Intravenous Busulfan in Hematopoietic Stem Cell Transplantation: Does Patient Specific Clearance Affect Outcomes?

by

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Intravenous Busulfan in Hematopoietic Stem Cell Transplantation: Do Patient Specific Factors Affect Clearance?

Purpose

The objective of this study is to ascertain factors that may be significantly associated with busulfan clearance (patient demographics (age, gender, race, weight), disease and donor type).

Methods

This retrospective study was conducted to determine population descriptive statistics (i.e. mean clearance, mean AUC, mean half-life, etc.). Additionally, the effect of independent variables age, gender, primary cancer diagnosis, ethnicity, prior transplant status, and transplant type were analyzed for effect on the dependent variable of normalized clearance. Institutional Review Board approvals were obtained as required for this retrospective data review. Patients included in this study were treated between September 2009 and December 31, 2012. Regression analysis was performed on patient specific variables (i.e. age, gender, disease) to determine which variables may significantly affect clearance. The association of patient factors with busulfan clearance was tested using linear regression. In this analysis, busulfan clearance is the dependent variable and is continuously distributed.

Results

752 patients were included in this analysis. Patients had a mean age of 46.2 years and a mean clearance of 101.2 ml/min/m^2, with a standard deviation of 17.5 and a range of 51.5 to 150 ml/min/m^2. With univariate analysis, only diagnosis ($p < 0.0001$) and transplant type ($p = 0.0002$) were statistically significant variables. Including all 6 variables produced a significant model (p-value < 0.0001) with an $R^2 = 0.0667$.

Conclusion

The complete six variable model only explains 6.7% of the variability in patient specific busulfan clearance. Given the large variability between patients, this is not a good model for predicting variation in clearances based on patient factors.

Intravenous Busulfan in Hematopoietic Stem Cell Transplantation: Do Patient Specific Factors Affect Clearance?

Objective

The objective of this study is to ascertain factors that may be significantly associated with busulfan clearance (i.e. patient demographics (age, gender, race, weight), disease, donor type).

Rationale

Busulfan, when used in combination with fludarabine or clofarabine, can be part of an effective chemotherapy based myeloablative preparative regimen for patients undergoing stem cell transplantation. Busulfan was originally available only as an oral formulation. The highly variable bioavailability of the oral formulation presented serious challenges when used as part of a high-dose combination regimen [1-4]. Low busulfan exposure is associated with a higher risk of graft rejection and relapse, while higher busulfan exposure is associated with a higher risk of liver toxicity and graft vs. host disease [2, 5-7]. Side effects include liver and lung toxicity, neurological disturbances (including grand mal seizures), and severe nausea and vomiting. Veno-occlusive disease (VOD) of the liver leading to fatal liver failure was the most serious side effect. Because of the safety concerns and highly variable bioavailability, an intravenous (IV) formulation of busulfan was developed. Intravenously administered busulfan has complete bioavailability with potentially a high level of dosing accuracy, thus, it has a greater potential to eliminate overdosing, minimizing a risk for either lethal toxicities or under dosing that may negatively influence tumor cell kill [8]. Using the IV formulation of busulfan has allowed exploration of other treatment strategies such as new dosing schedules and combination with other agents [9-11].

Initial experience with intravenous busulfan examined the pharmacokinetics (PK) of busulfan when delivered at a daily dose of 130 mg/m^2 for four days. This schedule was designed to approximate 80% of the total dose of the previous oral regimens to adjust for diminished oral bioavailability [12-13]. When dosed this way, the mean derived PK values were: clearance (Cl) 109 ml/min/m^2, maximum concentration (Cmax) 3.6 mcg/mL, volume of distribution (Vd) 22.6 L/m^2, and half-life (t ½) 2.73 hrs [12-14]. PK modeling was performed using the ADAPT II software program, version 4.0 [15]. While the mean daily AUC was 4891 micromol-min/ml, the range was 2900 to 8300 micromol-min/ml, demonstrating significant interpatient variability and the opportunity for better dose optimization based on target AUC. With tighter control of daily AUC, it was hypothesized that a more specific balance between acceptable toxicity and therapeutic effect could be achieved in individual patients. Historical data suggested that a daily AUC exposure of >6000 micromol-min was associated with excessive life-threatening or fatal toxicity. Busulfan has a steep dose response curve, thus, after starting at lower daily AUC targeted dosing, subsequent treatment plans were designed to explore the effectiveness and acceptability of giving higher average daily AUC targeted doses.

Target AUC dosing is achieved through PK- directed individualized dosing. Madden et al also demonstrated that daily busulfan dosing was pharmacokinetically similar (dose proportionality, unchanged estimated clearance) to every 6 hour dosing which had been the historical norm for oral and earlier IV busulfan studies [12]. Daily dosing greatly simplified treatment regimens through PK monitoring and allows for better optimization for PK directed therapy. Initial population Cl, Vd, C-Max, t½ were derived from 45 subjects [12]. The underlying hypothesis directing these studies is that pharmacodynamics effects (PD) are proportional to the total AUC exposure to busulfan, a generally accepted premise.

The MD Anderson Cancer Center Department of Pharmacy Research has provided PK monitoring services for patients on busulfan targeted therapy since September 2009. From September 2009 through December 31, 2012, approximately 1300 pharmacokinetic consults have been provided, representing approximately 750 unique patients, providing a large and robust data set for further study. This large sample provides the opportunity to verify the population estimates from the original study, as well as to determine other variables that could significantly affect busulfan kinetics. The objective of this study was to assess patient specific factors associated with busulfan clearance.

Methods

This retrospective study was conducted to determine population descriptive statistics (i.e. mean clearance, mean AUC, mean half-life, etc.). Additionally, the effect of independent variables age, gender, primary cancer diagnosis, ethnicity, prior transplant status, and transplant type were analyzed for effect on the dependent variable of normalized clearance. Institutional Review Board approvals were obtained as required for this retrospective data review.

