Improving Hematopoietic Cell Transplantation Therapeutics: Emphasis in Pharmacokinetic-Pharmacodynamic Relationships and Pharmacogenomics

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I would like to thank all the patients that contributed to this work. Their willingness to participate in these clinical studies will most certainly lead to better outcomes for many patients in the future and without their participation none of this would be possible.

Dedication

I would like to dedicate this work to my sister, Jennifer, and her two beautiful sons, Wyatt and Noah. It is her story that inspires me to work in pediatric oncology. Wyatt and Noah drive my passion for science, as they are constant reminders that miracles do happen.

Abstract

Treatment-related mortality and acute graft vs host disease remain prominent clinical problems in nonmyeloablative allogeneic hematopoietic cell transplantation (HCT). Hence, the need for improved preparative regimens and immunosuppressive strategies in HCT persists. The research presented in my dissertation will be focused on defining pharmacokinetic-pharmacodynamic relationships, and pharmacogenomics involving two antineoplastic agents, fludarabine and clofarabine, and the immunosuppressive agent, mycophenolate all of which are used in the setting of HCT. Fludarabine is a purine analog commonly used in both adult and pediatric nonmyeloablative allogeneic HCT. Although the pharmacokinetics of fludarabine have been extensively studied in a variety of malignant diseases, very little data is available in nonmyeloablative HCT and the relationship between fludarabine pharmacokinetic parameters and clinical outcomes such as treatment-related mortality have yet to be evaluated. Similarly, no PK data is available for clofarabine; a newer purine analog currently used pediatric patients undergoing HCT for non-hematologic malignancies. Finally, mycophenolic acid pharmacokinetics in HCT recipients displays wide inter- and intra-patient variability in plasma concentrations and low mycophenolate exposure is associated with lower rates of engraftment and greater risk of acute graft vs host disease. Patient characteristics such as weight or body surface area, or clinical markers for hepatic and renal function incompletely explain pharmacokinetic variability suggesting there may be

genetic factors influencing mycophenolate metabolism or transport. The methodologies and techniques employed to evaluate each individual agent will differ, including pharmacokinetic and statistical analyses. However, all projects share the common goal of improving patient outcomes and reducing toxicity in this very complex patient population.

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CHAPTER 1

INTRODUCTION

TREATMENT-RELATED MORTALITY IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOLLOWING NONMYELOABLATIVE CHEMOTHERAPY

1.1 Allogeneic Nonmyeloablative Hematopoietic Cell Transplantation

Allogeneic hematopoietic cell transplantation (alloHCT) is a proven effective treatment for patients with a variety of hematologic and non-hematologic malignancies. Defined as “similar, but not identical”, allogeneic refers to the source of donor stem cells and includes bone marrow, peripheral blood, and umbilical cord blood. Conventional or myeloablative alloHCT uses high dose chemotherapy prior to HCT resulting in ablation of the host immune system. These regimens have been associated with significant morbidity and treatment-related mortality (TRM), and are often limited to younger patients with good clinical status and organ function. In contrast, nonmyeloablative (NM) or reduced-intensity (RI) preparative regimens use lower doses of chemotherapy prior to stem cell rescue, allowing residue host immune cells to remain present. Hence, by reducing toxicity, more patients are able to undergo alloHCT that would otherwise not be suitable candidates for transplant. Additionally, it has been proposed certain hematologic malignancies may require immune-mediated assistance from donor lymphocytes for complete eradication of malignant cells termed the graft-versus-leukemia (GVL) effect.[1] Therefore, NM alloHCT aims to reduce TRM and improve efficacy by lowering the intensity of

chemotherapy administered and promoting eradication of the malignancy through the GVL effect.

Although major advances have been made with NM preparative regimens, high rates of TRM and aGVHD remain. The incidence of TRM varies between clinical trials and is approximately 25-30% at 6 months posttransplant.[2-3] Acute GVHD remains the leading cause of TRM in nonmyeloablative allogeneic HCT, followed by infection, multi-organ failure, and failure of stem cell engraftment.[4] Acute GVHD occurs when donor lymphocytes recognize the recipient or host tissue as foreign. The result is an attack of donor derived lymphocytes on normal, healthy tissue, most commonly the skin, gastro-intestinal track, and liver. The cumulative incidence of aGVHD grades II-IV in NM alloHCT is estimated to be approximately 67%, with more severe grades III-IV around 30%.[5] Mortality with grade IV aGVHD is nearly 100%. Today the mainstay of treatment for the prevention of the development of aGVHD remains prophylaxis with immunosuppressants to inhibit and minimize activity of donor T cells. Mycophenolate, in combination with a calcineurin inhibitor, is now one of the most common immunosuppression regimens used in NM alloHCT. There is wide variability in plasma concentrations of MPA, the active metabolite of mycophenolate, and low concentrations are associated with significantly lower rates of engraftment and increased development of aGVHD.[6] Personalized immunosuppression through pharmacogenomics is one strategy with the potential to reduce the incidence and severity of aGVHD leading to

increased survival and quality of life. Chapter 2 of this dissertation will evaluate the role of genetic variants on mycophenolate pharmacokinetics and clinical outcomes including engraftment and aGVHD.

Several independent factors have demonstrated an association with increased risk of TRM in NM alloHCT including pre-transplant high risk clinical features, positive recipient cytomegalovirus serostatus, age $\geq$45 years, and inclusion of antithymocyte globulin in preparative regimen.[2] Fludarabine, an antineoplastic agent with potent immunosuppressive activity against both B and T-cells, has recently become the back bone of most NM alloHCT regimens.[1] Because fludarabine is pharmacologically activite against a variety of hematologic malignancies, its utility in the preparative regimens of NM alloHCT is considered 2-fold. First, fludarabine provides sufficient activity leading to adequate tumor inhibition during high dose chemotherapy, resulting in the necessary environment required to facilitate stem cell engraftment. Secondly, the amount antitumor activity is not completely ablative, therefore supporting the GVL effect after stem cell rescue. Although extensive information is available regarding fludarabine pharmacokinetics and its toxicity profile outside the setting of HCT, fludarabine exposure and its potential contribution toward TRM in NM alloHCT has yet to be thoroughly evaluated and defined. Chapter 3 will explore the pharmacokinetics and pharmacodynamic relationships of fludarabine with clinical outcomes following NM alloHCT including TRM.

Allogeneic HCT is most commonly used for the treatment of hematologic malignancies. However, over the past 2 decades, the applicability of alloHCT has expanded to many nonmalignant diseases including immunodeficiencies and genetic inborn errors of metabolism. For several metabolic disorders such as mucopolysaccharidoses I (Hurler's syndrome), mucopolysaccharidoses II (Hunter's syndrome), and the many leukodystrophies neurodegeneration is a part of disease progression. These patients suffer symptoms including gait disturbances, visual impairment (including blindness), cognitive impairment, seizures, and death from their disease is inevitable. Treatment-related early and late neurological complications have been reported in NM alloHCT, most commonly in association with RI preparative regimens containing fludarabine.[7] Clofarabine is a next generation purine analog approved for refractory or relapsed acute lymphoblastic leukemia in pediatric patients.[8] Phase I/II clinical trials of clofarabine performed in adult and pediatric patients with variety of hematologic and solid tumor malignancies have yet to report any significant cases of neurotoxicity.[8-12] Hence, clofarabine may offer an advantage in children and adults at risk for neurologic complications following alloHCT. Chapter 4 of this dissertation will present the pharmacokinetics of clofarabine in adult and pediatric patients undergoing an innovative brain-sparing HCT (BS-HCT) as treatment for a variety of high risk metabolic disorders.

CHAPTER 2

MYCOPHENOLATE MOFETIL

Impact of UGT and MRP2 Genetic Variants on Mycophenolic Acid Exposure in Hematopoietic Cell Transplantation

Mycophenolate mofetil (MMF) is an immunosuppressant used to enhance engraftment and prevent acute graft vs host disease (GVHD) in allogeneic hematopoietic cell transplantation (HCT). This study investigates the influence of genetic variants of uridine diphosphate-glucuronosyltransferase enzymes (UGT) and the multidrug resistant protein-2 transporter (MRP2) on mycophenolate pharmacokinetics and clinical outcomes in 133 nonmyeloablative HCT recipients. In multivariate analysis, total mycophenolate troughs were lower in individuals carrying both *UGT1A8*2* and *UGT1A8*3* variants after oral administration ($p<0.01$). After intravenous dosing total mycophenolate troughs were increased in carriers of the *MRP2 3972C>T* variant ($p<0.01$). Unbound mycophenolate area under the curve $(AUC)_{0\text{-}tau}$ and trough concentrations were increased 2.26 and 2.89 times, respectively, in carriers of both *MRP2 3972C>T* and *UGT1A9-275T>A* variants after receiving intravenous MMF ($p<0.01$). As serum creatinine increased unbound mycophenolate $AUC_{0\text{-}tau}$ increased approximately 1.6 and 2.85 fold after oral and intravenous administration, respectively. Mycophenolate 7-O-glucuronide exposure was also increased 2-4.5 fold with increasing serum creatinine, irrespective of route of administration. These data demonstrate when combinations variants are considered, UGT and MRP2 variants may contribute to variability in mycophenolate exposure in HCT. However, given that multiple

other drug related factors such as renal function, route of MMF administration, and dosing schedule were also influential towards mycophenolate pharmacokinetics, genetic variants are not likely to be clinically useful. These data do not support routine genotyping for UGT and MRP2 variants as a guide to MMF dosing in HCT and demonstrate the complexity of drug disposition in highly complicated patient populations.

2.1 INTRODUCTION

Mycophenolate mofetil is an immunosuppressant used to enhance engraftment and prevent acute graft vs host disease (GVHD) in NM alloHCT.[2,13-17] Despite the inclusion of MMF in immunosuppressive regimens the development of acute graft vs host disease (GVHD) remains a prominent clinical problem. Hence, the need for improved immunosuppressive strategies in HCT persists. Mycophenolic acid (MPA) pharmacokinetics in HCT recipients displays wide inter and intra-patient variability in plasma concentrations[6,16,18-23] and low MPA exposure is associated with lower rates of engraftment and greater risk of acute GVHD.[6,23] Patient characteristics such as weight or body surface area, or clinical markers for hepatic and renal function are poorly correlated with MPA pharmacokinetics suggesting there may be genetic factors influencing MPA metabolism or transport.[6]

MMF is an ester prodrug formulated to enhance the bioavailability of the active moiety, MPA. MMF is rapidly and extensively hydrolyzed by esterases in the blood, gut wall, liver and tissues to MPA. MPA is then glucuronidated by

UDP glucuronosyltransferase (UGT) enzymes to the primary inactive metabolite, MPA 7-O-glucuronide (MPAG).[24] The glucuronide is then either excreted into the urine or carried back into the intestinal lumen via bile through multidrug resistant protein (MRP) transporters, specifically MRP2. In the intestine, MPAG may be converted back into MPA and reabsorbed back into systemic circulation through enterohepatic recycling.

The UGT enzymes responsible for MPA metabolism are well described. UGT1A9 is considered the primary enzyme involved in MPAG formation and is expressed in multiple tissues including the liver, kidney, and intestinal mucosa.[25] UGT1A8 and UGT1A10, expressed extrahepatically in the gastrointestinal tract, are also involved in the formation of MPAG.[25-27] UGT2B7, located in the liver and kidney, is responsible for the production of the acyl glucuronide metabolite, which constitutes approximately 5% of the total MPA metabolic pathway.[25]

Several genetic variants of UGT enzymes are known to alter *in vitro* enzymatic activity or modify MPA pharmacokinetics. *UGT1A9* exonic variants are associated with decreased *in vitro* catalytic activity towards MPA, specifically *UGT1A9*3*. This is in contrast to the high expression promoter variants *UGT1A9*, *-275T>A* and *-2152C>T,* which yield higher rates of MPA glucuronidation *in vitro* relative to wild type enzyme.[27-29] UGT1A9 variants have been shown to alter MPA pharmacokinetics in several clinical studies. In normal volunteers receiving a single dose of MMF, carriers of the *UGT1A9*3* have a

30% increase in total MPA exposure.[30] In ninety-five kidney transplant recipients, individuals carrying at least one UGT1A9-*275T*>or *2152C>T* allele had significantly lower total MPA exposure than those lacking a variant allele.[31] MPA glucuronidation is also mediated by the UGT1A8 enzyme which demonstrates the highest enzymatic activity for MPAG production *in vitro.*[27] Several *UGT1A8* variants have decreased *in vitro* catalytic activity, specifically *UGT1A8*3*.[27,32] Although *in vitro* experiments with the *UGT2B7*2* variant have found no change in functional activity, significant increases in both unbound MPA and MPAG exposures were seen in healthy volunteers.[30]

The transport of MPAG into the urine and bile is mediated by MRP2, an efflux pump located on the apical membrane of proximal renal tubular cells and the canalicular membrane of hepatocytes.[33-34] MRP2/ABCC2 belongs to the superfamily of ATP-binding cassette transporters and mediates the excretion of sulfated and glucuronidated organic anionic compounds formed in phase II conjugation in the liver.[35] The effect of MPR2 variants on MPA pharmacokinetic parameters has been evaluated in 95 renal allograft recipients[36] The *MRP2-24C>T* and *3972C>T* variant alleles provided protection from low MPA exposure in the presence of mild hepatic dysfunction when studied within one week posttransplant[36] However, at six weeks posttransplant carriers of the -*24C>T* variant allele had significantly higher MPA trough concentrations independent of hepatic function[36]

No mycophenolate pharmacogenetic data are available in HCT. Given the high pharmacokinetic variability in HCT and evidence of a pharmacogenetic effect in normal volunteers and solid organ transplant recipients it is reasonable to assume a similar pharmacogenetic relationship may exist in HCT. This study aimed to determine whether genetic variant alleles play a role in mycophenolate metabolism and if they are important determinants of neutrophil engraftment and acute GVHD.

2.2 METHODS

2.2.1 Patients

This was a single center analysis evaluating the relationship between mycophenolate pharmacokinetics and genetic variants in one hundred thirty-three HCT recipients treated with nonmyeloablative chemotherapy who received mycophenolate and cyclosporine for prophylactic immunosuppression. Mycophenolate pharmacokinetic data were utilized from three previously completed pharmacokinetic or pharmacodynamic studies.[6,37-38] Pretransplant recipient DNA was obtained from the Molecular Diagnostics Laboratory at the University of Minnesota. Patients were eligible if they had undergone a related or unrelated allogeneic transplant, were ≥ 18 years of age, and had both mycophenolate pharmacokinetic data, and recipient pretransplant DNA available. This study was approved by the University of Minnesota Institutional Review Board and Cancer Protocol Review Committee.

2.2.2 Preparative Regimen and Immunosuppression

All patients received cyclophosphamide 50 mg/kg/day IV day –6; plus fludarabine 40 mg/m^2/day IV on days –6 to –2 and TBI 200 cGy as a single fraction on day –1 as their preparative regimen. Patients not having received chemotherapy 6 months prior to transplant received equine antithymocyte globulin (ATG) 15 mg/kg IV every 12 hours for 3 days (either days -6, -5 and -4 or -3, -2, and -1) along with methylprednisolone 1 mg/kg every 12 hours. All patients received antibiotic prophylaxis/gut decontamination with a fluoroquinolone beginning on day –1. Fluconazole was initiated on day -3 in the majority of patients as antifungal prophylaxis, with voriconazole and micafungin provided in a few exceptions.

