Distribution Agreement

In presenting this Thesis as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my Thesis in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this Thesis. I retain all ownership rights to the copyright of the Thesis. I also retain the right to use in future works (such as articles or books) all or part of this Thesis.

_____

Hongzheng Zhang, Ph.D. Date

OUTCOMES OF ORAL VERSUS INTRAVENOUS DELIVERY OF BUSULFAN IN LYMPHOMA PATIENTS UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

APPROVED

_____

Joseph Lipscomb, Ph.D., Committee Chairperson Date

_____

Christopher R. Flowers, M.D., M.S., Field Advisor Date

_____

Walter Burnett, Ph.D., Healthcare Outcome Track Coordinator Date

_____

Melissa Alperin, MPH, CHES, Director, Career MPH program Date

OUTCOMES OF ORAL VERSUS INTRAVENOUS DELIVERY OF BUSULFAN IN LYMPHOMA PATIENTS UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

By

Hongzheng Zhang
M.P.H., Emory University, 2011
Ph.D., Mississippi State University, 1998
B.S., Jinan University, 1989

Thesis Committee Chair: Joseph Lipscomb, Ph.D.

An abstract of
A Thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements of the degree of
Master of Public Health in the Career MPH program
2011

Abstract

Improved survival with intravenous (IV) over oral delivery of Busulfan in autologous stem cell transplantation (ASCT) has been reported in a retrospective study for cohorts of lymphoma patients treated with high-dose chemotherapy and conditioning regimen of busulfan, cyclophosphamide (Cy) and etoposide (VP-16). However, the clinical advantage of pharmocokinetic-directed (PK)-based dosing on regimen-related mortality and overall survival remains unclear. To address this issue we performed a retrospective cohort study to compare the efficacy of PK-directed oral and IV busulfan-based conditioning regimen in lymphoma patients undergoing ASCT from 2000-2010 at Emory University. Sequential cohorts of patients included for analysis received oral (1.0mg/kg every 6 hours x 16, n=77), IV16 (0.9mg/kg every 6 hours x 16, n=103), or IV4 busulfan (3.6mg/kg daily x 4, n=40) followed by Cy (60mg/kg qd x 2), VP-16 (10 mg/kg qd x3) and infusion of previous collected autologous stem cells. PK-directed dosing was performed to achieve a predefined target area under the curve (AUC) range. For oral, IV16 and IV4 groups, respectively, the initial dose was 66, 63 and 255 mg, the $T_{1/2}$ was 224, 190 and 188 hours, and the percentage of patients reaching the target range was 42%, 89% and 88%, which were significantly different across groups ($p<0.001$). With a median follow-up of 1761, 895 and 392 days, the 100-day mortality was 2.6%, 2.9% and 5.3% for oral, IV16, and IV4, respectively ($p=0.76$). Five-year overall survival was 57.6% and 65.8%, for oral and IV administration, respectively. In multivariable Cox regression models, age (HR=1.34, $p=0.003$) but not the route of delivery had a significant effect on overall survival. **Conclusions:** PK-directed IV busulfan improves the consistency of delivering a predefined target AUC over oral PK-directed busulfan with similar early and late overall survival for lymphoma patients undergoing ASCT.

OUTCOMES OF ORAL VERSUS INTRAVENOUS DELIVERY OF BUSULFAN IN LYMPHOMA PATIENTS UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

By

Hongzheng Zhang
M.P.H., Emory University, 2011
Ph.D., Mississippi State University, 1998
B.S., Jinan University, 1989

Thesis Committee Chair: Joseph Lipscomb, Ph.D.

A Thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
In partial fulfillment of the requirements of the degree of
Master of Public Health in the Career MPH program
2011

ACKNOWLEDGMENTS

I would like to thank my husband, Ramesh who has supported me every step on the way with his love, wisdom, and dark humor. I am thankful to my daughter, who has brought me endless joy and sunshine.

I would like to express my sincere acknowledgment support and help of my professors, Dr. Christopher R. Flowers and Dr. Joseph Lipscomb. I would like to thank Dr. Edmund Waller for giving me the opportunity to work on the project.

I would like to extend my gratitude to Dr. Michael Graiser for going extra mile to help with the research and to Mrs. Melissa Krancer for making CMPH program better.

Table of Contents

Table 1. Committee Members

Study Committee	**Name**	**Institute**
SSP Field Advisor	Dr. Christopher Flowers	Hematology and Medical Oncology Department, Winship Cancer Institute
Project co-investigator	Dr. Edmund Waller	Hematology and Medical Oncology Department, Winship Cancer Institute
SSP Committee Chair	Dr. Joseph Lipscomb	Health Policy and Management, Rollins School of Public Health
SSP Healthcare Outcome Track Coordinator	Dr. Walter Burnett	Career MPH Outcome track, Health Policy and Management, Rollins School of Public Health
Career MPH director	Melissa Alperin	Rollins School of Public Health

Chapter I
Introduction

Hematopoietic stem cell transplantation (HSCT) is the transplantation of blood stem cells derived from the bone marrow or blood. At present HSCT provides the best chance of cure for many disease. It is used primarily for hematologic and lymphoid cancers such as multiple myeloma, NHL, HL, neuroblastoma, ovarian cancer, germ-cell tumors as well as other disorders including aplastic anemia, thalassemia major, sickle cell anemia (Copelan, 2006). Autologous HSCT requires the extraction of HSC from the patient and storage of the harvested cells in a freezer. Allogeneic HSCT requires healthy people as donors and the patient as the recipient. The patient is then treated with chemotherapy agents to suppress cancer cells as well as the patient's immune system sufficiently to allow engraftment (Little & Storb, 2002).

Issue statement

Busulfan is a chemotherapy agent originally used for the treatment of chronic myelogenous leukemia but was later used as a substitute for total body irradiation regimens in order to limit some of the induced toxicities. It also allowed the development of drug based protocols which could be used by centers without total body irradiation facility. Busulfan was usually given by mouth at 1mg/kg every 6 hours for 4 days. While effective, this regimen was quite toxic. The unpredictable absorption of oral Busulfan from the gastrointestinal tract and liver have led to the development a commercially available intravenous Busulfan, which is well tolerated and can give more predicable pharmacokinetics (Ciurea & Andersson, 2009). When the patient is treated with Busulfan, the amount of drug in the patient's circulation can be measured. Therapeutic drug monitoring is the process whereby the plasma concentrations

of the drug or area under the curve (AUC) are used to predict the therapeutic dose window (Alnaim, 2007). High AUC likely leads to regimen-related toxicity, specifically hepatic venoocclusive disease (HVOD), a significant cause of morbidity and early mortality following HSCT while low AUC has the risk of graft rejection and relapse (Kashyap et al., 2002).

Intravenous (IV) delivery of busulfan has become the preferred conditioning regimen over oral administration with the advantages of bypassing the gastrointestinal tract, eliminating delayed oral absorption, and reducing plasma exposure variability and inter dosing variability (McCune & Holmberg, 2009). However, the considerable inter-patient variability in the IV route administration highlights the need of therapeutic drug monitoring or pharmacokinetic-directed dosing to achieve the desired outcomes (McCune & Holmberg, 2009). With finer controlling of busulfan at the targeted range via PK-directed dosing, once-daily IV delivery maybe preferred due to its convenience. However, the comparison of clinical outcome of once daily IV with four times daily IV or oral administration of busulfan has been lacking.

Moreover, HSCT is a cost-intensive procedure (Copelan, 2006). Assessment of the clinical advantage and cost comparison of oral versus IV delivery through PK-directed dosing approach has never been investigated.

Theoretical Framework

Cancer is one of the main health care problems in the United States. In 2009, the National Institute of Health estimated the 2008 annual total costs of cancer are $228.1 billion. Although significant progress has recently been made, long-term survival is still disappointing for most common cancers. In the era of information exposure and advance in information

technology, to achieve improved cost-effective healthcare outcomes there is an urgent demand to create and sustain integration among population cancer prevention and control, personalized, evidence-based clinical practice and patient-reported outcomes (**Figure 1**). This has provided a great opportunity and posed challenges for outcomes research. For the current report, a conditioning regimen with personalizing busulfan dosing in patients undergoing hematopoietic stem cell transplant has provided an excellent example for the integral outcome research which intends to identify the right treatment, right patient, right time, right place and right cost. The institutional-based outcome study could provide a control environment to examine the benefit of practicing personalized medicine on one hand, while facing the limitation on the other hand.

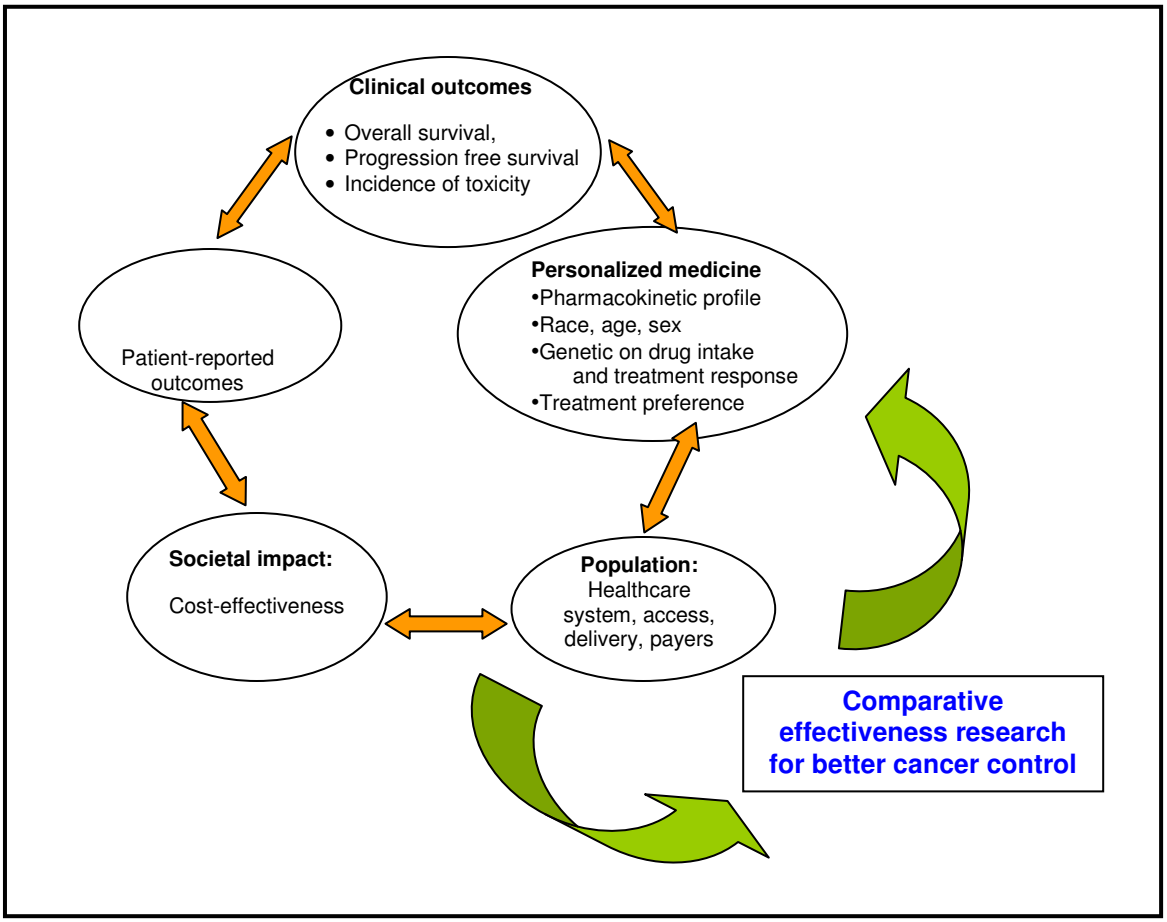


Figure 1. Theoretical framework for outcome research.

