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# Personalized Fludarabine Dosing To Reduce Non-Relapse Mortality In Hematopoietic Stem Cell Transplant Recipients Receiving Reduced Intensity Conditioning

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## Abstract

Patients undergoing hematopoietic cell transplantation (HCT) with reduced intensity conditioning (RIC) commonly receive fludarabine. Higher exposure of F-ara-A, the active component of fludarabine, has been associated with a greater risk of non-relapse mortality (NRM). We sought to develop a model for fludarabine dosing in adult HCT recipients that would allow for precise dose targeting and predict adverse clinical outcomes. We developed a pharmacokinetic model from 87 adults undergoing allogeneic RIC HCT that predicts F-ara-A population clearance (Clpop) accounting for ideal body weight and renal function. We then applied the developed model to an

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independent cohort of 240 patients to identify whether model predictions were associated with NRM and acute graft vs host disease (GVHD). Renal mechanisms accounted for 35.6% of total F-ara-A Clpop. In the independent cohort the hazard ratio of NRM at day 100 was significantly higher in patients with predicted F-ara-A clearance (Clpred) <8.50 L/hr ($p<0.01$) and area under the curve (AUCpred)>6.00 μg*hr/mL ($p=0.01$). A lower Clpred was also associated with more NRM at month 6 ($p=0.01$) and trended towards significance at 12 months ($p=0.05$). In multivariate analysis, low fludarabine clearance trended towards higher risk of acute GVHD ($p=0.05$). We developed a model that predicts an individual's systemic F-ara-A exposure accounting for kidney function and weight. This model may provide guidance in dosing in overweight individuals and those with altered kidney function.

### Keywords

fludarabine; pharmacokinetics; reduced intensity conditioning regimen; hematopoietic stem cell transplant; non-relapse mortality

## Introduction

Reduced intensity conditioning (RIC) and allogeneic hematopoietic cell transplantation (HCT) is commonly used in patients with preexisting comorbidities who do not qualify for myeloablative conditioning. Over the last decade, RIC regimens have allowed successful transplantation of patients who are heavily pretreated, older or have complicating comorbidities.(1-5) Fludarabine phosphate is an antitumor and immunosuppressive agent, and is a critical component of most RIC regimens in combination with other chemotherapeutics and/or total body irradiation (TBI).

Fludarabine has dose-dependent toxicities.(6-10) However, studies associating fludarabine dose and pharmacokinetics with toxicity and clinical outcomes are limited.(11-18) Data in HCT suggest that higher F-ara-A (the active component of fludarabine in the plasma) concentrations may be associated with greater mortality.(15) F-ara-A pharmacokinetic variability has also been demonstrated in HCT recipients although factors leading to variability are poorly understood.(16, 17, 19-21)

Body size in $m^2$ is the primary determinant of fludarabine doses in HCT despite there being a paucity of data and lack of understanding if body size alters pharmacokinetic disposition or contributes to variability. The ASBMT guideline for chemotherapy dose adjustment in obese HCT patients concluded that adjustments for weight have been mostly empiric or extrapolated from non-HCT populations and was not able to provide guidelines for fludarabine.(22) Approximately 35-60% of the fludarabine dose is recovered in the urine as F-ara-A or as fludarabine hypoxanthine within 24 hours after administration.(18, 20, 23-25) There is a high correlation between creatinine clearance (CrCl) and F-ara-A total body clearance.(10, 25) Therefore, renal function is a source of F-ara-A pharmacokinetic variability. Despite this dosing guidelines for renal dysfunction are limited. The recent ASBMT dosing guideline in patients with chronic kidney disease concluded that there are no clear dosing standards for renal impairment and that the available literature is insufficient.(26) Genetic variants may also contribute to pharmacokinetic variability. Important effects of

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variants have been observed towards cytarabine and gemcitabine but no data are available for fludarabine.(27-31) Our interests have centered on improving the safety and efficacy of conditioning regimens, through personalizing fludarabine dosing by accounting for clinical factors known to affect pharmacokinetic variability and to develop evidence based and validated models to guide dosing and reduce NRM.

## Subjects and Methods

### Patients and Pharmacokinetic Data for Model Development

Data for pharmacokinetic model development were obtained from 87 adult patients previously enrolled in a single center, prospective, observational, pharmacokinetic study undergoing RIC allogeneic HCT.(15) Subject characteristics are shown in Table 1. The research was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and informed consent was obtained from each patient. The study was approved by the Institutional Review Board and the Cancer Protocol Review Committee. The preparative regimen was i.v. cyclophosphamide (50 mg/kg/day) on day-6, i.v. fludarabine 40 mg/m$^2$/day on days -6 to -2 and TBI 200cGy single fraction on day -1. An empiric dose reduction of fludarabine to 30-35 mg/m$^2$ was given to 9 patients due to preexisting renal impairment per physician discretion. Patients who had not received intensive chemotherapy within last 6 months were administered equine antithymocyte globulin for 3 days. Post-HCT immunosuppression was mycophenolate and cyclosporine. Fludarabine phosphate was administered i.v. over 1 hour. Pharmacokinetic blood samples were collected with the first dose beginning immediately pre-dose and at 100 minutes, 2, 3, 4, 6, 8, 12 and 24 hours after start of infusion. F-ara-A detection and quantification in the plasma was performed with HPLC-UV as previously described.(15) The lower limit of quantification was 10 ng/mL with an assay accuracy of 93.5-100.1%. Age, gender, actual body weight, height, disease risk, serum creatinine, total bilirubin (obtained +/-48 hours of the first dose) and serum albumin data (obtained +/-48 hours of the first dose) were collected.

### DNA Collection, Variant Selection and Genotyping

Recipient genomic DNA was obtained pre-HCT from peripheral blood lymphocytes. DNA was quantified by measuring the absorbance at 260 nm. Genes potentially involved in fludarabine bioactivation and transport such as *NT5C2*, *NT5E*, *SLC28A3*, *SLC29A1*, *SLC29A2*, *DCK*, *ABCG2*, *ABCC4* were considered in the analysis. National Center for Biotechnology Information was searched for coding and promoter region variants. Our population was predominantly Caucasian therefore variants were identified from the Caucasian CEU population in the HapMap project (HapMart; schema: rel23a_NCBI_Build36, database: HapMap_rel23a). A total of 77 variants were identified. The Genetic Services Department, Sequenom, Inc., San Diego, CA, performed assay design and genotyping. All variants were in Hardy-Weinberg equilibrium. Sixty-six variants were monomorphic or had a minor allele frequency of less than 5% after genotyping and were eliminated. Eleven variants were analyzed (Supplementary Table S1).

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## Population Pharmacokinetic Model Building and Identification of Covariates Effecting Pharmacokinetics

F-ara-A plasma concentrations (n=768) were previously analyzed and reported.(15) The mean (standard deviation) of plasma concentrations at time 100 minutes, 2, 3, 4, 8, 12 and 24 hours after the start of infusion were 711 (163), 625 (145), 460 (113), 364 (82.5), 254 (61.7), 192 (50.0), 121 (33.3) and 57.3 (23.3) ng/mL, respectively. An equivalent weight of F-ara-A (MW 285) to that of fludarabine phosphate (MW 365) was used as an initial dose with the assumption of instantaneous and rapid conversion of the monophosphate form to F-ara-A in the plasma.