Intravenous MMF was started on day -3 and converted to oral therapy in week one posttransplant as tolerated. Intravenous cyclosporine was also initiated on day -3 at a dose of 2.5 mg/kg every 12 hours and adjusted to maintain whole blood trough concentrations of 200-400 ng/ml. Patients were converted to oral cyclosporine once they were able to tolerate oral medications.

2.2.3 Pharmacokinetics

Based on the original study of enrollment, each subject had steady-state mycophenolate pharmacokinetics performed one or two times within 15 days posttransplant. MMF was given at a dose of 1 g every 12 hours, 1.5 g every 12 hours or 1 g every 8 hours IV or PO. Samples were drawn at times 0, 2, 4, 6, 8 and 12 hours following an IV dose infused over 2 hours. Blood samples were obtained at time 0, 1, 2, 4, 6, 8 and 12 hours following the administration of oral

dosing. For patients receiving MMF every 8 hours the 12-hour sample was omitted. Clinical studies were obtained with each pharmacokinetic set including serum creatinine, blood urea nitrogen, albumin, total bilirubin, alanine aminotransferase, and alkaline phosphatase. All medications administered within 24 hours of pharmacokinetic sampling were recorded.

2.2.4 Bioanalysis

Plasma concentrations of total and unbound MPA and total MPAG were measured using a validated HPLC system equipped with variable wavelength UV detector as previously described [39]. The validated assay is linear in the range of 0.025-10 mcg/mL, 1-100 mcg/mL and 1-500 ng/mL for total MPA and MPAG, and unbound MPA, respectively.

2.2.5 Pharmacokinetic Analysis

For each patient, total and unbound MPA and MPAG plasma concentration-time data were analyzed using noncompartmental methods (WinNonLin Professional 5.2, Pharsight Corp, Mountain View, CA). Area-under-the-curve ($AUC_{0\text{-}tau}$) adjusted for dose was determined with the log/linear trapezoidal method. C_{max} was the highest observed plasma concentration. C_{last} (trough) was the 8-hour or 12-hour post dose concentration. Apparent clearance (Cl/F) was estimated by dose divided by $AUC_{0\text{-}tau}$.

2.2.6 Genotyping

Pretransplant recipient genomic DNA was extracted from peripheral blood lymphocytes either by PureGene DNA extraction kit (Gentra, MN) or

DNeasy DNA extraction kit (Qiangen, CA). DNA quantification was carried out by measuring the absorbance at 260nm.

All UGT and MRP2 single nucleotide polymorphisms were determined using TaqMan probes designed by Applied Biosystems (Foster City, CA) except for *UGT1A9-2152C>T* which was determined by a restriction fragment length polymorphism assay. The PCR and probe primers for the following UGT and MRP2 polymorphisms are shown in Table 2.1; *UGT1A8*2, UGT1A8*3, UGT1A8*4* (rs17862841)*, UGT1A8*8, UGT1A8*9, UGT1A9*2, UGT1A9*3, UGT1A9-275T>A* (rs6714486)*, UGT1A10*2* (rs10187694)*, UGT2B7*2* (rs7439366)*, MRP2-24C>T* (rs717620)*, MRP2 3972C>T* (rs3740066)*, MRP2 1249G>A* (rs2273697). The reaction components for each genotyping reaction were as follows: 2 μL of DNA (10 ng/μL), 12.5μL of TaqMan master mix (Applied Biosystems, Branchburg, NJ), 0.625 μL of primer/probe mix and water up to a total volume of 25μL. The thermocycler conditions were as follows: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 92°C for 15 seconds and 60°C for 1 min. The reaction was analyzed on an Applied Biosystems PRISM model 7500 sequence detection system and software. For the *UGT1A9 -2152C>T* polymorphism, the forward and reverse primers were 5'CAGGTCTCTCACCAATGTTT3' and 5'TCACACCTGTAGTCCCAGCA3', respectively and the assay has been previously described.[40]

2.2.7 Clinical Outcomes

Acute GVHD and neutrophil engraftment data were obtained through the University of Minnesota Bone Marrow Transplant Database. GVHD was staged and graded according to the standard University of Minnesota GVHD criteria based on clinical and pathological criteria.[41-42] Acute GVHD was defined as day of development of grades II-IV GVHD within day 100 of transplantation. Neutrophil engraftment was defined as the first of three consecutive days of an absolute neutrophil count > 500 cells/uL.

2.2.8 Statistical Analysis

All variants were evaluated for Hardy-Weinberg equilibrium by chi square testing. Pair-wise linkage disequilibrium (LD) between genotypes were estimated by scaled D and tested by Chi-square statistic. Analysis of variance performed by the Mann-Whitney U test was used to compare the effect of genotype of carriers (heterozygous or homozygous variant) vs non-carrier (homozygous wildtype) on MPA pharmacokinetic parameters. Previous studies have shown differences in MPA exposure due to route of MMF administration.[38] To control for these differences both univariate and multivariate analysis were performed separately for oral and intravenous MMF. Clinical covariates after intravenous and oral dosing were compared through a linear mixed model to account for the within-subject correlation for patients participating in pharmacokinetic sampling twice. Multivariate stepwise regression was used to model independent factors influencing MPA pharmacokinetics. The following variables were included in the regression model: genotype (carrier vs non-

carrier), bilirubin, albumin, serum creatinine, day pharmacokinetic sampling was performed in transplant course, age, gender, weight, dosing regimen (every 8 hours vs every 12 hours), and original study assignment. Exponentiated regression coefficients are reported and interpreted as the ratio in the original scale of the outcome variables per unit difference in a covariate. A p-value of 0.01 was considered significant.

2.3 RESULTS

2.3.1 Patients

Patient demographics and characteristics are listed in Tables 2.2 and 2.3. A total of 133 patients had both DNA and pharmacokinetic data available, resulting in 80 intravenous and 101 oral evaluable pharmacokinetic profiles. Median (range) serum creatinine and creatinine clearance were 0.90 mg/dL (0.49-2.5) and 84.6 ml/min (30.3-196.9), respectively, at time of pharmacokinetic sampling. No significant differences in clinical covariates including serum creatinine, total bilirubin, and albumin were observed between the intravenous and oral MMF groups. Patients receiving intravenous MMF had significantly lower cyclosporine troughs compared to the oral MMF group, 251 ng/mL (84-518) and 321 ng/mL (55-620), respectively ($p < 0.01$). There was no influence of cyclosporine route of administration or trough level on MPA pharmacokinetic parameters in univariate analysis (all $p \geq 0.21$).

2.3.2 Influence of Original Study Assignment and Dosing Regimen on Mycophenolate Pharmacokinetics

An initial analysis was performed to assess the influence of original study assignment, dosing interval (every 12 or 8 hours), and route of administration on MPA pharmacokinetic parameters. With both intravenous and oral administration of MMF, differences between total and unbound MPA trough concentrations were observed between the original study assignments ($p < 0.01$). However, differences in total and unbound MPA trough concentrations after intravenous administration of MMF were no longer detected between the study assignments when dosing interval was added into the regression model ($p \geq 0.06$). With oral administration, dosing MMF every 8 hours had a positive effect on total and unbound MPA troughs ($p \leq 0.04$).

2.3.3 Frequency of UGT and MRP2 Variants

Genotype frequencies for UGT and MRP2 variants are provided in Table 2.4. For UGT1A8**4, UGT1A8*8, UGT1A8*9, UGT1A9*2,* and *UGT1A10*2* no variant alleles were observed (data not shown). All allele frequencies were found to be in Hardy-Weinberg equilibrium. In two patients, DNA was limited and genotyping for each variant was not feasible. The *MRP2 1249G>A* genotype was indeterminate in fourteen patients. Alleles *UGT1A9-275T>A* and *UGT1A9-2152C>T* were in strong linkage disequilibrium (scale $D > 0.99$, $p < 0.0001$) as were *UGT1A8*3* / *UGT1A9-275T>A* (scale $D = 0.84$, $p < 0.0001$) and *MRP2-24C>T* / *MRP2 3972C>T* (scale $D = 0.96$, $p < 0.0001$).

2.3.4 Association Between UGT Variants and Mycophenolate Pharmacokinetics

The pharmacokinetics of mycophenolate in carriers and non-carriers of *UGT1A8*2*, *UGT1A8*3*, and *UGT1A9-275T>A* after oral and intravenous dosing are summarized in Table 2.5. No differences in pharmacokinetics were observed between carriers and non-carriers of *UGT2B7*2* or *UGT1A9*3* variants ($p > 0.01$) (data not shown). Although no significant differences were found in univariate analysis for the *UGT1A8*2* and *UGT1A8*3* variants, a trend of lower total MPA troughs was observed in carriers vs non-carriers after oral administration. Unbound MPA was significantly lower when patients received oral MMF and were carriers of the *UGT1A9-275T>A* variant; $AUC_{0\text{-}tau}$ 212.5 ng*hr/ml (132.0-592.3) vs 314.5 (136.6-518.3), ($p=0.01$). No other differences in pharmacokinetics were observed between carriers and non-carriers of *UGT1A8*2*, *UGT1A8*3,* and *UGT1A9-275T>A* variants with oral or intravenous dosing of MMF ($p > 0.01$) (data not shown).

2.3.5 Association of MRP2 Variants and Mycophenolate Pharmacokinetics

A summary of mycophenolate pharmacokinetics in carriers and non-carriers of *MRP2-24C>T* and *MRP2 3972C>T* after oral and intravenous MMF are summarized in Table 2.6. No differences were observed in MPA pharmacokinetic parameters between carriers and non-carriers of *MRP2 1249G>A* ($p > 0.01$) (data not shown). There were also no differences in MPA pharmacokinetics between carriers and non-carriers of the *MRP2-24C>T* variant after intravenous administration of MMF. However, patients receiving oral MMF who were carriers of the *MRP2-24C>T* variant had higher MPAG

trough concentrations, 54.5 ug/ml (16.1-99.6) vs 39.7(11.9-173.5), (p=0.01) when compared to non-carriers. MPAG trough concentrations were also increased after oral dosing in carriers of the *MRP2 3972C>T* variant vs non-carriers 49.6 ug/ml (11.9-118.3) vs 37.6 (12.8-173.5), (p=0.01). Following intravenous dosing, higher total MPA trough concentrations 0.6 ug*hr/ml (0.2-2.8) vs 0.3 (0.1-1.6), (p= 0.003) were observed in carriers vs non-carriers of the *MRP2 3972C>T* variant.

2.3.6 Multivariate Analysis

Table 2.7 summarizes the significant variables included in the final regression models toward MPA pharmacokinetics. After intravenous dosing, total MPA trough levels were increased by approximately 1.8 fold in carriers of the *MRP2 3972C>T* variant ($p < 0.01$). In contrast, total MPA trough concentrations were approximately 81% lower in patients who were carriers of both the *UGT1A8*2* and *UGT1A8*3* variant following oral dosing of MMF($p < 0.01$). No other variants or clinical covariates tested were significant towards total MPA exposure. Unbound MPA $AUC_{0\text{-}tau}$ and trough concentrations were increased 2.26 ($p < 0.01$) and 2.89 ($p < 0.01$) times, respectively, in carriers of both *MRP2 3972C>T* and *UGT1A9-275T>A* variants after receiving intravenous MMF (Table 7). Serum creatinine was also an independent predictor of unbound exposure after oral and intravenous dosing, increasing MPA $AUC_{0\text{-}tau}$ and troughs by 1.55 and 2.85 fold, respectively, with each 1 mg/dL increase in serum creatinine. None of the variants tested were found to be independent

predictors of MPAG pharmacokinetics (Table 7). However, MPAG exposure increased approximately 2-4.5 fold with each increase in serum creatinine by 1 mg/dL.

2.3.7 Relationship between SNPs and Clinical Outcomes

UGT and MRP2 SNPs were evaluated towards the development of aGVHD grades II-IV and engraftment. No single UGT or MRP2 variant was associated with acute GVHD ($p > 0.01$) or neutrophil engraftment ($p > 0.01$) (data not shown).

2.4 DISCUSSION

This is the first study to evaluate the influence of UGT and MRP2 variants on MPA pharmacokinetics in HCT. These data demonstrate UGT and MPR2 variants may contribute to the variability in MPA exposure, most notable when combinations of variants are considered. However, multiple other clinical variables were found to influence mycophenolate pharmacokinetics including renal function, route of MMF administration, and dosing schedule therefore it is unlikely that genetic variants will be an important clinical tool for dosing of MMF in HCT.

We hypothesized that genetic variants of *UGT1A8* and *1A9* may contribute to MPA pharmacokinetic variability, especially after oral dosing of MMF. In univariate analysis, a trend of lower total MPA trough concentrations after oral administration was noted with *UGT1A8*2* and UGT1A8*3. In multivariate analysis, individuals carrying both these variants had an 81%

reduction in MPA trough concentrations after oral administration. These data contrast with the *in vitro* data where *UGT1A8*2* results in no functional change in enzymatic activity and *UGT1A8*3* is associated with a significant reduction in MPA glucuronidation.[32,43] It is possible that the high expression of the *UGT1A9-275T>A* variant enzyme predominates over the *UGT1A8*3* variant as these are in strong linkage disequilibrium. The dosing schedule of MMF may have also influenced the trough levels. Total MPA tough concentrations were lower when MMF was administered 1-1.5 grams every 12 hours compared to 1 gram every 8 hours, irrespective of route of administration (p=0.04). Six of the seven patients with *UGT1A8*3* were receiving MMF at doses of 1-1.5 grams every 12 hours.

We found no influence of the UGT1A9*3 variant on MPA pharmacokinetic parameters. This is in contrast to previously published data in kidney transplantation suggesting carriers of the *UGT1A9*3* variant have higher MPA exposure compared to non-carriers.[31] In our population we found only 4 individuals heterozygous for the *UGT1A9*3* variant allele. The lack of effect of the *UGT1A9*3* variant in our population may be due the low allele frequency. Similarly, the *UGT2B7*2* variant showed no impact on pharmacokinetics in our study whereas in healthy volunteers the variant was associated with increased unbound and total MPA exposure.[30] Fluconazole, a known inhibitor of UGT2B7 [44], was used in the majority of our patients and may have masked the genotypic effect of UGT2B7 on MPA pharmacokinetics.

In our previous study in HCT recipients, we demonstrated unbound MPA AUC_{0-12} < 0.300 mcg hr/mL in week one posttransplant was associated with greater risk of developing acute GVHD.[6] Therefore, we were particularly interested in evaluating high expression variants, such as *UGT1A9-275T>A,* and the potential association with low concentrations of MPA. In univariate analysis, low unbound MPA exposure was associated with oral MMF administration and presence of the *UGT1A9-275T>A* variant. However, when the effects of multiple variants were considered, the combination of *UGT1A9-275T>A* and *MRP2 3972C>T* resulted in surprisingly higher exposures (unbound MPA AUC_{0-tau} and troughs) with intravenous dosing. MRP2 is responsible for the transport of MPAG into the bile.[33-34] However, in the absence of functional MRP2, MRP3 which is located on the basolateral membrane, may be up-regulated, providing a compensatory mechanism for drug transport into systemic circulation.[45-47] Additionally, at therapeutic concentrations, MPA and MPAG are highly protein bound to plasma albumin and *in vitro* data indicates that MPAG competes with MPA for albumin binding sites resulting in increased unbound fraction of MPA.[48] Rapid metabolism of MPA by hepatic *UGT1A9-275T>A*, coupled with impaired transport of MPAG into the bile by the *MRP2 3972C>T* variant may result in an increased shunting of MPAG into systemic circulation by MRP3. Increased systemic MPAG concentrations would then presumably lead to higher unbound MPA concentrations through competition for albumin binding sites.

