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Clofarabine-associated acute kidney injury in patients undergoing hematopoietic stem cell transplant

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Abstract

We examined clofarabine pharmacokinetics and association with renal toxicity in 62 patients participating in a phase I–II study of clofarabine–melphalan–alemtuzumab conditioning for hematopoietic stem cell transplant (HSCT). Pharmacokinetic parameters, including clofarabine area under the concentration–time curve (AUC), maximum concentration and clearance, were measured, and patients were monitored for renal injury. All patients had normal pretreatment creatinine values, but over half (55%) experienced acute kidney injury (AKI) after clofarabine administration. Age was the strongest predictor of AKI, with older patients at greater risk ($p = 0.002$). Clofarabine AUC was higher in patients who developed AKI, and patients with the highest dose-normalized AUCs experienced the most severe grades of AKI ($p = 0.01$). Lower baseline renal function, even when normal, was associated with lower clofarabine clearance ($p = 0.008$). These data suggest that renal-adjustment of clofarabine dosing should be considered for older and at-risk patients even when renal function is ostensibly normal.

Keywords: Clofarabine, pharmacokinetics, acute kidney injury, glomerular filtration rate

Introduction

Clofarabine is a nucleoside analog currently approved in the United States for the treatment of pediatric patients with relapsed or refractory acute lymphoblastic leukemia (ALL) after at least two prior regimens. It is also being studied as a novel therapy for a variety of other hematologic malignancies. Thus far, clofarabine has been shown to have activity in adult acute myelogenous leukemia, lymphoma and myelodysplastic syndrome (MDS) [1–3]. Recently, we reported results with clofarabine used in concert with melphalan and alemtuzumab as part of a conditioning regimen for hematopoietic cell transplant [4].

The established safety profile for clofarabine includes dose-limiting toxicities of hand–foot syndrome, liver function abnormalities, skin toxicity and systemic inflammatory response syndrome [1,5–7]. However, in our recent study we reported a significant incidence of renal toxicity (21%), a frequency not previously described in the literature [4]. Isolated prior reports have described cases of renal toxicity occurring in association with clofarabine administration in a variety of disease settings [8–12], but a detailed investigation of a large cohort, and examination of renal toxicity in relation to clofarabine exposure, have not to our knowledge been conducted previously.

The primary aim of the present study was to elucidate the relationship between clofarabine pharmacokinetics and renal toxicity in a cohort of patients undergoing hematopoietic stem cell transplant (HSCT).

Methods

Study participants

All patients included in this study are those from the previously reported phase I–II study of clofarabine–melphalan–alemtuzumab conditioning for allogeneic HSCT conducted at The University of Chicago (ClinicalTrials.gov identifier: NCT00943592) [4]. Institutional Review Board approval was obtained and all patients provided written informed consent. Eligibility criteria included intermediate and high-risk disease as per American Society for Blood and Marrow Transplant criteria, Zubrod performance status ≤ 2, < 75 years of age, adequate cardiac and pulmonary involvement, calculated baseline creatinine clearance > 50 mL/min, serum bilirubin < 2.0 mg/dL and serum glutamic pyruvic transaminase < 3 times the upper limit of normal [4,13]. Patients with evidence of chronic active hepatitis or cirrhosis, human immunodeficiency virus (HIV) positivity or a positive pregnancy test were excluded. Additionally,

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patients with severe infection that may have contributed to renal dysfunction during clofarabine administration or shortly thereafter were also excluded ($n = 3$). Finally, only patients from the original study for whom clofarabine pharmacokinetic samples were drawn were considered in this retrospective analysis.

Study procedures

Patients were treated according to the protocol described in the original study [4]. At the beginning of the study, all patients received intravenous clofarabine over 1 h on days −7 through −3; a subsequent protocol amendment revised the clofarabine infusion time to 3 h. The dose of clofarabine was escalated as per the phase I accelerated titration design from 10 mg/m^2 to 40 mg/m^2. All patients in the phase II cohort received doses of 30 or 40 mg/m^2. Hydration was given 4 h prior to and 2 h after each dose of clofarabine, with a goal to maintain urine output 100 mL/h. Alemtuzumab was administered at 20 mg intravenously on days −7 to −3 over 1 h. Patients received melphalan intravenously over 30 min on day −2. The dose of melphalan escalated as per the phase I protocol from 100 mg/m^2 to 120 mg/m^2. All patients in the phase II cohort received doses of 140 mg/m^2. Serum creatinine levels were drawn prior to therapy and checked daily.

Tacrolimus was prescribed to all patients for graft versus host disease (GVHD) prophylaxis beginning on day −2 with a target drug level of 5–15 ng/mL. Steroids were also used as first-line treatment for acute or chronic GVHD.

