Phosphoramide Mustard (PM) Population Pharmacokinetics to Estimate Inter-Individual Variability in Exposure and Identify Clinical Factors Associated with Variability After Reduced Intensity Conditioning (RIC)

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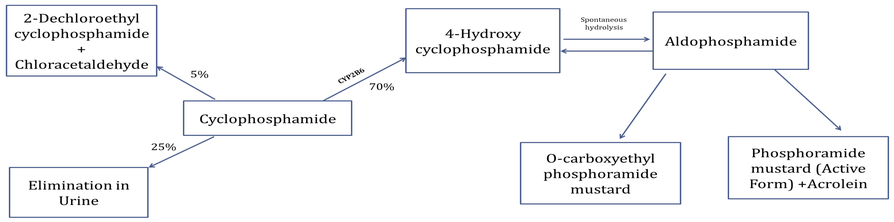
INTRODUCTION AND OBJECTIVES

RIC regimen used prior to hematopoietic cell transplant (HCT) has emerged as a successful treatment for hematologic malignancies among the elderly and patients with comorbid conditions. Although use of low dose conditioning regimens have substantially improved tolerability, treatment related mortality (TRM) still remains a significant problem.

Cyclophosphamide (CY) is an alkylating agent used in RIC regimens with other chemotherapeutic agents and radiation. CY is a prodrug that undergoes enzymatic biotransformation (mainly by hepatic CYP2B6) to an active metabolite, PM, which then forms nor-nitrogen mustard (Figure 1). Both PM and nor-nitrogen mustard alkylates the N-7 position of guanine on DNA, resulting in formation of G-NOR, G-NOR-OH and G-NOR-G adducts. These adducts prevent DNA replication, cell division, ultimately leading to cell death.(1)

There is high variability observed in the formation of DNA adducts. Low interstrand cross links are associated with lack of therapeutic response, and high cross links are associated with CY toxicity.(2,3) Also there are studies suggesting that pulmonary, cardio, hepatic and hemorrhagic cystitis may be associated with over exposure to CY metabolites which may lead to TRM.(4,5,6) Due to the complex metabolic pathway of CY, there is no clear understanding of which metabolite(s) is responsible for drug toxicity. Limited data are available for PM pharmacokinetics.(7,8) Therefore we have undertaken a series of studies to identify the relationships between PM and fludarabine pharmacokinetics and outcomes after transplant (abstracts 1153 and 3869). Understanding clinical factors associated with variability in PM exposure after RIC is necessary to implement strategies to control CY exposure.

Figure 1: Metabolic pathway of cyclophosphamide



The objective of our study was to characterize the population pharmacokinetics of PM and to identify clinical covariates associated with inter-individual variability in pharmacokinetics.

METHODS

Patients:

Forty-one adult allogeneic hematopoietic transplant recipients receiving fludarabine, CY and TBI were prospectively studied from March 2013 to May 2014 (Table 1). CY 50mg/kg x one dose was administered IV over 2 hr at constant rate on day -6 and pharmacokinetic sampling was conducted at 2, 4, 6, 21, 24 and 45 hrs after the end of infusion. PM was derivatized with diethyldithiocarbamate and measured by a validated HPLC assay with ultraviolet detection. The lower limit of quantification was 50ng/ml.

Model building:

A population pharmacokinetic analysis was conducted using non-linear mixed effects model (NONMEM) to obtain typical value of apparent clearance (TVCL/fm) of PM, apparent volume of distribution (TVV/fm) of PM and conversion rate constant (TVkf) from CY to PM.

Covariate testing:

Pretransplant actual and ideal body weight, age, gender, CrCl, total bilirubin, albumin, previous transplant, SCr, ALT, AST, and alkaline phosphatase were tested to explain the observed variability in kf, Cl/fm and V/fm. A step-wise covariate model building strategy of forward inclusion and backward elimination was used to identify the effect of clinical covariates on PM pharmacokinetics.

Model evaluation:

Visual predictive check was used to evaluate if the model adequately describes the observed data. A bootstrap method of sampling with replacement was then conducted to generate 1000 datasets and the model was applied to each dataset to evaluate the robustness and reliability of the estimated pharmacokinetic parameters.