Although we speculate that MRP3 compensates when MPR2 activity is impaired resulting in higher MPAG troughs and $AUC_{0\text{-}tau}$ in patients receiving oral MMF who are carriers of *MRP2-24C>T* or *MRP2 3972C>T* we recognize several limitations to this study regarding the evaluation of transporters. MPA exposure has been shown to be reduced when co-administered with broad-spectrum antibiotics, including fluorquinolones.[49] All patients included in this analysis began receiving a fluoroquinolone on day -1. Several published studies have demonstrated a lack of enterohepatic recycling of MPAG, when broad spectrum prophylactic antibiotic are used with MMF [6,37]. Additionally, fluoroquinolones are known substrates for MPR2 and may alter MPA hepatic clearance and renal excretion though competition with MPAG or MPA for binding sites.[50] The co-administration of the known MRP2 inhibitors specifically cyclosporine may also influence on MPA pharmacokinetics.[33-34] All subjects in this analysis were receiving cyclosporine. Finally, although differences in MPA pharmacokinetic parameters between intravenous and oral administration appear to be modified by genetic variants, this may be a reflection of the patient's clinical status, or differences in co-administered medications at the time of sampling.

In multivariate analysis, serum creatinine was highly associated with MPAG $AUC_{0\text{-}tau}$ and trough concentrations, and to a lesser extent unbound MPA exposure. This is consistent with data in solid organ transplant demonstrating significantly higher unbound MPA and MPAG concentrations

with impaired renal function.[48,51] Acute renal failure, defined as greater than 2-fold doubling of serum creatinine, is a common complication of both myeloablative and reduced intensity HCT, occurring to some degree in 33-56% of patients.[52-54] The incidence of severe acute renal failure is significantly higher after high-dose allogeneic HCT compared to autologous HCT (69% vs 21%) and typically occurs within the first three weeks of transplantation, during the time of mycophenolate aGVHD prophylaxis use.[55] For this reason, we believe changes in renal function are more likely lead to clinically relevant alterations in unbound MPA exposure when compared to genetic variants.

No relationship was found between UGT or MRP2 variants and neutrophil engraftment or incidence of acute GVHD grades II-IV. Acute GVHD and engraftment are complex processes and multiple factors are influential including stem cell source (i.e. peripheral blood, cord blood), age, donor-recipient HLA match, and other immunosuppressive therapy. We recognize a very heterogeneous study population relative to these important variables, and understand how this diversity poses significant limitations for evaluating outcomes and UGTs and MRP2 variants. Additionally, this study was limited by small sample size and included primarily a Caucasian population. Hence, multivariate analyses were not feasible to evaluate the relationship between clinical outcomes and genetic variants

In conclusion, our data demonstrate UGT and MPR2 variants may contribute to the wide variability in MPA exposure. However, the effect is likely

not clinically relevant given the greater importance of other clinical factors such as renal function, route of MMF administration, and MMF dosing interval. Serum creatinine was a strong predictor of unbound MPA and MPAG exposure. The impact of kidney dysfunction is more likely to result in relevant changes in MPA exposure when compared to genetic influences. Future studies are needed to evaluate the impact of co-administered medications on MPA pharmacokinetic parameters through induction or inhibition of UGT and MRP2. This is particularly relevant given that HCT recipients may receive 10-20 other medications concurrently with MMF. However, chemotherapy, infection, and tumor burden may also alter mycophenolate disposition through induction or inhibition and should be explored. Finally, we recognize several limitations to this study including co-administered medications, a very heterogeneous patient population regarding treatment course, and small sample size for some of the genetic variants. These data do not support routine genotyping for UGT and MRP2 variants as a guide to MMF dosing in the setting of HCT.

Table 2.1: TaqMan primers used for genotyping UGT and MRP2 variants

Variant	Forward amplification primer/ Reverse amplification primer	Allele 1 primer/Allele 2 primer
UGT		
1A9-275	5'CTTCAAGGTCCAAAAGCATTGGTTA3'	ATTCTGCT**T**CTAAACTT
	5'GCCCTGTGCTGCAATGTT3'	ATAATTCTGCT**A**CTAAACTT
*1A9*2*	5'CCCAGCTGCTTGCTCTCA3'	CTCTGATGGCTT**G**CACAG
	5'CAGCAGACACACACATAGAGGAA3'	TTCTCTGATGGCTT**A**CACAG
*1A9*3*	5'AGGCAGGGAAGCTACTGGTA3'	CCCATCC**A**TGGGCAC
	5'CCGACCTCATGGTGAACCA3'	CCATCC**G**TGGGCAC
*1A8*2*	5'CCCTCCCCTCTGTGGTCTT3'	TGGCAA**C**CTATTCC
	5'GCACTGTGCACCTTCTTCAAGATA3'	TGGCAA**G**CTATTCC
*1A8*3*	5'CCCGTGATGCCCAATATGATCTTC3'	TGGTATCAACT**G**CCATCAG
	5'GAGAGGTGACTTACCATAGGCAATG3'	TGGTATCAACT**A**CCATCAG
*1A8*4*	5'AGGAGTTTGTTTAATGACCGAAAATTAGTAGA3'	CAAGAAACACC**G**CATCAA
	5'AGCCACAGGCATCAAAAGGAT3'	AAGAAACACC**A**CATCAA
*1A8*8*	5'GGATGGGAGTCACTGGTTCAC3'	CCACCACC**G**ACTGCA
	5'TGCCCCCTGAGGATAAGTTTCT3'	CACCACC**A**ACTGCA
*1A8*9*	5'TCGGTGGTGGAGAAACTTATCCT3'	CACCTCAT**G**CCCCCTG
	5'CTCACCTCTGGCATGACTACAA3'	CCACCTCAT**T**CCCCCTG
*1A10*2*	5'GCAGGAGTTTGTTTAATGACCGAAA3'	AATACTTAAAG**G**AGAGTTC

	5'TCAAAAGGATCCAGAAACACTGCAT3'	TAGAATACTTAAAG**A**AGAGTTC
*2B7*2*	5'CTGACGTATGGCTTATTCGAAACTC3'	TTTTCAGTTTCCT**C**ATCCAC
	5'TGGAGTCCTCCAACAAAATCAACAT3'	ATTTTCAGTTTCCT**T**ATCCAC
MRP2		
C-24T	5'TTGATGAAACAAGTAAAGAAGAAACAACACAA3'	CTGGAAC**G**AAGACTC
	5'CAGAACTTCTCCAGCATGATTCCT3'	CTGGAAC**A**AAGACTC
3972C>T	5'TGGTCCTCAGAGGGATCACTT3'	ATGCTACC**G**ATGTCAC
	5'TCCTTCACTCCACCTACCTTCTC3'	ATGCTACC**A**ATGTCAC
1249G>A	5'GTCCATGGGTCCTAATTTCAATCCT3'	AGGAGTACACC**G**TTGGA
	5'CATCCACAGACATCAGGTTCACT3'	AAGGAGTACACC**A**TTGGA

Table 2.2: Patient Demographics

Characteristics		N (%)
No.		133
Gender		
	Female	50 (38%)
	Male	83 (62%)
Age (yrs)		
	Median (range)	52 (19-69)
Disease		
	Non-Hodgkin's	30 (23%)
	Hodgkin's	15 (11%)
	Chronic myelogenous leukemia	19 (14%)
	Acute leukemia	44 (33%)
	Myelodysplastic syndrome	19 (14%)
	Other	6 (5%)
Stem cell Source		
	Umbilical cord blood	82 (62%)
	Bone marrow	8 (6%)
	Peripheral blood stem cells	43 (32%)
Donor Type		
	Related sibling	44 (33%)
	Unrelated donor	89 (67%)

Table 2.3: Patient Characteristics[1]

	Intravenous MMF	Oral MMF	p-value
No.	80	101	
Serum Creatinine (mg/dL)	0.87 (0.49-2.06)	0.90 (0.50-2.50)	0.22
Creatinine Clearance (mL/min)	86.2 (32.6-185.7)	82.7 (30.3-196.9)	0.83
Total Bilirubin (mg/dL)	0.9 (0.2-20)	0.9 (0.1-7.5)	0.30
Albumin (mg/dL)	3.0 (2.1-4.1)	2.9 (2.0-4.3)	0.45
Cyclosporine (ng/mL)	251 (84-518)	321 (55-620)	<0.01

[1]data are represented by median (range)

Table 2.4: Allele Frequencies of UGT and MPR2 Polymorphisms

Gene	UGT Variant	Genotype	No. (genotype frequency)
UGT	**1A9 -2152C>T**	CC	121 (92.4%)
		CT	9 (6.8%)
		TT	1 (0.8%)
	1A9 -275T>A	T/T	119 (90.2%)
		T/A	12 (9.1%)
		A/A	1 (0.8%)
	1A9*3 98T>C	T/T	129 (97.0%)
		T/C	4 (3.0%)
		C/C	0
	1A8*2 518C>G	C/C	73 (55.3%)
		C/G	52 (39.4)
		G/G	7 (5.3%)
	1A8*3 830G>A	G/G	125 (94.7%)
		G/A	7 (5.3%)
		A/A	0
	2B7*2 802C>T	C/C	30 (22.9%)
		C/T	64 (48.9%)
		T/T	37 (28.2%)
MRP2	**-24C>T**	C/C	97 (72.9%)
		C/T	33 (24.8%)
		T/T	3 (2.3%)
	3972C>T	C/C	69 (52.3%)
		C/T	56 (42.2%)
		T/T	7 (5.3%)
	1249G>A	G/G	75 (64.1%)
		G/A	34 (29%)
		A/A	8 (9.9%)

Table 2.5: MPA pharmacokinetics in carriers and non-carriers of *UGT1A8*2*, *UGT1A8*3*, and *UGT1A9-275*

	Intravenous MMF			Oral MMF		
	1A8*2 (C/C)	**1A8*2 (C/G and G/G)**	**p-value**	**1A8*2 (C/C)**	**1A8*2 (C/G and G/G)**	**p-value**
No.	43	37		58	42	
Total MPA C_{last} [1]	0.5 [0.1-2.8]	0.4 [0.1-1.6]	0.40	0.7 [0.1-3.1]	0.5 [0.0-2.8]	0.02
Total MPA $AUC_{0\text{-}tau}$ [2]	22.4 [7.5-45.1]	24.3 [12.0-74.9]	0.17	22.3 [10.8-40.8]	19.1 [9.1-40.7]	0.32
Unbound MPA C_{last} [3]	7.8 [0.8-28.7]	4.6 [0.9-30.4]	0.06	9.6 [1.1-53.8]	6.8 [1.3-38.2]	0.16
Unbound MPA $AUC_{0\text{-}tau}$ [4]	395.8 [89.3-1000.8]	375.5 [185.1-1642.3]	0.58	321.7 [152.9-592.3]	279.1 [132.0-573.3]	0.10
MPAG C_{last} [1]	33.5 [6.4-127.2]	25.6 [6.7-103.6]	0.16	45.5 [13.6-173.5]	42.3 [11.9-99.6]	0.59
MPAG $AUC_{0\text{-}tau}$ [2]	622.7 [234.6-1871.3]	530.6[172-1691.4]	0.44	662.2 [287.0-2150.2]	769.7 [368.8-1459.9]	0.61
	1A8*3	**1A8*3 (G/A and A/A)**	**p-value**	**1A8*3 (G/G)**	**1A8*3 (G/A and A/A)**	**p-value**
No.	73	7		88	12	
Total MPA C_{last} [1]	0.4 [0.1-2.8]	0.5 [0.4-1.1]	0.44	0.7 [0.1-2.8]	0.4 [0.0-3.1]	0.05
Total MPA $AUC_{0\text{-}tau}$ [2]	23.3 [7.5-74.9]	23.2[10.8-41.0]	0.93	21.1 [9.1-40.8]	18.0 [14.4-25.3]	0.19
Unbound MPA C_{last} [3]	6.7 [0.8-30.4]	6.3 [4.1-15.4]	0.74	9.0 [1.1-38.2]	5.8 [1.6-53.8]	0.47
Unbound MPA $AUC_{0\text{-}tau}$ [4]	391.8 [89.3-1642.3]	462.5 [137.9-908.8]	0.82	299.5 [132.0-592.3]	216.6 [187.5-396.3]	0.07
MPAG C_{last} [1]	29.8 [6.4-127.2]	25.9 [21.1-43.9]	0.78	44.9 [11.9-173.5]	26.0 [19.4-67.6]	0.12
MPAG $AUC_{0\text{-}tau}$ [2]	559.6 [172.0-1871.3]	445.4 [234.6-664.2]	0.25	765.1 [287.0-2150.2]	491.5 [471.5-1192.2]	0.07

	1A9-275 (T/T)	1A9-275 (T/A and A/A)	p-value	1A9-275 (T/T)	1A9-275 (T/A and A/A)	p-value
No.	73	7		88	12	
Total MPA C_{last} [1]	0.4 [0.1-2.8]	1.1 [0.2-1.8]	0.02	0.7 [0.1-2.8]	0.5 [0.0-3.1]	0.18
Total MPA $AUC_{0\text{-}tau}$ [2]	23.1 [7.5-74.9]	32.5 [10.8-44.9]	0.17	21.4 [10.8-40.8]	19.3 [9.1-36.7]	0.25
Unbound MPA C_{last} [3]	6.5 [0.8-30.4]	11.1 [2.6-28.7]	0.07	9.0 [1.1-38.2]	7.0 [1.6-53.8]	0.36
Unbound MPA $AUC_{0\text{-}tau}$ [4]	383.7 [89.3-1642.3]	473.9 [137.9-1000.8]	0.55	314.5 [136.6-518.3]	212.5 [132.0-592.3]	0.01
MPAG C_{last} [1]	27.8 [6.4-127.2]	25.9 [15.1-82.3]	0.77	44.9 [11.9-173.5]	40.5 [19.4-67.6]	0.36
MPAG $AUC_{0\text{-}tau}$ [2]	557.3 [172.0-1871.3]	513.2 [234.6-1259.9]	0.56	765.1 [287.0-2150.2]	564.9 [439.1-1192.2]	0.15

Data are presented as median (range); C_{last}, 8-hour or 12-hour post dose concentration at steady state; $AUC_{0\text{-}tau}$, area under the curve from 0-8 hours for MMF dosed every 8 hours and 0-12 hours for MMF dosed every 12 hours; P-value determined by Mann-Whitney U test, [1] units = ug/mL, [2] units = ug*hr/mL, [3] units = ng/mL, [4] units = ng*hr/mL

