PERSONALIZING THERAPY IN TRANSPLANTATION: FOCUS ON PHARMACOKINETICS, PHARMACODYNAMICS AND PHARMACOGENOMICS OF DRUGS USED IN HEMATOPOEITIC STEM CELL AND KIDNEY TRANSPLANT

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Dedication

I want to dedicate my thesis to my grandparents who have taught me the value of hard work, sincerity and discipline that helped me all along my PhD

Abstract

Patients treated with a standardized dosing strategy often demonstrate a substantial variability in drug response. Number of factors influences systemic exposure of the drug and its effect on the biological targets. The central objective of this thesis was to identify biomarkers and develop personalized dosing of drugs used in hematopoietic stem cell transplant (HSCT) and kidney transplant to improve outcomes.

Fludarabine is a chemotherapeutic drug used in reduced intensity conditioning (RIC) HSCT. High fludarabine exposure is associated with greater treatment related mortality (TRM). Fludarabine dose reductions are commonly empirical for obese and/or those with renal dysfunction. We developed a dosing equation, accounting for creatinine clearance and body size. Using this model to make dose reductions will reduce the probability of fludarabine overexposure and reduce TRM. Cyclophosphamide (Cy) is another chemotherapeutic agent used in RIC HSCT, associated with high toxicity and TRM. Due to complex metabolic pathway it is unclear which metabolite is most important to predict Cy's efficacy and toxicity. We evaluated the association between the active metabolite, phosphoramide mustard (PM), exposure and TRM. We found that higher PM AUC of was associated with greater TRM. We further identified creatinine clearance and gender to influence PM clearance and volume of distribution respectively.

Tacrolimus is an immunosuppressant used in kidney transplant recipients. African Americans show very high variability in tacrolimus exposure and poor outcomes. We developed a tacrolimus dosing model, taking into account the clinical and genetic variants to individualize dose in African Americans that could help achieve the target

concentrations quicker and improve outcomes. Mycophenolic acid (MPA) is another immunosuppressant used in kidney transplant recipients. Enterohepatic recycling and high variability in trough concentrations make it very difficult to use MPA concentrations for routine therapeutic monitoring. We conducted an RNA sequencing analysis to measure gene expression to identify novel biomarkers to predict MPA efficacy and toxicity. We identified transient changes in gene expression post MPA administration and that expression of 3 genes out of ~20000 were significantly associated with MPA trough concentrations. Additional studies are required to identify if transient changes in gene expression are associated with MPA related outcomes.

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CHAPTER I

1 INTRODUCTION

1.1 HEMATOPOEITIC STEM CELL TRANSPLANT

Hematopoietic stem cell transplant (HSCT) has become a standard of care for patients with hematologic malignancies and congenital or acquired hematologic disorders. Advances in transplantation techniques, safer conditioning regimens, availability of alternative sources of hematopoietic stem cells has increased the applicability of HSCT to various indications and the annual number of HSCT recipients has increased from ~11,000 in 2001 to ~19000 in 2012 and is predicted to further increase in future.(1) The most common indications for HSCT (~57%) in the United States in 2012 were multiple myeloma and lymphoma.(2) The hematopoietic stem cells are obtained from the patient himself /herself (referred to as autologous), or another person (referred to as allogeneic). The choice of transplant procedure: autologous or allogeneic depends on several factors, such as type of hematologic malignancies, stage of disease, age, gender, karnovsky score and comorbidity score. The number of autologous transplant conducted in United States in 2012 was around 11,145(2) and was mainly used in treating hematologic malignancies such as lymphomas, myeloma and rare cancer of childhood. The most common indications were multiple myeloma and plasma cell disorders. Around 7,554 allogeneic transplant were conducted in United States in 2012 and the most common indication was acute myeloid leukemia and myelodysplastic syndrome (~51%).(2) The goal of allogeneic HSCT in hematologic malignancies is to eliminate malignant hematopoietic stem cells and to induce sufficient immunosuppression to prevent rejection of transplanted stem cells.

