Association of Phosphoramide (PM) and Fludarabine (FLU) Pharmacokinetics with Treatment Related Mortality (TRM) after Reduced Intensity Conditioning (RIC) Hematopoietic Cell Transplantation (HCT)

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INTRODUCTION

Over the last decade reduced intensity conditioning (RIC) has made allogeneic hematopoietic cell transplantation (HCT) possible among older patients and those with co-morbid conditions. Cyclophosphamide (CY) and fludarabine (FLU) with or without radiation is commonly used in many RIC regimens. Although RIC is safer relative to myeloablative conditioning, for these patients treatment related mortality (TRM) still remains high.(1),(2)

Fludarabine is associated with significant dose-dependent adverse effects most serious being neurotoxicity. Fludarabine is rapidly and non-enzymatically dephosphorylated to systemically circulating F-ara-A which is the active component. In a previous study conducted by our group in 87 HCT recipients we found that recipients with F-ara-A trough concentration >80 ng/mL were at a significantly higher risk of TRM at 6 months than those with <80 ng/mL (64% vs 15%, p<0.01). However, there were some individuals with high F-ara-A troughs that did not have TRM. TRM in these cases may be due to other agents in the conditioning.

CY is a prodrug which is enzymatically biotransformed to its active form, phosphoramide mustard (PM). PM is then further metabolized to nor-nitrogen mustard both of which form DNA adducts and halt cell replication. CY has been studied extensively for its side effects such as hemorrhagic cystitis, cardiotoxicity, pulmonary toxicity. However, due to complex metabolic pathway and high inter-individual variability in exposure, it is unclear whether the parent itself, PM or the DNA adducts are associated with adverse outcomes. Studies associating CY and/or its metabolites pharmacokinetics to clinical outcomes specifically in HCT settings are limited.(3),(4) We hypothesized that TRM is influenced by exposures to both agents and simultaneous analaysis of both agents is necessary.

OBJECTIVES

The objective of this study was to evaluate the relationships between clinical outcomes, TRM, acute graft vs host disease (GVHD) and engraftment and PM and F-ara-A pharmacokinetics.

METHODS

Patients: Forty adults undergoing allogeneic RIC HCT were prospectively studied from March 2013 to May 2014 (Table 1). All patients received FLU, CY and TBI as conditioning and cyclosporine or sirolimus, plus mycophenolate for posttransplant immunosuppression. CY 50mg/kg x 1 dose was administered IV over 2 hours at constant rate on day -6 and pharmacokinetic sampling was conducted at 2, 4, 6, 21, 24 and 45 hours after the end of infusion. PM was derivatized with diethyldithiocarbamate and measured by a validated HPLC assay with ultraviolet detection. Fludarabine was given at 30 mg/m2/d (n=35), 32 mg/m2/day (n=1) and 35 mg/m2/day(n=2) x 5 doses on days -6 to -2 and was administered as constant IV infusion over 1 hour. F-ara-A trough concentrations were obtained 24 hours after the start of the infusions on day -6 and -5. F-ara-A was quantified using HPLC-UV.

PM pharmacokinetics:
PM plasma concentration time data for each patient was analyzed using non-compartmental analysis (Phoenix WinNonlin Professional Version 6.3 software). Area under the curve [$AUC_{(0-24)}$ and $AUC_{(0-6)}$] were calculated using log/linear trapezoidal method.

Clinical outcomes:
TRM was defined as death due to any cause other than relapse or disease progression.
GVHD was staged and graded according to the standard GVHD criteria based on clinical and pathological criteria.
Day of neutrophil engraftment was the first of 3 consecutive days of an absolute neutrophil count > 500 cell/uL by day 42

Statistical analysis:
Recursive partitioning regression analysis was used to select optimal cut points of PM AUCs and F-ara-A trough concentrations towards the clinical outcomes. Once the optimal cutpoints for the pharmacokinetic parameters were determined then each of the outcomes were compared above and below the cutpoints. Cumulative incidence of engraftment, TRM and aGVHD (grades II-IV and III-IV) was calculated using death prior to event as a competing risk.
The proportional hazards model of Fine and Gray was used to assess the association of exposures towards TRM, acute GVHD and engraftment.

Table 1: Patient characteristics

	Median (range)/N (%)
Number of Patients (N)	40
Age (years)	62 (21-72)
Male / Female	22/18
Transplant Source	
Marrow/PBSC/Cord blood	12.5%/55%/32.5%
Unrelated / Sibling Donor	47.5% 52.5%
Primary Disease	
ALL	7.5%
AML	22.5%
Myelodysplasia	20%
Lymphoma	15%
Multiple Myeloma	10%
Other	25

RESULTS

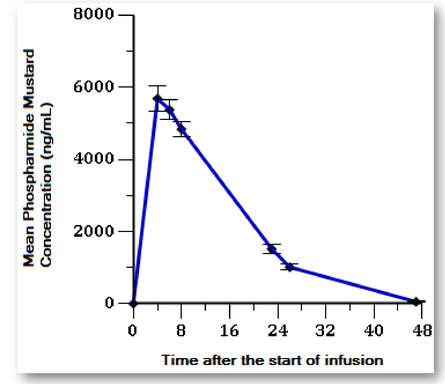
PM and F-ara-A pharmacokinetics: Summary of pharmacokinetic parameters is shown in table 2. The concentration time plot for PM is shown in figure 1. F-ara-A trough concentrations after the 1st and 2nd dose did not significantly differ indicating that there is minimal accumulation after 2 doses.