Patients included in this study were scheduled for a stem cell transplant as clinically appropriate treatment by the Department of Stem Cell Transplantation. The Division of Pharmacy provides a PK consult service that includes coverage for patients treated on busulfan targeted therapy on clinical treatment protocols. As part of the PK service, the pharmacist creates a patient specific worksheet based on the protocol defined target AUC. The worksheet contains patient specific information, such as height, weight, and BSA, that is critical to determining the normalized clearance and corresponding dose calculation to achieve the targeted AUC. The worksheet is also used to record blood draw time points (as documented by the phlebotomist), plasma busulfan levels, dose infused, infusion rate, and the infusion start and stop times. The plasma samples are analyzed by the Special Chemistry mass spectrometry lab technician in Laboratory Medicine for determination of busulfan levels. Using the ten time points from the blood draw worksheet, combined with the busulfan levels reported from Laboratory Medicine, the PK clinical pharmacy specialist calculates the patient specific clearance. Pharmacokinetic Data Analysis is performed using ADAPT II Release 4, a free pharmacokinetic/pharmacodynamic systems analysis software available from the University of Southern California, Biomedical Simulations Resource (Los Angeles, CA). ADAPT calculates the gross clearance (in liters per hour), the elimination constant (Ke), the volume of distribution (V) in liters, half-life in hours, and the daily AUC (micromoles*minutes). The results from the PK

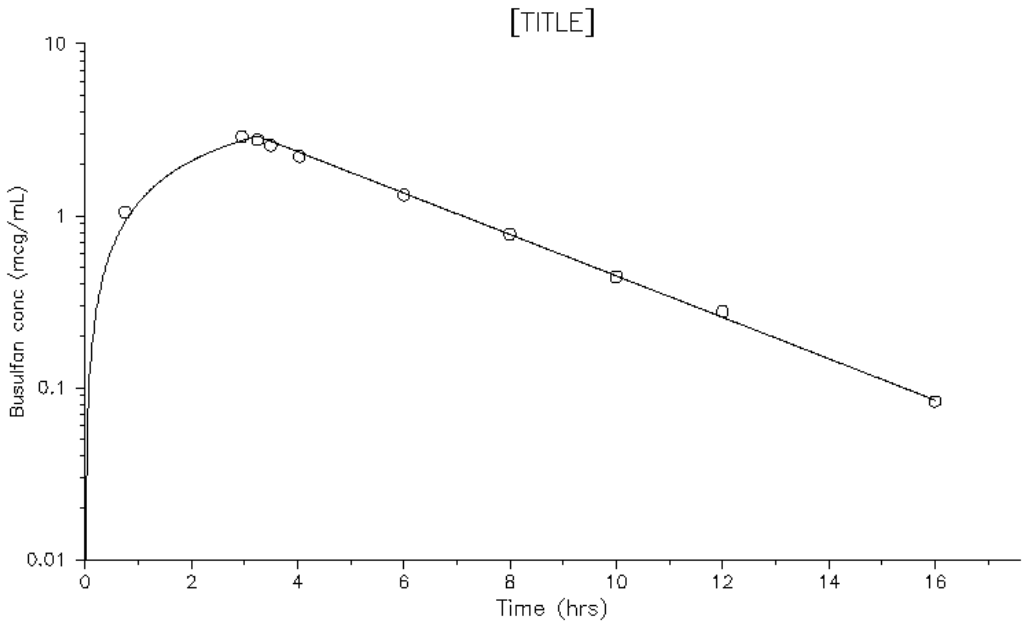
analysis are then communicated to the treating physician in a standardized format. The results include the gross clearance, normalized clearance, goodness of fit (R^2), half-life, AUC, the dose required to achieve the target AUC, and the pharmacist's initials who performed the PK analysis. The pharmacist information is included in case the clinicians providing clinical care for the patient have any questions or concerns with the results.

Example PK Report:

PK Results	Therapeutic Dose	
Gross Clearance:	126	ml/min
Clearance Normalized to BSA:	88	ml/min/m^2
Goodness of Fit (R^2)	0.992	
Half life:	2.5	hrs
AUC	3,880	µM•min
AUC and Dose Calculations		
Targeted Daily AUC:	4,000	µM•min
Targeted Course AUC:	16,000	µM•min
Adjusted Daily AUC Target =	4120	
((Course AUC) – (Current AUC X 2)) / 2		
Dose to achieve Adjusted Daily AUC Target:	128	mg

To perform PK calculations using the ADAPT software package, basic input criteria include the number of time points (observations) drawn, infusion rate (in mg per hour), and length of infusion. For busulfan PK, the estimation procedure used is weighted least squares (WLS), and the weighting option is inverse variance of the output error (linear). The number of desired weighting procedures, low measurement and high measurement of busulfan concentration in plasma, and the initial estimates for both elimination constant (Ke) and volume of distribution are also entered. The pharmacist then enters time points from the phlebotomy sheet along with the corresponding mean plasma concentration from laboratory medicine into ADAPT. ADAPT then calculates the patient specific clearance. Output from ADAPT includes patient specific Ke, volume, clearance, busulfan half-life, and goodness of fit (R^2), along with a graph depicting the patient clearance (busulfan concentration – time curve). The pharmacist enters output data into the report for the treating physician and copies the graph to the report.

Sample PK Graph:



Per standard operating procedure for the busulfan PK service, after consultation with Stem Cell Transplant leadership, in cases with a poor PK model fit, the pharmacist can drop outlying data points for calculation of patient clearance. Poor PK model fit is defined as an R^2 of less than 0.9, and outlying data points are time points with the largest residuals. As a note, outliers are usually the 2nd, 3rd, or 4th time points, and can be a result of incorrect documentation of sample draw time. As routine practice for patients with a poor model fit, the pharmacist will perform the calculation with all the time points included, and then drop the biggest outlier. While dropping a time point will improve the goodness of fit, it rarely results in a meaningful change in final clearance, or ultimately the patient's dose. In any event, all results are communicated to the treating physician for final decision on the patient's dose.