1.1.1 Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic HSCT uses stem cells from another individual (donor), which proliferates and replaces the diseased bone marrow and/or hematopoietic cells killed with chemotherapy. The conventional source of hematopoietic stem cells is the bone marrow that involves withdrawal of the bone marrow cells from the donor ilium. However due to greater simplicity of collection, mobilized peripheral blood stem cells (PBSCs) are now the preferred stem cell sources. Stem cells are mobilized out of the bone marrow and into the peripheral blood by an injection of granulocyte stimulating hormone few days before the harvest, resulting in stem cell counts similar to bone marrow harvests. Based on Centre of International Blood and Marrow Transplant Research (CIBMTR) reports, PBSCs was the primary graft source (~65%) used in HSCT and use of bone marrow as a source decreased by 24% from previous years based on data collected from 2008-2012.(2)

1.1.1.1 Allogeneic HSCT Donor

An ideal donor for a HSCT recipient is an HLA matched related donor due to lower risk of graft rejection and relapse compared to unrelated donor. With the advances in immunosuppressive therapies, using alternative sources for stem cell such as unrelated donor, or stem cells from umbilical cord is now possible and extends transplant to more individuals.

1.1.1.1.1 Related Donors

The best choice for a related donor is an HLA-matched sibling donor; although other family members may serve as donors if the sibling is not a good match. The aim is to

match the donors and recipients HLA-A, -B, -C and –DRB1 (8/8 HLA-match).(3) For a related donor transplant, one HLA mismatch is acceptable. However, a mismatch at HLA-B is found to be associated with higher graft vs host disease (GVHD) and thereby increasing the risk of treatment related mortality (TRM) risks.(4)

1.1.1.1.2 Unrelated Donors

Only around 30% of patients eligible for allogeneic HSCT have a matched related donor available.(5) Several unrelated donor registries may identify a suitable HLA matched donor. With improved immunosuppression regimens, studies have indicated that unrelated donors matched at HLA-A, -B, -C and –DRB1 allelic positions (8/8 match) with the donors have similar outcomes to those observed from a matched related donor.(6) However a single allele mismatch (7/8) in an unrelated HSCT may have inferior outcomes as compared to those with 8/8 matches.(7) Unfortunately, polymorphisms in the HLA region are very frequent and therefore a major hurdle in an unrelated source is the search for donors, which may take several months. Caucasians have a 75% chance of finding a fully matched donor and another 20% will find donors with a 1-allele mismatch, whereas in the black population (African Americans and those from South and Central America) chances are as low as 16-19% to find an optimal donor.(8)

1.1.1.1.3 Unrelated Umbilical Cord Blood

The use of stem cells from umbilical cord (UCB) is an alternative source of stem cells that has rapidly expanded over the last decade. Initially used in pediatric patients, its use is now extended to adults. In 2012, 10% of allogeneic HSCT were carried out using

UCB as donor source. Stem cells from UCB are preferred due to its ease of availability and less stringent HLA match requirements as compared to unrelated donors.(9) A major limitation of UCB transplant however, is low number of stem cells and as a result the time to engraftment is usually prolonged in UCB recipients. Many transplant centers are now using two units from 2 different sources of UCB transplant, to overcome the problem of low cell count. Several studies have compared outcomes such as overall survival, GVHD and TRM by donor source. Compared to unrelated donor source and UCB, matched donor source have slightly better outcomes, however results are contradictory as to whether UCB donor transplant is similar or superior to unrelated match donor.(10-16)