Table 2: Summary of Pharmacokinetic parameters for PM and F-ara-A

	PM $AUC_{(0-6)}$ µg*hr/mL	PM $AUC_{(0-24)}$ µg*hr/mL	PM peak conc. µg/mL	PM half-life (hrs)	F-ara-A trough conc. after the 1st dose (ng/mL)	F-ara-A trough conc. after the 2nd dose (ng/mL)
Mean (SE)	22.37 (1.33)	74.9 (3.09)	5.83 (0.35)	6.85 (0.38)	51.66 (2.95)	55.99 (3.32)
Min-Max	9.13-50.95	35.29-123.18	2.27-14.25	2.83-13.35	18.51-102.00	18.64-107.79
CV%	37.68	26.15	38.35	35.53	36.1	37.5

% CV indicates percent variability in the pharmacokinetic parameters

Figure 1: PM concentrations (means) after the start of infusion



Relationships Between Pharmacokinetics and Clinical Outcomes:

Treatment Related Mortality:
- The overall TRM rate was 13% at day 100 and 20% at 6 months
- The median (range) time to TRM was 115 days (37-319) days
- Cumulative incidence of TRM was significantly higher in recipients with higher PM AUC and higher F-ara-A troughs (Table 3)
- Figures 2 and 3 show the Kaplan-Meier plots for cumulative incidence of TRM by PM and F-ara-A pharmacokinetics at day 100

Acute Graft vs Host Disease:
- Grade II-IV GVHD and grade III-IV event rate was 38% and 25% at 6 months. PM AUC and F-ara-A trough concentrations did not significantly influence GVHD.

Engraftment:
- Neutrophil engraftment was achieved in 93%. In an univariate analysis, none of the pharmacokinetic parameters were associated with engraftment.

Table 3: Cumulative Incidence of TRM at day 100 and 6 months by pharmacokinetics

Pharmacokinetic measure	Cumulative incidence at day 100 (%)	p value	Cumulative incidence at 6 months (%)	p value
PM $AUC_{(0-6)}$				
< 20 µg*hr/mL	0	0.05	0	<0.01
≥ 20 µg*hr/mL	22 (5-38)		37 (16-59)	
PM $AUC_{(0-24)}$				
< 85 µg*hr/mL	4 (0-11)	<0.01	14 (0-28)	0.02
≥ 85 µg*hr/mL	36 (9-64)		47 (17-77)	
F-ara-A trough concentration after the second dose				
< 75 ng/mL	7 (0-15)	0.01	19 (4-34)	0.06
≥ 75 ng/mL	33 (4-63)		33 (4-63)	

Figure 2: Cumulative Incidence of Day 100 TRM with PM $AUC_{(0-24)}$ ≥ 85 µg*hr/mL vs < 85 µg*hr/mL

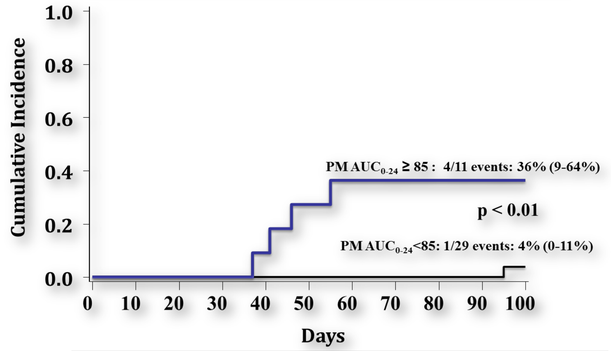
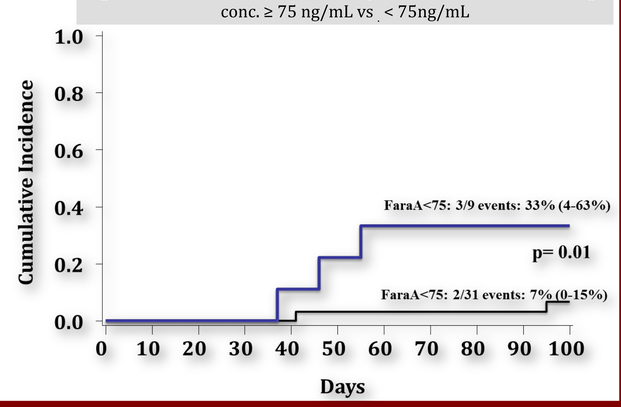


Figure 3: Cumulative Incidence of Day 100 TRM with F-ara-A trough conc. ≥ 75 ng/mL vs < 75ng/mL



CONCLUSIONS AND FUTURE DIRECTIONS

This is a first study to evaluate the relationship of PM AUC and risk to TRM following RIC-HCT. We also observed high inter-individual variability in PM exposure, possibly due to the highly complex metabolic pathway of CY. We also confirmed our previous results that higher F-ara-A trough concentrations are associated with higher risk of TRM in after RIC conditioning. Patient recruitment is ongoing and future analyses will develop multivariate models adjusting for clinical and genetic factors. We will also develop models combining PM and F-ara-A pharmacokinetics.

References:

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