Table 1: Patient Characteristics

Number of Patients	41
Age (years) median (range)	62 (21-72)
Male / Female	21/20
Actual body weight (kg) median (range)	83.7 (47.90-117.80)
Body surface area (m2) median (range)	2.01 (1.45-2.97)
BMI (kg/m^2) median (range)	29 (19-44)
SCr (mg/dL) median (range)	0.81 (0.3-1.58)
CrCl (mL/min) median (range)	109 (64-310)
BM/PBMC/CB %	12.19/53.6/34.14
Unrelated /sibling %	47.5/52.5

RESULTS

Base Pharmacokinetic Model: Metabolite kinetics of an IV administered parent drug was explained by a model similar to oral absorption. Exploring the plot of log of observed plasma PM concentrations vs time suggested a one compartment model best explained the observed data. Figure 2 shows diagnostic plots used during model development.

Important Covariates: Gender significantly influenced rate constant (TVkf), which is the conversion of CY to PM. Females had 55% higher rate of conversion than males.
A lower CrCl significantly reduced PM TVCL/fm.

Final Covariate Model: Table 2 are the final parameter estimates with %RSE.
TVkf is the typical population value of the conversion rate constant from CY to PM and was estimated by THETA(1) (eq.1).

TVKf = THETA(1)..... (eq. 1)

THETA(2) is the parameter estimate for proportional change in TVKf if the patient was female (eq. 2).

IF(SEX= FEMALE) TVKf= TVKf * THETA(2).....(eq. 2)

The typical value of the total clearance (TVCL) was modeled as a sum of non-renal (CLnr) and renal clearance (Cl_{slope} x RF_{std}) that changed with changes in CrCl. TVCL/fm was further allometrically scaled using total body weight (WT) (eq. 3 and eq. 4)

$TVCL/fm = (Cl_{nr} + Cl_{slope} \times RF_{std}) \times (WT/70)^{0.75}$ .....(eq. 3)
$RF_{std} = (CrCl/85) \times (70/WT)$ ..... (eq. 4)

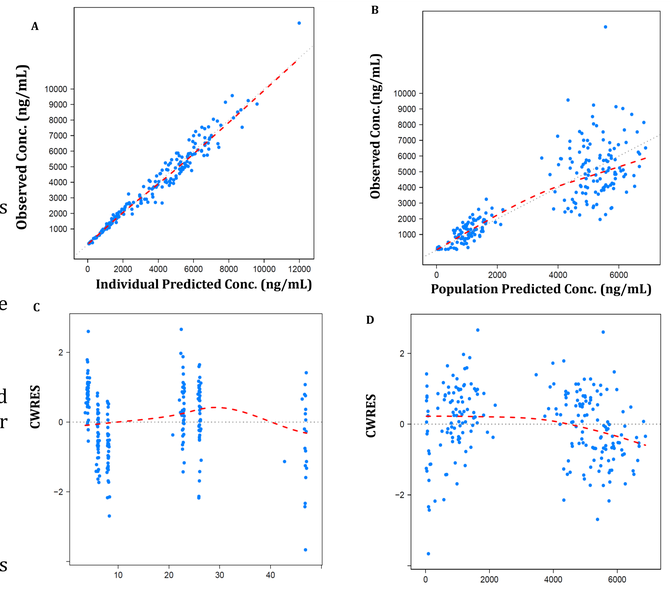
Typical value of volume of distribution TVV/fm was estimated from THETA(4) and was allometrically scaled using total body weight (WT) (eq. 5).