Objectives of the Study

Therefore, I hypothesized that 1) IV administered busulfan with a targeted AUC (dose range) may provide equivalent or better safety, efficacy and pharmacokinetics profile (correlation of AUC with efficacy and toxicity) than oral busulfan. 2) There could be difference of PK by racial/ethnicity, particularly African American versus non-African American. 3) The cost effectiveness analysis shows moderate advantage of intravenous over oral busulfan.

Hence, the study was aimed at investigating the safety, efficacy, pharmacokinetic profiles and cost-effectiveness of intravenous administered Busulfan in lymphoma patients undergoing autologous hematopoietic stem cell transplants.

Chapter II
Literature Review

The chapter presents the general description of lymphoma, review on hematopoietic stem cell transplant, significance of therapeutic drug monitoring and the evolution of conditioning regimens including oral and intravenous Busulfan. Current practice and relevance of study are discussed.

Lymphoma

Lymphomas, including Hodgkin lymphoma (also called Hodgkin's disease, HL) and non-Hodgkin lymphoma (NHL), are heterogeneous group of cancer that are characterized by abnormal growth of tissue in the lymphatic system. Symptoms include the painless enlargement of lymph nodes, spleen, or other immune tissues. Lymphoma represents approximately 5% of all cancers in the United States.

It is estimated in the year of 2010, there will be 65, 540 new cases and 20,210 death from NHL and 8,490 new cases and 1,320 death from HL. The incidence rate of NHL has increased significantly in the past two decades; mortality rates for NHL differ by the highly heterogeneous subtypes, are further complicated by race and gender factors (www.cancer.gov). On the other hand, due to the improvements in the treatment of HL, the mortality rate has decreased significantly over the past 25 years. Although the incidence rate for whites has remained relatively steady, the rates for African American have increased. It is estimated that approximately $4.6 billion is spent in the United States each year on treatment for lymphoma (http://pregressreport.cancer.gov in 2004).

NHL is a heterogeneous group of lymphoproliferative malignancies with differential behavior and responses to treatment (Flowers & Armitage). NHL usually originates in the lymphoid tissues and can spread to other organs. Unlike HL, NHL is much less predictable

and prone to disseminate to extranodal sites. The prognosis depends on the histologic type, stage and treatment. Nearly 80-90% of NHLs are B-cell origin. World Health Organization Classification published in 2001 and updated in 2008 group lymphomas by cell type, phenotypic, molecular and cytogenetic characteristics (Jaffe, 2009). Overall, NHL can be classified into subtypes including (Jaffe, 2009):

- chronic lymphatic leukemia and the related small lymphacytic lymphoma
- cutaneous T-cell lymphoma
- follicular lymphoma
- marginal zone lymphoma
- peripheral T-cell lymphoma
- mantle cell lymphoma
- primary mediastinal B-cell lymphoma
- Burkitt lymphoma
- Lymphophasmacytic lymphoma

NHL can be divided into two prognostic groups: the indolent and the aggressive lymphomas. Treatments for NHL are based on the type, stage, aggressiveness of the disease, health status and patient' age. For early stage and low grade disease, patients are given radiation therapy; for intermediate and high grade disease, chemotherapy is given. In general, 5-year overall survival is about 50-60%; of the patients with aggressive NHL, 30-60% can be cured. The vast majority of patients face relapses in the first 2 years after initial treatment. For indolent NHL, there is considerable success as long as disease histology remains low grade.

There are two types of HL: classical and nodular lymphocyte-predominant. Most HL are the classical type which can be further subgrouped into nodular scherosing, mixed

cellularity, lymphocyte depletion and lymphocyte-rich classical HL. Risks factors associated with HL include having age at 15-40 or age older than 55, male, infection with Epstein-Barr virus, and an inherited immune disorder. Treatment for HL depends primarily on the stage of the disease at diagnosis, the number and regions of lymph nodes affected, with one or both sides of diaphragm affected, patient's age, symptoms and health status. If HL is confined locally, radiation therapy may be used alone or in combination with chemotherapy. Chemotherapy can be given to patients if disease has progressed to additional lymph node areas or other organs. For both recurrent HL and NHL, additional high-dose chemotherapy with the option of hematopoietic stem cell transplant has provided improved long-term remission.

Hematopoietic stem cell transplant (HSCT)

HSCT has become the standard treatment approach for most patients with relapsed or refractory HL or NHL (Copelan, 2006; Hahn et al., 2003). Autologous HSCT (ASCT) is typically the primary transplant options, especially for chemotherapy-sensitive patients while allogeneic HSCT is reserved for particular patient subset. High doses of chemotherapy and extensive radiation therapy have been used to treat advanced and recurrent cancers. The limiting factor of this strategy is the toxic effect on bone marrow cells. It was recognized that if stem cells could be stored prior to treatment and be infused into the patient following treatment to allow restore bone marrow function, overcoming the resistant cancer then becomes possible with increasing doses of drug or radiation. In light of this, HSCT can be performed with allogeneic transplantation (cells from a family member or an unrelated donor) which may be vulnerable to the lack of a graft-versus-tumor effect in graft, or with autologous transplantation (ASCT) in which cells have been previously collected from the patient, has the

risk of contamination of malignant cells. In addition, the choice between more risky allogeneic and an autologous transplantation depends on patient's age, type of disease, and donor availability.

Over the past four decades important developments have made the stem cell transplantation common practice. These include

1) drugs that can be used safely in high concentrations;
2) safer administration of total body irradiation;
3) technology for collection and purification of stem cell from the bone marrow and peripheral blood has improved;
4) improved antibiotic and antiviral agents;
5) better use of marrow growth stimulating factors to promote early maturation of stem cells into functioning mature cells;
6) stem cells collected from peripheral blood cells can be effective and the procedures are faster than stem cells collected from bone marrow.

There are 4 components of transplantation, including conditioning, transplantation, engraftment and immunoreconstitution. Conditioning regimens consist of high dose chemotherapy, and in some cases, radiotherapy to eradicate the malignant disease and to suppress the recipient's immune system so that it will not reject the donor's stem cells. The process of stem cell transplantation begins with releasing stem cells mobilized into the peripheral blood by granulocyte colony stimulating factor, collection and storage of stem cells 2 weeks later, administration of high-dose chemotherapy, transplantation of stem cells, engraftment, and recovery from the toxicity of high dose chemotherapy within 30 days. Engraftment is the process wherein the donor cells begin to produce new blood components

within the recipient's bone marrow cavity, and usually occurs 10-20 days of post transplantation. Recovery of adequate blood counts allows discontinuation of antibiotics and release to home 2 weeks after transplantation. However, restoration of T-cell and B-cell, which takes more than 12 months, is critical to the recovery process. During this period, graft-versus-host disease (GVHD) can be acute occurring in the first 100 days or chronic.

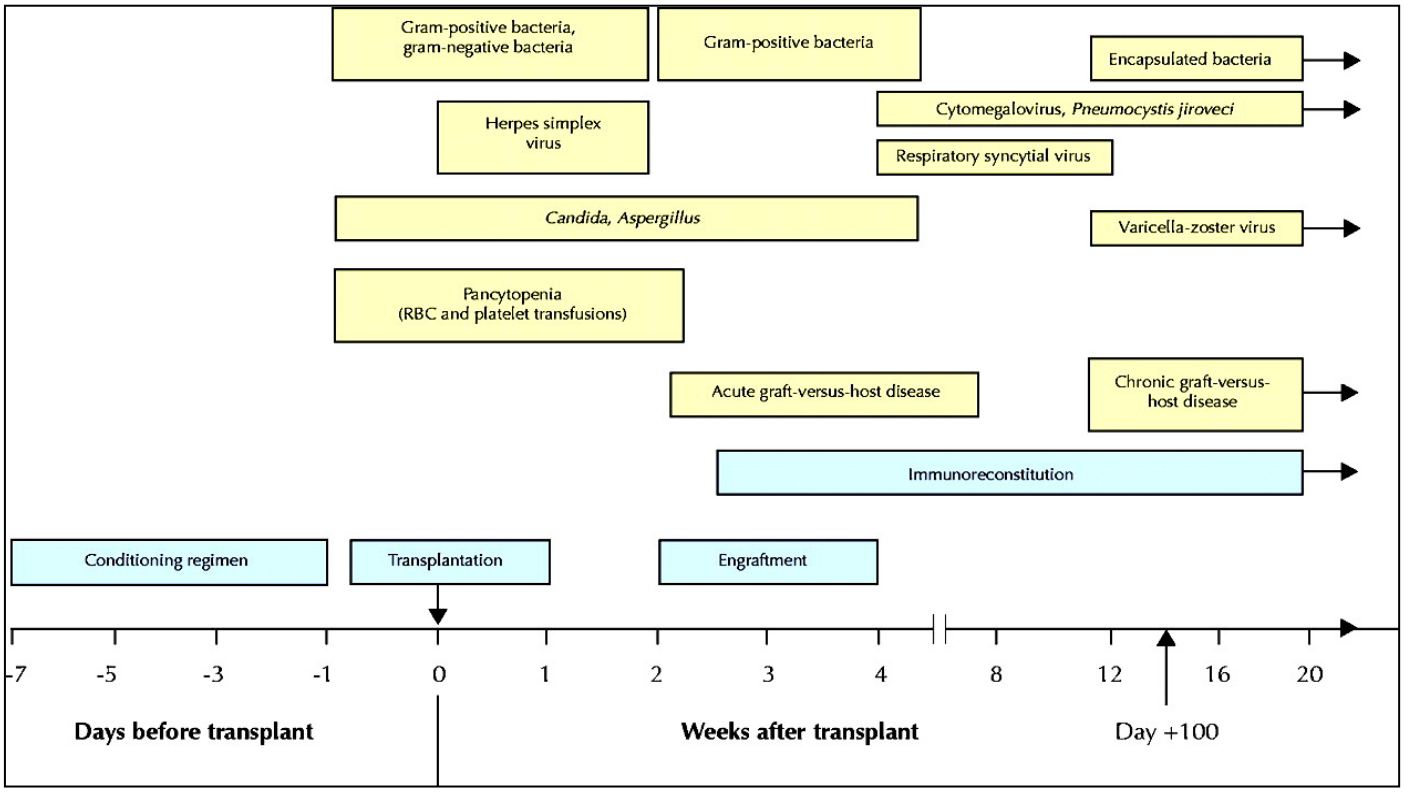


Figure 2. Four components (blue) and risks (yellow) associated with allogeneic transplantation. RBC=red blood cells. Copied from (Leger & Nevill, 2004).

For allogeneic transplantation with matched-sibling, treatment related mortality in the first 12 months maybe 20-30%, for unrelated donor, and it can reach up to 50%. For ASCT, the first three components are similar to allogeneic except that the donor and recipient are the same person. The other difference is that patient's immune recovery is more rapid and there is no GVHD; thus transplantation related mortality at day 100 is about 5-20% (Copelan, 2006). Hence, ASCT has become preferable to older patients. Although ASCT has a relatively low

toxicity compared with allogeneic HSCT, there is a persistent risk of tumor cells being present within the harvest. Consequently, patients who survive through allogeneic and autologous transplantation often face relapse within 2 years of transplantation.

