given as a percentage in parentheses. All grades of toxicity are included unless otherwise specified. Hematologic toxicity includes hemoglobin, neutrophil and platelet toxicity. *P* values are from the likelihood ratio test of a logistic regression model

Toxicity	All patients	$CrCl_{est}$ < 50 ml/min	$CrCl_{est}$ ≥50 ml/min	*P* value ($CrCl_{est}$ dichotomized)	*P* value ($CrCl_{est}$ continuous)
Any hematologic	147/186 (79)	31/40 (78)	116/146 (79)	0.79	0.84
Grade 3/4 hematologic	34/186 (18)	5/40 (13)	29/146 (20)	0.29	0.54
Platelet	102/184 (55)	21/40 (53)	81/144 (56)	0.67	0.58
Hemoglobin	75/183 (41)	22/40 (55)	53/143 (37)	0.04	0.01
Neutrophil	64/174 (37)	10/38 (23)	54/136 (40)	0.13	0.80
Infection	46/182 (25)	7/39 (18)	39/143 (27)	0.24	0.81

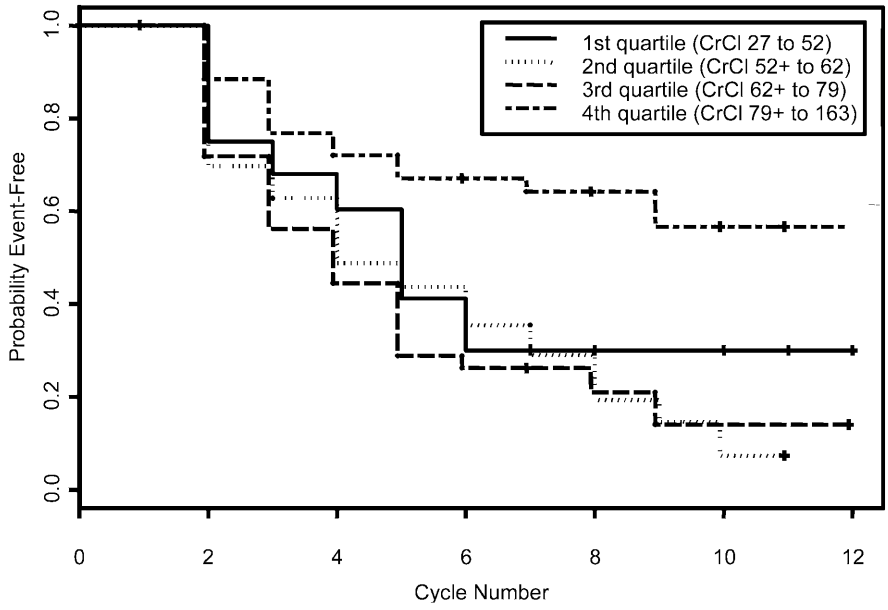

Fig. 1. Time to toxic event. Probability of remaining event-free by fludarabine cycle number and quartile of estimated creatinine clearance ($CrCl_{est}$) among 188 patients. There is a statistically significant association between time to toxic event and creatinine clearance ($P = 0.005$)

parameters. Pretreatment hemoglobin count was inversely correlated with neutrophil toxicity and grade 3/4 hematologic toxicity ($P < 0.0001$) during the first cycle of treatment. The median pretreatment hemoglobin of patients who experienced no neutrophil toxicity was 12.7 g/dl ($n = 109$), while that for patients who experienced neutrophil toxicity was 11.0 g/dl ($n = 65$). No other toxicity variables were correlated with the pretreatment hemoglobin. Pretreatment platelet count was inversely correlated with neutrophil toxicity and with grade 3/4 hematologic toxicity ($P < 0.0001$) during the first cycle of treatment. The median pretreatment platelet count of patients who experienced no neutrophil toxicity was 168,000/μl ($n = 111$), while that for patients who experienced neutrophil toxicity was 113,000/μl ($n = 65$). No other toxicity variables were correlated with the pretreatment platelet count. Pretreatment white blood cell count, neutrophil count and lymphocyte count showed no correlation with any measured toxicity outcome during the first cycle of treatment.