Infection prophylaxis included high-dose acyclovir, pre-transplant trimethoprim/sulfamethoxazole, and a quinolone during the initial neutropenic period and fluconazole 200 mg/day (or another broad-spectrum azole) or echinocandin until day 180. Trimethoprim/sulfamethoxazole was given from engraftment until 1 year post-transplant.

Patient or donor cytomegalovirus (CMV) seropositivity was treated with ganciclovir 5 mg/kg from day 28 until day 23, transitioning to acyclovir 10 mg/kg given every 8 h intravenously until discharge. Finally, after engraftment, these patients were switched to valacyclovir 2000 mg four times per day until day 210.

Other supportive care measures were as previously described [4].

Pharmacokinetic sampling and analysis

All patients provided blood samples on day −7 and day −5 for pharmacokinetic analyses, corresponding to dose 1 and dose 3 of clofarabine, respectively. Samples were collected prior to clofarabine infusion (t 0), at the end of the infusion (t 60), and at 0.5 h (t 90), 1 h (t 120), 2 h (t 180), 4 h (t 360), 6 h (t 480), 8 h (t 600) and 24 h (t 1500) after the end of the infusion. Each sample consisted of 10 mL of blood collected into sodium heparinized (green top) vacutainers. Green top tubes were centrifuged (2500 rpm, 20 min, 4°C) and plasma immediately separated and transferred as two aliquots into storage cryotubes and stored at −80°C until analysis. Samples were protected from light until analysis. Sample analysis was performed in the Genzyme Core Facility according to a modification of a previously published method [14]. Plasma pharmacokinetic parameters for clofarabine were calculated by standard non-compartmental methods with WinNonlin 4.0 (Pharsight Corporation, Mountain View, CA). The mean observed maximal concentration (C_{max}) was obtained by visual inspection of the plasma concentration–time data. The apparent terminal elimination rate constant (λ_z) was estimated as the slope of the linear regression of the log-linear portion of the plasma concentration–time plot as determined by visual inspection. The area under the plasma concentration–time curve extrapolated to infinity (AUC) was determined by the linear trapezoidal method. AUC values normalized to clofarabine dose were calculated, since patients received differing dose levels of clofarabine as dictated by the original clinical study. Half life ($t_{1/2}$) was calculated as $\ln(2)/\lambda_z$. Clearance (CL) was calculated as clofarabine dose/AUC.

Renal toxicity phenotype classification

Acute kidney injury (AKI) was defined according to the Acute Kidney Injury Network (AKIN) definition criteria [15]. AKI criteria were applied to injury occurring within 15 days of receiving clofarabine. Glomerular filtration rates (GFRs) were estimated by standard clinical laboratory measurement of serum creatinine using the Modification of Diet in Renal Disease (MDRD) formula, with correction of raw GFRs by a factor of 1.21 for patients identified as African-American [16].

Statistical analysis

Comparisons between groups with and without kidney injury were analyzed using a two-sample *t*-test for continuous risk factors and Pearson's χ^2 test for categorical risk factors. Use of the *t*-test for a skewed distribution has been previously validated in the literature [17,18]. For mean comparisons with more than two groups (i.e. comparing the no injury group and AKIN grades 1, 2 and 3), we used analysis of variance (ANOVA) [19]. All *p*-values are two-sided and observations with missing data were automatically removed from the analysis.

Role of the funding source

Individuals representing the funding source (Genzyme Corporation) had no involvement in the study design, collection of data or data analysis. These individuals were permitted to review the manuscript in draft form and provided comments on its final presentation.

Results

Patient characteristics

The analyzed patient cohort included 62 patients. Table I shows the baseline characteristics of all study patients. The majority of patients suffered from acute myelogenous leukemia ($n = 24$) or non-Hodgkin lymphoma ($n = 21$). All but two patients in the study received all five doses of clofarabine. One patient expired on day −3, and a single dose was held for one patient due to renal failure.

Table I. Baseline characteristics of study patients with normal renal function and AKIN criteria* grade 1–3 renal failure.

Characteristic	All patients	No renal toxicity	AKIN grade 1	AKIN grade 2	AKIN grade 3
n (%)	62 (100)	28 (45)	14 (22)	11 (18)	9 (15)
Mean weight (kg)	85	83	90	83	87
Mean age (years)	51	45	57	56	59
Gender, *n* (%)					
Male	36 (58)	18 (64)	10 (71)	3 (27)	5 (56)
Female	26 (42)	10 (36)	4 (29)	8 (73)	4 (44)
Mean baseline GFR (mL/min/1.73 m^2) (range)	94 (46–133)	99 (71–133)	88 (58–115)	91 (51–120)	90 (46–120)
Malignancy type, *n*					
CML	3	1	0	2	0
ALL	2	1	0	0	1
AML	24	11	7	4	2
NHL	21	11	4	4	2
MDS	6	1	2	1	2
Myelofibrosis	2	1	0	0	1
CLL	4	2	1	0	1

GFR, glomerular filtration rate; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia.
*Acute kidney injury (AKI), as defined by the Acute Kidney Injury Network (AKIN) criteria [15], includes any of the following: an increase in serum creatinine by ≥3 mg/dL within 48 h, increase in serum creatinine ≥1.5 times baseline or a urine volume <0.5 mL/kg/h for 6 h. Using the factor of creatinine increase, grade 1 AKI was defined as an increase in serum creatinine by a factor of 1.5–2. An increase in serum creatinine by greater than 2–3-fold qualified as grade 2 injury, and any increase >3 times baseline was considered to be grade 3 injury.