All other PK parameters were recorded as reported to the treating physician, with the exception of patients who had an unusually high normalized clearance. For safety reasons and per standard operating procedures in Stem Cell Transplant, any patient with a normalized clearance ≥ 150 ml/min/m^2, the clearance is capped at 150 ml/min/m^2. This capped clearance was used in calculations of mean clearance and dose determinations.

BMTWeb is a web-enabled Java application and Oracle database designed to collect detailed data for Stem Cell Transplantation and Cellular Therapy (SCTCT) operations in clinical research trials and for required reporting activities. As the main Informatics hub for SCTCT clinical research operations, BMTweb contains real-time interoperability architecture. BMTweb

supports Service Orientation Architecture (SOA) interfaces with institutional sources such as ADT (patient demographic), HLA Lab (Donor Typing), and SPIDR (Lab data) databases. The data storage design allows secured and roles based retrieval of data at any time, in any format and can be exported to other programs such as SAS for detailed analysis. This validated data is also used to generate reports with business intelligence and reporting tools (Crystal reports) in defined formats, for different purposes, ranging from patient admission (Flowsheets) to treatment to Outcome reports.

BMTWeb data included: birth date (used to calculate patient age at treatment date – this was absent from the de-identified data set); height, weight, and BSA; whether the patient had a prior transplant (yes/no); transplant type; grading for patients who developed GVHD, liver toxicity, CNS toxicity; survival status with date of last follow-up; and time to event for outcome variables (GVHD, liver toxicity, CNS toxicity, and death).

Complete PK data from the pharmacy worksheets was imported into BMTWeb by the database developers. Data importation linked pharmacy records to BMTWeb records by matching both fields for treatment date and patient's medical record number.

A de-identified dataset was created from this database under a retrospective data review protocol. The dataset included patient demographic information (gender, ethnicity, age at time of transplant, diagnosis, transplant type, and whether the patient had had a previous transplant), the complete PK data set as imported from the pharmacy PK service, and outcome data considered to be potentially associated with busulfan PK (GVHD, CNS toxicity, liver toxicity, and survival).

BMTWeb was considered the source record for all patient characteristics associated with the transplant service, while the pharmacy records served as the source record for all PK data.

Since PK data was imported from individual worksheets, the dataset was checked for importation errors. Before importation, records were matched on both medical record number and treatment date. Any discrepancies between pharmacy records and BMTWeb records were analyzed and resolved before importation. These errors were primarily treatment date issues and were fixed by confirming patient treatment date in the patient's electronic medical record. Patient MRNs were not present in the final de-identified dataset. Other data cleanup included verification of test dose versus therapeutic dose. Test doses are typically 0.8 mg/kg. To confirm dose type, the dataset was sorted by dose type and then dose. Scanning the dose field after this sort provided quick verification of accuracy of dose type. Additionally, the dataset was sorted by clearance. Since clearance was capped at 150 ml/min/m^2, any value above this cap was verified. The lowest clearance was estimated to be in the low 50's before the analysis (based on discussion with PK service providers). This estimation was confirmed during analysis.

Statistical Analysis

Regression analysis was performed on patient specific variables (i.e. age, gender, disease) to determine which variables may significantly affect clearance. All analyses were conducted with the individual patient as the unit of analysis. SAS version 9.3 was used to perform the analyses. Proc GLM univariate analysis was conducted with each of the 6 variables, followed by the full model analysis with all 6 variables included.

The association of patient factors with busulfan clearance was tested using linear regression. In this analysis, busulfan clearance is the dependent variable and is continuously distributed. Patient demographic and clinical measures at baseline were tested for unique association with busulfan clearance using linear regression. Assumptions of normality were tested. The level of statistical significance was set at 0.05 a priori.

Exposure – treatment with busulfan

A patient's exposure (plasma concentration/time data) to busulfan is used to calculate patient specific clearance, which is the independent variable of interest for this study. Patient's clearance is a continuous variable with a range of approximately 50 to 150 ml/min/m2. The assumption of normal distribution was verified.

Results:

Patients had a mean age of 46.2 years at time of transplant, with a range of 2-74 years (standard deviation 15.8). Other patient demographic information is included in the tables below:

Table: Patient Characteristics; Diagnoses

	Frequency	Percent
Male	450	59.4
Female	302	40.2
Prior transplant	49	6.5
Allogeneic transplant	504	67.0
Autologous transplant	245	32.6
Survival at 1 year	408	66.9
Diagnoses:		
ALL	100	13.4
AML	215	28.9
CML/MDS	146	19.6
Hodgkin's	110	14.6
Lymphoma	67	9
Ethnicity		
White	516	68.6
Asian	22	2.9
African American	58	7.7
Hispanic	121	16.1
Other	35	4.7

Patients included in the database had 9 different diagnoses. Since there were only 8 patients with chronic lymphocytic leukemia, these were excluded from the analysis. Chronic myelogenous leukemia patients (n = 15) were combined with myelodysplastic syndrome patients (MDS) for analysis purposes, since they are expected to have similar clinical outcomes.

Patients in this study were predominantly white. The other top ethnic groups are included in the table below. While there were only 22 Asian patients in the study (9 F, 13 M), there were analyzed as a separate group for comparison to the results of Choe et al [18].

Among all 752 patients in the dataset, patients had a mean clearance of 101.2 ml/min/m^2, with a standard deviation of 17.5 and a range of 51.5 to 150 ml/min/m^2. Male subjects had a mean clearance of 101.5 ml/min/m^2 while female subjects had a mean clearance of 100.7 ml/min/m^2, showing very minimal differences between genders.