Since anemia and thrombocytopenia define high-risk CLL, we assessed the association between Rai stage and treatment-related toxicity (Table 4). High-risk CLL (Rai stages III and IV) was strongly associated with neutrophil toxicity ($P = 0.0002$) and with grade 3/4 hematologic toxicity ($P < 0.0001$) during the first cycle of treatment. Of the patients with Rai stage I or II, 10% experienced grade 3/4 treatment-related hematologic toxicity, while 36% of patients with Rai stage III or IV experienced such toxicity. No other toxicity variables were associated with disease stage. Higher Rai stage was also associated with a shorter time to toxicity ($P = 0.03$). The hazard ratio for high- versus low-risk Rai stage was 1.53 (95% confidence interval 1.06–2.22); the median time to toxicity for high and low Rai stages was 4 versus 6 months, respectively.

Table 4. Proportion of patients experiencing selected toxicities during the first treatment cycle, by Rai stage. Data are presented as the number of patients experiencing toxicity/number of evaluable patients in each subgroup. This ratio is also given as a percentage in parentheses. All grades of toxicity are included unless otherwise specified. Hematologic toxicity includes hemoglobin, neutrophil and platelet toxicity. *P* values are from the likelihood ratio test of a logistic regression model

Toxicity	Stage I/II	Stage III/IV	*P* value
Any hematologic	85/112 (76)	63/72 (88)	0.05
Grade 3/4 hematologic	11/112 (10)	26/72 (36)	< 0.0001
Platelet	62/111 (56)	43/72 (60)	0.61
Hemoglobin	41/110 (37)	35/71 (49)	0.11
Neutrophil	28/106 (26)	36/66 (55)	0.0002
Infection	25/113 (22)	22/73 (30)	0.22

Discussion

The present study indicates that age is not an independent risk factor for fludarabine-related toxicity during the first cycle of treatment for CLL, nor for a time-to-toxicity endpoint. Cancer therapy in the elderly has not been well studied, yet the aging of the population has prompted increased interest in this issue. Many physiologic changes occur during aging that could potentially affect the pharmacokinetic and pharmacodynamic disposition of antineoplastic agents [26, 36]. Since cancer chemotherapy has traditionally been considered less well-tolerated in the elderly, the extent to which physiologic changes can be extrapolated to predict risk of toxicity is of much interest.

Overall poorer outcomes in older cancer patients complicates the specific issue of chemotherapeutic toxicity. In NHL and acute myeloid leukemia worse survival and increased complications have been found in the elderly [1, 15, 23, 43, 46, 53, 54]. Factors such as

disease biology, the presence of comorbid conditions, treatment history, performance status and organ function all impact significantly on morbidity and mortality during treatment of these patients [53, 57]. In fact, numerous studies in a variety of cancers have provided examples of chronological age by itself being independent of chemotherapy-related toxicities [4, 20, 22, 25, 49, 51, 57]. We focused our analysis by investigating previously untreated patients who were treated with a single agent that has a well-understood elimination pattern. We thoroughly evaluated specific toxicities in detail during the first cycle of treatment. Concisely evaluating the extent of treatment-related toxicity in subsequent treatment cycles is not straightforward since dose reductions and non-treatment-related comorbid conditions make the interpretation difficult. However, we defined a time-to-toxicity endpoint that has provided some insight to treatment-related toxicity after more than one cycle of fludarabine. Indeed, others have found first-cycle toxicity to be equivalent between older and younger patients being treated with CHOP for NHL, only to observe greater age-related toxicity in subsequent cycles [23]. Importantly, we found in this trial that age was not an independent predictor of development of toxicity in the first cycle or for time to toxicity event in subsequent cycles. One concern about clinical trials involving elderly patients is that only better-risk or more robust older patients are enrolled. However, we found that older patients had poorer performance status. Furthermore, there was no association between disease status and age. This study provides pertinent insights regarding fludarabine toxicity in older patients within the parameters of this evaluation.

Since decreased renal clearance is observed in the older population as a group, and systemic fludarabine clearance has been associated with renal function, we investigated whether surrogates of renal clearance would predict fludarabine toxicity [6, 18, 35, 42]. In particular, we estimated creatinine clearance, since serum creatinine by itself can be a poor indicator of renal function in older patients [17, 34, 42]. Although eligibility for this study was limited to patients with serum creatinine and blood urea nitrogen values less than 1.5 times normal, the study did include patients with a wide range of $CrCl_{est}$. As predicted, older patients were more likely to have diminished renal function based on this parameter. The correlation of $CrCl_{est}$ with age was consistent with that found by others using more accurate but more invasive methods of determining creatinine clearance. Such methods, such as 24-h urine creatinine determination or measuring the change in inulin clearance following an amino acid infusion, were not performed in this trial [6, 18, 34, 35].