Table 2.6: MPA pharmacokinetics of carriers and non-carriers of *MRP2-24C>T* and *MRP2 3972C>T*

		Intravenous MMF			Oral MMF	
	MRP2-24C>T (C/C)	**MRP2-24C>T (C/T and T/T)**	**p-value**	**MRP2-24C>T (C/C)**	**MRP2-24C>T (C/T and T/T)**	**p-value**
No.	61	19		71	30	
Total MPA C_{last} [1]	0.1 [0.1-2.8]	0.6 [0.2-1.2]	0.25	0.6 [0-3.1]	0.7 [0.2-2.3]	0.17
Total MPA $AUC_{0\text{-}tau}$ [2]	21.9 [7.5-74.9]	25.0 [14.9-39.4]	0.22	19.4 [9.1 -40.7]	24.4 [11.6-40.8]	0.07
Unbound MPA C_{last} [3]	6.3 [0.8-30.4]	8.7 [0.9-22.8]	0.52	7.8 [1.1-53.8]	9.2 [1.9-38.2]	0.49
Unbound MPA $AUC_{0\text{-}tau}$ [4]	375.3 [89.3-1642.3]	419.1 [227.4-896.2]	0.68	286.1 [132.0-592.3]	320.5 [155.2-579.1]	0.23
MPAG C_{last} [1]	27.5 [6.6-127.2]	39.1 [6.2-91.5]	0.97	39.7 [11.9-173.5]	54.5 [16.1-99.6]	0.01
MPAG $AUC_{0\text{-}tau}$ [2]	546.9 [172.0-1871.3]	577.1 [335.3-1179.7]	1.0	645.1 [287.0-2150.2]	851.3 [409.7-1459.9]	0.04
	MRP2 3972C>T (C/C)	**MRP2 3972C>T (C/T and T/T)**	**p-value**	**MRP2 3972C>T (C/C)**	**MRP2 3972C>T (C/T and T/T)**	**p-value**
No.	38	41		50	51	
Total MPA C_{last} [1]	0.3 [0.1-1.6]	0.6 [0.2-2.8]	<0.01	0.6 [0-3.1]	0.7 [0.1-2.8]	0.21
Total MPA $AUC_{0\text{-}tau}$ [2]	20.4 [7.7-43.2]	25.0 [7.5-74.9]	0.04	20.0 [9.1-37.9]	21.1 [11.3-40.8]	0.11
Unbound MPA C_{last} [3]	5.9 [0.8-30.4]	7.4 [1.1-28.7]	0.27	7.8 [1.1-53.8]	9.0 [1.6-38.2]	0.34
Unbound MPA $AUC_{0\text{-}tau}$ [4]	392.4 [137.9-777.9] 27.7 [9.7-127.2]	386.0 [89.3-1642.3] 31.9 [6.4-119.3]	0.60 0.83	280.0 [123.0-581.3] 37.6 [12.8-173.5]	320.5 [155.2-592.3] 49.6 [11.9-118.3]	0.03 0.01

MPAG C_{last} [1]						
MPAG $AUC_{0\text{-}tau}$ [2]	608.6 [234.6-1871.3]	503.0 [172.0- 1304.7]	0.40	631.1 [287.1-2150.2]	796.4 [368.8-1459.9]	0.08

Data are presented as median (range); C_{last}, 8-hour or 12-hour post dose concentration at steady state; $AUC_{0\text{-}tau}$, area under the curve from 0-8

hours for MMF dosed every 8 hours and 0-12 hours for MMF dosed every 12 hours; P-value determined by Mann-Whitney U test.

Table 2.7: Significant variables included in the final regression models for MPA pharmacokinetics

	Intravenous MMF		Oral MMF
Total MPA C_{last}		**Total MPA C_{last}**	
MRP2 3972[a]	1.83 (< 0.01)	UGT1A8*2/UGT1A8*3[b]	0.19 (< 0.01)
Unbound MPA C_{last}		**Unbound MPA C_{last}**	
SCr[c]	2.85 (< 0.01)		
UGT1A9-275/MRP2 3972	2.89 (< 0.01)		
Unbound MPA $AUC_{0\text{-}tau}$		**Unbound MPA $AUC_{0\text{-}tau}$**	
UGT1A9-275/MRP2 3972	2.26 (< 0.01)	SCr	1.55 (< 0.01)
MPAG C_{last}		**MPAG C_{last}**	
SCr	4.54 (< 0.01)	SCr	2.50 (< 0.01)
MPAG $AUC_{0\text{-}tau}$		**MPAG $AUC_{0\text{-}tau}$**	
SCr	2.98 (<0.0001)	SCr	2.03 (<0.0001)
		Albumin	1.34 (0.008)

[a] Exponentiated regression coefficients are reported and should be interpreted as a ratio in the original scale of the outcome variable per unit difference for each covariate. For example, patients carrying at least one MRP2 3972 variant allele have total MPA trough levels approximately 1.83 fold higher than individuals' homozygous wild type. [b] individuals carrying at least one variant allele for both genes, [c] serum creatinine. For example, with each increase in serum creatinine by 1mg/dL, unbound MPA trough concentrations are approximately 2.85 fold higher after intravenous dosing of mycophenolate.

CHAPTER 3

FLUDARABINE

High fludarabine exposure and relationship with treatment related mortality after nonmyeloablative hematopoietic cell transplantation

Despite its common use in nonmyeloablative preparative regimens, the pharmacokinetics of fludarabine are poorly characterized in hematopoietic cell transplantation (HCT) recipients and exposure-response relationships remain undefined. Our objective of this study was to evaluate the association between systemic F-ara-A exposure, the active moiety of fludarabine, and engraftment, acute graft vs host disease (GVHD), treatment-related mortality (TRM) and survival after HCT. The preparative regimen consisted of cyclophosphamide 50 mg/kg/day i.v. day –6; plus fludarabine 30-40 mg/m^2/day i.v. on days –6 to –2 and TBI 200 cGy on day –1. F-ara-A pharmacokinetics were performed with the first dose of fludarabine in 87 adult patients. Median (range) F-ara-A $AUC_{(0-\infty)}$ was 5.0 ug*hr/mL (2.0-11.0), clearance 15.3 L/hour (6.2-36.6), C_{min} 55 ng/mL (17-166), and concentration on day_{zero} 16.0 ng/mL (0.1-144.1). Despite dose reductions, patients with renal insufficiency had higher F-ara-A exposures. There was strong association between high plasma concentrations of F-ara-A and increased risk of TRM and reduced overall survival. Patients with an $AUC_{(0-\infty)}$ greater than 6.5 ug*hr/mL had 4.56 greater risk of TRM and significantly lower survival. These data suggest that clinical strategies are needed to optimize dosing of fludarabine to prevent overexposure and toxicity in HCT.

3.2 INTRODUCTION

Conventional myeloablative HCT is associated with significant treatment related morbidity and mortality, and therefore often limited to younger patients with good clinical status and organ function. Nonmyeloablative or reduced-intensity preparative regimens reduce toxicity and risk of TRM, allowing more patients to undergo allogeneic HCT that would otherwise not be suitable for traditional transplantation.

Fludarabine is often included in the conditioning regimen of allogeneic nonmyeloablative HCT.[56] Fludarabine (9-β-D-arabinofuranosyl-2-fluoroadenine monophosphate) is a purine analog with potent antitumor and immunosuppressive activity.[57-58] Fludarabine phosphate (Fludara®) is a prodrug that undergoes rapid dephosphorylation to the systemically circulating active compound, F-ara-A. F-ara-A is then phosphorylated intracellularly by several kinases to the 9-beta-D-arabinofuranosyl-2-fluoroadenine triphosphate, which is responsible for inhibition of DNA synthesis and RNA production, ultimately resulting in apoptosis.[57-59] Typically, fludarabine is administered for three to five days prior to transplantation at doses ranging from 25-40mg/m^2/day.[56] Rates of hematopoietic engraftment, TRM, and graft vs host disease (GVHD) are highly variable and the reasons for variability remain unclear.[2,16,60] Several factors that may contribute to differences in outcomes using fludarabine-based preparative regimens include underlying disease, concomitant antineoplastics, stem cell source, GVHD prophylaxis, and variability in fludarabine exposure.

The pharmacokinetics of fludarabine has been studied extensively in a variety of malignant diseases but limited data are available in HCT.[61-71] Fludarabine pharmacokinetics in the setting of nonmyeloablative HCT has been evaluated when combined with busulfan at doses of 30 mg/m^2 given daily over 4 days.[72-73] In two published reports sample size was limited and the relationship between F-ara-A pharmacokinetic parameters and clinical outcomes such as engraftment and toxicity were not evaluated.[72-73] The goal of this study was to characterize the pharmacokinetics of F-ara-A in subjects receiving a nonmyeloablative fludarabine, cyclophosphamide, and total body irradiation (TBI) preparative regimen and evaluate the relationship between fludarabine exposure and engraftment, acute GVHD, TRM and survival.

3.2 METHODS

3.2.1 Patients

This was a single center, pharmacokinetic-pharmacodynamic study of fludarabine in 87 patients who underwent allogeneic nonmyeloablative HCT. Patients were eligible to participate if they met protocol eligibility criteria for transplantation and were to undergo a related or unrelated nonmyeloablative allogeneic HCT containing fludarabine, cyclophosphamide and TBI along with cyclosporine and mycophenolate mofetil (MMF) for posttransplant immunosuppression. This study was approved by the Institutional Review Board and the Cancer

3.2.2 Preparative Regimen and Immunosuppression

The preparative regimen consisted of cyclophosphamide 50mg/kg/day i.v. day –6 (total dose 50mg/kg); fludarabine 40mg/m^2/day i.v. on days –6 to –2 (total dose 200mg/m^2); and TBI 200cGy single fraction on day –1 (total dose 200cGy). Fludarabine was dose-reduced to 30-35mg/m^2/day in 9 patients with pre-existing mild to moderate renal impairment at the discretion of the clinical team. Patients without intensive chemotherapy in the prior 6 months received equine antithymocyte globulin 15mg/kg i.v. every 12 hours for 3 days (total dose 90 mg/kg) , with methylprednisolone 1mg/kg i.v. every 12 hours.

Intravenous MMF and cyclosporine was started on day -3 and converted to oral as tolerated. Cyclosporine doses were adjusted to maintain whole blood troughs of 200-400ng/ml.

3.2.3 Fludarabine Pharmacokinetics

F-ara-A pharmacokinetic sampling was prospectively performed with the first dose of fludarabine. Fludarabine was administered intravenously over one hour at a constant rate and samples were obtained at times 0, 1.6 (100min), 2, 3, 4, 6, 8, 12, and 24 hours after start of infusion. On day of transplant a sample was obtained prior to stem cell infusion. Five mL of blood was collected at each sampling time and placed in a heparinized green-top tube for F-ara-A analysis. All samples, within 60 minutes of collection, were centrifuged at 3400 rpm for 10 minutes at 4 degrees C, and the plasma removed and stored at -80 degrees C until analysis. Plasma serum creatinine (SCr) was obtained on day of pharmacokinetics and creatinine clearance

3.2.4 Bioanalysis

Plasma concentrations of F-ara-A were measured on an Agilent 1100 series (Agilent Technologies, Wilmington, DE) HPLC system equipped with variable wavelength UV detector and a Phenomenex Polar-RP, 4.6 x 250mm, 4µM reverse phase C-18 column (Phenomenex, Torrance, CA). F-ara-A standard and the internal standard 5, 6 dimethylbenzimidazole (DMB) were obtained from Sigma-Aldrich (Milwaulkee, WI). The mobile phase was sodium phosphate in water adjusted to a pH 1.59 with H_3PO_4 and acetonitrile (90%, 10% v/v). Plasma (750 µL) was added to tubes containing internal standard (DMB, 20 µL of a 1 µg/mL solution in methanol, evaporated to dryness). Phosphoric acid solution 4%, 0.8 ml was added to the tubes and samples applied to a 3ml Oasis MAX solid phase extraction cartridge (Waters Corp, Milford, MA). The plasma (750 µL) was then added to the prepared cartridge followed by 2 mL of 4% NH_4OH/water solution. Samples were then eluted with 2 mL of 4% NH_4OH/methanol solution, spun for 2 minutes, and evaporated to dryness under nitrogen at 37^{o}C and then reconstituted with 50uL of mobile phase. Twenty five uL were injected and monitored at 267 nm. The column flow-gradient was 0.75 mL/min for 0-8 min, 1.2 mL/min for 9-22 min and 0.75 mL/min for 22.5 min. The column and sample temperatures were maintained at 25°C and 10°C, respectively. Run time was 23 minutes. The validated assay was linear in the range of 10-3000 ng/mL. Assay accuracy, intraday and interday variability were 93.5-100.1%, 1.6-3.6% and 1.3-3.3%, respectively.

3.2.5 Pharmacokinetic Analysis

For each patient, F-ara-A plasma concentration-time data was analyzed using noncompartmental methods (WinNonLin Professional 5.2). Area-under-the-curve $AUC_{(0-\infty)}$ was estimated by the log/linear trapezoidal method as $AUC_{(0-t^*)} + C(t^*)/Ke$ where $C(t^*)$ was the last concentration and Ke is the terminal first order elimination rate constant. Ke was calculated from the slope of the log-linear portion of the plasma-concentration time curve using linear regression analysis. The terminal half-life was 0.693/Ke. Clearance was determined by dose/$AUC_{(0-\infty)}$. Volume of distribution was estimated from terminal phase of the time-concentration data. C_{max} was as the largest observed concentration. C_{min} (trough) was the 24 post dose concentration.

3.2.6 Statistical Analysis

The primary objective of this study was to determine the relationship between F-ara-A systemic exposure and neutrophil engraftment. Secondary objectives were to evaluate the relationship between F-ara-A exposure and incidence of acute GVHD, TRM, and overall survival. Neutrophil engraftment, acute GVHD, TRM, and survival data were collected through the transplant database. GVHD was staged and graded according to the standard GVHD criteria based on clinical and pathological criteria.[41-42] Day of neutrophil engraftment was defined as the first of 3 consecutive measures of an absolute neutrophil count (ANC) >500 cells/uL. Graft failure defined as survival without an ANC >500 cells/uL at day 42 or with autologous reconstitution. TRM was

defined as death without disease progression or relapse. For survival analysis, death due to any cause was considered an event. Pharmacokinetic data was summarized by descriptive statistics. Normal renal function was defined as a CrCl >70ml/min. Renal impairment was defined as CrCl< 70ml/min.[74]

Probabilities and 95% confidence intervals (CI) of acute GVHD and TRM were calculated using the cumulative incidence function while neutrophil engraftment and overall survival were calculated using the Kaplan-Meier method. Recursive partitioning regression was used to determine optimal significant cutpoints for pharmacokinetic parameters ($AUC_{(0-\infty)}$, C_{min}, clearance, Day_{zero}) towards clinical endpoints. Univariate analysis was performed evaluating each pharmacokinetic parameter and other clinical factors towards neutrophil engraftment, TRM and overall survival at 6 months and at day 100 for acute GVHD.

Cox proportional hazards regression was used to model independent predictors of TRM at 6 months posttransplantation and included the following: F-ara-A $AUC_{(0-\infty)}$, age, use of ATG in preparative regimen, recipient CMV status, donor source, comorbidity score[75-76], CrCl, and acute GVHD grades II-IV.