Data analysis was conducted using population pharmacokinetics with nonlinear mixed effects modeling (NONMEM) (version 7.2, ICON Development Solutions, Hanover, MD, USA). Inspection of the pharmacokinetic data, model diagnostics, and covariate testing, bootstrapping and visual predictive check were performed using Perl Speaks Nonmem (PSN) and Xpose version (version 4.3.2) through Pirana workbench (2.7.2), Amsterdam, The Netherlands. First-order conditional estimation with interaction (FOCEI) was utilized for model development. Pharmacokinetic parameters estimated were typical values of clearance (referred to as Clpop in this paper) and volume of distribution (referred to as Vpop in this paper).

Between-subject variability (BSV) was modeled exponentially to pharmacokinetic parameters as shown in equation 1.

$$\mathrm{Pj}=\mathrm{TVP}\times\exp(\eta\mathrm{j}) \quad \text{(equation 1)}$$

where, Pj is the parameter estimate for jth individual, TVP is the typical value of the parameter in a population. $\eta$j is the estimate of deviation of individual j from the TVP and is assumed to be normally distributed with the mean of zero and variance of omega$^2$ (population variability). Residual unexplained variability (RUV) is the unexplained variability between the observed and the predicted value. A combined proportional and additive error model was chosen to describe the RUV (equation 2).

$$\mathrm{Cobs,ij}=\mathrm{Cpred,ij}\times(1+\varepsilon\mathrm{ij})+\varepsilon\mathrm{ij}(\text{proportional and additive RUV model}) \quad \text{(equation 2)}$$

where, Cobs,ij is the j$^{th}$ observed concentration in the i$^{th}$ individual, Cpred,ij is the j$^{th}$ predicted concentration in the i$^{th}$ individual and $\varepsilon$ij is the residual error that is assumed to be independent and normally distributed with a mean zero and variance of $\sigma_{\varepsilon}^2$.

Base model and covariate selection was based upon inspection of the diagnostic plots, a significant decrease in the objective function value (OFV) and a physiological plausible relationship to pharmacokinetic parameter relative to competing models.

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Empirical Bayes estimates (ebe) for parameters were plotted against each covariate to identify the relationships between the parameters and the covariates. Covariates were tested for significant effects on F-ara-A Clpop using a forward inclusion and backward elimination procedure. In NONMEM, minimization of -2 log likelihood is used as a model statistic and is given by the objective function value (OFV); a measure of goodness of fit similar to sum of squares. Covariates were deemed statistically significant if their inclusion in a nested model resulted in OFV decrease ≥ 3.84 ($X^2$, df=1, $p<0.05$) and their exclusion from the full model resulted in an OFV increase ≥ 6.63 ($X^2$, df=1, $p<0.01$). The effect of continuous covariates; age, CrCl calculated using Cockcroft and Gault equation using ideal body weight (IBW) (32), height, actual body weight, IBW calculated using Devine formula (33), adjusted body weight calculated as IBW+0.4 (actual body weight-IBW), body surface area (BSA) calculated using actual body weight, serum albumin and bilirubin were tested towards their effect on F-ara-A Clpop and volume in the central compartment (V1pop). Gender and genotypes were evaluated as categorical covariates. Genotypes were tested as 3 categories (homozygous for major allele, heterozygotes and homozygous for the minor allele). If the number of individuals homozygous for the minor allele was less than 5% then it was combined with the heterozygous group. We did not study the influence of coadministered drugs since there were no known drug interactions occurring with fludarabine in our protocol. All subjects presented with normal aspartate aminotransferase and therefore, it was not studied. The final model was then evaluated using a non-parametric bootstrap approach that evaluated the precision of the final estimated parameters. This approach used random sampling with replacement from the original dataset to generate new datasets (n=1000). The final model was fit to each of these datasets and estimates of parameters were obtained. Bootstrap parameter estimates and their 95% confidence interval were compared to parameter values obtained from the original dataset. Predictive performance of the model was also assessed using visual predictive checks. One thousand datasets were simulated from the final model using a design similar to the original dataset. The 5th, 50th and 95th percentile bands of the simulated predictions along with their 95% prediction intervals were then plotted with superimposed observed concentrations.

### Validation of the Utility of the Pharmacokinetic Clearance Model in an Independent Cohort

We tested the utility of our model in an independent cohort by examining the association between model predicted first dose F-ara-A clearance (Clpred) and predicted $AUC_{0\rightarrow\infty}$ (AUCpred) towards clinical outcomes. Two hundred and forty patients who underwent allogeneic RIC HCT at University of Minnesota from 2008-2014 who received i.v. fludarabine (25-40 mg/m2/day) on days -6 to day -2, i.v. cyclophosphamide 50 mg/kg/day on day -6 and total body irradiation on day -1 were studied. Subject characteristics are shown in Table 1. Approval by the Institutional Review Board and the Cancer Protocol Review Committee was obtained. Patients who had received prior autologous HCT more than a year prior to allogeneic HCT and had not received intensive chemotherapy within the past 3-6 months were administered equine antithymocyte globulin for 3 days. Mycophenolate and cyclosporine were given as maintenance immunosuppression. The administered fludarabine dose, actual body weight, height and serum creatinine on day of admission, demographic data were obtained on each subject. For each patient, F-ara-A Clpred was calculated using the developed model (using equation 9 described later in results

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section) and then using the administered fludarabine dose in F-ara-A equivalents, the AUCpred was determined (using equation 10 described later in results section). Non-relapse mortality was defined as death due to any cause other than relapse or disease progression. Acute GVHD to month 6 was staged and graded according to the standard acute GVHD criteria based on clinical and pathological criteria. Day of neutrophil engraftment was the first of 3 consecutive days of an absolute neutrophil count >500 cells/uL by day 42.

Recursive partitioning regression analysis was performed in the independent cohort to select optimal cut points for model predicted F-ara-A Clpred and F-ara-A AUCpred towards NRM and acute GVHD. Once optimal cut points were identified the cumulative incidence of engraftment, NRM and acute GVHD (grades II-IV) above and below each of the cut points was calculated using death prior to event as a competing risk. An *apriori* two sided log rank test at an alpha level of 0.05 was conducted and a sample size of 240 subjects would detect a 10% or more difference in hazard of NRM in patients above and below F-ara-A Cl cut point with a power of 0.98-1.00

Recipient gender, age, use of ATG in preparative regimen, recipient CMV status, donor source, recipient HLA type (match/mismatch), disease risk, Karnofsky score, BMI, comorbidity score were univariately tested for their influence on NRM (day 100, months 6 and12), acute GVHD (grades II-IV) (month 6). Additionally acute GVHD (grades II-IV) was tested as a time-dependent covariate towards NRM. Allele or antigen mismatch at one (7/8) of the loci (HLA-A, -B, -C and DRB1) was defined as HLA mismatch. HLA mismatch was identified under low resolution for 235 patients and on high resolution for 5 patients. Standard disease risk was defined as acute leukemia in first or second remission, CML in chronic phase, NHL and other malignancies in first and second remission and non-malignant diseases; all other malignancies were classified as high risk. Comorbidity score was defined as described in Sorror et al.(34)

Fine and Gray regression was used to estimate the effect of model predicted F-ara-A Clpred and F-ara-A AUCpred towards time to NRM at day 100, months 6 and 12, time to acute GVHD (grades II-IV) at month 6, and time to neutrophil engraftment at day 42 adjusting for clinical covariates that were significant in the univariate analysis (full models).(35). Reduced models for each endpoint was then created using backward selection method by eliminating covariates from the full model with a p-value of >0.20. An a priori p-value of 0.025 was set to identify significant covariates in the reduced model accounting for multiple testing. Statistical analysis was performed with SAS 9.3 (SAS Institute, Cary, NC) system) and R Statistical Software (Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org).