Characteristics of acute kidney injury

All patients in the study had creatinine levels within normal limits (as defined by 0.5–1.4 mg/dL at the study institution) on day −8 before receiving clofarabine. Over half of patients in the study ($n = 34$, 55%) experienced some degree of AKI according to AKIN criteria within 15 days of clofarabine exposure. The largest number experienced grade 1 AKI ($n = 14$, 22%), but a sizable proportion of patients experienced grade 3 AKI ($n = 9$, 15%). The average age of those who did not experience renal toxicity (45 years) was younger than those who did experience renal injury (57 years) ($p = 0.0002$). Gender was not statistically associated with kidney injury. Five patients had comorbid diabetes mellitus (of whom one developed grade 1 AKI, one developed grade 3 AKI and three were without AKI). Despite the fact that all patients had normal creatinine levels at study entry, baseline GFR was lower in those who subsequently developed AKI after clofarabine compared to those who did not develop AKI (89 vs. 99 mL/min/1.73 m^2; $p = 0.06$). Five of the six patients with MDS experienced AKI. Although the small absolute number of patients with MDS limits definitive conclusions to be drawn about this possible factor, this represented the highest proportion observed across the various malignancies in our study.

Acute kidney injury is associated with clofarabine exposure

Table II shows the detailed pharmacokinetic results obtained after the first and third doses of clofarabine for the study cohort. Patients who experienced renal injury consistently had higher clofarabine AUC and C_{max} levels after both dose 1 and dose 3. We observed a compelling relationship between AUC and severity of kidney injury. Figure 1 depicts the distribution of dose-normalized AUCs for patients grouped according to AKI severity grade.

Table II. Pharmacokinetic parameters for clofarabine for all patients, and association with development of AKIN criteria grade 1–3 renal injury.

Parameter	All patients ($n = 62$) (SD)	No renal toxicity ($n = 28$)	AKIN grade 1 ($n = 14$)	AKIN grade 2 ($n = 11$)	AKIN grade 3 ($n = 9$)
Dose* (mg), mean	66.4	64.2	66.6	68.7	69.6
AUC dose 1 (ng*h/mL)	1831	1538	1590	2354	2480
AUC dose 3† (ng*h/mL)	1925	1578	2009	1981	3045
Mean AUC (ng*h/mL)	1878 (904)	1558	1799	2167	2762
C_{max} dose 1 (ng/mL)	456	410	456	543	490
C_{max} dose 3† (ng/mL)	477	407	558	504	543
Mean C_{max} (ng/mL)	466 (161)	408	507	523	516
CL dose 1 (mL/h)	42 668 (17 769)	44 004	46 921	42 512	32 084
Dose-normalized‡					
AUC dose 1 (ng*h/mL/mg)	28.0	24.9	24.6	32.9	36.6
AUC dose 3† (ng*h/mL/mg)	29.4	25.4	29.4	28.7	44.8
Mean AUC (ng*h/mL/mg)	28.7 (12.9)	25.2	27.0	30.8	40.7
C_{max} dose 1 (ng/mL/mg)	7.0	6.6	7.0	7.7	7.1
C_{max} dose 3† (ng/mL/mg)	7.0	6.3	8.0	7.4	7.7
Mean C_{max} (ng/mL/mg)	7.0 (2.3)	6.4	7.5	7.5	7.4

AKIN, Acute Kidney Injury Network; AUC, area under the curve; C_{max}, maximal concentration; CL, clearance; SD, standard deviation.
*Two patients were excluded from these calculations – one who was treated with two different doses, and another who was treated with a low dose of clofarabine outside of the planned protocol dosing.
†Day −5 (dose 3) data were not available for 15 patients.
‡AUC and C_{max} values were dose-normalized by dividing by the dose (mg) received by each patient.