Clearance between different ethnicities only showed a 6.2% difference between means from high to low.

Table: Clearance by Ethnicity

Analysis Variable : Normalized Clearance					
Ethnicity	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
White	516	101.9	17.3	56.7	150
Asian American	22	95.6	16.7	62.7	126.8
African American	58	97.9	16.9	51.5	150
Hispanic	121	100.8	17.9	53.9	150
Other	35	100.7	20.3	67.9	150

Patients who did not survive at least 1 year from transplant had a mean clearance that was 2.2% lower than patients who did survive. Patients with an autologous transplant had a mean clearance that was 5.4% lower than patients with an allogeneic transplant. Patients who had a prior transplant had a mean clearance that was 4% lower than patients who did not receive a previous transplant. Mean clearances, standard deviations, and range of clearance for these patients are included in the table below.

Mean clearance between diagnoses showed the greatest variability. The clearance of the myeloma patients was 15.6% lower than the highest mean clearance (MPD patients). Mean clearances, standard deviations, and range of clearance for patients based on diagnosis are included in the table below.

Table: Clearance by Diagnosis:

Analysis Variable : Normalized Clearance					
Diagnosis	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
ALL	100	96.9	16.7	68.1	139.8
AML	215	104.6	16.4	61.8	150
CML/MDS	146	102.7	18.0	53.9	150
Hodgkin's	110	97.6	15.0	51.5	131.9

Lymphoma	67	99.5	15.1	68.7	150
MPD	27	113.8	26.9	68.2	150
Myeloma	79	96.1	17.6	56.8	150

Table: Clearance by Gender; Clearance by Transplant Type

Analysis Variable : Normalized Clearance					
SEX	**N**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
F	302	100.7	17.2	51.5	150
M	450	101.5	17.7	53.9	150
Transplant Type:					
ALLO	504	103.0	18.1	53.9	150
AUTO	245	97.4	15.7	51.5	150
SYNG	3	99.5	6.2	95.7	106.8

Overall mean clearance for all 752 patients was 101.2 ml/min/m^2, with a standard deviation of 17.5, a minimum clearance of 51.5 ml/min/m^2 and a maximum clearance of 150 ml/min/m^2.

Below are the summary results of the analysis of busulfan clearance in the 752 stem cell transplant patients included in the dataset. The only significant patient factors associated with clearance were diagnosis and transplant type.

AIM 1: Proc GLM univariate analysis

Dependent variable Normalized Clearance:

Independent Variable	p-value
Age	0.7481
Age (decile)	0.7404
Diagnosis	<0.0001
Ethnicity	0.2725
Gender	0.5760
Prior transplant	0.1100
Transplant type	0.0002

Including all 6 variables produced a significant model (p-value < 0.0001) with an R^2 = 0.0667, with four variables (one ethnicity and 3 diagnoses) in the full model were statistically significant.

Discussion

It has been well documented that BSA is a significant factor in busulfan clearance. Dividing a patient's gross clearance by their BSA produces that patient's normalized clearance. For clinical services and routine communications, clearances are routinely discussed relative to BSA.

The mean clearance of 101 ml/min/m^2 reported in this study is similar to the results (109 ml/min/m^2) reported by de Lima et al. One difference between the two studies is the lab where the busulfan plasma levels were determined. The de Lima results were analyzed in a research setting while the results for this study were done in the CLIA certified Special Chemistry mass spectrometry lab in Laboratory Medicine. The method for analysis of busulfan levels in plasma was transferred from the research laboratory to the special chemistry laboratory and is a highly consistent method, so this transition would be expected to have minimal impact on mean clearance. In addition to the transition in labs, the patients in the de Lima study were AML and MDS patients. Similar patients in this study had a mean clearance of approximately 104 ml/min/m^2, making the difference the means in the de Lima study and this study even smaller. While there were 96 patients in the de Lima study in total, only 45 patients volunteered for the optional PK assessment, making direct comparisons between the studies more difficult.

Other data about potential patient factors that affect busulfan clearance are either conflicting or less clear. For example, Choe et al reported gender as a significant finding; however, this study was only 60 subjects and all were Asian. While this study did not confirm these results (based on the limited sample size of Asians included in the study), there was a 10% difference in clearance between Asian females and Asian males. Finally, the issue of genetic polymorphisms is beyond the scope of this study, since this information was neither collected nor available in either BMTWeb or the patient's electronic medical record.

There is large interpatient variability in busulfan clearance. Given the large sample size for this study, it was anticipated finding patient characteristics that would be meaningful in predicting a patient's clearance. This would have been very useful for other cancer centers that do not have a formalized pharmacokinetic service. While there are two statistically significant findings from this study, there is little clinical value in these findings being predictors of a specific patient's clearance. For example, when including all subjects, there is less than a 1% difference in clearance between male and female patients. By comparison, when using the Cockcroft-Gault equation to calculate renal function, there is a 15% decrease in creatinine clearance in females compared to males. Likewise, there is only a 6% difference between the highest and lowest clearance ethnicities. Even patients who had a prior transplant, and thus who been greatly treated systemically with chemotherapy previously, there was only a 4% difference in clearance.

The significant finding of diagnoses was unexpected. Excluding myeloproliferative disorder patients with a clearance of 113, the other diagnoses were all within an 8% range from high to low. Even MPD patients only had a clearance that is 13% higher than the median clearance. While the finding of diagnosis is a significant finding, it isn't substantial enough to improve the strength of the final model.

One of the biggest strengths of this study is the very large sample size. 16 publications reviewed prior to this study had an average sample size of 61 (range 12-145). With 752 patients accumulated over a 3 year period, there is very good representation of 7 different diagnoses, patients with both allogeneic and autologous transplants, patients with prior transplant, and several different ethnicities. Furthermore, the relatively short duration of the

study insured relatively constant treatment standards. Finally, the plasma concentrations of busulfan were all conducted in a single CLIA certified laboratory, where the special chemistry lab analysis is performed by very few specially trained personnel, insuring high consistency between the critical determinations of plasma levels.