## Results

### Development of F-Ara-A Clearance Model (Clpop) and Covariates Influencing Clearance

A two-compartment model with i.v. administration best described F-ara-A pharmacokinetics. Typical value of pharmacokinetic parameters, between subject variability and residual unexplained variability estimates are provided in Table 2.

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Creatinine clearance significantly influenced F-ara-A Clpop. We calculated Clpop as a sum of the renal and nonrenal Cl. Body size measures (actual body weight, IBW, adjusted body weight and BSA) also significantly influenced Clpop. We conservatively chose IBW for further scaling with a power of 0.75 (equation 7) and weight standardization (equation 8). Our previous work showed that a high AUC was associated with more treatment related mortality, and using total body weight in obese patients increases the dose administered thereby placing patients at higher risk of high AUC.(15) Age, as a continuous covariate on Clpop, significantly reduced the OFV during forward inclusion, but was not significant during backward elimination. None of the other tested covariates were significant towards Clpop including the genetic variants. The final model for F-ara-A Clpop is shown in equation 9.

$$\text{F-ara-A Clpop} = (\text{Cl}_{\text{nr}} + \text{Cl}_{\text{slope}} \times \text{RenFunc}_{\text{std}}) \times (\text{IBW}/70)^{0.75} \quad \text{(equation 7)}$$

$$\text{RenFunc}_{\text{std}} = (\text{CrCl}/85) \times (70/\text{IBW}) \quad \text{(equation 8)}$$

$$\text{F-ara-A Clpop(L/hr)} = [7.04 + 3.90 \times \{(\text{CrCl}/85) \times (70/\text{IBW})\}] \times (\text{IBW}/70)^{0.75} \quad \text{(equation 9)}$$

$\text{Cl}_{\text{nr}}$ is the nonrenal clearance of F-ara-A; $\text{Cl}_{\text{slope}}$ is the change in renal clearance with a unit change in standardized renal function (*RenFunc*$_{std}$). $\text{RenFunc}_{\text{std}}$ is the CrCl as calculated by Cockcroft and Gault using IBW, and then standardized by IBW (equation 8). The Cockcroft and Gault equation included IBW and therefore CrCl and IBW were highly correlated in the model. To effectively eliminate this correlation, renal clearance was first IBW-standardized as described by Mould et al.(36) The renal function was centered using the mean CrCl (85 ml/min) observed in the study population. Clpop was scaled using IBW (equation 7).

The estimates of $\text{Cl}_{\text{slope}}$ and $\text{Cl}_{\text{nr}}$ were 3.90 L/hr per standardized CrCl (CrCl/85 ml/min × 70kg/IBW) per 70 kg IBW and 7.04 L/hr per 70 kg IBW, respectively. Using these estimates the Clpop for a standard 70 kg IBW subject with a CrCl of 85 mL/min was 10.9 L/hr (3.90 L/hr + 7.04 L/hr). For this standard subject, renal clearance accounts for 35.6% of Clpop and for every 10 unit decrease in CrCl, total F-ara-A Clpop decreases by 0.46 L/hr.

The diagnostic plots (Supplementary Figures S1A-D) were used to examine the goodness of fit of the model and demonstrated that the model adequately explained the observed data and there was no evidence of model misspecification.

The visual predictive check (Supplementary Figure S2) shows that the model reasonably describes the data and that no systematic deviation between observed and simulated data was

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observed. Our final model was also evaluated for its reliability by non-parametric bootstrap. Out of 1000 datasets generated, 930 minimized successfully. Table 2 shows the median of each estimate with 95% confidence intervals obtained from the bootstrap datasets. Estimates for pharmacokinetic parameters, inter- and intraindividual variability are similar and lie within 5% of the estimates obtained from the final model, indicating that the model is robust and reproducible.

## The Relationships between F-ara-A Clpred and AUCpred with Clinical Outcomes in an Independent Cohort

For each individual in the independent cohort, CrCl and IBW obtained on day -7 pre-HCT were used in equation 9 to predict F-ara-A Clpred. The F-ara-A AUCpred was then estimated for each individual by the formula;

$$\text{F-ara-A AUCpred} = \text{F-ara-A equivalent dose(mg)} / \text{F-ara-A Clpred} \quad \text{(equation 10)}$$

F-ara-A equivalent dose was calculated as administered fludarabine phosphate dose /1.28. Creatinine clearances were capped at 150 ml/min since values greater than those seemed implausible. The median (range) F-ara-A Clpred, F-ara-A AUCpred and the administered fludarabine dose were 10.9 (7.51-15.4) L/hr, 4.85 (2.82-7.52) μg*hr/mL and 67 (42-100) mg in the independent cohort.

The cumulative incidence of NRM was 8, 13 and 19% at day 100, 6 and 12 months, respectively, in the independent cohort. The median (range) time to NRM was 165 (18-1518) days. Optimal cut points towards NRM for F-ara-A Clpred and AUCpred were 8.50 L/hr and 6.00 μg*hr/mL, respectively.

More rapid F-ara-A Clpred was associated with less NRM. In univariate analysis, the cumulative incidence (95% CI) of NRM at day 100 in patients with F-ara-A Clpred <8.50 L/hr was 25% (6-43) as compared to 6% (3-10) in patients with F-ara-A Clpred ≥8.50 L/hr ($p<0.01$) (Figure 1A). In univariate analysis, donor source, HLA mismatch, high-risk disease, comorbidity score ≥3 and acute GVHD (grades II-IV) before NRM were each significant towards higher NRM and were chosen for adjustment in the full model. Fludarabine dose was not associated with NRM (25-40mg/$m^2$/day). The multivariate regression reduced models after backward elimination are shown in Tables 3 and 4. At day 100 the hazard ratio (HR) of NRM in patients remained significantly lower in patients with F-ara-A Clpred ≥8.50 L/hr as compared to <8.50 L/hr [HR (95% CI) 0.1 (0.02-0.42), $p<0.01$], after adjusting for donor source, disease risk, comorbidity score, and acute GVHD (grades II-IV) before NRM (Table 3). A lower F-ara-A Clpred was also associated with greater NRM at month 6 (Table 3). Cumulative incidence of NRM (95% CI) at day 100 was significantly higher in patients with F-ara-A AUCpred ≥6.00 μg *hr/mL as compared to <6.00 μg*hr/mL [22% (8-37) vs 6% (3-9)($p<0.01$)] (Figure 1B). Results of multivariate regression of F-ara-A AUCpred and NRM at day 100, months 6 and 12, adjusted for clinical covariates are shown in Table 4. The total number and percent of patients with NRM events

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at day 100, months 6 and 12 in each covariate group are shown in supplementary Tables 2A and B.