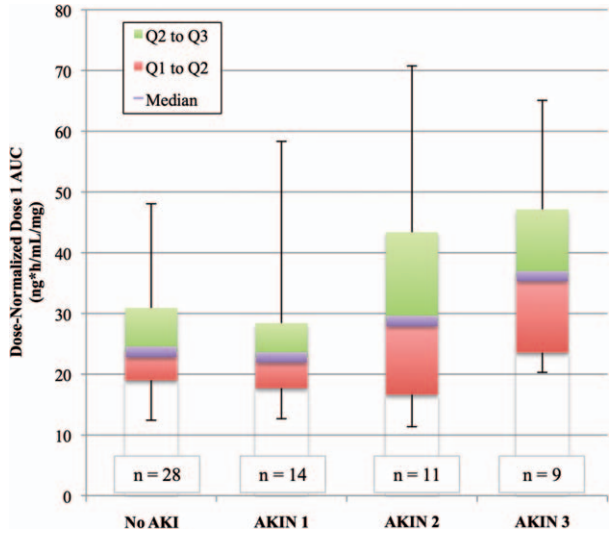

Figure 1. Dose-normalized AUC after dose 1 in patients with different levels of kidney injury. Higher levels of clofarabine exposure were significantly associated with increasingly severe AKI ($p = 0.01$). Q1, Q2 and Q3 refer to the quartiles of data distribution with Q1 = 25th percentile, Q2 = 50th percentile and Q3 = 75th percentile.

These data demonstrate that higher measured AUCs were significantly associated with increasingly severe AKI ($p = 0.01$). For those who did not experience AKI, the mean dose-normalized AUC was 25.15 ng*h/mL per mg of administered clofarabine. In comparison, those who developed grade 2 AKI had an average dose-normalized AUC of 30.78 ng*h/mL per mg of administered clofarabine, and those with grade 3 AKI exhibited average normalized AUC levels of 40.70 ng*h/mL per mg of administered clofarabine – levels 62% higher than in patients who did not develop renal injury. Expressed another way, Figure 2 shows that greater AUC levels were significantly associated with a greater decline in GFR after receipt of clofarabine ($p = 0.00002$). Even irrespective of AKI severity (and using dose 1 AUC values as a benchmark), the clofarabine levels in patients developing any-grade AKI were significantly higher than in patients who did not develop AKI ($p = 0.02$). Normalization for the administered clofarabine dose attenuated the statistical significance of this comparison ($p = 0.08$ for dose-normalized AUC comparison between the AKI and no AKI groups) but not the relationship. There were no statistically significant differences in the mean dose 1 CL or mean C_{max} for those experiencing AKI compared to those who did not.

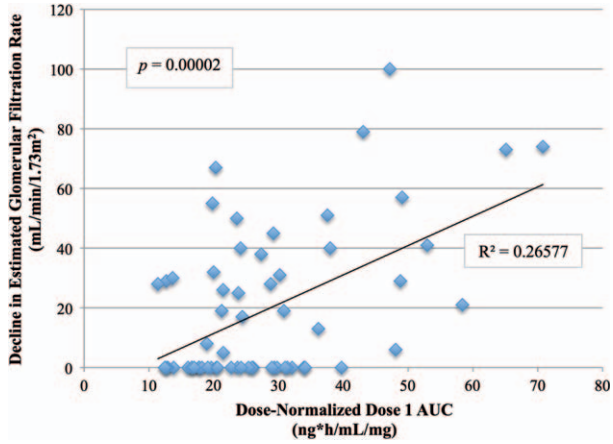

Figure 2. Decline in GFR from day −8 to day 0 as a function of clofarabine AUC. Greater AUC levels were significantly associated with a greater decline in GFR after receipt of clofarabine ($p = 0.00002$).

Since this study included dose escalation as a part of its design, patients received different dose levels of clofarabine, ranging from 10 to 40 mg/m^2. The majority of patients were treated with 30 or 40 mg/m^2 of clofarabine (31 and 24 patients, respectively). Those patients treated at 30 mg/m^2 had a mean C_{max} of 369.70 ng/mL, mean AUC of 1503.54 ng*h/mL and mean CL of 44555.64 mL/h for dose 1. For patients treated at 40 mg/m^2, the corresponding values were 547.67 ng/mL, 2106 ng*h/mL and 43235.21 mL/h, respectively.

Clofarabine AUC and clearance are dependent upon baseline renal function, but baseline renal function did not reliably predict AKI risk

Although all patients in this study had baseline creatinine values that were considered normal, we found that patients with higher baseline GFRs exhibited lower dose-normalized dose 1 AUCs, and correspondingly, greater clofarabine clearances. Figure 3(A) shows that higher baseline GFR was statistically significantly associated with lower dose-normalized dose 1 AUC levels ($p = 0.0002$). Figure 3(B) illustrates the statistically significant positive association between baseline GFR and dose 1 clofarabine clearance ($p = 0.008$). The effect of reduced GFR on clofarabine CL has previously been documented [20].