3.3 RESULTS

Patient characteristics are described in Table 3.1. A total of 87 adult patients had fludarabine pharmacokinetic sampling performed; nine patients

received dose reductions of fludarabine due to pre-existing renal impairment [30mg/m^2/day (n=1), 32mg/m^2/day (n=5) and 35mg/m^2/day (n=3)].

3.3.1 F-ara-A Pharmacokinetics

F-ara-A pharmacokinetics parameters are shown in Table 3.2. The concentration-time profiles for patients receiving the standard dose of fludarabine (40mg/m^2) and modified doses of fludarabine (30-35mg/m^2) are shown in Figure 3.1. F-ara-A exposure was variable with up to a 5.7 fold difference in $AUC_{(0-\infty)}$ among patients receiving the standard fludarabine dose of 40mg/m^2/day. Individuals with preexisting mild to moderate renal impairment, despite dose reductions of 20-25%, had higher F-ara-A exposures including $AUC_{(0-\infty)}$ and trough concentrations (Table 3.2). F-ara-A clearance was reduced by ~25% in patients with decreased renal function. F-ara-A was detectable on day of transplant in all patients. F-ara-A dose adjusted $AUC_{(0-\infty)}$ was poorly correlated with CrCl (r^2=0.22, p< 0.01) and SCr (r^2=0.02, p=0.18).

3.3.2 Relationship between F-ara-A Exposure and Clinical Outcomes

3.3.3 Engraftment of Donor Cells

Primary neutrophil recovery occurred in 86% of patients at a median (range) of 11 days (1-38) following HCT. Graft failure occurred in 14 (16%) patients. In univariate analysis, no F-ara-A pharmacokinetic parameters were associated with engraftment.

3.3.4 Acute Graft vs Host Disease

Cumulative incidences of grades II-IV and III-IV acute GVHD at day 100 were 50% and 17%, respectively. In univariate analysis, patients with F-ara-A $AUC_{(0-\infty)}$ >6.5ug*hr/mL had significantly lower incidence of acute GVHD grades II-IV (14%) compared to those ≤6.5ug*hr/mL (52%), p=0.04. No other F-ara-A pharmacokinetic parameters were associated with development of grades II-IV or III-IV acute GVHD.

3.3.5 Treatment-Related Mortality

The incidence of TRM at 6 months was 21% (95% CI, 12-29%), occurring at a median of 1.5 months (range, 0.2-6.1) posttransplant (Figure 3.2). A total of 18 treatment-related deaths occurred with multi-organ failure (n=8) the most common cause. Pulmonary and/or cardiac involvement was present in the majority of multi-organ failure deaths. Of the 18 patients that died of TRM, 7 patients had confirmed alveolar hemorrhage. Three patients died from encephalopathy, 2 were directly attributed to fludarabine and 1 considered multifactoral. Other causes of TRM included infection (n=3), bleeding/thrombosis (n=3), and acute GVHD (n=1). In univariate analysis, patients with F-ara-A $AUC_{(0-\infty)}$ >6.5ug*hr/mL had significantly higher cumulative incidence of TRM at 6 months (50%) compared to patients with F-ara-A $AUC_{(0-\infty)}$ ≤6.5ug*hr/mL (15%), p<0.01 (Figure 3.2). The cause of death in the 7 patients with TRM and F-ara-A $AUC_{(0-\infty)}$ >6.5ug*hr/mL included multi-organ failure (n=4), encephalopathy (n=1), infection (n=1) and bleeding/thrombosis (n=1). TRM at 6 months was also significantly higher in patients with F-ara-A

clearance ≤12.5L/h when compared to patients with an F-ara-A clearance >12.5L/h (45% vs 12%, p<0.01). F-ara-A C_{min}>80ng/mL was associated with higher cumulative incidence of TRM at 6 months vs F-ara-A C_{min} ≤80ng/mL (64% vs 15%, p<0.01). F-ara-A concentrations on day of transplant ≤30ng/mL were associated with reduced TRM (12%) compared to patients with F-ara-A >30ng/mL (53%), p<0.01. Of the 87 patients, 14 patients (16.1%) had F-ara-A $AUC_{(0-\infty)}$ >6.5ug*hr/mL, 65 patients (74.7%) had troughs >80ng/mL, and 12 patients (10.44%) had an F-ara-A clearance <12.5L/h. Seventeen (14%) had F-ara-A concentrations ≥30ng/mL on the day of stem cell infusion. In univariate analysis other clinical factors associated with higher risk of TRM at 6 months were ATG in the preparative regimen (30% vs 11%, p=0.02) and CrCl <70ml/min compared to ≥70ml/min (22% vs 2%, p<0.01).

The Cox regression analysis for F-ara-A $AUC_{(0-\infty)}$ is given in Table 3.3. At 6 months following transplantation the only independent predictor of TRM was F-ara-A $AUC_{(0-\infty)}$. Patients with an F-ara-A $AUC_{(0-\infty)}$ >6.5ug*hr/mL had a 4.56 greater risk of TRM than individuals with an F-ara-A $AUC_{(0-\infty)}$ ≤6.5ug*hr/mL (p=0.02).

3.3.6 Overall Survival

Six month overall survival was (71%, 95% CI: 60-80%) with 47 patients alive at a median of 12 months (range, 0.2-29.5) (Figure 3). The most frequent causes of death were disease relapse or progression (n= 7), multi-organ failure (n=8), encephalopathy (n=3), infection (n=3), bleeding/thrombosis (n=3), and

acute GVHD (n=1). In univariate analysis, patients with F-ara-A $AUC_{(0-\infty)}$ >6.5ug*hr/mL had significantly lower overall survival (36%) at 6 months compared to patients with F-ara-A $AUC_{(0-\infty)}$ ≤6.5ug*hr/mL (78%), p<0.01 (Figure 3.3). Similarly, overall survival at 6 months was lower in patients with F-ara-A clearance ≤12.5L/h compared to clearance >12.5L/h, 41% vs 81%, p<0.01, respectively. Elevated F-ara-A trough >80ng/mL was associated with reduced overall survival 6 months posttransplantation (27% vs 76%), p<0.01. On the day of transplant, patients with F-ara-A concentrations >30ng/mL had significantly lower overall 6 month survival compared to patients with concentrations ≤30ng/mL (41 vs 80%), p<0.01.

3.4 DISCUSSION

This is the first study to evaluate F-ara-A exposure and its relationship to stem cell engraftment, GVHD, TRM, and survival in nonmyeloablative allogeneic HCT. We showed that F-ara-A exposure is highly variable with 6-10 fold differences in AUC and troughs in patients receiving fludarabine at 40mg/m^2. Our data demonstrates that elevated F-ara-A concentrations are associated with a greater risk of TRM and decreased survival. Finally, in preparative regimens that use 40mg/m^2 of fludarabine, dose reductions greater than 20-25% should be considered in patients with renal impairment as F-ara-A clearance is reduced and exposure is higher even despite dose reductions.

Fludarabine pharmacokinetics have been previously reported in HCT patients receiving fludarabine 30mg/m^2 intravenously over 30 minutes days -6

to -3 along with busulfan 1 mg/kg PO every 6 hours day -5 to day -2.[72-73] The pharmacokinetic parameters in our study were comparable to those previously described. It is evident from these data that renal insufficiency contributes to the variability in F-ara-A exposure. Previous studies demonstrate F-ara-A clearance correlates with CrCl, resulting in urinary excretion values for F-ara-A as high as 60% of the total administered dose.[66] In phase I trials of fludarabine a decline in renal function was associated with a decrease in total body clearance and volume of distribution.[71] In our study, the nine patients with pre-existing mild to moderate renal impairment (median CrCl 57.1ml/min) despite receiving a 20-25% dose reduction had higher plasma concentrations, reduced clearance of F-ara-A, and longer half-life compared to patients not receiving dose reductions (median CrCl 85.9ml/min). A trend towards a decrease in volume of distribution was also noted in our patients with renal insufficiency. Fludarabine dose reductions of 20-25%, as recommended by the manufacturer's guidelines[77], are routinely performed at our institution in patients with renal insufficiency. However, our data suggests that dose reductions >20-25% may be required in HCT patients with mild to moderate renal impairment in preparative regimens that use fludarabine 40mg/m^2.

Interestingly we report a weak correlation between CrCl and F-ara-A $AUC_{(0-\infty)}$ (r^2=0.22). This is lower than what has been reported in patients with refractory hematologic malignancies, where a moderate correlation between CrCl and F-ara-A clearance has been demonstrated (r^2=0.68).[67] Creatinine

clearance estimates in our study were determined by the Cockcroft and Gault equation and may not provide a true estimate of renal function.[78-79] At our institution, fludarabine doses are clinically determined based on this equation: however; they are occasionally determined by using a CrCl derived from a pre-HCT 24-hour urine collection. The poor correlation between CrCl and $AUC_{(0-\infty)}$ is therefore likely due to differences by which CrCl was estimated and doses determined.

Higher doses of fludarabine (40-100 mg/m^2) have been associated with delayed onset severe neurotoxicity including progressive multifocal leukoencephalopathy (PML), coma and death. Early phase I/II trials conducted in relapsed leukemia, estimated an 18% incidence of severe fludarabine neurotoxicity at these doses.[80-81] In the setting of allogeneic HCT the incidence of neurotoxicity and risk factors are not well defined. Fludarabine has been suggested as a potential risk factor for neurotoxicity in the setting of allogeneic umbilical cord transplantation HCT.[82] The large F-ara-A volume of distribution, (median 1.90L/kg), supports previous reports of high binding of fludarabine in the peripheral tissues.[67] Hence, patients with elevated systemic plasma concentrations of F-ara-A may experience higher drug concentrations in the central nervous system. This may be potentiated in patients with renal insufficiency where drug clearance would be delayed due to redistribution of F-ara-A from peripheral tissues. Several confounding variables must be considered including JC virus status and co-administration of other neurotoxic

agents. PML resulting from opportunistic infection with JC virus has been reported in chronic lymphocytic leukemia (CLL) patients treated with fludarabine.[83-86] More recently MMF has been associated with cases reports of PML.[87] Finally, all patients were treated with cyclosporine. Neurotoxicity, including posterior reversible leukoencephalopathy, may occur in up to 30% of patients receiving cyclosporine.[88] Irreversible encephalopathy with the use of cyclosporine in pediatric patients have been reported.[89] In each of these six case reports none of the children received fludarabine.[89]

Pulmonary toxicity associated with the use of fludarabine in the treatment of CLL is characterized by fibrosing interstitial pneumonitis, mononuclear cell infiltrates, and fibrosing alveolitis.[90-91] Fludarabine-induced apoptosis has been demonstrated *in vitro* in human alveolar epithelial cell lines at pharmacologically relevant concentrations.[92-93] It is reasonable to assume patients with higher systemic F-ara-A may experience increased exposure at the site of alveolar epithelial cells, therefore contributing to the pulmonary toxicity we observed. Of the 18 patients that died of TRM, 7 patients had confirmed alveolar hemorrhage, of which 3 had F-ara-A $AUC_{(0-\infty)}$ >6.5ug*hr/mL.

We found no influence of F-ara-A pharmacokinetic measures on neutrophil engraftment. Interestingly, detectable F-ara-A levels were still present on day of transplant in all patients. Studies in relapsed CLL patients comparing responders vs non-responders suggest sufficient systemic F-ara-A plasma concentrations are needed to maintain a critical minimum inhibitory F-

ara-ATP concentration in tumor cells.[65] It is possible the long terminal half-life, combined with the 5 day dosing regimen leads to intracellular F-ara-ATP accumulation, resulting in all patients at or above this threshold. In our analysis intracellular F-ara-ATP concentrations were not evaluated however pharmacodynamic studies involving intracellular concentrations of F-ara-ATP and engraftment are needed. Additionally, variability in intracellular drug concentrations may be important in predicting stem cell engraftment and pharmacogenomic studies including transporters, kinases and targets involved in the fludarabine metabolic pathway should also be considered.[94-96] Finally, we acknowledge engraftment is a complex process and is influenced by several factors including cell dose, donor source, and MMF exposure which are not accounted for in these analyses.[6]

We found a strong association between higher plasma concentrations of fludarabine and increased the risk of TRM and reduced overall survival following nonmyeloablative HCT. Whether these relationships hold true for other preparative regimens, especially those containing lower doses of fludarabine have yet to be established. Renal insufficiency led to higher concentrations of fludarabine and dose reductions are warranted to avoid excessive systemic exposure. Future studies are needed to more clearly define the relationship between the level of immunosuppression required for stem cell engraftment and toxicity.

Table 3.1: Patient Demographics

	Median (range) / N (%)
N	87
Age (years)	55(20-69)
Fludarabine dose (mg/m^2)	40(30-40)
Weight (kg)	82.6(41.5-139.5)
Male	56 (64%)
Female	31(36%)
Serum creatinine (mg/dL) [1]	0.9(0.4-1.5)
Creatinine clearance (mL/min) [1]	82.1(49.5-153.2)
Recipient CMV positive	53(61%)
Comorbidity Score [2]	
0	9 (10%)
1-2	32 (37%)
$\geq$3	46 (53%)
Disease	
Acute lymphoblastic leukemia	6(7%)
Acute myelogenous leukemia	26(30%)
Chronic myeloid leukemia	1(1%)
Other leukemia	6(7%)
Myelodysplastic syndrome	14(16%)
Non-Hodgkin's lymphoma	17(20%)
Hodgkin's lymphoma	8(9%)
Other	9(10%)
Graft Source	
Bone marrow	2(2%)
Peripheral blood stem cell	21(24%
Cord Blood	64(74%)
1 umbilical cord	3(5%)
2 umbilical cords	61(95%)
Donor	
HLA Matched-related	22(25%)
Unrelated	65(75%)
Disease Risk[3]	
Standard risk	28(32%)
High risk	59(68%)
ATG in the conditioning regimen	40(46%)

[1]obtained on day of pharmacokinetic sampling, [2] Sorror et al.[75], [3] Brunstein et al.[2]

Table 3.2: F-ara-A pharmacokinetic parameters with first dose of fludarabine[1]

	Standard Dose	Dose-Reduced
N	78	9
Fludarabine dose	40mg/m^2	30-35mg/m^2
Creatinine Clearance, mL/min	85.9(49.5-153.2)	57.1(50.5-65.1)
C_{max}, ng/mL	958 (384-2046)	798 (694-1073)
C_{min} trough, ng/mL	52 (17-166)	69 (39-93)
$AUC_{(0-\infty)}$, ug*hr/mL	4.9 (2.0-11.5)	5.5 (4.3-7.0)
Volume of distribution, L/kg	1.95 (0.89-4.78)	1.72 (1.40-2.51)
Clearance, L/hr	16.0 (6.2-36.6)	11.5 (6.9-15.2)
Half-life, hours	8.53 (3.75-22.18)	10.32 (7.81-14.48)
Day_{zero}[2], ng/mL	15.0 (0.1-144.1)	19.0 (4.0-63.6)