The cumulative incidence of acute GVHD (grades II-IV) at month 6 was 43% in the independent cohort. F-ara-A Clpred and AUCpred optimal cut points were 13.0 L/hr and 6.00 μg*hr/mL towards acute GVHD, respectively. In univariate analysis, F-ara-A Clpred ≥13.0 L/hr was associated with lower cumulative incidence (95% CI) of acute GVHD (grades II-IV) at month 6 as compared to F-ara-A Clpred <13.0 L/hr [23% (7-39) vs [45% (38-52), p=0.04] (Figure 1C). In multivariate analysis, F-ara-A Clpred ≥13.0 L/hr also had a lower the hazard of acute GVHD at month 6 after adjusting for clinical factors but was not significant [HR (95% CI) 0.44 (0.19-1.02), p=0.05] (Table 5). F-ara-A AUCpred was not associated with acute GVHD (grades II-IV) in univariate analysis (p=0.05). The total number and percent of patients with acute GVHD events at month 6 in each covariate group are shown in supplementary Table 2C.

Ninety seven percent of patients engrafted within day 42 and therefore, none of the F-ara-A pharmacokinetics or clinical variables were significant towards engraftment given the small event rate (data not shown).

## Discussion

Reduced intensity conditioning for allo-HCT is increasingly common. These patients often present with comorbid conditions such as compromised renal function and obesity. Comorbid conditions may affect drug clearance leading to over or under dosing of chemotherapy and poor outcomes. Non-relapse mortality remains high in RIC HCT(37-42), which may in part be due to our inability to predict an individual's capacity for chemotherapy clearance. An understanding of the clinical factors associated with drug clearance and conditioning regimen intensity is critical in improving outcomes. In this study we identified factors affecting fludarabine clearance and developed an individualized dosing model from 87 adult patients undergoing RIC HCT that accounts for these factors. We then evaluated the utility of our model and identified F-Ara-A clearance and AUCs associated with poor clinical outcomes in a large independent cohort.

We found that CrCl and body weight significantly influenced F-ara-A clearance. In chronic lymphocytic leukemia patients who received fludarabine (25 mg/m$^2$ for 5 days every 28 days) with a CrCl less than 80 ml/min had a significantly greater probability of toxicity as compared to those greater than 80 ml/min (p<0.001).(43) The fludarabine package insert recommends a dose reduction of 20% for a CrCl of 30-70 ml/min and avoidance if CrCl <30 ml/min.(44) These dose reductions for renal function are important but unfortunately are quite imprecise since a patient with a CrCl of 70 ml/min would receive the same dose reduction as an individual with a CrCl of 30 ml/min. Our data showed that renal clearance accounts for over one third of total clearance and that for every 10 unit decrease in CrCl in the typical patient the total F-ara-A clearance decreases by ~5% therefore small changes in CrCl are relevant towards elimination. Because we modeled CrCl as a continuous variable, precise dose reductions for any CrCl are possible.

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Dosing of chemotherapy in obese patients is a growing clinical problem given the increasing number of overweight and obese patients presenting for HCT. The 2014 ASBMT guidelines on chemotherapy dose adjustments in HCT found insufficient data to support level 1 or 2 recommendations in overweight individuals.(22) A review of fludarabine studies in the guideline found that trials mainly used total body weight to estimate BSA and fludarabine doses; however, evidence for the basis of using total body weight was lacking. A recent ASCO guidance recommended for solid tumors that actual body weight be used for chemotherapy dose calculation.(45) In our study, 33.3% of subjects were overweight (BMI 25.0-29.9), 23.0% obese (BMI 30.0-34.9) and 11.5% morbidly obese (BMI>35.0), therefore, weight is an problem for many patients. In clinical practice the use of total body weight in patients with obesity results in higher chemotherapy doses than IBW. In our analyses all body size measures were associated with F-ara-A clearance and since many of our patients presenting for HCT are obese we chose a conservative approach and used IBW to develop the final model. We previously found that high F-ara-A concentrations in our RIC protocol were associated with greater treatment related mortality and our goal is to improve the safety.(15) We found that as IBW increased, F-ara-A clearance also increases thereby increasing dose requirements. Our data are consistent with a previous population pharmacokinetic analysis in HCT recipients, which also found that all tested body size measures (BSA using adjusted IBW, height, actual body weight, adjusted IBW) were associated with pharmacokinetic parameters.(21) Our developed model adequately explained the observed data as shown in the diagnostic plots and visual predictive checks, with robust parameter estimates obtained though bootstrap model evaluations.

We also evaluated our pharmacokinetic clearance model in an independent cohort of 240 RIC HCT recipients and determined if model predicted F-ara-A clearance and predicted AUC were associated with clinical outcomes. In multivariate analysis patients with a predicted F-ara-A clearance <8.50 L/hr had a 10 times higher hazard of NRM as compared to clearance ≥8.50 L/hr at day 100. F-ara-A predicted clearance remained associated with NRM at month 6 (Table 3). In addition, F-ara-A predicted AUC >6.00 μg*hr/mL had a 5.30 times greater hazard of NRM at day 100 (Table 4). We also observed a higher hazard of acute GVHD (grades II-IV) in those with high predicted F-ara-A clearance in univariate analysis but it was not significant.

These data are consistent with our previous study where HCT recipients receiving RIC with fludarabine (40mg/$m^2$ × 5 days), cyclophosphamide and TBI with an F-ara-A AUC >6.50 μg*hr/mL had a 4.56 greater risk of treatment related mortality.(15) In a small study of 16 patients receiving fludarabine 50mg/$m^2$/day × 5 days with pharmacokinetic guided busulfan and rATG, an F-ara-A AUC above the mean (24.8 μM*hr or 7.07 μg*hr/mL) trended towards a higher hazard for non-relapse mortality (HR=5.2, p=0.10) and overall mortality (HR=3.4, p=0.12). Unfortunately, the study was closed early due to high toxicity.(17) In a small study of 42 subjects receiving fludarabine 30mg/$m^2$ days -6 to -3 and concentration-controlled busulfan dosing, no association was observed between mean F-ara-A AUC (19.1 μM/hr or 5.44 μg*hr/mL) and engraftment or T-cell chimerism but NRM was not evaluated. (11) A recent study by same group in 102 patients receiving fludarabine 30mg/$m^2$/day for 4 consecutive days followed by TBI on day of HCT, found no association between F-ara-A AUC and NRM and acute GVHD (grades II-IV).(16) A letter to the editor reported on 166

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HCT recipients receiving fludarabine 50mg/m$^2$ days -6 to -2 and busulfan with or without TBI.(12) F-ara-A concentrations on day of HCT were not associated with risk of acute GVHD, CMV reactivation, risk of relapse, or death due to any cause. These data may suggest that when fludarabine is combined with busulfan or given in a conditioning regimen using four or fewer doses of fludarabine the exposure response relationships may be modest. However, when fludarabine (30-40 mg/m2/day) is given as 5 consecutive days in combination with cyclophosphamide and TBI there may be a strong concentration dependent effect on outcomes.

Central to individualizing fludarabine doses is an understanding of the therapeutic blood target range for RIC. Considering our results, a first dose F-ara-A AUC >6.00 μg*hr/mL carries an unacceptable risk of mortality. Therefore, it is likely that an upper limit AUC is between 4.50-5.50 μg*hr/mL for 5 days when combined with cyclophosphamide and TBI. Future studies should be directed at defining the lowest plasma exposure required to minimize toxicity without compromising efficacy.