The plots in Figure 4 show individual trends for each patient in the study, divided into groups according to AKI severity. These plots demonstrate how the GFRs of each patient changed during, and just following, exposure to clofarabine. For most patients with AKI, the significant decline in GFR occurred just after the course of clofarabine was completed (but before HSC infusion), suggesting a strong temporal relationship with clofarabine exposure. In most cases, the delay in the appearance of AKI precluded investigators from holding subsequent infusions. Indeed, for only one patient (AKIN grade 2) was a dose of clofarabine held due to renal failure. Also notably, the average baseline GFR for patients in the four phenotype groups (no AKI, grade 1, grade 2 and grade 3 AKI) were not significantly different from each other (98, 88, 91 and 90 mL/min/1.73 m^2, respectively; ANOVA $p = 0.30$), suggesting that baseline GFR, although associated with clofarabine AUC, was not a reliable predictor of subsequent kidney injury risk.

Multivariate model of risk factors for clofarabine-associated renal injury

We fit a multiple logistic regression model to interrogate kidney injury (no AKI vs. AKIN = 1, 2, 3) including all risk factors reported above that were even marginally significant in the model (age, AUC and baseline GFR). In the multiple logistic regression model, in the presence of either AUC or dose-normalized AUC and baseline GFR, age remained a significant risk predictor ($p < 0.007$). Conversely, AUC or dose-normalized AUC and baseline GFR were not

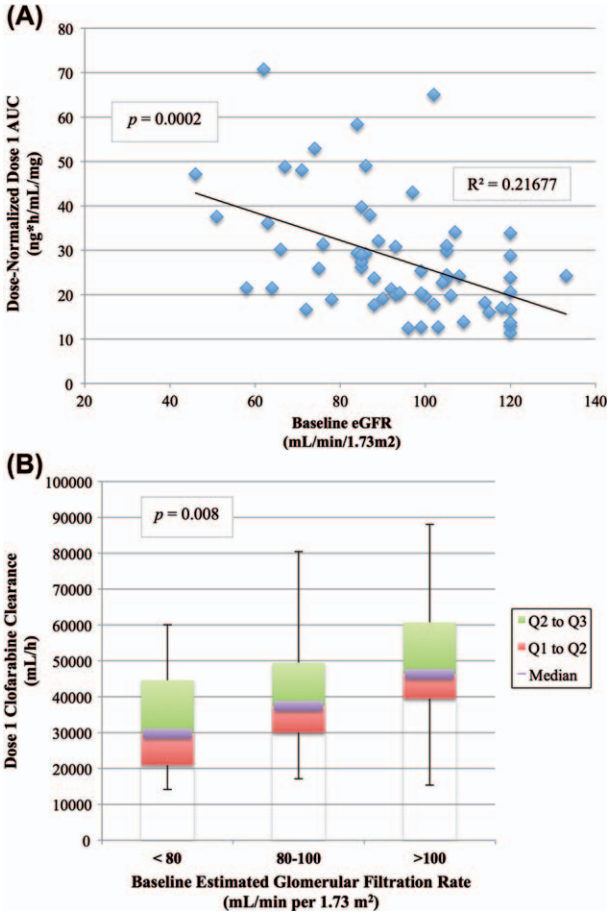

Figure 3. (A) Dose-normalized dose 1 AUC as a function of baseline GFR. Patients with higher baseline GFRs had lower levels of clofarabine ($p = 0.0002$), although there were several clear patient outliers. (B) Corresponding relationship between baseline GFR and dose 1 clofarabine clearance. Consistent with (A), patients with higher baseline GFRs had higher levels of clofarabine clearance ($p = 0.008$). The direct relationship existed even between groups of patients having normal baseline GFRs. Q1, Q2 and Q3 refer to the quartiles of data distribution with Q1 = 25th percentile, Q2 = 50th percentile and Q3 = 75th percentile.

significant predictors when considered along with age as a risk factor (both $p > 0.05$). Given the well-known interrelatedness of GFR and age, we additionally ran the model with only age and AUC included, with no substantial change in the findings.

Discussion

Drawing upon pharmacokinetic and prospectively collected toxicity data from a large cohort of patients with HSCT, our study provides, to our knowledge, the most compelling evidence to date that clofarabine-induced renal injury may be underappreciated in certain clinical settings. Furthermore, such renal injury appears to be directly related to greater clofarabine exposure. The reasons for significant inter-individual variation in exposure even between patients with normal and similar baseline renal function and despite using body surface area (BSA)-based dosing are not completely understood, with the strong exception that greater clofarabine exposure was more likely to occur in older patients.

All patients in our study had normal serum creatinine levels at baseline (before drug administration), yet over 50% of patients experienced some degree of renal toxicity. The degree of risk of renal injury appeared to be directly associated with higher clofarabine AUCs (and correspondingly, with decreased clofarabine clearance). Indeed, those patients with AKIN 2 and 3 had consistently higher clofarabine AUC levels. This suggests that high clofarabine exposure has a directly cytotoxic effect on the nephron. We amended the study protocol after partial enrollment to mandate longer (3 h) clofarabine infusion times as a step to try to decrease the rates of AKI we were observing. This intervention did not reverse the AKI rate, and, relatedly, we did not find that clofarabine C_{max} was associated with risk of AKI.