Busulfan as conditioning regimen and therapeutic drug monitoring

To eradicate the patient's underlying disease and to suppress immune reactions through ablating bone marrow cells, a conditioning regimen such as total body irradiation is commonly employed prior for either autologous or allogeneic transplantation. Busulfan, 1,4-dimethanesulphonyloxybutane, (Myleran®) can alkylate DNA, has potent suppressive properties on a myelogenous cell population. Although Busulfan alone is toxic to bone marrow, it has minimal toxicity for mature lymphocytes, and therefore, can be used alone for autologous transplantation. Busulfan in combination with other cytotoxic agents such as cyclophosphamide is used to prevent the graft rejection. Preclinical and clinical studies lead by Santos and Tuschka (Glazier, Tutschka, Farmer, & Santos, 1983; Hassan et al., 1994; Santos et al., 1983) showed that a combination of Busulfan with cyclophosphamide (BU/CY) was an effective conditioning regimen alternative to the standard total body irradiation with CY, with the advantages of ease of administration and the lack of need for a total body irradiation facility.

Therapeutic drug monitoring (TDM) has been incorporated into clinical practice since 1960s under the premise that the measurement and interpretation of drug concentration in biological fluid and personalizing dosing and/or scheduling could maximize therapeutic outcomes and minimize toxicities. However, TDM for anticancer drugs is limited by 1) incomplete knowledge about pharmacokinetics of cancer drugs; 2) the relationship of plasma concentration of the drug with the amount of drug in the target tissue; 3) a time lag between

the measurement of drug in plasma and the time when the effect takes place; 4) the difficulty in determining the effective dose of the drug in the case of combination of treatment; and 5) difficulty in determining the effective dose due to the heterogeneous nature of cancer. Despite the limitations of TDM application in cancer drugs, there is a vast potential for improvement in cancer therapy due to two key reasons: the current adopted principle of application of maximum tolerated dose, and wide variability of anticancer drugs in pharmacokinetics and narrow therapeutic windows.

In a relatively controlled environment, the benefit of TDM as a form of personalized medicine in improving individual cancer outcomes can be tested. Busulfan clearly fits the criteria for TDM as it can be measured in the plasma, and there is significant intrapatient and inter-patient variability (Hassan et al., 1994), importantly, there is a relationship between plasma exposure and narrow therapeutic index, i.e., Busulfan levels that are too high are associated with more organ toxicity, whereas lower levels are associated with more relapse. Busulfan was usually given by mouth at 1mg/kg every 6 hours for 4 days. It is rapidly absorbed with peak plasma concentration at 1.5-2.5 hour post administration. A study reported that up to 26% of patients had delayed absorption or prolonged elimination of oral Busulfan (McCune & Holmberg, 2009). In multivariate analysis, the use of the oral formulation was the strongest predictor of the development of liver toxicity like HVOD (Ciurea & Andersson, 2009). The unpredictable absorption of oral Busulfan from the gastrointestinal tract and liver has led to the development a commercially available intravenous Busulfan, approved by U.S. FDA in 1999, which is well tolerated and can give more predicable pharmacokinetics and lower incidence of severe hepatic venooclusive disease

(HOVD) (Ciurea & Andersson, 2009), (Kashyap et al., 2002), as well as better efficacy according to the latest study without PK monitoring (Dean et al., 2010).

Both oral and intravenous Busulfan are usually dosed based on body weight, which leads to appreciable variability in Busulfan exposure. Busulfan exposure in terms of pharmacokinetics is expressed as area under curve (AUC, steady-state plasma concentration). It has been established that the clinical outcome of Busulfan exposure is associated with the conditioning regimen, the age of the HSCT recipient and the underlying disease (McCune & Holmberg, 2009) . In addition, total course AUC in excess of 24,000μM/min was associated with inferior survival (Geddes et al., 2008) whereas low BU AUC levels (below 900μM/min) have been correlated with graft failure and disease recurrence (Ciurea & Andersson, 2009), suggesting the need of PK-directed dosing to ensure the optimal clinical outcome. As for all PK sampling, obtaining reliable PK information require proper attention to the entire infusion process and accurate recording of infusion and sampling times. This includes priming the tubing with drug, not saline, all the way to patient; infusion by controlled-rate pump; and, finally, disconnecting all the tubing and pump cassette at the end of infusion without the use of saline chasers to clean the line.

HSCT is a highly costly procedure at present, more than $80,000 for autologous, and $150,000 for allogeneic transplantation (Copelan, 2006), demanding investment and integrative strategies for success. It involves days of hospitalization, physicians and staffs, the use of the laboratory for preparing stem cells, the use of diagnostic tests, and also transplantation follow-up outpatient visits. The large financial burden on the health care system justifies economic evaluation. Multiple reports have highlighted the importance of initial hospital stay in the total cost of HSCT (Kline, Meiman, Tarantino, Herzig, & Bertolone,

1998). The peripheral blood stem cells have replaced bone marrow, with the advantages of delaying neutropenia and shortening the length of hospital stay (Hiddemann et al., 2005). With respect to the conditioning regimen, reduced toxicity and better clinical efficacy associated with intravenous Busulfan can achieved only at a much higher cost of drug (Ciurea & Andersson, 2009). For example, based on the most current average whole sale price, oral Busulfan is $4.46/2mg vial, intravenous Busulfan is $1150.82/60mg vial. The cost of intravenous Busulfan factored into drug, administration and hospitalization, PK-directed dosing, lab test, treating liver and lung toxicity has not been investigated and thus has not been incorporated into decision making equation. In addition, conducting TDM is very resource-intensive, to date, there is no report of cost analysis comparing oral and intravenous formulation. Furthermore, ethnicity influences on PK profiles and thus efficacy, which has been largely understudied in cancer drugs (Wilkinson, 2005), has not been incorporated into the prognostic factor modeling (Hari et al., 2010).

There are no prospective randomized clinical trials for determining the optimal conditioning regimens for HSCT. Furthermore, the majority of investigations have been conducted with retrospective studies addressing the toxicity between oral and IV busulfan administration. Comparisons of progression free survival and overall survival outcomes between the two routes and the need of PK-directed dosing have not been clearly defined (Ciurea & Andersson, 2009). PK-directed dosing provides an effective way to deliver busulfan. Thus, once daily IV administration of busulfan may offer convenience over four times daily dosing. However, the efficacy of this regimen has not been investigated.

Hypothesis of the study

IV administered Busulfan with a targeted AUC (dose range) may provide equivalent or better safety, efficacy and PK profile (correlation of AUC with efficacy and toxicity) than oral Busulfan.

Significance of the study

Examining the safety, efficacy, and cost analysis of conditioning regimen through PK-directed dosing with either oral or intravenous Busulfan is essential for personalized treatment and healthcare planning (**Figure 1**).

Chapter III
Methodology

The primary endpoint of study was to determine the overall survival difference between the two routes of Busulfan delivery. The secondary endpoint is to examine the toxicity and pharmacokinetic profiles of these two drugs using measures of incidence of HVOD, and length of stay (days). A simplified cost analysis was also conducted.

Design

This retrospective observational cohort was conducted with a preexisting database populated with Hodgkin lymphoma and non-Hodgkin lymphoma patients undergoing the first ASCT at Emory Winship Cancer Institute between September 5, 2000 and May 19, 2010 (n=220). The data analysis was approved by the Emory Institutional Review Board. Patients received either oral (1mg/kg every 6 hours x 4 days from day-8 to day-5, n=77) or IV16 (0.9mg/kg every 6 hours for 16 doses from day-8 to day-5, n=103), or IV4 (3.6mg/kg daily x 4 from day-8 to day-5, n=44) followed by Cy (60mg/kg qd x 2 on day-3 and -2), etoposide (10 mg/kg qd x3 on day -4 to day -2). For oral busulfan administration, half or full doses were re-administered if vomiting occurred within 30 or after 30 minutes of any single dose, respectively. Following one day of no chemotherapy (day-1), patients received autologous bone marrow transplantation or granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells that had been previously collected, frozen, and thawed immediately prior to infusion as previously reported (Lonial et al., 2004). Minimum dose of CD34+ at 2 x 10^6/kg was required for transplantation (Lonial et al., 2004). Hepatic venoocclusive disease (HVOD) was diagnosed based on Baltimore criteria (Jones et al., 1987).

Pharmacokinetic-directed dosing

In this study, following the initial IV or oral dose, PK monitoring of busulfan plasma levels was accomplished using gas chromatography method with mass selective detection at clinical lab at Emory University. The AUC was calculated and used to adjust the subsequent doses. PK-directed delivery was based on an institutional protocol: if the targeted AUC was not achieved with initial dosing, dose adjustments were made and repeated PK sampling at dose 3, 5, 9 and 11 was performed for oral or IV16, at dose 2 for IV4. Prior to 2003, PK-directed dosing modifications were applied to patients with starting dose at AUC<1500μM-min. Since 2003, regardless of IV16 or IV4, all patient doses were adjusted to deliver an average AUC of 1150-1350μM-min (total AUC 18,400-21,600μM-min). Pharmacokinetic analysis was performed using the TOPFIT program.

Table 2. Treatment response classification used in the current study based on American society for bone marrow transplantation (ASBMT) and center for international blood and marrow transplant research (CIBMTR)

ASBMT classification	CIBMTR classification and definition
Low risk: **CR1/CRU1**	**CR1 confirmed:** complete disappearance of all known disease for >=4 weeks. **CRU1:** CR1 with the exception of persistent scan abnormalities of unknown significance.
Intermediate risk: **CR2+** **CRU2+** **PR without prior CR (PR1)** **PR without prior CR (PR2) includes any sensitive relapse)**	**CR2+ confirmed:** the recipient relapsed, then achieved complete absence of disease for >=1 month without radiographic evidence of disease. **CR2+ confirmed:** the recipient has achieved a second or subsequent complete response but has persistent radiographic abnormalities of unknown significance. **Partial remission (PR):** reduction of >=50% in greatest diameter of all sites of known disease and no new sites.
High risk: **Primary refractory (PIF)** **Relapse untreated (any number)** **Relapse resistant (any number)**	**Primary refractory:** less than partial response to initial therapy or PR not maintained at the time of HSCT, and recipients who achieved a prior PR but never CR and are not currently in either PR. **Relapse:** obtained CR/CRU but relapsed (any sensitivity including PR with prior CR), recurrence of disease after CR; patients who have any relapse and have resistant or untreated or unknown sensitivity to chemotherapy.

Statistical Methods

The median value of the continuous variables was used as a cut-off point for exploratory analysis. Categorical variables including sex, race, diagnosis, and disease status at transplantation were compared by regimens and route of administration using Chi-square tests. Multiple comparison corrections were conducted with the Bonferroni adjustment. Post-transplant disease status was incomplete due to missing data and therefore progression free survival could not be accurately obtained. The 100-day transplant-related mortality was defined as death within 100 days post-transplantation without relapse or disease progression. Overall survival was defined as the time from transplantation to last follow-up or death irrespective of the cause of death. Probabilities of overall survival were calculated using the Kaplan-Meier estimate; the log-rank test was used for univariate comparisons. Association of patients' characteristics with outcomes were evaluated with stepwise Cox proportional hazards regression models. Factors associated with a *P* value less than 0.15 by univariate analysis and factors with clinical relevance were included in the final model. All tests were two-sided with α=0.05 for determination of statistical significance. Statistical analysis was performed with SAS version 9.2 (SAS Institute, Cary, NC).