An example of how our model can be applied to the clinical setting in a patient with a low CrCl is as follows. Consider an adult patient with an IBW of 53 kg, height of 161 cm, CrCl of 45 ml/min and a BSA of 2m$^2$ calculated using actual body weight. Assume a desired F-ara-A AUC of 5.0 μg*hr/mL. The fludarabine dose would be estimated as follows:

**Step 1: Determine F-ara-A clearance using equation 9**

$$\text{F}-\text{ara}-\text{A Clpred}(\text{L}/\text{hr})=[7.04+3.90\times\{(\text{CrCl}/85)\times(70/\text{IBW})\}]\times(\text{IBW}/70)^{0.75}$$

F-ara-A Clpred for the example = [7.04 +3.90 × {(45/85) × (70/53)}] × (53/70)$^{0.75}$ = 7.90 L/hr

**Step 2: Determine F-ara-A predicted dose using equation 11**

Once the F-ara-A Clpred is estimated for an individual and a target F-ara-A AUC is selected by the clinician, the optimal dose to achieve the AUC target for any individual can be estimated.

$$\text{Predicted daily F}-\text{ara}-\text{A dose}(\text{mg})=\text{Desired AUC}(\mu\text{g}*\text{hr}/\text{mL})\times\text{F}-\text{ara}-\text{A Clpred}(\text{L}/\text{hr})$$

Predicted daily F-ara-A dose (mg) = 5 μg*hr/mL × 7.90 L/hr = 39.5 mg/day

**Step 3: Determine fludarabine phosphate dose using the following**

Since the drug is administered as fludarabine phosphate, the F-Ara-A estimate (MW 285.23) must be converted to an equivalent of fludarabine phosphate (MW 365.2).

$$\text{Final daily fludarabine phosphate dose mg}=\text{Predicted F}-\text{ara}-\text{A dose}(\text{mg})*1.28$$

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Fludarabine phosphate dose for the example = 39.5 × 1.28 = 50.5 mg/day i.v.

For this individual, traditional dosing based on BSA alone at 35mg/m$^2$/day would give a dose of 70 mg. Due to the patients renal dysfunction a dose reduction of 20% may be made, if manufacturer's recommendations were followed, and the final dose would be 56 mg/day. Our model estimated a dose of 50.5 mg/day, which is lower and better accounts for reduced renal function.

One of the limitations of our study is that no patient had a CrCl<45 ml/min in our model development cohort and it is not known if the model is sufficient for lower CrCls; however, patients with CrCl lower than 45 ml/min are generally excluded from HCT. Due to the low frequency of some of our genetic variants our samples size may have been too small to detect changes in the pharmacokinetics especially given the strong effect of renal function and weight. Therefore, future larger studies should reevaluate these and other genetic variants. Most of our patients engrafted by day 42 and we were unable to assess its relationship to F-ara-A pharmacokinetics. The minimum F-ara-A target AUC required to maintain efficacy towards engraftment will require a larger analyses and consideration of the immunosuppressive drugs comprising the conditioning regimens to sustained immunosuppression.

We provide evidence that body size and CrCl significantly influences F-ara-A pharmacokinetics and have developed an individualized fludarabine dosing equation to personalize fludarabine dose using IBW and accounting for CrCl. This equation would be most useful in overweight individuals and in those with renal dysfunction where traditional BSA dosing may overestimate their dosing requirements. Finally we evaluated the model in an independent cohort that found that predicted F-ara-A clearance and AUC are highly significant towards NRM even after adjusting for clinical variables. This model offers a method to personalize fludarabine dosing and control systemic exposure to reduce adverse clinical outcomes. Future studies using the equation should focus on refining the model prospectively, considering the effect of cyclophosphamide exposure on outcomes, and creating pediatric models. It is time to reconsider our long standing practice in HCT of one size fits all dosing.

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## References

1. Abdul Wahid SF, Ismail NA, Mohd-Idris MR, et al. Comparison of reduced-intensity and myeloablative conditioning regimens for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia and acute lymphoblastic leukemia: a meta-analysis. Stem Cells and Development. 2014; 23(21):2535–52. [PubMed: 25072307]

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2. Aoki J, Kanamori H, Tanaka M, et al. Impact of age on outcomes of allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning in elderly patients with acute myeloid leukemia. Am J Hematol. 2015 Epub ahead of print.

3. Oran B, Weisdorf DJ. Survival for older patients with acute myeloid leukemia: a population-based study. Haematologica. 2012; 97(12):1916–24. [PubMed: 22773600]

4. Schneidawind D, Federmann B, Buechele C, et al. Reduced-intensity conditioning with fludarabine and busulfan for allogeneic hematopoietic cell transplantation in elderly or infirm patients with advanced myeloid malignancies. Ann Hematol. 2016; 95(1):115–24. [PubMed: 26411736]

5. Tomblyn M, Brunstein C, Burns LJ, et al. Similar and promising outcomes in lymphoma patients treated with myeloablative or nonmyeloablative conditioning and allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2008; 14(5):538–45. [PubMed: 18410896]

6. Avramis VI, Champagne J, Sato J, et al. Pharmacology of fludarabine phosphate after a phase I/II trial by a loading bolus and continuous infusion in pediatric patients. Cancer Res. 1990; 50(22): 7226–31. [PubMed: 1699658]

7. Beitinjaneh A, McKinney AM, Cao Q, Weisdorf DJ. Toxic leukoencephalopathy following fludarabine-associated hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2011; 17(3):300–8. [PubMed: 20399878]

8. Chun HG, Leyland-Jones BR, Caryk SM, Hoth DF. Central nervous system toxicity of fludarabine phosphate. Cancer Treat Rep. 1986; 70(10):1225–8. [PubMed: 2428492]

9. Grever M, Leiby J, Kraut E, et al. A comprehensive phase I and II clinical investigation of fludarabine phosphate. Semin Oncol. 1990; 17(5 Suppl 8):39–48. [PubMed: 1699282]

10. Malspeis L, Grever MR, Staubus AE, Young D. Pharmacokinetics of 2-F-ara-A (9-beta-D-arabinofuranosyl-2-fluoroadenine) in cancer patients during the phase I clinical investigation of fludarabine phosphate. Semin Oncol. 1990; 17(5 Suppl 8):18–32. [PubMed: 1699279]

11. Bornhauser M, Storer B, Slattery JT, et al. Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. Blood. 2003; 102(3):820–6. [PubMed: 12676781]

12. Griffiths CD, Ng ES, Kangarloo SB, et al. Fludarabine metabolite level on day zero does not affect outcomes of hematopoietic cell transplantation in patients with normal renal function. Bone Marrow Transplant. 2014; 49(4):589–91. [PubMed: 24464143]

13. Hersh MR, Kuhn JG, Phillips JL, et al. Pharmacokinetic study of fludarabine phosphate (NSC 312887). Cancer Chemother Pharmacol. 1986; 17(3):277–80. [PubMed: 2427240]

14. Knebel W, Davis JC Jr, Sanders WD, et al. The pharmacokinetics and pharmacodynamics of fludarabine in rheumatoid arthritis. Pharmacotherapy. 1998; 18(6):1224–9. [PubMed: 9855320]