The clinico-pathologic risk factors associated with clofarabine-associated renal injury in our study included greater age, higher AUC and, marginally, lower baseline GFR. Of these, age and AUC were most strongly associated with the development of AKI in our cohort. It is understood that these variables are interrelated, since GFR declines with age, and clofarabine clearance is related to GFR. However, age appeared to have an independent risk effect in our study, even after adjusting for baseline GFR. This could potentially be explained by decreased metabolic clearance of clofarabine in older patients or even possibly decreased non-renal excretion of the drug. Given that clofarabine binds to albumin, age-related hypoalbuminemia could also have had an impact on exposure differences (albumin levels were not available for analysis for the majority of study patients). It is certainly possible that currently unspecified pharmacogenomic biomarkers may also explain a portion of this inter-individual variation. Interestingly, patients with MDS had a higher rate of developing AKI when compared to other hematologic malignancies, although we did not have power to detect true differences between hematologic malignancies, and these data about disease type affecting AKI risk are therefore hypothesis-generating.

The clinical outcomes of patients with clofarabine-associated kidney injury in this cohort varied. Of the nine patients who experienced grade 3 AKI, five patients progressed to dialysis; two remained on dialysis until death, although the need for dialysis may not have been directly related to clofarabine-exposure. Indeed, three patients from the grade 3 group also became septic during conditioning or shortly after transplant, two of whom required dialysis. One patient underwent a renal biopsy, which showed acute tubular injury, with regenerative nuclear atypia. While acute tubular injury can be seen in the setting of HSCT [22], the pattern of nuclear atypia specifically has been described after exposure to some medications, such as cyclophosphamide and busulfan [23]. Finally, at the end of the follow-up period, only one of these patients was alive without relapse.

It would be useful for clinicians to be able to recognize AKI at early stages so that patients who would benefit from dose-reduction or dose-tailoring (beyond BSA-based dosing) could be identified. In tracking GFR values over time in those who experienced the most severe renal toxicity, however, there were no obvious trends to suggest that early cessation would prevent renal injury. Indeed, the peak timing of greatest renal injury from a drug administration standpoint occurred several days after the completion of clofarabine, likely reflecting as much the inadequacies