The limitations of the study include it was conducted on patients from a single cancer center. Given the large sample size and diverse patient population, though, results would not expected to differ significantly from similar patient populations treated at other cancer centers. Finally, this study did not analyze any potential genomic factors, since this data was not included in the BMTWeb dataset.

Variables analyzed in this study were not good predictors of busulfan clearance. Thus, changes in busulfan clearance are probably most likely attributed to other clinical factors (albumin level, liver function, explanation of busulfan uptake, busulfan covalently charged, etc). A comprehensive review of these potential clinical factors is beyond the scope of this paper.

Conclusion

The complete six variable model only explains 6.7% of the variability in patient specific busulfan clearance. Given the large variability between patients, this is not a good model for predicting variation in clearances based on patient factors.

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19.

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Purpose

The objective of this study is to assess correlation of busulfan clearance with outcomes (GVHD, liver toxicity, CNS toxicity, and survival).

Methods

This retrospective study was conducted to determine population descriptive statistics (i.e. mean clearance, mean AUC, mean half-life, etc.). Additionally, the effect of independent variables age, gender, primary cancer diagnosis, ethnicity, prior transplant status, and transplant type were analyzed for effect on the dependent variable of normalized clearance. Institutional Review Board approvals were obtained as required for this retrospective data review. Patients included in this study were treated between September 2009 and December 31, 2012. Logistic regression was performed on the three toxicities of interest plus survival. The toxicities of interest included CNS, GVHD, and Liver. Toxicities with severity of ≥ Grade 3 were included in the analysis. In addition to the logistic regression, a Kaplan-Meier regression analysis was performed on the same three toxicities and survival. The cutoff for the Kaplan-Meier regression analysis was set at one year. All analyses were conducted with the individual patient as the unit of analysis, with normalized busulfan clearance as the dependent variable.

Results

752 patients were included in this analysis. Patients had a mean age of 46.2 years and a mean clearance of 101.2 ml/min/m^2, with a standard deviation of 17.5 and a range of 51.5 to 150 ml/min/m^2. In the logistic analysis, clearance was associated with liver toxicity and survival, with increased clearance having a protective effect on both. In the survival analysis, lower clearance was associated with an increased risk of dying in three of the four (out of 10 groups) lowest clearance groups.

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Objective

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Rationale

Busulfan, when used in combination with fludarabine or clofarabine, can be part of an effective chemotherapy based myeloablative preparative regimen for patients undergoing stem cell transplantation. Busulfan was originally available only as an oral formulation. The highly variable bioavailability of the oral formulation presented serious challenges when used as part of a high-dose combination regimen [1-4]. Low busulfan exposure is associated with a higher risk of graft rejection and relapse, while higher busulfan exposure is associated with a higher risk of liver toxicity and graft vs. host disease [2, 5-7]. Side effects include liver and lung toxicity, neurological disturbances (including grand mal seizures), and severe nausea and vomiting. Veno-occlusive disease (VOD) of the liver leading to fatal liver failure was the most serious side effect. Because of the safety concerns and highly variable bioavailability, an intravenous (IV) formulation of busulfan was developed. Intravenously administered busulfan has complete bioavailability with potentially a high level of dosing accuracy, thus, it has a greater potential to eliminate overdosing, minimizing a risk for either lethal toxicities or under dosing that may negatively influence tumor cell kill [8]. Using the IV formulation of busulfan has allowed exploration of other treatment strategies such as new dosing schedules and combination with other agents [9-11].

Initial experience with intravenous busulfan examined the pharmacokinetics (PK) of busulfan when delivered at a daily dose of 130 mg/m^2 for four days. This schedule was designed to approximate 80% of the total dose of the previous oral regimens to adjust for diminished oral bioavailability [12-13]. When dosed this way, the mean derived PK values were: clearance (Cl) 109 ml/min/m^2, maximum concentration (Cmax) 3.6 mcg/mL, volume of distribution (Vd) 22.6 L/m^2, and half-life (t ½) 2.73 hrs [12-14]. PK modeling was performed using the ADAPT II software program, version 4.0 [15]. While the mean daily AUC was 4891 micromol-min/ml, the range was 2900 to 8300 micromol-min/ml, demonstrating significant interpatient variability and the opportunity for better dose optimization based on target AUC. With tighter control of daily AUC, it was hypothesized that a more specific balance between acceptable toxicity and therapeutic effect could be achieved in individual patients. Historical data suggested that a daily AUC exposure of >6000 micromol-min was associated with excessive life-threatening or fatal toxicity. Busulfan has a steep dose response curve, thus, after starting at lower daily AUC targeted dosing, subsequent treatment plans were designed to explore the effectiveness and acceptability of giving higher average daily AUC targeted doses. Target AUC dosing is achieved through PK- directed individualized dosing. Madden et al also

demonstrated that daily busulfan dosing was pharmacokinetically similar (dose proportionality, unchanged estimated clearance) to every 6 hour dosing which had been the historical norm for oral and earlier IV busulfan studies [12]. Daily dosing greatly simplified treatment regimens through PK monitoring and allows for better optimization for PK directed therapy. Initial population Cl, Vd, C-Max, t½ were derived from 45 subjects [12]. The underlying hypothesis directing these studies is that pharmacodynamics effects (PD) are proportional to the total AUC exposure to busulfan, a generally accepted premise.

The MD Anderson Cancer Center Department of Pharmacy Research has provided PK monitoring services for patients on busulfan targeted therapy since September 2009. From September 2009 through December 31, 2012, approximately 1300 pharmacokinetic consults have been provided, representing approximately 750 unique patients, providing a large and robust data set for further study. This large sample provides the opportunity to verify the population estimates from the original study, as well as to determine other variables that could significantly affect busulfan kinetics. The objective of this study was to assess patient specific factors associated with busulfan clearance.