15. Long-Boyle JR, Green KG, Brunstein CG, et al. High fludarabine exposure and relationship with treatment-related mortality after nonmyeloablative hematopoietic cell transplantation. Bone Marrow Transplant. 2011; 46(1):20–6. [PubMed: 20383215]

16. McCune JS, Mager DE, Bemer MJ, et al. Association of fludarabine pharmacokinetic/dynamic biomarkers with donor chimerism in nonmyeloablative HCT recipients. Cancer Chemother Pharmacol. 2015; 76(1):85–96. [PubMed: 25983023]

17. McCune JS, Woodahl EL, Furlong T, et al. A pilot pharmacologic biomarker study of busulfan and fludarabine in hematopoietic cell transplant recipients. Cancer Chemother Pharmacol. 2012; 69(1): 263–72. [PubMed: 21909959]

18. Yin W, Karyagina EV, Lundberg AS, Greenblatt DJ, Lister-James J. Pharmacokinetics, bioavailability and effects on electrocardiographic parameters of oral fludarabine phosphate. Biopharm Drug Dispos. 2010; 31(1):72–81. [PubMed: 19862681]

19. Bemer MJ, Sorror M, Sandmaier BM, O'Donnell PV, McCune JS. A pilot pharmacologic biomarker study in HLA-haploidentical hematopoietic cell transplant recipients. Cancer Chemother Pharmacol. 2013; 72(3):607–18. [PubMed: 23907443]

20. Bonin M, Pursche S, Bergeman T, et al. F-ara-A pharmacokinetics during reduced-intensity conditioning therapy with fludarabine and busulfan. Bone Marrow Transplant. 2007; 39(4):201–6. [PubMed: 17211431]

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21. Salinger DH, Blough DK, Vicini P, et al. A limited sampling schedule to estimate individual pharmacokinetic parameters of fludarabine in hematopoietic cell transplant patients. Clin Cancer Res. 2009; 15(16):5280–7. [PubMed: 19671874]
22. Bubalo J, Carpenter PA, Majhail N, et al. Conditioning chemotherapy dose adjustment in obese patients: a review and position statement by the American Society for Blood and Marrow Transplantation practice guideline committee. Biol Blood Marrow Transplant. 2014; 20(5):600–16. [PubMed: 24462742]
23. Kemena A, Fernandez M, Bauman J, Keating M, Plunkett W. A sensitive fluorescence assay for quantitation of fludarabine and metabolites in biological fluids. Clin Chim Acta. 1991; 200(2-3): 95–106. [PubMed: 1723357]
24. Kuo GM, Boumpas DT, Illei GG, et al. Fludarabine pharmacokinetics after subcutaneous and intravenous administration in patients with lupus nephritis. Pharmacotherapy. 2001; 21(5):528–33. [PubMed: 11349741]
25. Lichtman SM, Etcubanas E, Budman DR, et al. The pharmacokinetics and pharmacodynamics of fludarabine phosphate in patients with renal impairment: a prospective dose adjustment study. Cancer Invest. 2002; 20(7-8):904–13. [PubMed: 12449721]
26. Bodge MN, Reddy S, Thompson MS, Savani BN. Preparative regimen dosing for hematopoietic stem cell transplantation in patients with chronic kidney disease: analysis of the literature and recommendations. Biol Blood Marrow Transplant. 2014; 20(7):908–19. [PubMed: 24565993]
27. Baker JA, Wickremsinhe ER, Li CH, et al. Pharmacogenomics of gemcitabine metabolism: functional analysis of genetic variants in cytidine deaminase and deoxycytidine kinase. Drug Metab Dispos. 2013; 41(3):541–5. [PubMed: 23230131]
28. Farrell JJ, Bae K, Wong J, et al. Cytidine deaminase single-nucleotide polymorphism is predictive of toxicity from gemcitabine in patients with pancreatic cancer: RTOG 9704. Pharmacogenomics J. 2012; 12(5):395–403. [PubMed: 21625252]
29. Khatri A, Williams BW, Fisher J, et al. SLC28A3 genotype and gemcitabine rate of infusion affect dFdCTP metabolite disposition in patients with solid tumours. Br J Cancer. 2014; 110(2):304–12. [PubMed: 24300978]
30. Wong AL, Yap HL, Yeo WL, et al. Gemcitabine and platinum pathway pharmacogenetics in Asian breast cancer patients. Cancer Genomics Proteomics. 2011; 8(5):255–9. [PubMed: 21980041]
31. Yue L, Saikawa Y, Ota K, et al. A functional single-nucleotide polymorphism in the human cytidine deaminase gene contributing to ara-C sensitivity. Pharmacogenetics. 2003; 13(1):29–38. [PubMed: 12544510]
32. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976; 16(1):31–41. [PubMed: 1244564]
33. Pai MP, Paloucek FP. The origin of the “ideal” body weight equations. Ann Pharmacother. 2000; 34(9):1066–9. [PubMed: 10981254]
34. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005; 106(8): 2912–9. [PubMed: 15994282]
35. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999; 94(446):496–509.
36. Mould DR, Holford NH, Schellens JH, et al. Population pharmacokinetic and adverse event analysis of topotecan in patients with solid tumors. Clin Pharmacol Ther. 2002; 71(5):334–48. [PubMed: 12011819]
37. Brunstein CG, Eapen M, Ahn KW, et al. Reduced-intensity conditioning transplantation in acute leukemia: the effect of source of unrelated donor stem cells on outcomes. Blood. 2012; 119(23): 5591–8. [PubMed: 22496153]
38. Verneris MR, Lee SJ, Ahn KW, et al. HLA Mismatch Is Associated with Worse Outcomes after Unrelated Donor Reduced-Intensity Conditioning Hematopoietic Cell Transplantation: An Analysis from the Center for International Blood and Marrow Transplant Research. Biol Blood Marrow Transplant. 2015; 21(10):1783–9. [PubMed: 26055300]

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39. Warlick ED, Peffault de Latour R, Shanley R, et al. Allogeneic hematopoietic cell transplantation outcomes in acute myeloid leukemia: similar outcomes regardless of donor type. Biol Blood Marrow Transplant. 2015; 21(2):357–63. [PubMed: 25452032]

40. Weisdorf D, Eapen M, Ruggeri A, et al. Alternative donor transplantation for older patients with acute myeloid leukemia in first complete remission: a center for international blood and marrow transplant research-eurocord analysis. Biol Blood Marrow Transplant. 2014; 20(6):816–22. [PubMed: 24582782]

41. Weisdorf D, Zhang MJ, Arora M, et al. Graft-versus-host disease induced graft-versus-leukemia effect: greater impact on relapse and disease-free survival after reduced intensity conditioning. Biol Blood Marrow Transplant. 2012; 18(11):1727–33. [PubMed: 22766220]

42. Raj K, Pagliuca A, Bradstock K, et al. Peripheral blood hematopoietic stem cells for transplantation of hematological diseases from related, haploidentical donors after reduced-intensity conditioning. Biol Blood Marrow Transplant. 2014; 20(6):890–5. [PubMed: 24650678]

43. Martell RE, Peterson BL, Cohen HJ, et al. 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. Cancer Chemother Pharmacol. 2002; 50(1):37–45. [PubMed: 12111110]

44. Fludara(R). Wayne, NJ: Bayer HealthCare Pharmaceuticals Inc.; 2008. http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020038s032lbl.pdf

45. Griggs JJ, Mangu PB, Anderson H, et al. Appropriate chemotherapy dosing for obese adult patients with cancer: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol. 2012; 30(13):1553–61. [PubMed: 22473167]

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**Background**

High fludarabine plasma concentrations are associated with greater non-relapse mortality. Patients undergoing reduced intensity hematopoietic cell transplant are older, more likely to have mild to moderate renal dysfunction, and over the past decade, more likely to be obese. Fludarabine dose reductions are common for obese and/or those with renal dysfunction; however, there are no data to guide these reductions.