Risk adjustment

Risk adjustment is a strategy for reducing the effect of confounding factors in studies where patients are not randomly assigned to different treatments. Observational studies provide an alternative to randomized control trial, the gold standard of research design, the use of which is often limited by cost, feasibility, practicality, resources, and subjects that are not representative of the population (external validity). However, observational studies assessing treatment effect or exposure are subject to sources of bias that can be difficult to

eliminate using standard analytic techniques. For example, there may be large differences between two arms, and the investigator may have no control over which subjects were allocated to each treatment group, which may lead to biased estimates of treatment effect. In the current study, patient came to the clinic starting from the year of 2005 were given IV busulfan rather than oral administration (**Table 4**) simply because the application of IV busulfan had become new clinical practice. Thus, the two treatment groups were not necessarily comparable because the treatment was not given randomly, but rather was decided by the time when the patient presented. This means that direct treatment comparison is not appropriate.

There are two major strategies to control for selection bias in observational studies, standard and advanced. The standard methods ensure comparability and reduce bias: stratification and multivariate regression. This is based on the assumption that after adjusting for confounding variables, there is an equal chance that every subject will have a certain treatment despite nonrandom treatment assignment in the original database. It is generally assumed that adding observed confounders to the model minimizes any differences within treatment group. Hence, the observed differences in outcome can be attributed to the treatment (i.e., causal inference) rather than to prognostic differences between the treatment groups. The advanced methods include propensity score and instrumental variables.

Propensity Score The propensity score can be described as a probability of treatment assignment conditional on observed baseline characteristics (Rosenbaum, 1983), e.g., in the current case, each patient has his/own propensity score derived from the treatment and all the outcome related covariates. There are three approaches of propensity score analysis of which the pros and cons are enlisted in **Table 3.**

Instrumental variables The concept and application of instrumental variables have been well known in economics (Bowden RJ, 1984; Greene, 1990). Two fundamental assumptions are required: 1) the instrumental variable should be highly associated with treatment; 2) it has no direct effect on the outcome measure. In addition, it should be a randomly assigned factor which is unrelated to patient characteristics. Hence, in the current study, the year of transplantation could be an instrumental variable which may affect the likelihood of receiving a particular treatment (oral or IV administration); however, it did not directly affect the overall survival (**Table 4**). It can be estimated how much the variation in the treatment variable is accounted for by the year of transplantation (exogenous variation). Typically, the instrument is used in a two-stage least squares regression; the first creates the predicted probability of each subject receiving a particular treatment, which reveals whether there is bias in treatment assignment. The second step is to estimate treatment difference. The two-stage process can be done in one step using the Qualitative Limited Dependent Model (QLIM) procedures in SAS.

Compared with propensity scoring, the instrumental variable approach relies on selected instrument to account for measured as well as unmeasured factors. The challenges to the approach include the difficulty of identifying a proper instrumental variable and validity of the instrument. This demands adequate knowledge to hypothesize the relationship between treatment and instrumental variable, which is hard to test empirically. On the other hand, propensity scoring builds on the assumption that all factors that affect treatment assignment and outcome are used in the modeling.

Table 3. Methodology of propensity score analysis

Methodology	**Pros**	**Cons**
Matching	A most efficient approach of integrating the score.	Requires a large pool of controls to select from and eliminate subjects who are unable to be matched, which is not practical.
Stratification	Assessing the treatment effect based on score quintles.	Hindered by the number of subclasses growing exponentially with increasing number of covariates. However, this is not an issue in the current study.
Regression adjustment	Propensity score serves as a covariate to reduce bias.	More covariates are associated with closer resemblance to the conventional logistic regression model.

Chapter IV
Results

Patient characteristics

Route of busulfan differed by year of transplantation, with each regimen occurring in serial cohorts of patients (**Table 4**). Median age for oral, IV16 and IV4 were 47, 47 and 45, respectively. Patients' median age, race, lymphoma diagnosis, mean weight, body surface area (BSA), and CD34+ cell count were comparable across the regimens except that IV16 patients had a significantly higher BMI than the other two groups (p=0.03, **Table 5**). In addition, the distribution of disease status at transplantation was different across three regimens (p=0.0001) with a greater proportion of patients receiving oral busulfan having primary refractory disease, and a greater proportion of IV4 patients being in CR1. Because treatment strategies were selected in a serial fashion, patients treated with oral Bu have twice and five times as long follow-up as with IV4 and IV1 Bu, respectively (p=0.0001).

Table 4. Route of busulfan delivery by year of transplantation

	2000-2001	2002-2004	2005-2006	2007-2008	2009-2010	Total
Oral	17	60	0	0	0	77
IV16	0	16	71	16	0	103
IV4	0	0	0	36	4	40

Toxicity

Among patients who had elevated maximum bilirubin (>=2mg) by day30 post-transplantation (n=11 in oral and n=38 in IV group), none of them developed severe HVOD by Baltimore criteria. Grade 3-4 neurotoxicities were not observed in any of the patients.

Patients receiving IV Bu generally had to stay in the hospital one extra day longer than those patients receiving oral Bu, IV4 had significantly longer hospital stay than oral group ($p<0.05$).

Table 5. Characteristics of the transplanted patients and main clinical findings (Average with minimum and maximum)

	Oral (N= 77)	IV16 (N=103)	IV4 (N=40)	p-value
Median age (years)	47 (19-66)	47 (17-69)	45 (21-66)	NS
Older >=60, n=43	10 (13%)	22 (21%)	11 (28%)	0.065
Male/Female	51/26	68/35	24/16	NS
Race				NS
White, n=161	56 (73%)	78 (73%)	27 (68%)	
African American (AA), n=46	18 (23%)	21 (19%)	7 (18%)	
Asian, Hispanic, other, n=10	3 (4%)	4 (5%)	3 (8%)	
BSA	1.97 (1.56-2.60)	2.03 (1.47-2.84)	2.03 (1.44-2.53)	NS
Body mass index (kg/m^2)	27.9 (17.9-53.6)	30.4 (17.4-62.6)	29.0 (20.7-45.3)	0.03
Diagnosis				NS
HL (n=87)	30 (39%)	41 (39%)	16 (40%)	
NHL (n=134)	47 (61%)	63 (61%)	24 (60%)	
CD34+ count ($x10^6$/kg bodyweight)	9.6 ± 13.3 (1.7-95.9)	10.6 ± 10.5(3.0-68.2)	13.5 ± 15.0 (4.3-71.7)	NS
Length of hospitalization (days)	20.8 ± 6.1 (8-42)[a]	22.4 ± 4.2 (9-48)[ab]	23.1 ± 4.3 (12-42)[b]	0.02
Median follow-up (days)	1761	895	392	0.0001
Disease status at transplantation**				0.0001
I: CR1/CR_U1, n=39	9 (12%)	14 (14%)	16 (40%)	
II: CR2/CR_U2, n=45	19 (25%)	22 (21%)	4 (10%)	
III: PR1/PR2/CR3, n=87	25 (32%)	45 (43%)	17 (43%)	
IV: Primary refractory, n=49	24 (31%)	22 (21%)	3 (7%)	

*: Length of hospitalization was calculated by including patients with stay at least 18 days.
**: See the definition of disease status at transplantation in Table 2.

Outcomes

There were 2 deaths in the oral group (2/76=2.6%) and 3 deaths (5/104=2.9%) in the IV4 group, and 2 deaths (2/40=5%) in IV4 group in the 100 days post-transplantation (p=0.74, **Table 6**), 1-year and 2-year overall were comparable regardless route of delivery or regimen, 5-year survival in IV groups (combining IV16 and IV4) was 8% greater than that by oral route (p=0.10).

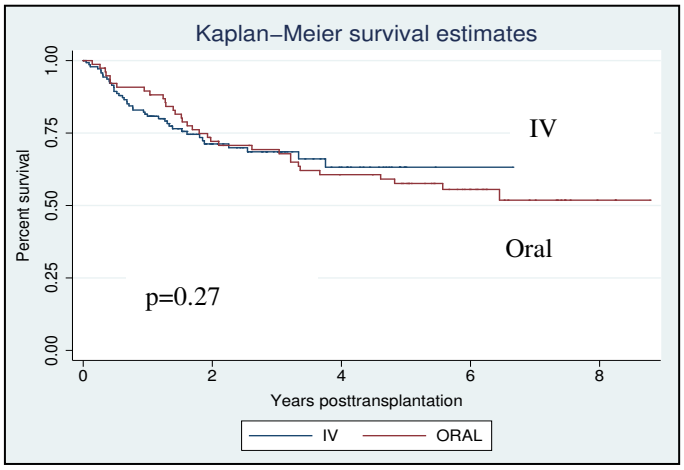
Table 6. Overall survival between routes of administration

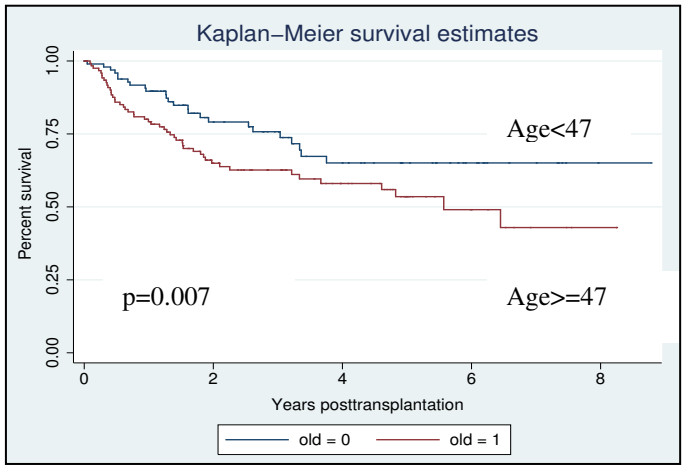
Regimen	100-day mortality Mean (95% CI)	Overall survival Mean (95% CI)		
		1-year	2-year	5-year
Oral	2.6 (0.6, 10)	89.5 (80.1, 94.6)	72.1 (60.5, 80.8)	57.6 (45.4, 68.0)
IV16	2.9 (0.9, 8.7)	82.7 (73.9, 88.7)	74.0 (64.2, 81.5)	65.8 (53.4, 75.6)
IV4	5.3 (1.3, 19.4)	76.1 (59.1, 86.8)	N/A	N/A

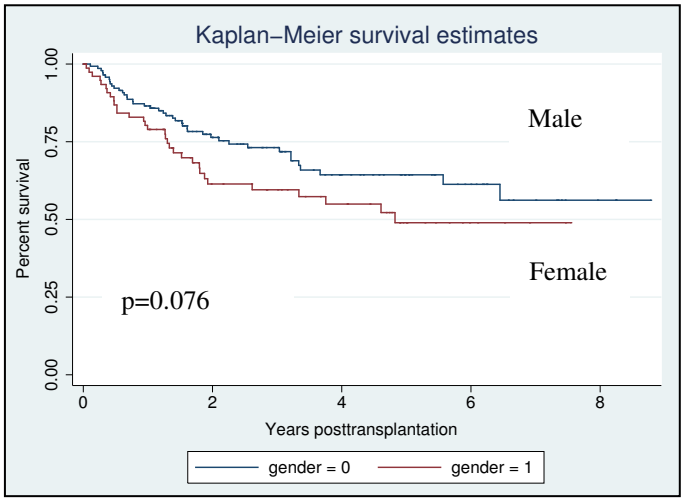
As the group receiving once daily busulfan (IV4) had relatively short median follow-up, both univariate and multivariate analysis were performed between the two groups receiving oral and IV busulfan (combing IV16 and IV4) groups. Univariate analysis reveals that patients with age greater at 45 had significant higher mortality (HR=1.71, p=0.029) than those with age 45 and younger (**Figure 3**). In addition, female gender was associated with greater mortality risk than male gender (HR=1.51, p=0.076); a diagnosis of HD had better overall survival than a diagnosis of NHL (HR=0.67, p=0.108), however, these differences were not statistically significant. There were no differences in overall survival by route of administration with or without adjusting with age, sex, race, diagnosis, disease status at transplantation, transplantation time and the level of CD34+ counts. As nature of HL and