TVV/fm = THETA(4) x WT/70 ..... (eq. 5)

Table 2: PM parameter estimates model estimated parameters and bootstrap estimates

Parameter Estimates	Original Dataset (%RSE)	Bootstrap Estimates (median 95% C.I.)
TVKf (hr^{-1})	0.155 (11)	0.162 (0.106-0.2658)
$Cl/_{non\text{-}renal}$ (L/hr)	26.9 (21)	27.92 (13.41-38.52)
$Cl/_{renal}$ (L/hr)	17.6 (34)	16.77 (7.02-31.70)
TVV/fm (L)	271 (10)	301.37(186.23-356.61)
If gender female	1.55 (15%)	1.64 (1.13-2.46)
Between subject variability (BSV)		
BSV on TVKf (CV%)	0.068 (43%) CV% =38.9	0.137 (0.025-0.351)
BSV on TVCl/fm (CV%)	0.061 (31%) CV% = 21.9	0.045 (0.026-0.079)
BSV on TVV/fm (CV%)	0.075 (33%) CV% = 28.1	0.045(0.020-0.209)
Residual unexplained variability (RUV)		
RUV proportional	0.0239 (23%) CV%= 22.36	0.0226 (0.0141-0.0372)

Figure 2: Diagnostic plots used during model development



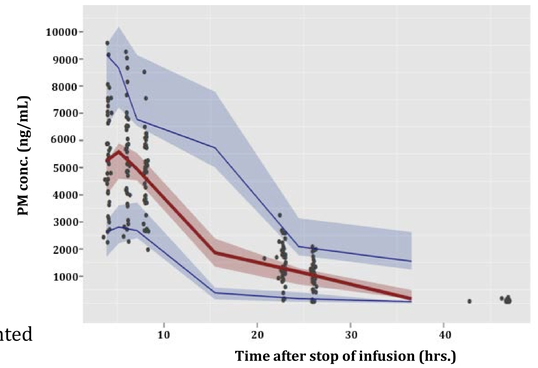
A) Observed concentration (DV) vs Population predicted concentration (PRED),
B) Observed Concentration(DV) vs Individual predicted concentration,
C) Conditional weighted residuals (CWRES) vs TIME and
D) CWRES vs PRED.

Model Evaluation:

1. Non-parametric bootstrap: Final population pharmacokinetic model was evaluated for its reliability with non-parametric bootstrap. Out of 1000 datasets generated, 905 minimized successfully (Table 2). Estimates for fixed and random effects are comparable, indicating that the model is robust and reproducible.

2. Visual Predictive Check: Figure 3 shows that the observed data (black dots) lie within the prediction intervals (shaded areas). Therefore the model adequately explains the observed data.

Figure 3: Visual Predictive Check



The solid red line represents the median of the observed plasma concentrations obtained from the 41 subjects.
The pink block around the solid red line is the 95% confidence interval for the median, obtained from the simulation prediction. The 97.5th and 2.5th percentiles of the observations are represented by blue lines above and below, respectively.
The blue shaded areas represent 95% confidence intervals for corresponding 97.5th and 2.5th prediction intervals obtained from the simulations. Finally the black dots represent the observed concentrations from the 41 subjects.

CONCLUSIONS AND FUTURE DIRECTIONS

Population pharmacokinetics of PM was explained by one compartment model. Renal clearance contributed 39.5% of total clearance. Therefore renal impairment reduces PM clearance and possibly increases the risk of toxicity. Our results suggests that females could be at higher risk of PM related toxicity due to higher conversion rate from CY to PM. Effect of genetic variants associated with PM kinetics also need to be explored. In future studies correlating PM exposure to clinical outcomes (poster #1153) may support personalizing CY doses to improve efficacy and prevent toxicity.

References: **1** de Jonge ME el al.,Clin Pharmacokinet. 2005;44(11):1135-64 **2**. Souliotis VL , Clin Cancer Res. 2003 Oct 1;9(12):4465-74 **3.** Reed E et al. , Proc Natl Acad Sci U S A. 1987 Jul;84(14):5024-8 **4.** Kachel DL, et al. J Pharmacol Exp Ther. 1994 Jan;268(1):42-6. **5.** Fraiser LH et al. Drugs. 1991 Nov;42(5):781-95. **6.** McDonald GB, et al. Blood. 2003 Mar 1;101(5):2043-8. **7**.de Jonge, Ther Drug Monit 2005;27:756–765 **8.** Juma et al. Br. J. Clin. Pharmac. (1980), 10, 327-335