**Translational Significance**

We developed a dosing model, which estimates a dose, taking into account creatinine clearance and body size. Accurately accounting for kidney function and obesity will reduce the probability of overexposure to fludarabine and reduce non-relapse mortality.

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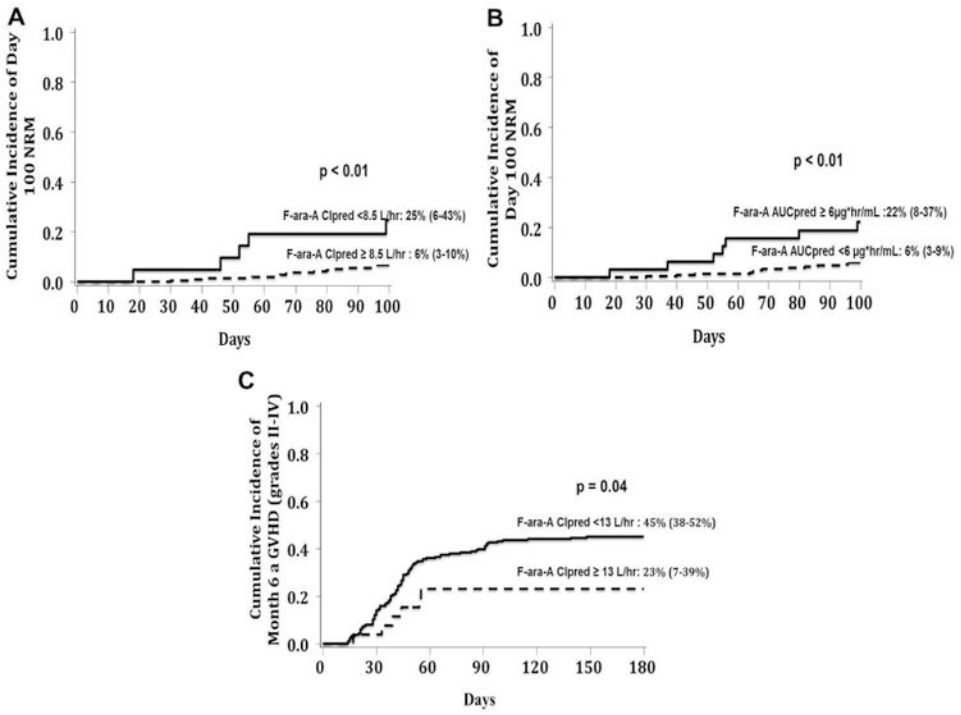


**Figure 1.**

A) Cumulative incidence of NRM day 100 after RIC HCT in patients with first dose F-ara-A Clpred <8.50L/hr (cumulative incidence [95%CI] 25% [6-43%]) compared to patients with F-ara-A Clpred ≥ 8.50 L/hr (cumulative incidence [95%CI]: 6% [3-10%]).

B) Cumulative incidence of NRM day 100 after RIC HCT in patients with first dose F-ara-A AUCpred ≥ 6.00 µg*hr/mL (cumulative incidence [95%CI] 22% [8-37%]) compared to patients with F-ara-A AUCpred <6.00 µg*hr/mL (cumulative incidence [95%CI] 6% [3-9%]).

C) Cumulative incidence of acute GVHD (grades II-IV) at month 6 after RIC HCT in patients with first dose F-ara-A Clpred <13.0 L/hr (cumulative incidence [95%CI] 45% [38-52%]) compared to patients with F-ara-A Clpred ≥13.0 L/hr (cumulative incidence [95%CI] 23% [7-39%]).

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## Table 1

### Subject Characteristics

| | Development Cohort Median (range)/N (%) | Independent Cohort Median (range)/N (%) |
|---|---|---|
| Number of Patients | 87 | 240 |
| Administered single day dose (mg), median (range) | 75 (46-100) | 67 (42-100) |
| Age (years), median (range) | 55 (20-69) | 59 (19-75) |
| Males, N (%) | 56 (64.4) | 139 (57.9) |
| Actual body weight (kg), median (range) | 82.5 (41.5-140) | 84.4 (46.6-183) |
| Ideal body weight (kg), median (range) | 65.9 (40.6-81.0) | 67.8 (97.7-42.0) |
| Body surface area (m2), median (range) | 1.95 (1.30-2.50) | 1.98 (1.38-3.12) |
| BMI, N% ($kg/m^2$) | | |
| <25 | 28(32.2%) | 64(26.7%) |
| 25-29.9 | 29(33.3%) | 81(33.8%) |
| 30-34.9 | 20(23.0%) | 58(24.2%) |
| >35 | 10(11.5%) | 37(15.4%) |
| Serum creatinine (mg/dL), median (range) | 0.90 (0.40-1.50) | 0.82 (0.32-1.96) |
| Creatinine clearance (ml/min), median (range) | 82.1 (45.0-153) | 88.0 (29.3-206)[a] |
| Total bilirubin (mg/dL), median (range) | 0.40 (0.10-1.20) | Not collected |
| Recipient CMV positive, N (%) | 45 (51.7) | 135 (56.3) |
| Disease, N (%) | | |
| Acute lymphoid leukemia | 6 (7.00%) | 22 (9.16%) |
| Acute myeloid leukemia | 26 (30.0%) | 71 (29.6%) |
| Chronic myeloid leukemia | 1 (1.00%) | 6 (2.50%) |
| Other leukemias[b] | 6 (7.00%) | 21 (8.75%) |
| Myelodysplastic syndrome | 14 (16.0%) | 40 (16.7%) |
| Non-Hodgkin's lymphoma | 17 (20.0%) | 36 (15.0%) |
| Hodgkin's lymphoma | 8 (9.00%) | 15 (6.25%) |
| Other Malignancies[c] | 9 (10.0%) | 29 (12.1%) |
| Donor Source, N (%) | | |
| Cord blood | 64 (73.3%) | 104 (43.3%) |
| Related | 22 (25.3%) | 35 (14.6%) |
| Unrelated | 1 (0.01%) | 101 (42.1%) |

[a] Estimated creatinine clearances are shown but were capped at 150 ml/min in the analysis since values greater than those seemed implausible

[b] Other leukemias are acute myeloid leukemias not otherwise specified and chronic lymphocytic leukemias, not otherwise specified as per standard WHO criteria.