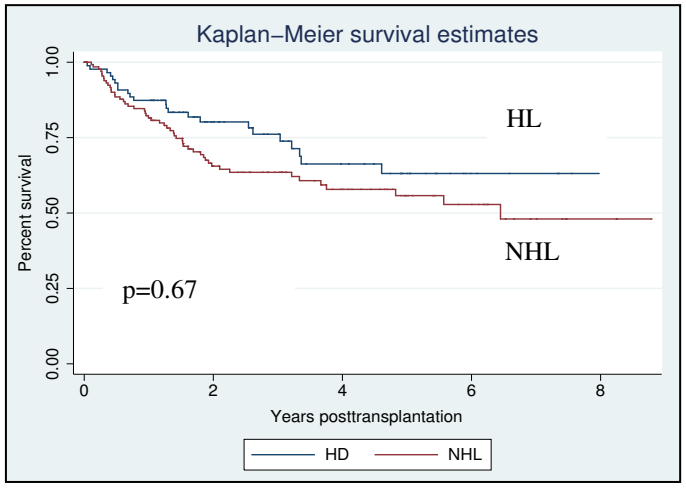
NHL are not combinable, overall survival analysis was performed by HL and NHL separately. There was no significant difference by route of delivery (**Figure 3**).

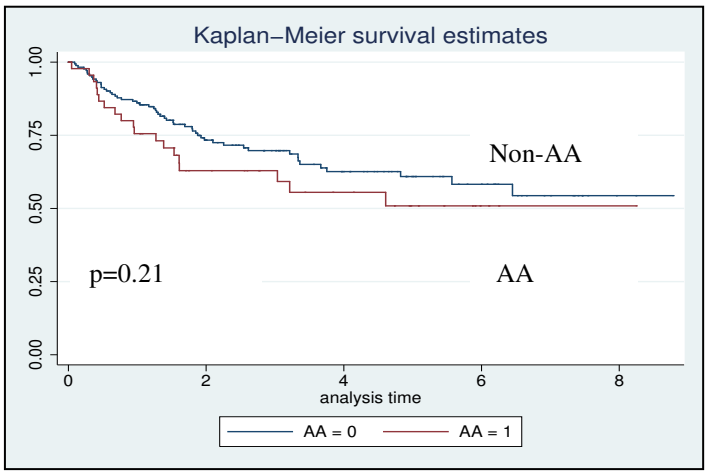
Multivariable analysis by Cox proportional hazards regression model (**Table 7**) showed that age (increasing per 10 years, HR=1.34, p=0.003) was a predictor of survival in this cohort. African American race (HR=1.66, p=0.068) and less responsive disease state at transplantation (primary refractory, HR=1.68, p=0.058) were associated with higher risk of mortality. It is noteworthy that the change of targeted AUC via PK-directed dosing, i.e., AUC of less than 24,000 µM-min prior to 2003 versus AUC at 18,000-22,000µM-min post 2003 did not have an impact on overall survival.

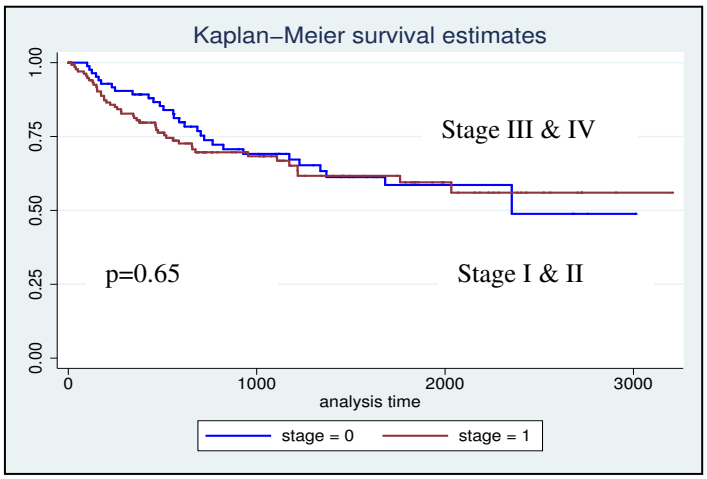




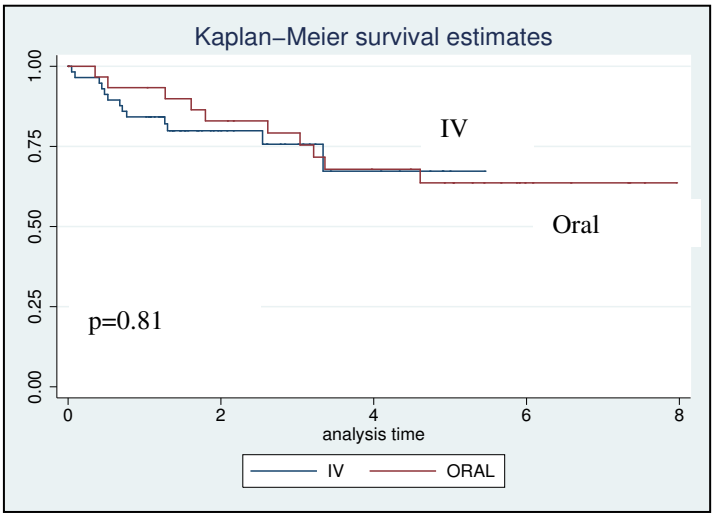








HL only



NHL only

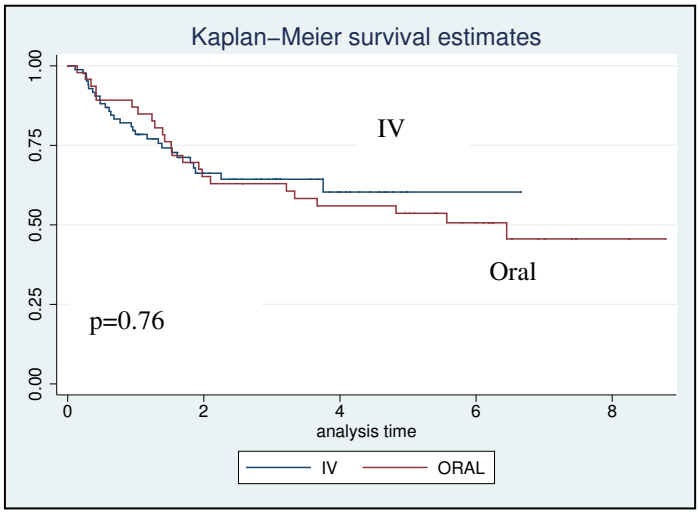


Figure 3. Univariate analysis of prognostic factors on overall survival.

Table 7. Univariate and multivariate analysis of prognostic factors for overall survival

	Univariate		Multivariate	
Variable	HR (95% CI)	p-value	HR (95% CI)	p-value
Route: IV vs. Oral	0.99 (0.62-1.60)	0.98	0.61 (0.27-1.38)	0.24
Age: >=47 vs. <47	1.71 (1.05-2.77)	0.027	-	-
Per 10 year increase	1.34 (1-1.48)	0.002	1.34 (1.1-1.71)	0.003
Sex: Female vs. male	1.51 (0.95-2.40)	0.076	1.44 (0.90-2.32)	0.13
Race: AA vs. non-AA	1.40 (0.83-2.36)	0.21	1.66 (0.96-2.87)	0.068
Diagnosis: HL vs. NHL	0.67 (0.41-1.10)	0.108	0.95 (0.52-1.72)	0.87
Disease status : III+IV vs. I+II	1.11 (0.70-1.78)	0.65	1.68 (0.98-2.87)	0.058
IV vs. non-IV (I, II and III)	1.39 (0.84-2.30)	0.21		
BMTyear: Per 1 year increase	1.04 (0.93-1.16)	0.49	1.16 (0.96-1.41)	0.13
Post 2003 vs. prior 2003	0.95 (0.57-1.59)	0.82	-	
CD34+ x10^6/kg: >=median 7 vs. <7	0.81 (0.51-1.28)	0.37	1.09 (0.66-1.78)	0.74

Pharmacokinetics

At the initial dose, by design IV4 had significantly higher dose than the other two groups ($p<0.0001$). $T_{1/2}$ by oral route was significantly longer than regimen with IV16 (**Table 8**, $p=0.002$). Total AUC were not significantly different among three regimens; however, the oral groups had a wider range of variation than the other groups. The histogram shows that majority of IV delivery reached targeted AUC (IV16 79%, IV4 85%) while only 47% of oral delivery remained at the targeted AUC between 18000-22000μMol-min (**Figure 4 left**). When repeated dosing assessment was performed, $T_{1/2}$ and total AUC no longer differ among all groups. The majority of IV16 (81%) consistently achieved the target window while 63% oral and 50% of IV4 patients had achieved the target range ($p=0.072$). Note that in spite of majority of IV16 and IV4 patients had reached targeted AUC at the initial dose, nearly half of

IV16 and 25% of IV4 still received dose adjustment, whereas only a third of oral group received dose adjustment (**Table 8**).

The percentage reaching to target AUC window was not affected by race, gender or age. Overall survival was not associated with the failure of reaching to the target total AUC.

Both univariate and multivariate analysis reveal that age is independent prognostic factor for the overall survival. There was no significant association of age (older or younger than 47) with the distribution of total AUC and year of transplantation, and though age was a associated with disease status at transplantation (**Table 9**).