Methods

This retrospective study was conducted to determine population descriptive statistics (i.e. mean clearance, mean AUC, mean half-life, etc.). Additionally, the effect of independent variables age, gender, primary cancer diagnosis, ethnicity, prior transplant status, and transplant type were analyzed for effect on the dependent variable of normalized clearance. Institutional Review Board approvals were obtained as required for this retrospective data review.

Patients included in this study were scheduled for a stem cell transplant as clinically appropriate treatment by the Department of Stem Cell Transplantation. The Division of Pharmacy provides a PK consult service that includes coverage for patients treated on busulfan targeted therapy on clinical treatment protocols. As part of the PK service, the pharmacist creates a patient specific worksheet based on the protocol defined target AUC. The worksheet contains patient specific information, such as height, weight, and BSA, that is critical to determining the normalized clearance and corresponding dose calculation to achieve the targeted AUC. The worksheet is also used to record blood draw time points (as documented by the phlebotomist), plasma busulfan levels, dose infused, infusion rate, and the infusion start and stop times. The plasma samples are analyzed by the Special Chemistry mass spectrometry lab technician in Laboratory Medicine for determination of busulfan levels. Using the ten time points from the blood draw worksheet, combined with the busulfan levels reported from Laboratory Medicine, the PK clinical pharmacy specialist calculates the patient specific clearance. Pharmacokinetic Data Analysis is performed using ADAPT II Release 4, a free pharmacokinetic/pharmacodynamic systems analysis software available from the University of Southern California, Biomedical Simulations Resource (Los Angeles, CA). ADAPT calculates the gross clearance (in liters per hour), the elimination constant (Ke), the volume of distribution (V) in liters, half-life in hours, and the daily AUC (micromoles*minutes). The results from the PK analysis are then communicated to the treating physician in a standardized format. The results

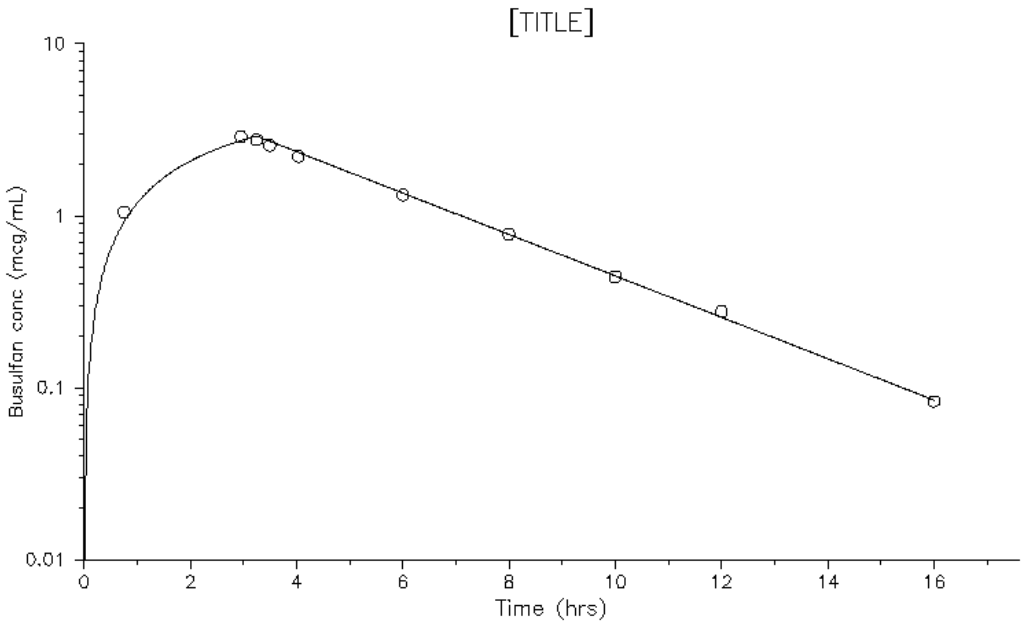
include the gross clearance, normalized clearance, goodness of fit (R^2), half-life, AUC, the dose required to achieve the target AUC, and the pharmacist's initials who performed the PK analysis. The pharmacist information is included in case the clinicians providing clinical care for the patient have any questions or concerns with the results.

Example PK Report:

PK Results	Therapeutic Dose	
Gross Clearance:	126	ml/min
Clearance Normalized to BSA:	88	ml/min/m^2
Goodness of Fit (R^2)	0.992	
Half life:	2.5	hrs
AUC	3,880	µM•min
AUC and Dose Calculations		
Targeted Daily AUC:	4,000	µM•min
Targeted Course AUC:	16,000	µM•min
Adjusted Daily AUC Target =	4120	
((Course AUC) – (Current AUC X 2)) / 2		
Dose to achieve Adjusted Daily AUC Target:	128	mg

To perform PK calculations using the ADAPT software package, basic input criteria include the number of time points (observations) drawn, infusion rate (in mg per hour), and length of infusion. For busulfan PK, the estimation procedure used is weighted least squares (WLS), and the weighting option is inverse variance of the output error (linear). The number of desired weighting procedures, low measurement and high measurement of busulfan concentration in plasma, and the initial estimates for both elimination constant (Ke) and volume of distribution are also entered. The pharmacist then enters time points from the phlebotomy sheet along with the corresponding mean plasma concentration from laboratory medicine into ADAPT. ADAPT then calculates the patient specific clearance. Output from ADAPT includes patient specific Ke, volume, clearance, busulfan half-life, and goodness of fit (R^2), along with a graph depicting the patient clearance (busulfan concentration – time curve). The pharmacist enters output data into the report for the treating physician and copies the graph to the report.