1.1.2 Conditioning Regimens Used In Allogeneic Transplant

Conditioning regimens usually consists of chemotherapy and radiation given prior to transplant to eliminate cancer, and to provide adequate immunosuppression to enable stem cell engraftment, with minimal toxicity. Conditioning regimens are broadly classified as myeloablative (high dose chemotherapy and/or radiation), reduced intensity (intermediate dose chemotherapy and/or radiation) and non-myeloablative (low dose chemotherapy and/or radiation). There are several factors that govern the choice of conditioning regimen.(17) A Pretransplantation Assessment of Mortality (PAM) scale has been developed to predict 2 year survival which includes 8 pre-transplantation clinical variables: age, donor type, disease risk, conditioning regimen, FEV1, carbon monoxide diffusion capacity, serum creatinine and serum alanine aminotransferase.(18) Another predictive model of TRM is the comorbidity score as described by Sorror M.(19) Genetic variability in HLA alleles, drug targets and in genes encoding transporters, metabolizing

enzymes and drug targets could additionally affect the choice of conditioning regimen although are not routinely used in practice.

1.1.2.1 Myeloablative Conditioning

Myeloablative conditioning regimens cause irreversible pancytopenia and require stem cell support.(20) A myeloablative-conditioning regimen is expected to fully ablate marrow hematopoiesis and not allowing for autologous hematologic recovery. Historically total body irradiation (TBI) of 10-12 Gy was the main agent used in myeloablative conditioning. It has immunosuppressive effects and also access to deeper tissues in the body. TBI based regimens are widely used for hematologic malignancies with autologous and allogeneic transplant. Alkylating agents, cyclophosphamide (Cy) 120-mg/kg and busulfan (Bu) 12-16 mg/kg, were later introduced as an alternative myeloablative regimen in those that were unable to receive TBI.(21) The Cy-TBI (120 mg/kg and 12 Gy) regimen was also tested towards HSCT outcomes(22) and due to its superior outcomes, the combination became the standard myeloablative conditioning regimen for most allogeneic HSCT. Attempts to increase the dose of TBI to 15.75 Gy reduced the risk of relapse, however, significantly increased TRM therefore TBI is not used at this increased dose.(23) Other drugs used in combination with TBI are melphalan (Mel), cytarabine and etoposide.(20) Based on the latest 2012 CIBMTR report myeloablative conditioning regimen are characterized as regimens with TBI doses of ≥ 500 cGY, single fractionated doses of ≥ 800 cGY, Bu doses of > 9mg/kg, or Mel doses of >150 mg/m^2 given as single agents or in combination with other drugs.(2)Although myeloablative regimens provide rapid stem cell engraftment, acceptable disease free survival and relapse risk, there are several fatal complications associated with these high

dose regimens. Acute complications such as nausea, vomiting, diarrhea, skin reactions are common. Fatal complications with one or multi organ failures, infections often occur. Interstitial pneumonitis, pulmonary fibrosis, renal failure, sinusoidal obstruction syndrome are also common. Treatment related mortality is as high as 50%, in those with high risk factors such as increased age, poor disease risk, alternative donor source and multiple comorbidities. These high intensity regimens result in a proinflammatory milieu that increases the risk of acute GVHD, which further increases the risk of TRM.(17, 24, 25)

1.1.2.2 Non-Myeloablative And Reduced Intensity Conditioning

Due to higher rates of morbidity and mortality, older (>50 years) patients with comorbidities are typically ineligible for myeloablative conditioning.(26, 27) Reduced intensity conditioning/ non-myeloablative conditioning, consist of lower dose chemotherapy and/or radiation as compared to myeloablative and the transplant therapies have now extended to older and comorbid patients. Reduced intensity/ non-myeloablative conditioning regimens are less immunosuppressive than high dose cytotoxic agents. The goal is not to eradicate cancer with high dose chemotherapy, which ablates the bone marrow, but rather it depends on the engraftment of donor stem cells, which eradicate the tumor cells through a graft vs tumor effect.(24, 27-30)

Following transplant, donor and the recipient stem cells coexist in the recipient and the phenomena is called mixed chimerism.(27) The donor immune cells, primarily lymphocytes, eradicate residual malignant cells within the recipient, that have escaped the cytotoxic effect of chemotherapeutic agents and TBI.(25) Full donor chimerism eventually occurs post-transplant is required to eradicate recipient's residual normal and

malignant stem cells. Figure 1.1 shows the development of mixed and full donor chimerism following conditioning regimen and transplant in HSCT recipients. The extent of donor engraftment and chimerism can be measured using molecular mechanisms.