Within the range of renal function studied here, we did not find a significant correlation between estimated renal function and markers of myelosuppression during the first cycle of treatment. However, there was a strong relationship between $CrCl_{est}$ and the time-to-toxicity endpoint. This finding suggests that the cumulative toxicity from fludarabine is greater in patients with lower renal function. This analysis was designed to explore the incidence of treatment-related toxicity in patients who may have had subclinical renal impairment. Patients with serum creatinine greater than 1.5 times the upper limit of normal were specifically excluded from this study. Therefore, our analysis did not address any effect that might exist in patients with even greater renal impairment, a group that would presumably be at risk for fludarabine-related toxicity. Evaluation of an association between $CrCl_{est}$ and time to treatment failure, disease progression or survival were not pursued in the present analysis due to potentially confounding variables including the crossover design of the study and toxicity-related dose reductions. However, investigation of such relationships in future studies may provide important clinical guidance. Finally, calculation of $CrCl_{est}$ may provide a more useful surrogate for predicting multi-cycle toxicity compared to serum creatinine. Since $CrCl_{est}$ was unable to predict toxicity during the first cycle of treatment, this study does not support a priori dose reduction in the first cycle of treatment based on the $CrCl_{est}$ alone. However, this study does indicate that patients with $CrCl_{est}$ below 80 ml/min are at a higher risk of developing toxicity after multiple cycles of fludarabine.

We found significant relationships between surrogates of poor renal function and hemoglobin toxicity in the first cycle of treatment. A decline in hemoglobin of more than 10% during the first cycle of treatment was seen in 40% of CLL patients receiving fludarabine. It is not clear why hemoglobin toxicity was associated with poor renal function during this period when a more common dose-limiting toxicity, such as neutropenia, was not associated. The decline in hemoglobin levels during the 28-day period analyzed in this study was more rapid than one might have expected based purely on a myelosuppressive action of fludarabine. Perhaps patients with poorer renal function had impaired erythropoietin production, resulting in a reduced red blood cell regenerative reserve. Erythropoietin levels were not measured during this study. Fludarabine-associated anemia has been found in previous studies, but whether this side effect is dose-related or if this toxicity represents an exacerbation of a disease-related process was not determined [13, 28, 56, 58].

Autoimmune hemolytic anemia (AIHA) has been widely reported in CLL treatment studies, and is included in the package labeling of fludarabine [13, 28, 31, 40, 44, 52, 56, 58]. Indeed, CLL, fludarabine treatment and renal failure have all been associated with an altered CD4:CD8 T-cell ratio, which represents a possible mechanism for initiation of AIHA [7, 13, 21, 56, 58]. We did not find that reports of AIHA or laboratory values supported this mechanism as the etiology of the hemoglobin toxicity observed. However, we cannot rule out subclinical AIHA as playing a role in the increased hemoglobin toxicity seen in patients with poor renal function. We are also not aware of studies in which

selective accumulation of fludarabine or fludarabine metabolites has been found in red blood cells. Further investigation is needed to determine the mechanism(s) underlying hemoglobin reduction following the first cycle of fludarabine. Clinicians should be aware of this increased risk during treatment of CLL patients with fludarabine.

The pretreatment factors most clearly associated with toxicity during the first cycle of treatment with fludarabine were low pretreatment hemoglobin and platelet counts. Interestingly, anemia and thrombocytopenia are also the parameters that define high-risk CLL (Rai stage III and IV). Pretreatment anemia and thrombocytopenia probably reflect impaired bone marrow functional capacity. We suspect that this decreased myeloproliferative reserve manifests in decreased ability of neutrophils to recover following treatment with fludarabine. Others have also reported that pretreatment thrombocytopenia is associated with fludarabine toxicity during treatment for CLL [16]. Our findings that CLL at an advanced stage is the single most predictive factor for serious treatment-related toxicities during the first cycle of treatment and that disease stage is associated with short time to toxicity pose a challenging dilemma. It is not known whether dose reduction would avoid such toxicities, or whether such a strategy would diminish the efficacy of fludarabine.

This study supports the initiation of fludarabine treatment at full dose in older patients with CLL. Patients, families and physicians often use age as a factor in making treatment decisions, even in the absence of other comorbidities. The older patients enrolled in this study were not more likely to suffer from fludarabine-related toxicities compared to a younger cohort. We conclude that older patients who meet the criteria of this study should not be excluded from treatment with fludarabine for CLL based on age alone, nor should appropriate doses be modified solely because of concern for age-related toxicities. Alternatively, $CrCl_{est}$ with a threshold below 80 ml/min may be used as an independent risk factor for fludarabine-related toxicity.

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