Sample PK Graph:



Per standard operating procedure for the busulfan PK service, after consultation with Stem Cell Transplant leadership, in cases with a poor PK model fit, the pharmacist can drop outlying data points for calculation of patient clearance. Poor PK model fit is defined as an R^2 of less than 0.9, and outlying data points are time points with the largest residuals. As a note, outliers are usually the 2nd, 3rd, or 4th time points, and can be a result of incorrect documentation of sample draw time. As routine practice for patients with a poor model fit, the pharmacist will perform the calculation with all the time points included, and then drop the biggest outlier. While dropping a time point will improve the goodness of fit, it rarely results in a meaningful change in final clearance, or ultimately the patient's dose. In any event, all results are communicated to the treating physician for final decision on the patient's dose.

All other PK parameters were recorded as reported to the treating physician, with the exception of patients who had an unusually high normalized clearance. For safety reasons and per standard operating procedures in Stem Cell Transplant, any patient with a normalized clearance ≥ 150 ml/min/m^2, the clearance is capped at 150 ml/min/m^2. This capped clearance was used in calculations of mean clearance and dose determinations.

BMTWeb is a web-enabled Java application and Oracle database designed to collect detailed data for Stem Cell Transplantation and Cellular Therapy (SCTCT) operations in clinical research trials and for required reporting activities. As the main Informatics hub for SCTCT clinical research operations, BMTweb contains real-time interoperability architecture. BMTweb

supports Service Orientation Architecture (SOA) interfaces with institutional sources such as ADT (patient demographic), HLA Lab (Donor Typing), and SPIDR (Lab data) databases. The data storage design allows secured and roles based retrieval of data at any time, in any format and can be exported to other programs such as SAS for detailed analysis. This validated data is also used to generate reports with business intelligence and reporting tools (Crystal reports) in defined formats, for different purposes, ranging from patient admission (Flowsheets) to treatment to Outcome reports.

BMTWeb data included: birth date (used to calculate patient age at treatment date – this was absent from the de-identified data set); height, weight, and BSA; whether the patient had a prior transplant (yes/no); transplant type; grading for patients who developed GVHD, liver toxicity, CNS toxicity; survival status with date of last follow-up; and time to event for outcome variables (GVHD, liver toxicity, CNS toxicity, and death).

Complete PK data from the pharmacy worksheets was imported into BMTWeb by the database developers. Data importation linked pharmacy records to BMTWeb records by matching both fields for treatment date and patient's medical record number.

A de-identified dataset was created from this database under a retrospective data review protocol. The dataset included patient demographic information (gender, ethnicity, age at time of transplant, diagnosis, transplant type, and whether the patient had had a previous transplant), the complete PK data set as imported from the pharmacy PK service, and outcome data considered to be potentially associated with busulfan PK (GVHD, CNS toxicity, liver toxicity, and survival).

BMTWeb was considered the source record for all patient characteristics associated with the transplant service, while the pharmacy records served as the source record for all PK data.

Since PK data was imported from individual worksheets, the dataset was checked for importation errors. Before importation, records were matched on both medical record number and treatment date. Any discrepancies between pharmacy records and BMTWeb records were analyzed and resolved before importation. These errors were primarily treatment date issues and were fixed by confirming patient treatment date in the patient's electronic medical record. Patient MRNs were not present in the final de-identified dataset. Other data cleanup included verification of test dose versus therapeutic dose. Test doses are typically 0.8 mg/kg. To confirm dose type, the dataset was sorted by dose type and then dose. Scanning the dose field after this sort provided quick verification of accuracy of dose type. Additionally, the dataset was sorted by clearance. Since clearance was capped at 150 ml/min/m^2, any value above this cap was verified. The lowest clearance was estimated to be in the low 50's before the analysis (based on discussion with PK service providers). This estimation was confirmed during analysis.

Statistical Analysis

The statistical analysis was conducted using two different approaches. The first approach was to perform a logistic regression for the three toxicities of interest plus survival. The toxicities of interest included CNS, GVHD, and Liver. Toxicities with severity of ≥ Grade 3 were included in the analysis. In addition to the logistic regression, a Kaplan-Meier regression analysis was performed on the same three toxicities and survival. The cutoff for the Kaplan-Meier regression analysis was set at one year. All analyses were conducted with the individual patient as the unit of analysis. SAS version 9.3 was used to perform the analyses. In this analysis, normalized busulfan clearance is the dependent variable and is continuously distributed. The level of statistical significance was set at 0.05 a priori.

Exposure – treatment with busulfan

A patient's exposure (plasma concentration/time data) to busulfan is used to calculate patient specific clearance, which is the independent variable of interest for this study. A patient's normalized clearance is a continuous variable with a range of approximately 50 to 150 ml/min/m^2. The assumption of normal distribution was verified.

Results:

Patients had a mean age of 46.2 years at time of transplant, with a range of 2-74 years (standard deviation 15.8). Other patient demographic information is included in the tables below:

Table: Patient Characteristics

	Frequency	Percent
Male	450	59.4
Female	302	40.2
Prior transplant	49	6.5
Allogeneic transplant	504	67.0
Autologous transplant	245	32.6
Survival at 1 year	408	66.9
(died)	202	33.1

Patients included in the database had 9 different diagnoses. Since there were only 8 patients with chronic lymphocytic leukemia, these were excluded from the analysis. Chronic myelogenous leukemia patients (n = 15) were combined with myelodysplastic syndrome patients (MDS) for analysis purposes, since they are expected to have similar clinical outcomes.