Table 8. Pharmacokinetic characteristics: a discrepancy between the subgroup reaching the targeted AUC and the subgroup receiving dose-adjustment

	Oral	IV16	IV4	p value
Dose 1	n=77	n=103	n=40	
Starting dose (mg)	65.7 ± 6.6	63.9 ± 10.5	254.1 ± 51.5	<0.0001
$T_{1/2}$ (hr)	227.6 ± 115.4[a]	186.3 ± 39.8[b]	200.9 ± 74.9[ab]	<0.0001
Total AUC (μM-min)	20,021 ± 2,799	19,350 ± 1,372	19,994 ± 1,552	0.08
% at targeted 18000<=AUC<=22000	46.7	78.6	85	<0.0001
Dose >=3 (dose>=2 for IV4)*	n=24 (31%)	n=47 (45.6%)	n=10 (25%)	
Starting dose (mg)	65.4 ± 14.6	66.2 ± 17.8	288.1 ± 78.1	<0.0001
$T_{1/2}$ (hr)	223.9 ± 57.9	204.6 ± 40.6	199.8 ± 31.4	NS
Total AUC (μM-min)	19,850 ± 2,284	19,619 ± 1,098	19,830 ± 2,099	NS
% at targeted 18000<=AUC<=22000	62.5	80.8	50	0.072

***Note: patient who received dose adjustment was only counted once.**

Initial dose **Repeated dose≥3**

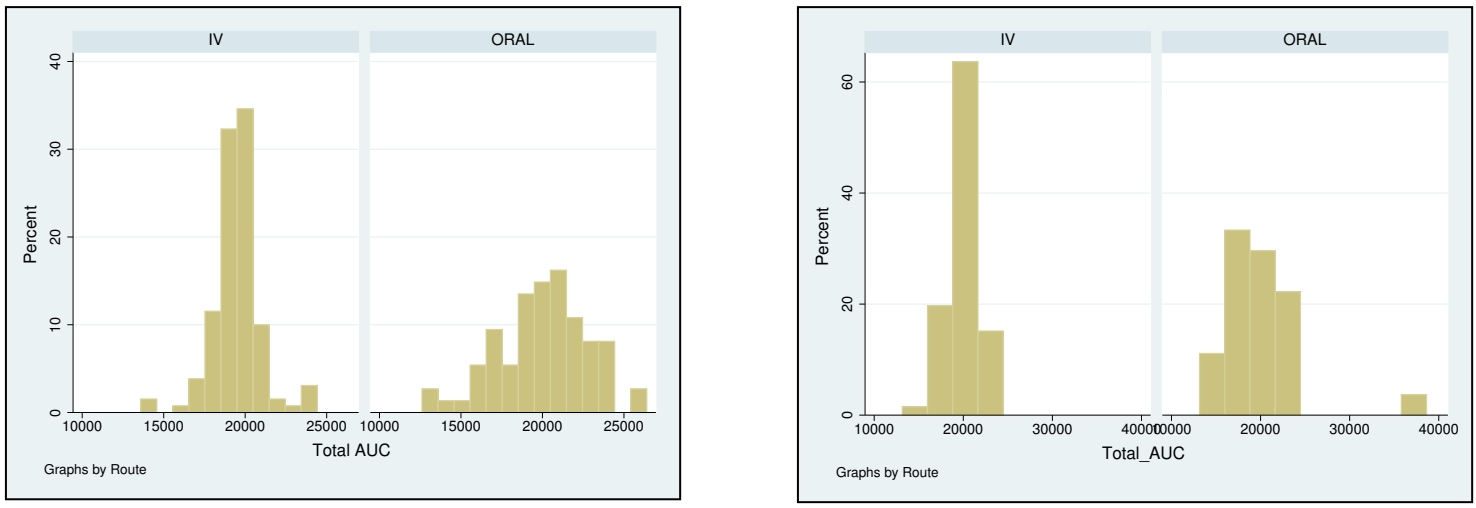


Figure 4. Histogram of targeted total AUC (18,000-22,000μM-min) at initial (left) and repeated dose (right).

Table 9. Distribution of disease status by age (p= 0.058, Pearson Chi-square)

Disease status at transplantation	**Age<47 N=107 (%)**	**Age >=47 N=113 (%)**
I: CR1/CR_U1	14 (13%)	25 (22%)
II: CR2/CR_U2	19 (18%)	26 (23%)
III: PR1/PR2/CR3	43 (40%)	44 (39%)
IV: Primary refractory	31 (29%)	18 (16%)

Cost analysis

To perform a simplified cost comparison, direct costs derived from whole sale prices of drugs, the costs of administration and monitoring, and hospital stay were obtained. There are two major high cost expenditures for using IV route of delivery. One is the cost of drug itself, based on the most current average whole sale price, oral Busulfan is $4.46/2mg-vial, intravenous Busulfan is $1150.82/60mg-vial. The other is the cost of hospital stay; the average of length of hospital stay for IV route is 22.6 days (combining IV16 and IV4, **Table 2**), whereas the average for oral administration of busulfan is 20.8 days. As both routes of

administration used PK-directed dosing and about 25-50% of the patient population in either oral or IV route needed repeated dosing, the cost for PK-dosing including labor and testing were cancelled out, and they were not included in the cost-effective analysis (**Table 10**). As there was no incidence of severe HVOD and neurotoxicities, no cost for toxicity treatment was identified.

Data on utility (quality of life or patient preferences) were not collected. Consequently, a full cost-utility analysis (cost per quality-adjusted life years (QALY) gained can not be provided.

Since there was no clinical advantage of using IV over oral busulfan in terms of overall survival, the incremental cost of using IV over oral busulfan delivery would be the differences of cost between IV and oral busulfan shown in **Table 10**:

= Δ of direct cost of IV-oral busulfan = $22,549

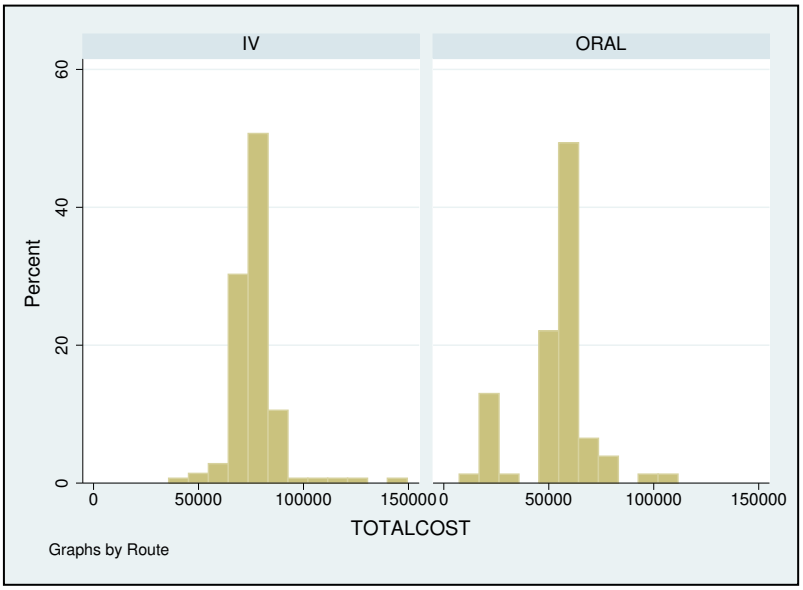


Figure 5. Distribution of cost by route.

Table 10. Average of direct cost ($)/patient during the transplantation up to discharge from the hospital

Route of delivery	Oral n=77	IV n=143
Total drug used	$4.46/2 mg $2,248 (1,266-3,158)	$1150.82/60 mg vial $20,297 (11,815-30,688)
Administration	Patient self	2 hours of monitoring with drug pump
Test with blood samples	$52/sample x 10 = $520	$52/sample x 6 = $312
Transplantation	$80,000	$80,000
Hospitalization	20.8 days X $2500/day = $52,000	22.6 days X $2500/day = $56,500
Total (not including PK-dosing) = total drug used + transplantation + hospitalization	$134,248	$156,797

.

Chapter IV
Discussion

The current study reveals that PK-directed dosing approach resulted in equivalent overall survival among oral and IV delivery of busulfan, with no incidence of HVOD and low 100-day mortality. Our report is largely comparable to previously reported series using other regimens, as summarized in **Table 11.** The chapter will discuss the clinical outcome, the significance of prognostic factors for HSCT, and limitation of the study. In addition, the chapter will briefly touch upon the issue of quality of life and socioeconomic aspects and quality improvement in the HSCT program.

Outcomes

Consistent with the early findings (Hassan et al., 1994; McCune & Holmberg, 2009), oral administration of busulfan generated long serum half life and wide variation in terms of total AUC in the initial dose, 47% of patients reaching the targeted AUC whereas nearly 80% of patients receiving IV busulfan reached the target range. Dose adjustment led to narrower target ranges than the initial dosing in the oral treatment group. Thus, careful and frequent monitoring of drug level could avoid extremely high or low level of the drug. This emphasizes the need for busulfan monitoring with oral administration. On the other hand, the majority of patients receiving IV treatment group consistently obtained the target total AUC. However, this did not confer an overall survival advantage over oral delivery. The necessity of PK-directed dosing in IV administration may require the comparison with non-PK-directed approach. Nearly 46% of patients in IV group received dose adjustment, suggesting that we may have chosen a very conservative, tightly targeted AUC which contributed to the lower incidence of 100-day mortality and zero incidence of severe HVOD (**Table 11**).

Our 1- and 5-year survival of IV group (combining IV16 and IV4) was remarkably similar to previous studies (Dean et al., 2010) and (Aggarwal et al., 2006). However, our study has shown comparable efficacy and the absence major toxicity difference between oral and IV delivery of busulfan, which is in large contrast with other studies. This could be due to several reasons: 1) PK-directed dosing adjustment may result in optimal clinical outcome; 2) our institutional protocol with conservative tightly defined target AUC; 3) younger patients with median age of 47 compared with the reported studies (**Table 11**); 4) mixed patient population with 61% of NHL and 39% of HL; or 5) treatment with once daily delivery of busulfan had numerically higher 100-day mortality rate and lower 2-year overall survival, which could undermine the advantage of IV16. The current study used a narrowly defined targeted AUC (1150-1350μM-min) from the year of 2003 compared with the study where a broad targeted AUC at 1000-15000 μM-min captured 96% of patients (Aggarwal et al., 2006). The discrepancy between the subgroup reaching targeted AUC and those receiving dose adjustment could be due to the under-adjustment prior 2003 and/or possibly over-adjustment from the year 2003 (**Table 8**).

The monoclonal chimeric anti-CD20 antibody rituximab has changed the landscape of treatment strategies for B-cell NHL in the last decade. Prospective randomized phase III studies performed during 1999-2002 with rituximab combined with chemotherapy as the first-line therapy, the relapse treatment or rituximab maintenance therapy all showed significant improvement in overall survival over treatments without rituximab, with impressive 80-93% 5-year overall survival from relapse patients receiving both autologous HSCT and rituxiumab (David, Mauro, & Evens, 2007; Wrench & Gribben, 2008) (Khouri et al., 2005). The significant disease control by rituximab may mask the difference between two conditioning

regimens, assuming that since 2003, the majority of the patients in B-cell NHL, which was remarkably similar across all three regimens (**Table 5**) had received rituximab as the first-line, salvage therapy and even at post ASCT. Due to lack of performance status and comorbidity data, we could not identify the elements associated with numerically higher 100-day mortality and lower 2-year survival in IV4. It is speculated that these patient population may have failed from the first-line and salvage therapies with rituximab, thus underperforming relatively to the oral and IV16 groups.

In addition to the evolved treatments, the widespread use of positron emission tomography scans, immunohistochemistry and molecular markers have required the reassessment of treatment response that was initially established by an international working group in 1999 (Cheson et al., 1999; Cheson et al., 2007). The identification of essential prognostic factors such as performance status, treatment history and comorbidity could be valuable to the current investigation.