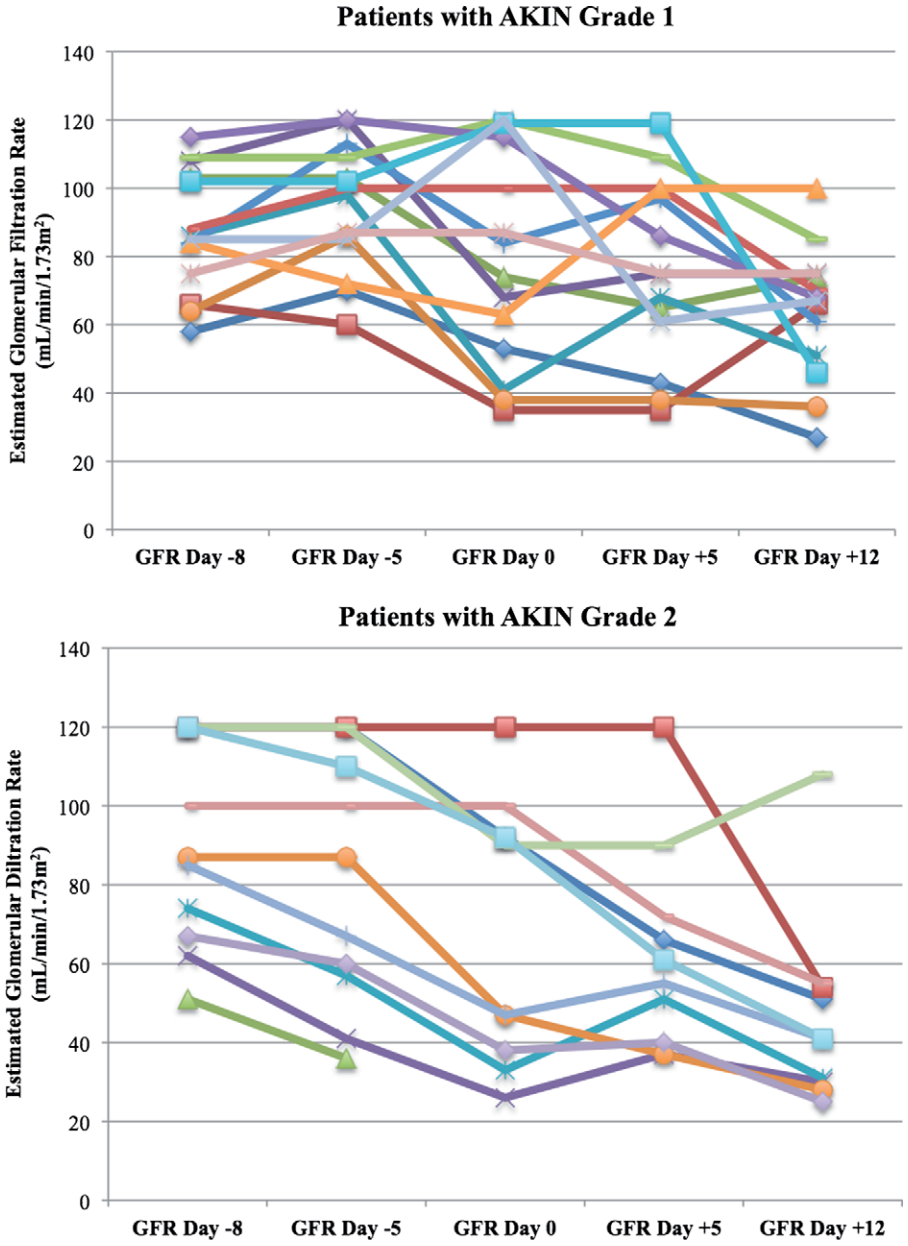

Figure 4. Individual patient GFRs measured at times day −8, −5, 0, +5 and +12, separated by level of kidney injury. Kidney injury was classified using AKIN criteria, which are dependent on the factor of serum creatinine increase. AKIN grade was defined using the single highest creatinine value measured from day −8 to day +12. For most patients with AKI, the significant decline in GFR occurred just after the course of clofarabine was completed (but before HSC infusion), suggesting a strong temporal relationship with clofarabine exposure. The average baseline GFR of patients across the four phenotype groups was not significantly different.

of serum creatinine as an early marker of AKI as it does a cumulative effect of multiple doses of clofarabine administered sequentially over 5 consecutive days. Because this schedule was necessary as conditioning for transplant, and because the observation of the serum creatinine rise is delayed up to 48 h or more after a nephrotoxic injury [21], it is difficult with current clinical tools to recognize those patients who are at greatest risk of AKI once clofarabine administration has begun. While the current drug label for clofarabine recommends a 50% dose reduction for renal impairment, this only applies to patients with a baseline creatinine clearance of 30–60 mL/min [6]. There are currently no guidelines for those patients with ostensibly "normal" creatinine clearance values. Therefore, we would argue that pre-identification of such patients is paramount.

Thoughtful characterization of clinical markers of risk, and perhaps even pharmacokinetic monitoring after the first dose of clofarabine, may be useful measures. More practically, development of dosing algorithms which consider renal function in the dosing equation may be warranted if the tight relationship between GFR and exposure that we observed in our study and that which others have documented can be demonstrated in other cohorts of patients [24]. This type of renally adjusted dosing (even when baseline renal function is within normal limits) would of course not be foreign to oncology, as it is routinely used in the administration of carboplatin [25].