[c] Other Malignancies are myelofibrosis, prolymhocytic leukemia, and renal cell carcinoma

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## Table 2

**F-ara-A pharmacokinetic parameter estimates of model estimated parameters and bootstrap estimates in the development cohort**

| Parameters | Original Dataset (%RSE) | Bootstrap Estimates (95% C.I.) |
|---|---|---|
| Final Pharmacokinetic Parameters | | |
| $Cl_{nr}$ (L/hr) [a] | 7.04 (14.1%) | 6.95 (5.01-9.01) |
| $Cl_{slope}$ (L/hr) [a] | 3.90 (25.2%) | 4.02 (1.99-5.98) |
| V1pop (L) | 65.9 (2.90%) | 65.9 (62.2- 70.1) |
| Qpop (L/hr) | 9.52 (6.20%) | 9.58 (8.41-10.9) |
| V2pop (L) | 67.2 (6.70%) | 66.7 (56.7-77.5) |
| Between Subject Variability (BSV) | | |
| BSV on Cl | 0.07 | 0.07 (0.04-0.09) |
| | CV% =26.5 | CV% =26.4 (20.0-30.0%) |
| BSV on V1 | 0.06 | 0.06 (0.03-0.09) |
| | CV% = 24.5 | CV% =24.5 (17.3-30.0%) |
| Residual unexplained variability (RUV) | | |
| RUV proportional | 0.05 (15.4%) | 0.05 (0.03-0.07) |
| | CV% =22.3 | CV% = 22.3 (17.3-26.5%) |
| RUV additive | 11.2 ng/ml (27.9%) | 10.5 ng/mL (3.73-16.5) |

[a] F-ara-A Clpop is 10.94 L/hr which is a sum of estimate of $Cl_{nr}$ (7.09 L/hr) for 70 kg IBW individual and $Cl_{slope}$ (3.90 L/hr) for 70 kg IBW individual with CrCl of 85 ml/min.

$Cl_{nr}$: is an estimate of non-renal clearance;

$Cl_{slope}$ is an estimate of the change in renal clearance with a unit change in standardized renal function ($RenFunc_{std}$);

V1pop: Estimate of typical volume of distribution in central compartment;

Qpop: Estimate of typical inter-compartmental clearance;

V2pop: Estimate of typical volume of distribution in peripheral compartment.

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**Table 3**

**Multiple Regression Analysis of NRM at Day 100, Months 6 and 12 with Predicted F-ara-A Clearance (Clpred) in the Independent Cohort**

| Variable | Hazard Ratio (95% CI) at day 100 | p-value | Hazard Ratio (95% CI) at month 6 | p-value | Hazard Ratio (95% CI) at month 12 | p-value |
|---|---|---|---|---|---|---|
| **F-ara-A Clpred** | | | | | | |
| <8.50 L/hr | 1.00 | | 1.00 | | 1.00 | |
| ≥8.50 L/hr | 0.10 (0.02-0.42) | <0.01 | 0.19 (0.05-0.70) | 0.01 | 0.41 (0.17-1.00) | 0.05 |
| **Donor source** | | | | | | |
| Related | 1.00 | | 1.00 | | 1.00 | |
| Unrelated (UR) | 4.13 (1.03-16.6) | 0.05 | 2.84 (1.05-7.69) | 0.04 | 3.24 (1.53-6.99) | <0.01 |
| UR cord blood | 3.92 (1.28-12.0) | 0.02 | 2.30 (0.93-5.69) | 0.07 | 1.60 (0.75-3.39) | 0.22 |
| **Disease risk** | | | | | | |
| Standard | 1.00 | | 1.00 | | | |
| High | 3.98 (0.80-19.7) | 0.09 | 2.94 (0.95-9.12) | 0.06 | NA | NA |
| **Comorbidity score** | | | | | | |
| 0 | 1.00 | | 1.00 | | 1.00 | |
| 1-2 | 3.12 (0.93-10.5) | 0.07 | 2.77 (1.19-6.48) | 0.02 | 2.08 (1.02-4.25) | 0.04 |
| ≥3 | 2.20 (0.70-6.96) | 0.18 | 1.48 (0.58-3.80) | 0.41 | 1.66 (0.82-3.36) | 0.16 |
| **Acute GVHD (grades II-IV) before NRM** | | | | | | |
| No | 1.00 | | 1.00 | | 1.00 | |
| Yes | 2.40 (0.92-6.30) | 0.07 | 2.62 (1.22-5.60) | 0.01 | 3.23 (1.69-6.18) | <0.01 |

NA is not applicable and indicates that the covariate was not significant in the multivariate full model (p >0.20) and was eliminated in the final reduced model.

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**Table 4**

**Multiple Regression Analysis of NRM at Day 100, Months 6 and 12 with Predicted F-ara-A AUCpred in the Independent Cohort**

| Parameter | Hazard Ratio (95% CI) at 100 days | p-value | Hazard Ratio (95% CI) at 6 months | P value | Hazard Ratio (95% CI) at 12 months | p-value |
|---|---|---|---|---|---|---|
| **F-ara-A AUCpred** | | | | | | |
| <6.00 (μg*hr/mL) | 1.00 | | 1.00 | | 1.00 | |
| ≥6.00 (μg*hr/mL) | 5.30 (1.59-17.7) | 0.01 | 2.42 (0.87-6.77) | 0.09 | 2.67 (1.31-5.43) | 0.01 |
| **Donor source** | | | | | | |
| Related | 1.00 | | 1.00 | | 1.00 | |
| Unrelated (UR) | 4.32 (1.16-16.06) | 0.03 | 2.79 (1.06-7.31) | 0.04 | 3.35 (1.59-7.05) | <0.01 |
| UR cord blood | 1.91 (0.55-6.67) | 0.31 | 1.71 (0.70-4.13) | 0.24 | 1.33 (0.61-2.86) | 0.47 |
| **Disease risk** | | | | | | |
| Standard | 1.00 | | 1.00 | | | |
| High | 2.84 (0.87-9.33) | 0.08 | 2.27 (0.91-5.65) | 0.08 | NA | NA |
| **Comorbidity score** | | | | | | |
| 0 | 1 | | 1 | | 1 | |
| 1-2 | 2.27 (0.72-7.13) | 0.16 | 2.42 (1.04-5.62) | 0.04 | 1.93 (0.93-4.01) | 0.08 |
| ≥3 | 2.83 (0.83-9.57) | 0.10 | 1.72 (0.66-4.48) | 0.27 | 1.80 (0.88-3.68) | 0.11 |
| **Acute GVHD (grades II-IV) before NRM** | | | | | | |
| No | 1.00 | | 1.00 | | 1.00 | |
| Yes | 1.89 (0.71-5.00) | 0.20 | 2.35 (1.07-5.15) | 0.03 | 3.04 (1.62-5.73) | <0.01 |

NA is not applicable and indicates that the covariate was not significant in the multivariate full model (p >0.20) and was eliminated in the final reduced model.

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## Table 5

**Multiple Regression Analysis of acute GVHD (grades II-IV) at month 6 with Predicted F-ara-A Clearance (Clpred) in the Independent Cohort**

| Variable | Hazard Ratio (95% CI) of acute GVHD (grade II-IV) at 180 days | P-value |
|---|---|---|
| **F-ara-A Clpred** | | |
| <13.0 L/hr | 1.00 | |
| ≥13.0 L/hr | 0.44 (0.19-1.02) | 0.05 |
| **Stem Cell Source** | | |
| Related | 1.00 | |
| Unrelated (UR) | 1.76 (1.08-2.87) | 0.02 |
| UR Cord Blood | 0.75 (0.69-1.66) | 0.75 |