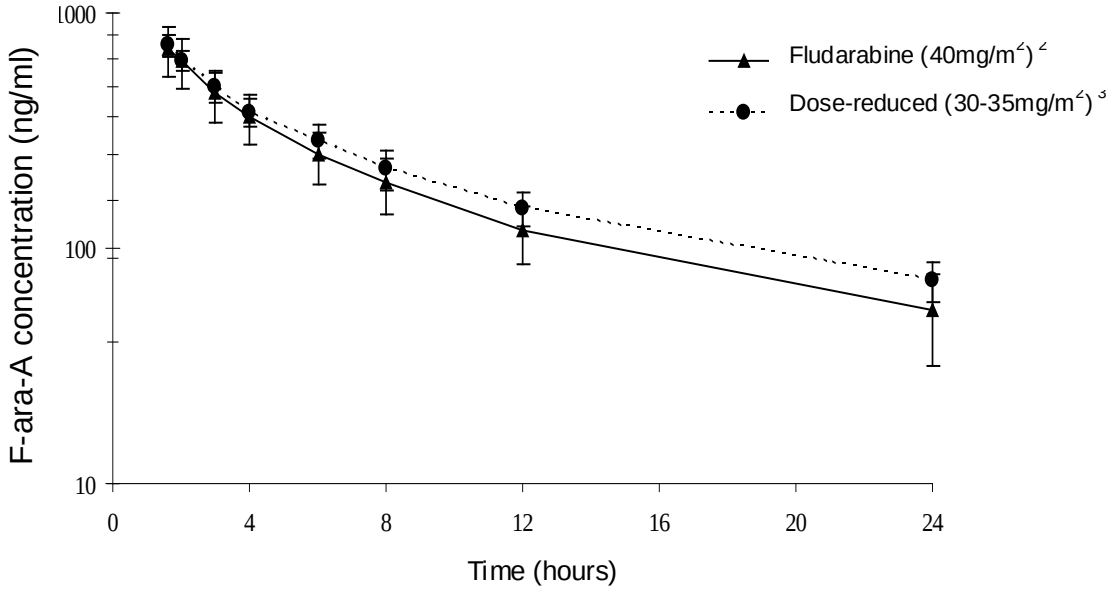
[1]data are median (range), [2]single concentration obtained on day of transplant

Table 3.3: Multiple regression analysis of TRM at 6 months following nonmyeloablative HCT

Factor	Relative Risk of TRM (95% CI)	P-value
F-ara-A $AUC_{(0-\infty)}$		**.02**
≤ 6.5 ug hr/mL*	1.00	
> 6.5 ug hr/mL	4.56 (1.22-17.14)	
Age (yrs)		.89
< 55*	1.00	
≥ 55	0.92 (0.26-3.20)	
ATG in conditioning regimen		.09
Yes*	1.00	
No	0.36 (0.11-1.19)	
Recipient CMV status		.93
Negative*	1.00	
Positive	0.94 (0.27-3.34)	
Donor Source		.97
Matched-related*	1.00	
Unrelated	1.03 (0.31-3.40)	
Comorbidity Score		.18
0*	1.00	
1-2	0.22 (0.04-1.25)	
≥3	0.60 (0.12-2.98)	
Creatinine clearance		.41
≥ 70 ml/min*	1.00	
< 70 ml/min	1.63 (0.52-5.13)	
aGVHD grades III-IV		.15
No*	1.00	
Yes	2.91 (0.67-12.60)	

* reference group

Figure 3.1: F-ara-A Time vs Concentration Profile with first dose of fludarabine[1]



[1]data are mean (standard deviation), [2]patients receiving standard doses of fludarabine with CrCl median (range) of 85.9ml/min (49.5-153.2), [3]patients receiving dose modifications of fludarabine based on pre-existing mild to moderate renal insufficiency with CrCl median (range) of 57.1ml/min (50.5-65.1).

Figure 3.2: Cumulative incidence of TRM for patients 6 months after nonmyeloablative HCT.

(A) Overall TRM. (B) TRM for patient with F-ara-A $AUC_{(0-\infty)}$ $\leq$6.5ug*hr/mL compared to patients with $AUC_{(0-\infty)}$ >6.5ug*hr/mL.

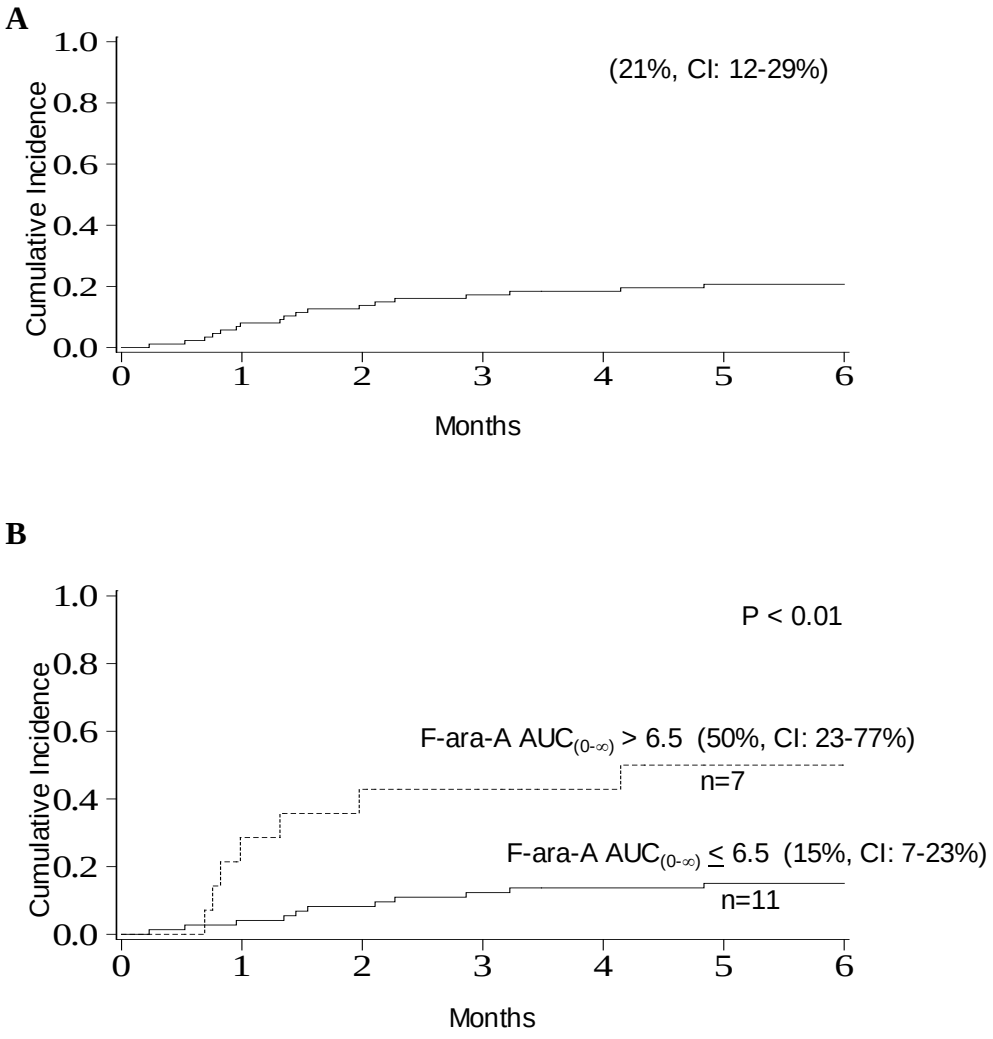
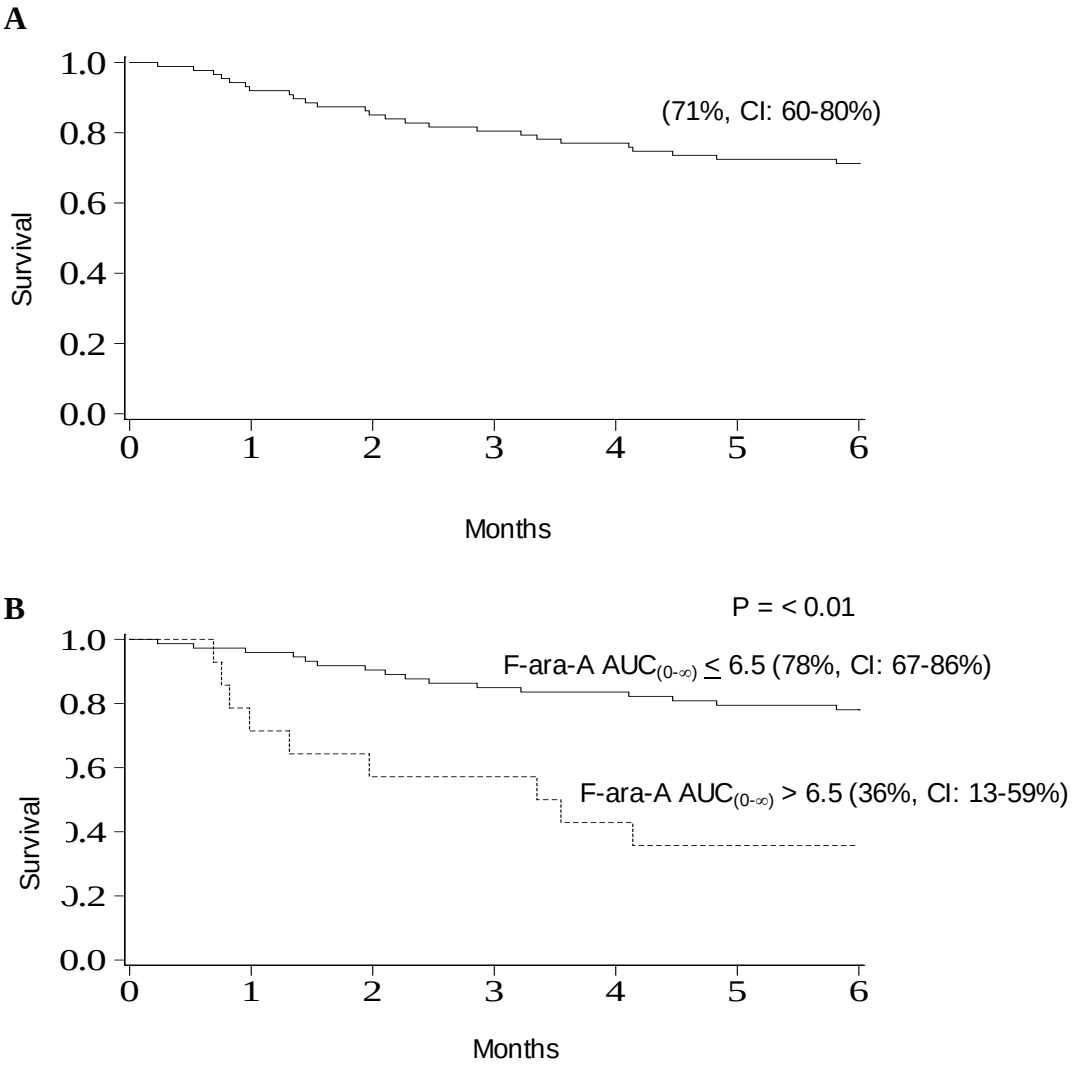


Figure 3.3: Cumulative proportion of overall survival for patients 6 months after nonmyeloablative HCT.

(A) Overall survival. (B) Overall survival for patient with F-ara-A $AUC_{(0-\infty)}$ $\leq$6.5ug*hr/mL compared to patients with $AUC_{(0-\infty)}$ >6.5ug*hr/mL.



CHAPTER 4

CLOFARABINE

Pharmacokinetics of Clofarabine in Patients with High Risk Inherited Metabolic Disorders Undergoing Brain Sparing Hematopoietic Cell Transplantation (BS-HCT)

Clofarabine, a newer purine analog with reduced central nervous system (CNS) toxicity may prove advantageous in HCT in patients where neurotoxicity is a natural part of disease progression. This study aimed to evaluate clofarabine pharmacokinetics in adult and pediatric patients undergoing BS-HCT for the treatment of high risk, inherited metabolic disorders. Clofarabine (40 mg/m^2/day) was administered intravenously on days -7 to -3. Kinetic sampling occurred with dose 1 and 5, along with a single level collected on day of transplant (day_0). Sixteen patients were studied with a median (range) age and BSA of 7.5 years (0.5-43) and 0.94 m^2 (0.31-2.3), respectively. Clofarabine $AUC_{0-\infty}$ was 930ng·hr/ml (684-1875), C_{max} 225ng/ml (162-600), and C_{min} 3.17ng/ml (1.74-5.64). Clofarabine clearance (CL) was 1.6L/h/kg (0.7-2.4) and inversely correlated with wt (r^2=0.33) and BSA (r^2=0.26). No difference in plasma concentrations was found between dose 1 and 5 (all $p>0.05$). All levels were below the limit of quantification (1ng/ml) on day_0 in patients with normal renal function. Variability in clofarabine clearance was approximately 2-3 fold and only moderately explained by covariates such as weight and age. In patients with adequate renal function, no drug accumulation occurs with consecutive daily dosing.

4.1 INTRODUCTION

Hematopoietic cell transplantation has been shown to effectively stop disease progression in a variety of inherited metabolic disorders (i.e. X-linked adrenoleukodystrophy) provided it is performed in early stages of disease development.[97-101] However, in patients with aggressive or advanced disease including significant CNS involvement, the benefits of HCT remain controversial. In these patients, neurological deterioration may be accelerated by HCT, the result of a combination of chemotherapeutic agents used in preparative regimens with known neurotoxic side-effects and underlying disease-related CNS inflammation.[102-104] Strategies have been developed to improve outcomes and decrease treatment-related toxicity in patients with advanced disease, including the use of reduced-intensity (RI) chemotherapy.[105] The nucleoside analog, fludarabine, is often included in RI conditioning regimens because of its potent antitumor and immunosuppressive properties. However, both early and delayed CNS toxicity has been associated with RI protocols which incorporate fludarabine into the preparative regimen.[7] The avoidance of chemotherapeutic agents in preparative regimens with known neurotoxic side-effects is especially important for patients with metabolic disorders where pre-existing CNS damage might be further potentiated by chemotherapy.

Designed to retain the anti-tumor properties of fludarabine and cladrabine but improved safety profile, clofarabine (2-chloro-2'-fluoro-deoxy-9-β-D-arabinofuranosyladenine) a newer generation nucleoside analog, was

developed. Clofarabine has demonstrated anti-tumor activity against a variety of hematologic and solid tumor malignancies[12,106] and exerts its anti-cancer activity through intracellular generation of the triphosphate species, which inhibits DNA synthesis, ribonucleotide reductase, and induces apoptosis.[12,107-109] Clinical studies with clofarabine in adult and pediatric subjects report no serious cases of neurotoxicity, demonstrating an improved safety profile compared to older generation nucleoside analogs.[8-12] The absence of neurotoxicity observed with clofarabine may be the result of reduced CNS penetration, demonstrated by lower drug concentrations in the cerebrospinal fluid (CSF) of non-human primates receiving therapeutic doses of clofarabine.[110] The median percent of clofarabine in the CSF was 5%, compared to 20% for cytarabine and cladrabine, which are associated with significant neurotoxicity.[110] Clofarabine's potential for lower CNS toxicity, and retention of immunosuppressive activity may be advantageous in HCT, particularly in patients where neurodegeneration is part of disease progression. To date, no pharmacokinetic data is available on clofarabine in the setting of HCT. The goal of this study was to characterize the plasma pharmacokinetics of clofarabine in patients with high risk inherited metabolic disorders receiving a brain-sparing preparative regimen.