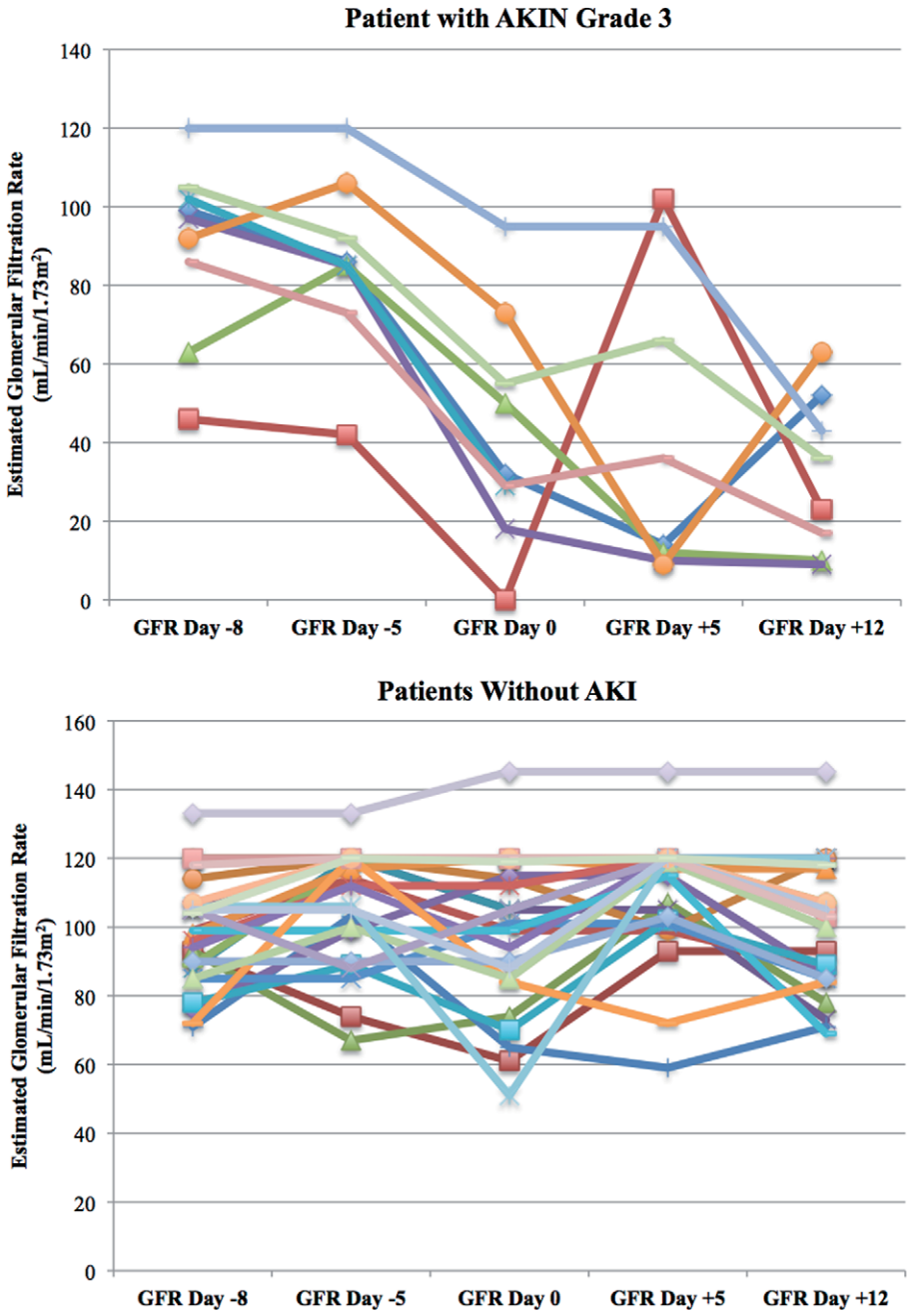

Figure 4. (*Continued*)

As a limitation, our study is complicated by the fact that HSCT itself and HSCT-associated infections are known risk factors for AKI, as are co-administered medications in the peri-transplant period. Of note, all patients in our study received tacrolimus beginning on day −2 and levels were carefully monitored. Three patients who developed peri-transplant sepsis while receiving this transplant regimen were excluded from the present analysis, since in those cases sepsis was felt to be the definitive primary cause of renal injury. At least two patients who suffered grade 3 AKI also experienced CMV viremia, but both instances occurred greater than 1 month after transplant (55 000 copies/mL detected on day 36, and 125 000 copies/mL detected greater than 3 months after day 0), which makes CMV reactivation a less likely potential cause of AKI. It is true that some patients required the use of other potentially nephrotoxic drugs during the study period, as all patients received peri-transplant administration of ganciclovir, trimethoprim/sulfamethoxazole and an oral quinolone. However, these are standard prophylactic agents used at our transplant center and are unlikely to be significantly related to the AKI rate seen in this cohort, because our prior studies with other conditioning regimens have not revealed this frequency or severity of renal injury [26,27]. Additionally, melphalan and alemtuzumab have been used successfully in patients with renal failure and are not associated with renal toxicity [28]. Though we cannot completely rule out the concomitant role of these factors in some of our patients' development of AKI, even with these potential confounders, the novel

relationship between clofarabine exposure and AKI risk was robustly demonstrated in our cohort.

A minor potential limitation to this study is the use of creatinine and estimated GFR as markers of renal function. Creatinine's utility as a marker for kidney function is limited by variations in creatinine secretion, creatinine excretion and issues with measurement. Indeed, it is possible that clofarabine impedes tubular secretion of creatinine, without jeopardizing total renal function. Furthermore, it is acknowledged in the literature that the range of normal GFR varies amongst healthy individuals, and for some, GFR may decline with age without evidence of renal disease [29,30]. This "normal" decline in GFR may begin at approximately age 30, but is thought to accelerate after age 65 [30]. The age range in our study cohort was 21–73 years with a mean of 51 years, suggesting that many of our patients would not have yet suffered significant decline in GFR from normal aging at the time of the study. Direct measurement of GFR via 24 h urine collection, cystatin C measurement or inulin clearance provide more accurate representations of renal function and would answer this question, but these measurements were not performed in our study. Indeed, only one patient had cystatin C measured, but this occurred 2 months after HSC infusion and is therefore not applicable to this study.

In summary, greater clofarabine exposure is associated with increased risk of clofarabine-related renal toxicity, especially in older adults. In order to decrease this risk and pursue personalized dosing when using clofarabine, further research is needed to understand inter-individual factors beyond baseline renal function and age that result in impaired clofarabine clearance and resulting over-exposure. Finally, renal function-adjusted dosing based on baseline GFR even among patients with apparently normal serum creatinine levels deserves consideration in the future use of clofarabine. We suggest careful monitoring for renal toxicity and minimizing the presence of other renal insults in older patients and those with lower baseline GFRs who will be receiving clofarabine before HSCT.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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