Prognostic factors

As overall survival is a non-disease specific outcome measure, there are many prognostic factors that potentially contributed to the outcomes. Unlike NHL or acute and chronic leukemias, no robust molecular, genetic, immunocytologic or other biologic risk factors exist yet in HL. Salvage chemotherapy is administered prior to high dose chemotherapy to reduce disease bulk and determine chemosensitivity. Chemosensitivity to salvage treatment and time to relapse are important factors in predicting a patient's response to high dose chemotherapy and autologous HSCT (reviewed by (Murphy, Sirohi, & Cunningham, 2007)). The International Prognostic Score (IPS), developed by Senclever and Diehl (Hasenclever & Diehl, 1998) identified seven prognostic factors in advanced HL which

includes stage IV, male, age older than 45, hemoglobin, white blood count, lymphocyte count, albumin, each of which contributed a 7% reduction in freedom from progression at 5 years. Furthermore, well-known late complications following conventional therapy for HL include second cancers, with incidence up to 21% at 10 years and increased cardiovascular events (Brown et al., 2005; Deeg & Socie, 1998). The late complications could undermine the advantage of disease control gained via ASCT. For aggressive NHL, the International Prognostic Index (IPI) has defined the risks factors including age older than 60, LDH greater than normal, ECOG performance score greater than 2, Ann Arbor stage III-IV, and greater than 1 extranodal site as predictors of worse survival (Blay et al., 1998). Consistent with these findings, our report shows that age was an independent prognostic factor either by univariate or multivariate analysis (**Table 7**), it did not interact with disease status, totalAUC distribution. Furthermore, the average age of deceased patients was 49.2 compared with the patients who were alive (age 42.7, $p<0.0001$). The association of age with distribution of disease status ($p=0.058$, **Table 9**) indicates the likelihood of patient selection bias by physician referral. This hypothesis warrants future investigation.

Quality of life and socioeconomics

Over the past decade, application of ASCT has become common in the treatment of hematological malignancies and some solid tumors (Gratwohl & Baldomero, 2009). However, little is known about the effects on quality of life (QOL), such as physical, emotional, social and role functioning. It has been shown that better post-HSCT adaptation and QOL are predicted for younger age, male, higher educational level, better QOL and social support at the time of HSCT, longer time since HSCT and absence of late complication including

GVHD (reviewed by (Carlson & Macrae, 2002) and (Smith, Zimmerman, Williams, & Zebrack, 2009). These factors could better help physicians with patient counseling.

The current study reveals that the combination of PK-directed approach and our institutional protocol has resulted in comparable overall survival for oral route with IV route of busulfan administration. In light of this, the cost in the treatment via IV delivery has become prominent due to its higher cost of drug, longer hospital stay in spite of its numerically greater 5-year survival advantage than oral administration. The cost of IV delivery can be reduced if PK-directed dose adjustment is eliminated. On the other hand, the study has suggested an alternative for patients who could not afford IV administration of busulfan. With a small additional cost of PK-directed dosing, patient could achieve similar outcomes to IV administration without paying large amount of expense on drug and hospital stay.

The current study has performed simplified estimates of cost comparison during HSCT. The report did not include the cost associated with the processing of peripheral blood or bone marrow. ASCT using peripheral blood has been reported to be associated with lower costs and a better QOL than using bone marrow in patients with relapsed NHL or Morbus Hodgkin (van Agthoven, Vellenga, Fibbe, Kingma, & Uyl-de Groot, 2001). In addition, the current study did not access the post-transplantation cost, such as complications that may have significant impact on the cost-effectiveness of the two routes. This underscores the difficulty in predicting cost of HSCT and patient counseling. The tremendous cost has led to the exploring of home care after HSCT, where psychosocial and economic issues were examined. Home care could provide better QOL and lower costs, including a lower incidence of complications than standard hospital care (Miano et al., 2003; Svahn et al., 2002).

The patient population was remarkably similar across either oral or IV treatment group in terms of age, mean weight, BSA, distribution of sex and race, diagnosis and CD34+ counts. The percentages of male patients and White/Caucasians patients in this population may reflect the incidence of HL and NHL and the risk factors associated with the disease (www.cancer.gov). In addition, disparity in access of HSCT could represent either underutilization in female and/or American Americans or overutilization in Whites. A study with center for international blood and marrow transplant research (CIBMTR) database reported that African Americans and women are less likely to receive autologous HSCT for reasons unexplained by age or disease status (Joshua et al.). It would be interesting to investigate what other factors of patient population studied are associated with this disparity in accessing healthcare.

Quality improvement in HSCT

The paramount cost associated with HSCT and increases in demand has lead to the issues related to quality-of-care. Defining outcome measures is not straightforward for a variety of reasons. For example, 1) each HSCT center may have a different volume of performing HSCT per year; 2) patient populations can be hard to compare across centers as survival outcomes varies according to disease, disease stage, age, type of graft, type of transplantation; 3) each center may practice different preparative regimens and transplantation strategies, (for example, the current report used institutional defined targeted AUC for PK-directed dosing adjustment); 4) difference in the ratio of patients to physician. A study by IBMTR has shown that low 100-day mortality after allogeneic HSCT is associated with a high patient-to-physician ratio and centers where physicians answer emergency calls; increased mortality was associated with the incidence when students and residents were

present without fellow supervision in the center affiliated with a medical school (Loberiza et al., 2005).

The regulatory mechanism for quality assessment has been voluntary accreditation. The U.S. FDA has established rules governing the collection, processing and storage of cells and tissues, most of the standard-setting and compliance has been voluntary by the HSCT community itself (LeMaistre & Loberiza, 2005). The establishment of the Foundation for the Accreditation of Cellular Therapy has played a pivotal role in pushing voluntary accreditation movement. To standardize terminology and reporting to payers, the standardized request for information (RFI) initiated ASBMT has been adopted in the HSCT community since 2003. The standardized RFI includes descriptive data about the transplantation and treatment outcome data, which provides the benefit of comparison with uniform datasets. Hence, it is critical to select the appropriate outcomes and measures that are meaningful to patients, programs and the society.

Limitations

Limited by retrospective observational study in nature, the report is based on single institutional experience, respective patient-population, modest sample size, practice-based protocol for PK-directed dosing and suffers from the lack of important prognostic factors such as treatment history, performance status, comorbidity scores and the key outcome measures such as response duration and progression free survival. The study was performed with the assumption that during year of 2000-2010, there were no changes in the institutional factors such as patient-to-physician ratio, diagnostic practice, patient referral patterns. Due to these large unmeasured and uncontrollable variables, the study has limited implications. Alternatively, if large observational databases and pooled trials are available, a broaden and

deepen treatment comparison could help derive the optimal healthcare outcome by integrating the elements of personalized medicine (the right treatment and right patient population), right time (climate of clinical practice and policy), right place (institute, healthcare structure) and the right cost (socioeconomic factors, **Figure 1**).

In conclusions, PK-directed IV delivery of Busulfan had yielded a well defined targeted AUC compared with oral administration. PK-directed dosing approach resulted in comparable early and late overall survival between IV and oral busulfan delivery for lymphoma patients undergoing ASCT. The study could contribute to clinical, patient and policy decision-making.

Table 11. Comparison with other similar autologous conditioning regimens

Authors	N & median age	Regimen	PK-directed dosing	Disease	Median Follow-up, years	HVOD	100-day mortality	Progression free survival	Overall survival
(Kashyap et al., 2002)	N=100 IV: 38 Oral: 50	BuCy2: Oral : 1mg/kg x 16 IV: 0.8mg/kg x 16	No	CML, AML, HL Allogeneic	No	IV: 5% Oral: 20%	IV: 13% Oral: 33%	No	No
(Aggarwal, et al., 2006)	N=49 IV: 51 Oral: 53	BuCyVP Oral : 1mg/kg x 16 IV: 1mg/kg x 16	IV only	NHL, ASCT	IV: 2-4 Oral: 7-11	No	No	IV: 50% Oral: 17%	5-year: IV:58% Oral: 28%
(Dean, et al., 2010)	N=604 IV: 58 Oral: 51	BuCyVP Oral : 1mg/kg x 16 IV: 0.8mg/kg x 16	No	NHL	IV: 1.2 Oral: 5.7		IV: 2.9% Oral: 5.8%	1-year: IV: 73% Oral: 6.%	1-year: IV: 84% Oral: 76%
Current study	N=295 IV: 47 Oral: 47	BuCyVP Oral : 1mg/kg x 16 IV: 0.9mg/kg x 16	Oral and IV	NHL61%, HL 39% ASCT	IV: 2.4 Oral: 5.4	None	IV16: 3% IV4: 5% Oral: 3%	No	1-year: IV: 82.7% Oral: 89.5% 5-year: IV: 66% Oral: 58%

References

Aggarwal, C., Gupta, S., Vaughan, W. P., Saylors, G. B., Salzman, D. E., Katz, R. O., et al. (2006). Improved outcomes in intermediate- and high-risk aggressive non-Hodgkin lymphoma after autologous hematopoietic stem cell transplantation substituting intravenous for oral busulfan in a busulfan, cyclophosphamide, and etoposide preparative regimen. *Biol Blood Marrow Transplant, 12*(7), 770-777.

Alnaim, L. (2007). Therapeutic drug monitoring of cancer chemotherapy. *J Oncol Pharm Pract, 13*(4), 207-221.

Blay, J., Gomez, F., Sebban, C., Bachelot, T., Biron, P., Guglielmi, C., et al. (1998). The International Prognostic Index correlates to survival in patients with aggressive lymphoma in relapse: analysis of the PARMA trial. Parma Group. *Blood, 92*(10), 3562-3568.

Bowden RJ, T. D. (1984). Instrumental variables. *Cambridge: Cambridge Univ. Press*.

Brown, J. R., Yeckes, H., Friedberg, J. W., Neuberg, D., Kim, H., Nadler, L. M., et al. (2005). Increasing incidence of late second malignancies after conditioning with cyclophosphamide and total-body irradiation and autologous bone marrow transplantation for non-Hodgkin's lymphoma. *J Clin Oncol, 23*(10), 2208-2214.

Carlson, L. E., & Macrae, J. H. (2002). Quality of life issues following autologous bone marrow transplantation. *Expert Rev Pharmacoecon Outcomes Res, 2*(2), 129-146.

Cheson, B. D., Horning, S. J., Coiffier, B., Shipp, M. A., Fisher, R. I., Connors, J. M., et al. (1999). Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol, 17*(4), 1244.

Cheson, B. D., Pfistner, B., Juweid, M. E., Gascoyne, R. D., Specht, L., Horning, S. J., et al. (2007). Revised response criteria for malignant lymphoma. *J Clin Oncol, 25*(5), 579-586.

Ciurea, S. O., & Andersson, B. S. (2009). Busulfan in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant, 15*(5), 523-536.

Copelan, E. A. (2006). Hematopoietic stem-cell transplantation. *N Engl J Med, 354*(17), 1813-1826.

David, K. A., Mauro, L., & Evens, A. M. (2007). Relapsed and refractory Hodgkin lymphoma: transplantation strategies and novel therapeutic options. *Curr Treat Options Oncol, 8*(5), 352-374.

Dean, R. M., Pohlman, B., Sweetenham, J. W., Sobecks, R. M., Kalaycio, M. E., Smith, S. D., et al. (2010). Superior survival after replacing oral with intravenous busulfan in autologous stem cell transplantation for non-Hodgkin lymphoma with busulfan, cyclophosphamide and etoposide. *Br J Haematol, 148*(2), 226-234.

Deeg, H. J., & Socie, G. (1998). Malignancies after hematopoietic stem cell transplantation: many questions, some answers. *Blood, 91*(6), 1833-1844.

Flowers, C. R., & Armitage, J. O. A decade of progress in lymphoma: advances and continuing challenges. *Clin Lymphoma Myeloma Leuk, 10*(6), 414-423.

Geddes, M., Kangarloo, S. B., Naveed, F., Quinlan, D., Chaudhry, M. A., Stewart, D., et al. (2008). High busulfan exposure is associated with worse outcomes in a daily i.v.

busulfan and fludarabine allogeneic transplant regimen. *Biol Blood Marrow Transplant, 14*(2), 220-228.