4.2 PATIENTS AND METHODS

A total of 18 patients between February 2007 and December 2008 were enrolled in this single center, open-label, non-randomized study evaluating the

efficacy and safety of clofarabine as part of a brain sparing preparative regimen for the treatment of high risk inherited lysosomal and peroxisomal disorders. Patients were eligible to participate in pharmacokinetic sampling if they met protocol specific eligibility criteria for transplantation and were to undergo a related or unrelated BS-HCT containing clofarabine, melphalan, alemtuzumab, and TBI for the treatment of a high risk, inherited metabolic disorder which included significant CNS involvement. This study was approved by the University of Minnesota Institutional Review Board and all patients provided written informed consent. Of the 18 patients enrolled in this study, 16 subjects elected to participate in pharmacokinetic sampling.

The preparative regimen consisted of alemtuzumab 0.3mg/kg/day IV days -12 to -8; clofarabine 40mg/m^2/day IV on days -7 to -3; melphalan 140mg/m^2 IV on day -2; and TBI 200cGy single fraction on day -1. Clofarabine (Clolar®, Genzyme Corporation) was purchased from a commercially available vender as 20mg vials (1mg/ml). Just prior to administration, each dose was removed from a stock vial, filtered though a 0.22 micron filter, and diluted with 0.9% sodium chloride to a yield a final concentration of 0.15-0.4mg/ml.

4.2.1 Pharmacokinetic Sampling

Intensive pharmacokinetic sampling was performed with the first dose of clofarabine therapy. Clofarabine (40mg/m^2) was administered intravenously over two hours at a constant rate and samples were obtained at 2, 3, 4, 6, 8, and 24 hours after start of infusion. To evaluate drug accumulation over the

entire five day course of treatment less intensive pharmacokinetic sampling was performed on day 5 of therapy, with sample collection occurring just prior to administration of the 5th dose, followed by 4 and 8 hours after start of infusion. On day of transplant ($day_{0,}$ 72 hours post dose 5) a single sample was obtained just prior to stem cell infusion. Three (3mL) of blood was collected at each sampling time through a central venous catheter and placed in a heparinized tube for clofarabine analysis. All samples, within 60 minutes of collection, were centrifuged at 3400 rpm for 10 minutes at 4ºC, and the plasma removed and stored at -80ºC until analysis. Clinical studies were obtained with dose 1 pharmacokinetics and included markers for renal function (serum creatinine (Scr), blood urea nitrogen (BUN)). Creatinine clearance (CLCR) was estimated in adults by the Cockcroft-Gault equation using ideal body weight[111] and in pediatric patients by the Schwartz method.[112]

Plasma samples were analyzed by MicroConstants (San Diego, California) using a validated reverse phase high performance liquid chromatography with mass spectrometry as previously described.[113] The assay was linear in the range of 1 to 500ng/ml. Samples with concentrations above the upper limit of linearity were diluted and re-assayed. Samples with clofarabine levels reported below the lower limit of quantification (1ng/ml) were entered into pharmacokinetic analysis as having a concentration of 0.5ng/ml (half the lower limit of quantification). Assay accuracy, intra-day, and inter-day variability were range 95-96.2%, 5.1-7.4%, and 6.7-14.4%, respectively.

4.2.2 Pharmacokinetic Analysis

For each patient, clofarabine plasma concentration-time data from dose 1 was analyzed using standard noncompartmental methods (WinNonLin Professional 5.2, Pharsight Corp, Mountain View, CA). Area-under-the-curve $AUC_{0-\infty}$ was estimated by the log/linear trapezoidal method as $AUC_{(0-t^*)}$ + $C(t^*)/Ke$ where $C(t^*)$ was the last quantifiable concentration and Ke is the terminal first order elimination rate constant. The elimination phase rate constant (Ke) was calculated from the slope of the log-linear portion of the plasma-concentration time curve using linear regression analysis. The terminal half-life ($T_{1/2}$) was calculated as 0.693/Ke. Clearance (CL) was determined by dose/$AUC_{0-\infty}$. Volume of distribution (V_{ss}) was estimated from terminal phase of the time-concentration data. C_{max} was as the largest observed plasma concentration of clofarabine after completion of infusion. C_{min} was the minimum concentration 24 hours after dose 1.

4.2.3 Statistical Analysis

All statistical analysis was performed using Microsoft Excel 2007 software for Windows (Microsoft Corporation, Seattle, WA). Patient demographics and pharmacokinetic data were summarized by descriptive statistics. To evaluate the relationship between pharmacokinetic parameters and clinical covariates including body surface area (BSA), weight (wt), and markers for renal function, correlations were performed by simple linear regression using the least squared method. All r-squared (r^2) values reported

represent the squared product of the Pearson's coefficient, along with associated p-values. The paired student's t-test was used to compare clofarabine plasma concentrations between dose 1 and dose 5 using both C_{min} and the 4-hour concentration. The C_{min} used for dose 5 was the concentration obtained just prior to administration of the dose. For all statistical analyses a p-value of less than 0.05 was considered statistically significant.

4.3 RESULTS

Patient demographics are presented in Table 4.1. Intensive pharmacokinetic sampling was performed in a total of 16 patients (1 adult, 15 children) following dose 1 of clofarabine. Among the 16 study subjects, 13 patients completed less intensive pharmacokinetic sampling with dose 5. Eleven patients had a single plasma sample available for analysis on day_0. The majority of subject were male (97%) and had a diagnosis of adrenoleukodystrophy. All patients had normal renal function starting with the first dose of clofarabine with CRCL of 152.6ml/min (118.9-238.9).

Clofarabine pharmacokinetic parameters determined following the first dose of clofarabine are presented in Table 4.2. Maximum plasma concentrations were observed just following completion of infusion with median (range) C_{max} of 225.3ng/ml(162-600). Trough concentrations 24-hours post start of infusion were low with C_{min} 3.2ng/ml (1.74-5.64). There was 2-3 fold variability in $AUC_{0-\infty}$ and CL at 930.9ng·hr/ml (684.5-1875.6) and 1.6L/h/kg (0.7-2.4), respectively. Volume of distribution was large at 8.8L/kg (4.1-12.1) and

also variable (~3 fold) among subjects, despite a relatively short $T_{1/2}$ of 4.9h (4.0-5.6). Only 1 out of 11 patients had clofarabine plasma concentrations above the limit of quantification on day_0 (6.35ng/ml). Notably, this subject (6-month-old male weighing 6.2 kg, with diagnosis of osteopetrosis) developed acute renal dysfunction over the course of treatment, beginning with the second dose of clofarabine. Acute renal failure was indicated by elevated BUN and Scr and ultimately peritoneal dialysis was required and initiated on day of stem cell infusion (day_0). Figure 4.1 displays clofarabine plasma concentrations collected over the course of treatment in all patients. Additionally, individual plasma concentrations for the single patient experiencing renal dysfunction during clofarabine course of treatment are distinguished in Figure 4.1.

To determine if clofarabine accumulation occurred over the 5 day course of treatment, plasma concentrations were compared between dose 1 and dose 5 of therapy (n=13). Although generally higher, plasma concentrations 24-hour post dose 1 of clofarabine (C_{min}) were not statistically different when compared to trough levels collected just prior to Dose 5 (p=0.10) (Table 4.3). Similarly, no differences were seen between 4-hour plasma concentrations collected with dose 1 and dose 5 (p=0.67).

To evaluate interpatient variability in clofarabine pharmacokinetics, correlation analysis was performed with clinical parameters. Despite known renal elimination of clofarabine, the correlation between Scr (r^2=0.24) and CL was poor and no linear relationship was identified between BUN (r^2=0.11),

CLCR (r^2=0.05), and clofarabine CL. Similarly, no correlations between other pharmacokinetic parameters ($AUC_{0-\infty}$, C_{max}, C_{min}) and clinical markers for renal function were found. Clofarabine CL was moderately inversely correlated with wt (r^2=0.33) and age (r^2=0.33), and less than 30% variability in CL could be explained by BSA (r^2=0.26) (figure 4.2). Observed variability in clofarabine exposure or V_{ss} did not correlate with any clinical parameters tested including wt, BSA, age, or renal function.

4.4 DISCUSSION

Clofarabine has demonstrated potent antitumor activity and efficacy towards a variety of hematologic malignancies in both adult and pediatric patients and extensive information is available regarding clofarabine pharmacokinetics.[12,114-119] We are the first to report clofarabine pharmacokinetics as part of a brain-sparing preparative regimen in patients undergoing HCT for the treatment of high risk inherited metabolic disorders. The parameter estimates for $AUC_{0-\infty}$ and C_{max} presented in this analysis are consistent with the dose-proportional pharmacokinetics of clofarabine characterized in phase I and phase II trials of pediatric patients receiving clofarabine at doses ranging from 11.25-52mg/m^2 daily for 5 days for treatment of refractory hematologic malignancies.[115] Clofarabine CL (median 1.6L/h/kg) was slightly higher compared to 32.8L/h reported by Bonate et al and likely attributed lower median age (7.5 yrs vs 12.3) and weight (25.1kg vs 45.4) of patients enrolled in our study.[115] Linear regression identified a moderate

inverse correlation between clofarabine Cl and increasing body weight and age. Weight has been previously identified through population pharmacokinetic analysis as a significant covariate influencing clofarabine clearance in pediatric leukemia patients.[115] Furthermore, when clofarabine pharmacokinetics where evaluated in adults with solid tumors, clearance was lower and volume of distribution smaller, compared to pediatric patients.[12] The effect of weight and age on clearance is not unexpected, as younger children often require higher doses compared to older adolescence or adults due to increased renal clearance when eliminated occurs by glomerular filtration, as well through tubular secretion via transporters.[120] Although their impact on renal elimination has not yet been evaluated *in vivo*, several transporters have been shown to influence clofarabine distribution.[121-122]

Clofarabine inhibits DNA synthesis in a variety of leukemic and solid tumor cell lines *in vitro* at IC_{50} values ranging from 0.028-0.29μM.[106,123] Despite a systemic beta half-life of only 4.9 hours, all patients maintained systemic C_{min} concentrations well above the *in vitro* concentrations required for tumor inhibition. When drug levels were compared between the 1st dose and 5th dose, we did not find any evidence supporting accumulation of systemic clofarabine over the 5 day treatment course. These results are similar to what has been reported when fludarabine is given daily at doses ranging from 30 to 40mg/m^2 in adult HCT patients with adequate renal function.[72,124] However, discussion is warranted regarding the single patient included in this analysis that developed

acute renal failure beginning with dose 2 of the 5 day treatment regimen. Clofarabine concentrations were highest in this patient, approximately 2.7- 4 fold higher at each pharmacokinetic sampling time with dose 5 compared to the median. Additionally, this was the only patient with a quantifiable clofarabine concentration on day of hematopoietic cell infusion, indicating prolonged half-life and reduced clearance. Clofarabine is up to 60% renally eliminated unchanged in the urine and currently there is no pharmacokinetic data available in patients with renal dysfunction.[8] The high volume of distribution of clofarabine represents extensive peripheral binding in the tissues. Prolonged drug exposure in the peripheral compartment resulting from impaired renal elimination could lead to increased risk of treatment-related-toxicity, including CNS toxicity. To ensure appropriate dosing and prevent over-exposure in HCT, futures studies are needed to clearly define the influence of renal function on clofarabine pharmacokinetics.

Reduced-intensity conditioning regimens in HCT attempt to minimize toxicity while ensuring sufficient immunosuppression necessary to facilitate engraftment of stem cells. Unfortunately, higher rates of graft failure have been reported with RI conditioning regimens in patient with nonmalignant disorders.[125-127] Systemic clofarabine concentrations on day_0 (72 hours post dose 5) were below the limit of detection (1ng/ml) in all patients with normal renal function. Recently we have shown significant systemic fludarabine concentrations are detectable on day of stem cell infusion in adult patients with

hematologic malignancies undergoing nonmyeloablative HCT receiving fludarabine 40mg/m^2/day IV on days -6 to -2.[124] In this study, primary neutrophil recovery was high at 86%, however no association was found between systemic drug concentrations on day_0 and engraftment.[124] It is reasonable to consider a minimum inhibitory concentration of intracellular fludarabine triphosphate is required to maintain the level of immunosuppression needed for stem cell engraftment on day_0 and perhaps systemic fludarabine is acting as a surrogate marker. Hence, exposure to clofarabine on day of stem cell infusion may enhance engraftment. Intracellular clofarabine triphosphate concentrations were not a part of this analysis. When evaluated in 62 patients with relapsed or refractory leukemia, responders to clofarabine therapy accumulated more intracellular triphosphate compared with nonresponders, and yet no correlation was observed between systemic clofarabine and intracellular triphosphate concentrations.[114] Future studies should include quantification of intracellular clofarabine triphosphate as these analyses may provide for a better correlation with clinical outcomes.

Variability of 2-3 fold was observed in several clofarabine pharmacokinetic parameters including CL, $AUC_{0-\infty}$, and V_{ss}. Correlation coefficients were low indicating weight, age, and to a lesser extent BSA accounted for only about 30% of the variability in clofarabine clearance. No clinical covariates tested in this analysis adequately explained variability in $AUC_{0-\infty}$ and $V_{ss.}$ Clofarabine undergoes extensive renal elimination but clinical

markers of renal dysfunction are less likely to influence variability in Cl and exposure in this analysis since all patients were considered to have adequate renal function (>70ml/min) when intensive pharmacokinetic sampling occurred with administration of the first dose of clofarabine. This was evident in the poor correlation between Scr, BUN, CLCR and clofarabine CL and $AUC_{0-\infty}$. Clofarabine is transported across cell membranes by human equilibrative and concentrative nucleoside transporters, specifically, hENT1, hENT2, hCNT2 and hCNT3.[121-122] Localized in several tissues, including the kidney, transporters may influence clofarabine CL, exposure, and distribution. In particular, co-administration of drugs commonly used in the setting of HCT including anti-viral prophylaxis medications, antibiotics, and immunosuppressants excreted through the same renal transporters may lead to altered renal elimination and reduced clearance of clofarabine. Several genetic variants of hENT and hCNT transporters have recently been identified and may influence clofarabine pharmacokinetic parameters through altered activity or expression.[128-129]

This is the first study to report clofarabine pharmacokinetics in the setting of HCT as part of a brain-sparing preparative regimen including clofarabine, melphalan, alemtuzumab, and total body irradiation for treatment of an inherited lysosomal or peroxisomal disorder affecting the CNS. Due to the small sample size evaluation of pharmacokinetic-pharmacodynamic relationships were not feasible. Median age of subjects was 7.5 years and therefore the

pharmacokinetic parameters reported in this analysis not be similar to those of small children (less than 10kg) or adults undergoing HCT.

In conclusion, clofarabine pharmacokinetics displayed biphasic elimination and Cl similar to previously reported data in pediatric patients receiving single agent nucleoside monotherapy with clofarabine for refractory acute leukemia.[115-116] Drug accumulation over the five day treatment course did not occur and by day of stem cell infusion, systemic clofarabine drug levels were below the limit of quantifcation on day_0 in all patients with normal renal function. Variability in clofarabine clearance was observed and was only moderately explained by the covariates such as weight and age.