Table: Diagnoses and Ethnicities:

	Frequency	Percent
ALL	100	13.4
AML	215	28.9
CML/MDS	146	19.6
Hodgkin's	110	14.6
Lymphoma	67	9
MPD	27	3.6

Myeloma	79	10.7
Ethnicity		
White	516	68.6
Asian	22	2.9
African American	58	7.7
Hispanic	121	16.1
Other	35	4.7

Patients in this study were predominantly white. The other top ethnic groups are included in the table above. While there were only 22 Asian patients (9 F, 13 M) in the study, there were analyzed as a separate group for comparison to the results of Choe et al.[18]

Among all 752 patients in the dataset, patients had a mean clearance of 101.2 ml/min/m^2, with a standard deviation of 17.5 and a range of 51.5 to 150 ml/min/m^2. More detailed information about patient factors that affect clearance is available in Intravenous Busulfan in Hematopoietic Stem Cell Transplantation: Do Patient Specific Factors Affect Clearance?

Outcome Variables:

Event	Number	Percent
GVHD	46	6.1
CNS Toxicity	56	7.4
Liver Toxicity	116	15.4
Died	202	33.1

AIM 2: Association of Clearance and outcomes:

Probability modeled is Event=1.

Logit Model	**Number**	**Likelihood ratio**	**Sig. variable**	**p-value**	**Odds Ratio**	**Confidence Intervals**	
GVHD	46	<0.0001	Dx - AML	0.0323	0.388	0.163	0.923
CNS	56	0.0126	Age (years)	0.0090	0.974	0.955	0.993
Liver Toxicity	116	<0.0001	Clearance	0.0223	0.985	0.973	0.998
			Age (years)	0.012	0.981	0.966	0.996
			Dx – AML	<0.0001	0.284	0.153	0.528
			Dx – CML/MDS	0.0064	0.374	0.184	0.758
Died	202	<0.0001	Clearance	0.0013	0.988	0.972	0.993
			Race – Asian	0.0440	2.729	1.027	7.253
			Age (years)	0.0001	1.022	1.014	1.042
			Prior Transplant	0.0015	3.389	1.623	7.768

Diagnoses vs (ALL) Prior TP vs 0 (no prior TP)

Survival Analysis	**Chi-square**	**Significant variables**	**p-value**	**Hazard Ratio**
GVHD	0.3119	Clearance – 0	0.0014	23.426
CNS	0.6612	Sex (M)	0.0247	2.867

		Dx – AML	0.017	6.960
Liver Toxicity	0.0504	Age	0.0021	0.979
D	<0.0001	Clearance – 0	0.0087	2.240
		Clearance – 1	0.0194	2.042
		Clearance – 3	0.0087	2.237
		Race - Asian	0.0199	2.269
		Age	0.0001	1.021
		Prior Transplant	0.0005	2.487
Note:		Clearance – 2	0.1711	1.345

(included because Clearance 0, 1 and 3 are significant)
Clearance versus highest clearance (decile 9); clearance was ranked 0-9 in ascending order.
Race 1 (Asian) versus 0 (white).

Discussion

Logistic results

Busulfan clearance was associated with liver toxicity and survival, with higher clearance being associated with both a decreased risk of liver toxicity and improved chance of survival. The improved survival with higher clearance can be a direct result of the higher therapeutic dose given to patients with a higher clearance (in order to hit the target AUC).

The one significant finding with GVHD was Dx-AML, which had a protective effect (odds ratio of 0.388). This may have been a serendipitous finding as there were only 46 total cases of GVHD, with XX of these cases in the patients with AML.

In the logistic analysis, there was no significant finding in relation to CNS toxicity regarding clearance, but increasing age had a protective effect against CNS toxicity. Other factors associated with liver toxicity included age, which had a protective effect, and a diagnosis of AML or CML/MDS, which had a protective effect versus that of ALL.

Asian patients had a 2.7 fold increase risk associated with death, compared to whites, although this study had a limited sample size of Asian patients. Other factors associated with an increased risk of death include increasing age and prior transplant (odds ratios of 1.022 and 3.389 respectively).

Surival analysis results

Kaplan-Meier time to event model, the only significant finding associated with clearance was survival. Three of the four lowest groups (out of 10 groups) had a significantly increased risk of death (hazard ratio of around 2 for each group).

Patients with the lowest clearance were significantly more likely to develop GVHD. With CNS toxicity, males were 2.9 times as likely to develop CNS toxicity as females, and Dx-AML patients 7 times as likely to develop CNS toxicity (compared to ALL). With liver toxicity, age had a slightly protective effect (hazard ratio 0.98). There were other factors that were significantly

associated with survival included race, age, and prior transplant. For race, Asian patients had a hazard ratio of 1.7 compared to white patients, and most significantly patients with a prior transplant had a hazard ratio of 2.3 compared to patients without a prior transplant. Age was also significant, with a hazard ratio of 1.02.

One of the biggest strengths of this study is the very large sample size. 16 publications reviewed prior to this study had an average sample size of 61 (range 12-145). With 752 patients accumulated over a 3 year period, there is very good representation of 7 different diagnoses, patients with both allogeneic and autologous transplants, patients with prior transplant, and several different ethnicities. Furthermore, the relatively short duration of the study insured relatively constant treatment standards. Finally, the plasma concentrations of busulfan were all conducted in a single CLIA certified laboratory, where the special chemistry lab analysis is performed by very few specially trained personnel, insuring high consistency between the critical determinations of plasma levels.

The limitations of the study include it was conducted on patients from a single cancer center. Given the large sample size and diverse patient population, though, results would not expected to differ significantly from similar patient populations treated at other cancer centers. Finally, this study did not analyze any potential genomic factors, since this data was not included in the BMTWeb dataset.

It should also be noted that this paper did not address potential drug-drug PK interactions or differences in clinical impact of patients being treated on different therapeutic protocols. The therapeutic treatment protocol was coded in the dataset, but was not included as a covariate in this analysis since the stated objective was to look for predictors of clearance and the effect of clearance on outcomes.

Conclusion

In the logistic analysis, clearance was associated with liver toxicity and survival, with increased clearance having a protective effect on both. In the survival analysis, lower clearance was associated with an increased risk of dying in three of the four (out of 10 groups) lowest clearance groups.

7.0 References

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