Glazier, A., Tutschka, P. J., Farmer, E. R., & Santos, G. W. (1983). Graft-versus-host disease in cyclosporin A-treated rats after syngeneic and autologous bone marrow reconstitution. *J Exp Med, 158*(1), 1-8.

Gratwohl, A., & Baldomero, H. (2009). Trends of hematopoietic stem cell transplantation in the third millennium. *Curr Opin Hematol, 16*(6), 420-426.

Greene, W. (1990). Econometric Analysis. *New York: Macmillan.*

Hahn, T., Wolff, S. N., Czuczman, M., Fisher, R. I., Lazarus, H. M., McCarthy, P. L., et al. (2003). The role of cytotoxic therapy with hematopoietic stem cell transplantation in the treatment of diffuse large cell B-cell non-Hodgkin's lymphoma. *Biol Blood Marrow Transplant, 9*(10), 667.

Hari, P. N., Majhail, N. S., Zhang, M. J., Hassebroek, A., Siddiqui, F., Ballen, K., et al. (2010). Race and outcomes of autologous hematopoietic cell transplantation for multiple myeloma. *Biol Blood Marrow Transplant, 16*(3), 395-402.

Hasenclever, D., & Diehl, V. (1998). A prognostic score for advanced Hodgkin's disease. International Prognostic Factors Project on Advanced Hodgkin's Disease. *N Engl J Med, 339*(21), 1506-1514.

Hassan, M., Ljungman, P., Bolme, P., Ringden, O., Syruckova, Z., Bekassy, A., et al. (1994). Busulfan bioavailability. *Blood, 84*(7), 2144-2150.

Hiddemann, W., Buske, C., Dreyling, M., Weigert, O., Lenz, G., Forstpointner, R., et al. (2005). Treatment strategies in follicular lymphomas: current status and future perspectives. *J Clin Oncol, 23*(26), 6394-6399.

Jones, R. J., Lee, K. S., Beschorner, W. E., Vogel, V. G., Grochow, L. B., Braine, H. G., et al. (1987). Venoocclusive disease of the liver following bone marrow transplantation. *Transplantation, 44*(6), 778-783.

Joshua, T. V., Rizzo, J. D., Zhang, M. J., Hari, P. N., Kurian, S., Pasquini, M., et al. Access to hematopoietic stem cell transplantation: effect of race and sex. *Cancer, 116*(14), 3469-3476.

Kashyap, A., Wingard, J., Cagnoni, P., Roy, J., Tarantolo, S., Hu, W., et al. (2002). Intravenous versus oral busulfan as part of a busulfan/cyclophosphamide preparative regimen for allogeneic hematopoietic stem cell transplantation: decreased incidence of hepatic venoocclusive disease (HVOD), HVOD-related mortality, and overall 100-day mortality. *Biol Blood Marrow Transplant, 8*(9), 493-500.

Khouri, I. F., Saliba, R. M., Hosing, C., Okoroji, G. J., Acholonu, S., Anderlini, P., et al. (2005). Concurrent administration of high-dose rituximab before and after autologous stem-cell transplantation for relapsed aggressive B-cell non-Hodgkin's lymphomas. *J Clin Oncol, 23*(10), 2240-2247.

Kline, R. M., Meiman, S., Tarantino, M. D., Herzig, R. H., & Bertolone, S. J., Jr. (1998). A detailed analysis of charges for hematopoietic stem cell transplantation at a children's hospital. *Bone Marrow Transplant, 21*(2), 195-203.

Leger, C. S., & Nevill, T. J. (2004). Hematopoietic stem cell transplantation: a primer for the primary care physician. *Cmaj, 170*(10), 1569-1577.

LeMaistre, C. F., & Loberiza, F. R., Jr. (2005). What is quality in a transplant program? *Biol Blood Marrow Transplant, 11*(4), 241-246.

Little, M. T., & Storb, R. (2002). History of haematopoietic stem-cell transplantation. *Nat Rev Cancer, 2*(3), 231-238.

Loberiza, F. R., Jr., Zhang, M. J., Lee, S. J., Klein, J. P., LeMaistre, C. F., Serna, D. S., et al. (2005). Association of transplant center and physician factors on mortality after hematopoietic stem cell transplantation in the United States. *Blood, 105*(7), 2979-2987.

Lonial, S., Hicks, M., Rosenthal, H., Langston, A., Redei, I., Torre, C., et al. (2004). A randomized trial comparing the combination of granulocyte-macrophage colony-stimulating factor plus granulocyte colony-stimulating factor versus granulocyte colony-stimulating factor for mobilization of dendritic cell subsets in hematopoietic progenitor cell products. *Biol Blood Marrow Transplant, 10*(12), 848-857.

McCune, J. S., & Holmberg, L. A. (2009). Busulfan in hematopoietic stem cell transplant setting. *Expert Opin Drug Metab Toxicol, 5*(8), 957-969.

Miano, M., Manfredini, L., Garaventa, A., Fieramosca, S., Tanasini, R., Morreale, G., et al. (2003). Home care for children following haematopoietic stem cell transplantation. *Bone Marrow Transplant, 31*(7), 607-610.

Murphy, F., Sirohi, B., & Cunningham, D. (2007). Stem cell transplantation in Hodgkin lymphoma. *Expert Rev Anticancer Ther, 7*(3), 297-306.

Rosenbaum, P. a. R., DB. . (1983). The central role of the propensity score in observational studies for casual effects. *Biometrika, 70*(41-55.).

Santos, G. W., Tutschka, P. J., Brookmeyer, R., Saral, R., Beschorner, W. E., Bias, W. B., et al. (1983). Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. *N Engl J Med, 309*(22), 1347-1353.

Smith, S. K., Zimmerman, S., Williams, C. S., & Zebrack, B. J. (2009). Health status and quality of life among non-Hodgkin lymphoma survivors. *Cancer, 115*(14), 3312-3323.

Svahn, B. M., Remberger, M., Myrback, K. E., Holmberg, K., Eriksson, B., Hentschke, P., et al. (2002). Home care during the pancytopenic phase after allogeneic hematopoietic stem cell transplantation is advantageous compared with hospital care. *Blood, 100*(13), 4317-4324.

van Agthoven, M., Vellenga, E., Fibbe, W. E., Kingma, T., & Uyl-de Groot, C. A. (2001). Cost analysis and quality of life assessment comparing patients undergoing autologous peripheral blood stem cell transplantation or autologous bone marrow transplantation for refractory or relapsed non-Hodgkin's lymphoma or Hodgkin's disease. a prospective randomised trial. *Eur J Cancer, 37*(14), 1781-1789.

Wilkinson, G. R. (2005). Drug metabolism and variability among patients in drug response. *N Engl J Med, 352*(21), 2211-2221.

Wrench, D., & Gribben, J. G. (2008). Stem cell transplantation for non-Hodgkin's lymphoma. *Hematol Oncol Clin North Am, 22*(5), 1051-1079, xi.

Appendix Copy of IRB Approval or Letter of Exemption

Institutional Review Board
1256 Briarcliff Road, 307-N
Atlanta, GA 30306

Phone (404) 712-0720
Fax (404) 727-1358
http://www.emory.edu/IRB

RECEIVED
FEB 24 2010
EMORY INSTITUTIONAL REVIEW BOARD

REQUEST FOR MODIFICATION

Modification #: _____

Section I. Investigator Information

IRB Number 0732-2004	Title Clinical Database for Bone Marrow and Peripheral Blood Stem Cell Transplant Outcomes	
Principal Investigator Edmund Waller, MD, PhD, FACP	Interoffice Address (Mail Code, Department, Bldg, Room or mail stop number) Winship Cancer Institute 1365 Clifton Road, Bldg. C, Ste. 3012 Atlanta, GA 30322	
Contact Name Nyesha Smith		
Phone 404-778-3610	Fax 404-778-4389	Email nsmit25@emory.edu

Section II. Type of Modification (Select ALL that apply)

☐	Amendment	(Attach a Narrative and Supporting documentation) Amendment # _____ Date of Amendment _____	
☐	New Procedures	Describe how the change affects the risk/benefit: (Attach a description of the procedures)	
☒	Change in Study Personnel	☒ add ☐ delete ☐ change	Include role of personnel, address, phone, fax, email for additions – REMEMBER, all persons on a study must have current CITI certification. Hongzheng Zhang WCI/EUH. Atlanta, GA 30322 Phone 404-778-5672 Email: hzhan27@emory.edu
☐	Change of Site	☐ add ☐ delete ☐ modify (Attach a narrative that lists the resulting sites)	
☐	Change in Enrollment	(Attach narrative justifying the change) increase #____ decrease #_____ resulting total ____ to be enrolled	
☐ ☐	Consent Change New Consent	Version Date:_____ **Highlighted changes** and **A clean copy must be attached** Date: _____ Targeted Population: _____	
☐	Advertisement	Select All that apply and attach copies of ad or announcement ☐ Newspaper Ad – Name of Paper _____ ☐ Radio Announcement – Station _____ ☐ Internet Posting - Web-site _____ ☐ Post on Clinical Trials Web site (www.emoryhealthcare.org/clinicaltrials)	
☐	Change of Site		
☐	Change in Enrollment	(Attach narrative justifying the change) increase #____ decrease #_____ resulting total ____ to be enrolled	
☐ ☐	Consent Change New Consent	Version Date:_____ **Highlighted changes** and **A clean copy must be attached** Date: _____ Targeted Population: _____	
☐	Advertisement	Select All that apply and attach copies of ad or announcement ☐ Newspaper Ad – Name of Paper _____ ☐ Radio Announcement – Station _____ ☐ Internet Posting - Web-site _____ ☐ Post on Clinical Trials Web site (www.emoryhealthcare.org/clinicaltrials) ☐ Television Announcement – Station _____ ☐ Flyer – Distributed where _____ ☐ Information Brochure - Distributed how _____ ☐ Other - Describe:_____ Has this ad been approved by the sponsor? ☐ Yes ☐ No ☐ N/A	
☐	Clinical Investigator's Brochure	Select one: ☐ Addendum ☐ Updated ☐ New Date: Date: Date:	Should consent be changed based upon this revision? ☐ Yes ☐ No
☐	Funding	☐ Add Agency Name: ☐ Delete Agency Name:	

Page 1 of 2

☐	Site	List all sites this amendment applies to:
☐	Other	(e.g., Annual Report, Package Insert, General Correspondence) Describe and attach a narrative.

☐ Supporting documentation is attached. (e.g., Narrative, **highlighted consent**, form 1572, etc.) MANDATORY

PI Signature _____ Date 2/22/10

Faculty Advisor (if PI is student) _____ Date _____

NARRATIVE: Please add Zhang, Hongzheng to this study.

Section III. IRB USE ONLY

*** Protocol expiration is not changed by the approval of this modification***

✓ The Modification has been approved.

___ The Correspondence has been acknowledged.

___ Consent(s) and/or HIPAA Authorization dated_____ has been approved.

___ Subjects currently enrolled must sign the new consent.

Regina Waller
IRB Committee Member Staff

Approval Date: 2-25-10 Approval Type: ___ Full ✓ Expedited

Section below for Research Studies Performed at the Atlanta VA

Section IV. RESEARCH & DEVELOPMENT COMMITTEE USE ONLY

___ Modification has been approved by the R&D Committee

R&D Committee Chair Approval Date

Page 2 of 2 Rev. 07/28/05