Table 4.1: Patient demographics

	Median (range) / N (%)
No.	16
Age (years)	7.5(0.5-43)
Male/Female	14(87%)/2(13%)
Weight (kg)	25.1(6.2-113.6)
BSA (m^2)	0.9(0.3-2.3)
Scr (mg/dl)[1]	0.4(0.2-1.0)
CLCR (ml/min)[1]	152.6(118.0-238.9)
Diagnosis	
Adrenoleukodystrophy	11(68%)
Metachromatic leukodystrophy	2(13%)
Mucolipidosis Type II	1(6%)
Gangliosidosis	1(6%)
Osteopetrosis	1(6%)
Donor	
HLA matched-related	4(25%)
Unrelated	12(75%)
Graft Source	
Bone marrow	4(25%)
Cord blood	12(75%)

[1]obtained on day 1 of intensive pharmacokinetic sampling with first dose of clofarabine

Table 4.2: Clofarabine pharmacokinetic parameters with first dose of clofarabine[1]

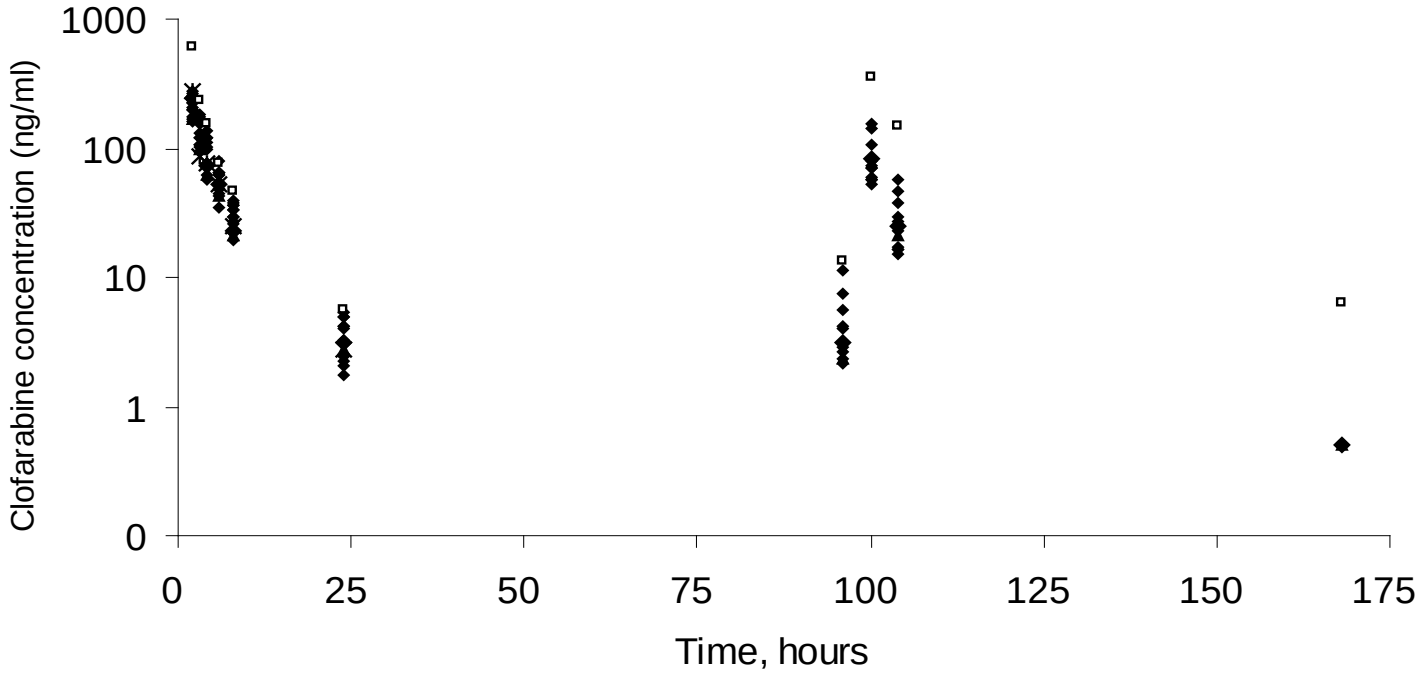
Pt No.	Weight, kg	Clofarabine dose, mg	Cmax, ng/ml	Cmin, ng/ml	$T_{1/2}$, hours	$AUC_{0-\infty}$, ng·hr/ml	CL, L/h/kg	Vss, L/kg	day_0,[2] ng/ml
1	8.1	16	194.4	1.74	4.1	823.5	2.4	10.3	*
2	113.6	90	224.5	5.02	5.2	1132.1	0.7	4.1	*
3	9.7	18	237.1	2.48	4.1	932.0	2.0	10.0	*
4	31.7	44	168.9	5.02	5.6	977.3	1.4	8.7	*
6	6.2	13	600.0	5.64	5.0	1875.6	1.1	4.7	6.35
7	25.5	38	250.0	2.50	4.0	1024.6	1.5	6.9	bql
8	44.6	52	242.0	3.12	4.9	914.8	1.3	6.2	bql
9	25.2	38	210.0	4.01	5.4	856.6	1.7	10.3	bql
10	25.8	40	198.0	3.22	4.6	929.8	1.7	8.9	bql
11	17.7	30	162.0	5.38	5.3	929.1	1.8	12.1	bql
12	29.3	44	226.0	4.21	4.8	969.3	1.5	9.0	bql
13	24.9	37	274.0	4.20	5.0	1151.1	1.3	6.1	bql
14	22.2	35	172.0	2.26	4.3	761.3	2.1	10.4	bql
16	24.4	36	169.0	2.76	4.9	684.5	2.2	11.5	bql
17	30.3	42	272.0	2.82	4.5	898.5	1.5	7.2	*
18	20.1	30	262.0	2.03	4.2	935.9	1.6	6.3	bql
median	25.1	37.3	225.3	3.2	4.9	930.9	1.6	8.8	-
range	(6.23-113.6)	(13-90)	(162-600)	(1.74-5.64)	(4.0-5.6)	(684.5-1875.6)	(0.7-2.4)	(4.1-12.1)	-

[1]data are median (range), [2]single concentration obtained on day of transplant, *no drug level obtained on day of transplant, blq=below level of quantification (1ng/ml)

Table 4.3: Comparison of clofarabine concentrations with dose 1 and dose 5

Patient No.	C_{min} concentration (ng/ml)		4-hr concentration (ng/ml)	
	Dose 1	Dose 5	Dose 1	Dose 5
1	1.74	2.13	77.6	52.8
2	5.02	11.5	121.0	156.2
3	2.48	4.01	56.9	108.4
4	5.02	7.36	99.9	142.6
6	5.64	13.6	152.0	351.0
7	2.50	2.70	121.0	56.5
8	3.12	3.07	74.4	83.1
9	4.01	3.14	61.9	74.3
10	3.22	2.86	112.0	71.1
11	5.38	5.51	103.0	59.5
13	4.20	4.12	136.0	58.1
14	2.26	2.36	75.0	69.7
16	2.76	2.37	61.3	80.5
mean	3.64	4.98	96.3	104.9
std	1.29	3.61	30.3	79.2
		p = 0.10		p = 0.67

Figure 4.1: Clofarabine time vs plasma concentration profile

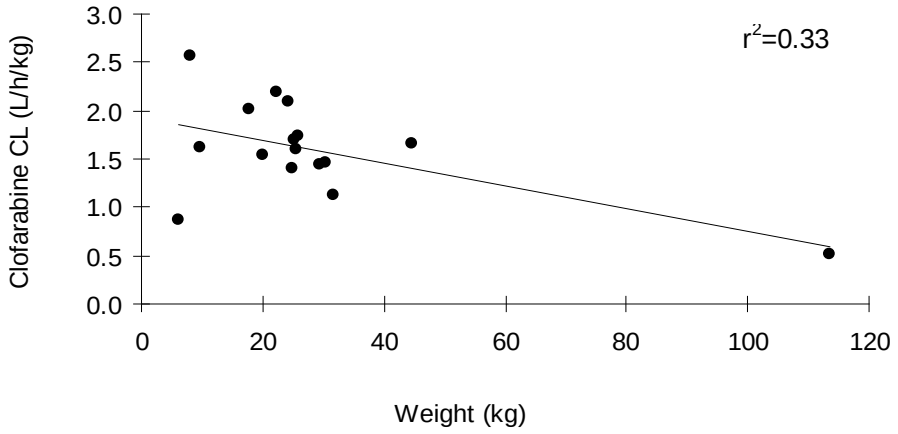


◆ Closed, black diamond represent clofarabine plasma concentration in patients with normal renal function (>70ml/min) during entire course of the clofarabine therapy through day_0

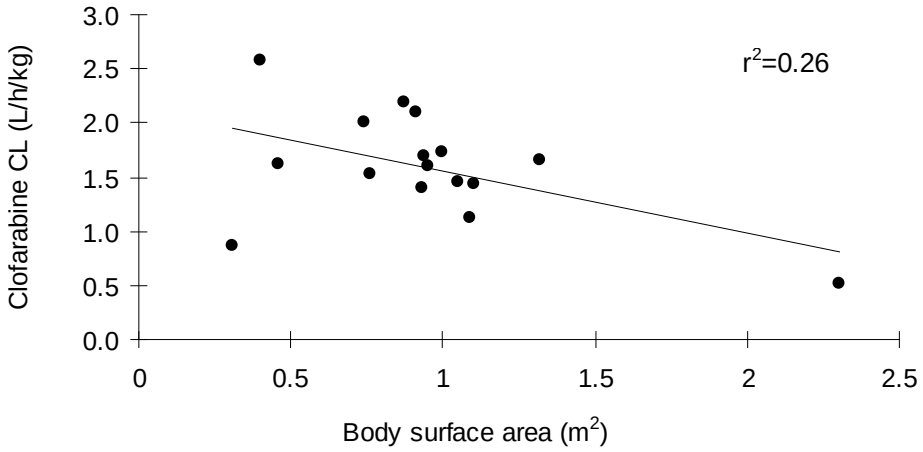
□ Open box represents plasma concentration in single subject that developed acute renal dysfunction starting with dose 2 of clofarabine therapy, demonstrating accumulation over the duration of the 5 day treatment course therapy through day_0

Figure 4.2: Linear regression plots for weight and body surface area vs clofarabine clearance

(a)



(b)



(a) Weight plotted vs clofarabine clearance (b) Body surface area plotted vs clofarabine clearance

CHAPTER 5

CLOSING THOUGHTS

5.1: WHERE DO WE GO FROM HERE?

The common objectives shared between each of the three clinical studies presented in this dissertation were to improve patient outcomes and reduce toxicity in patients undergoing NM alloHCT. However, with all the data analyzed and conclusions drawn it is easy for one to ask the question, "Where do we go from here?" In the last few pages of this dissertation, I will discuss how this research translates to the patient's bedside, as well provide future considerations for clinical and laboratory studies regarding MMF, fludarabine, and clofarabine in the setting of NM alloHCT.

Pharmacogenomics offers the ability to predict drug response based on genetic variability. The concept of selecting the best medication or dose for an individual based on their genetics with the goal of improving drug therapy is a grand notion. However, for many drugs, including MMF, the influence of non-genetic factors such as drug interactions, hepatic function, and renal function must also be considered in explaining both inter and intra-patient variability. For the clinician, the analysis of MMF provides some insight into the clinical covariates influencing the clearance and exposure of MPA in NM alloHCT, specifically renal function. However, with no established relationship between high MPA exposure and toxicity in HCT, the interpretation of creatinine clearance and MPA exposure must handled with caution. Empiric dose reductions of MMF in patients that develop renal dysfunction should be carefully considered at the present time. Close monitoring of white blood cell counts

should be performed and used in conjunction to guide dose modifications of MMF in patients that develop renal impairment.

In the current analysis, genetic variants proved unlikely to provide a better dosing strategy for MMF in NM alloHCT. However, population pharmacokinetic analysis of the MMF dataset could potentially lead to a better understanding of the genetic and non-genetic covariates influencing MPA and MPAG exposure. Population pharmacokinetic analyses are well designed to handle datasets with multiple doses and schedules, routes of administration, co-administered drugs, clinical covariates, and genetic variants, especially in comparison to traditional regression analysis. All of these variables mentioned are readily available for analyses in these patients. Several population pharmacokinetic models for MPA and MPAG have already been developed in solid organ transplantation and could likely be adapted for this dataset.

Analysis of fludarabine in patients undergoing NM alloHCT revealed variability in systemic F-ara-A concentrations and a strong association between higher plasma concentrations and increased risk of TRM and reduced overall survival. Additionally, it was revealed that F-ara-A exposures are still higher despite the recommend dose reduction of 20% in patients with renal insufficiency, when compared to patients with normal renal function receiving standard dose of 40mg/m^2 of fludarabine. I believe this is the first study confirming dose reductions of fludarabine in patients with renal dysfunction are warranted in NM alloHCT in order to prevent excessive systemic F-ara-A

exposure and toxicity. Currently, empiric dose reductions of fludarabine are guided by estimates for creatinine clearance, most commonly calculated by the Cockcroft and Gault equation. The accuracy of this method to estimate renal function in HCT remains controversial, especially among clinical pharmacists. In a separate analysis of the fludarabine dataset, population pharmacokinetic methods were used to formulate a covariate model for fludarabine clearance (data not shown, collaborative project with Pharmacometrics group). This covariate model has lead to the development of a test dose and limited pharmacokinetic sampling strategy that could be applied in the clinic setting prior to HCT. The recommended dose of fludarabine each patient would receive at the time of transplant would be compiled using a Bayesian approach, taking into consideration both the pre-developed covariate model for fludarabine clearance and 3 individual pharmacokinetic samples collected during the test dose administered in clinic prior to transplant. The goal of the test dose strategy is to ensure every patient is receiving the optimal dose on the first day of treatment. Additionally, intracellular drug concentrations may be important in predicting stem cell engraftment and disease relapse. Gathering information about intracellular species prior to transplant may lead to further optimal drug therapy. Pharmacokinetic-pharmacodynamic relationships would be evaluated between circulating systemic concentrations of fludarabine and the intracellular species through incorporation into the population pharmacokinetic model, along

with genetic variants that might influence intracellular active triphosphate concentrations.

Presented in chapter 4 is the first study to report clofarabine pharmacokinetics in the setting of HCT. With only 16 subjects, this clinical study was small in sample size. However, embedded in the dataset was a single patient telling a very important story to clinicians. Clofarabine is approximately 50-60% renally eliminated unchanged in the urine and currently there is no pharmacokinetic data available in patients with renal dysfunction. The single patient that developed renal failure over the course of clofarabine treatment provides important insight to clinicians regarding the impact of renal function and clofarabine exposure. Prior to this analysis, dose reductions of clofarabine at our institution were not considered in patients with renal impairment due to the absence of any pharmacokinetic data available in any patient population. This pharmacokinetic study is currently still enrolling subjects. Additional pharmacokinetic data is needed to confirm the results found in these analyses, identify additional sources of variability surrounding clofarabine pharmacokinetics, and eventually evaluate pharmacokinetic relationships with clinical outcomes.

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