Several nonmyeloablative regimens have been studied, the most common includes low dose TBI, alone or in combination with fludarabine (Flu) and or rituximab.(20, 25, 31-34) Initial preclinical studies conducted in dog models, demonstrated that a reduced dose of 200cG of TBI was associated with sufficient engraftment.(29) Similarly, dose of alkylating agents such as Bu(35, 36), Mel(37), and Cy(38) were also reduced in these regimens. Examples of non-myeloablative regimens include, TBI<2 Gy alone(39), TBI (<2 Gy)/Flu (30 mg/m^2 for 4 days) (39), Flu (30 mg/m^2 for 4 days) /Bu (3.3 mg/day for 2 days)/anti-thymocyte globulin (ATG) (2.5 mg/kg)(40), Flu/Cy/ATG, Flu (25 mg/m^2 for 5 days) /Cy (200 mg/m^2 /day) /idarubicin (12 mg/m^2 for 5 days)/etoposide (250 mg/m^2/day for 2 days)(41), Flu (30 mg/m^2 for 4 days)/ cytarabine (2 mg/m^2 for 4 days)/ idarubicin (12 mg/m^2 for 3 days) (42), Flu (30 mg/m^2) daily for 3 days), intravenous Cy (750 mg/m^2 daily for 3 days), and rituximab(43), cisplatin (25 mg/m^2 continuous infusion daily for 4 days), Flu(30 mg/m^2 daily for 2 days), and cytarabine (1,000 mg/m^2 daily for 2 days)(43)

Regimens that do not fit the criteria for myeloablative and non-myeloablative are classified as reduced intensity regimens. The dose of alkylating agents in reduced intensity conditioning regimen is reduced by 30% or more as compared to myeloablative regimens.(20) The CIBMTR and National Marrow Program have used the following criteria to define a reduced intensity conditioning as any regimen that consists one or more of the following combinations:

- Total body irradiation of less than 500cG

- Total dose of Bu should not exceed 9 mg/kg
- Total dose of Mel should not exceed 140 mg/kg
- Total dose of thiotepa should not exceed 10mg/kg
- The regimen includes purine analog; Flu, cladribine, or pentostatin. (44)

Commonly used reduced intensity conditioning regimens are reported in CIBMTR and were recently reviewed (Table 1.1) adapted from (45).

1.1.3 Mechanism of Action of Drugs Used In Reduced Intensity Conditioning

1.1.3.1 Purine analogues

Fludarabine phosphate is a prodrug that is rapidly and completely dephosphorylated to F-ara-A (9-β-D-arabinofuranosyl-2-fluroadenine). F-ara-A is actively transported intracellularly where it undergoes several phosphorylation steps via kinases into its active F-ara-ATP form. Figure 1.2 shows the activation pathway of Flu phosphate to its active form F-ara-ATP. The active form is incorporated into the growing DNA strand, and thereby prevents elongation of the DNA strand and cell proliferation. It also inhibits DNA polymerases, DNA ligases, ribonucleotide reductase in addition to its effect on the DNA. Fludarabine is exclusively used only in reduced intensity and non-myeloablative conditioning regimens. Fludarabine exerts a synergistic effect by inhibiting the DNA repair enzymes and preventing repair of DNA adducts formed by alkylating agent such as Mel, Bu and Cy. Thus addition of Flu to regimens with alkylating agents enhances immunosuppression.(46) However, in addition to being an effective immunosuppressant, Flu is also associated with rare but fatal neurotoxicity.(47-50) A second-generation purine analogue, clofarabine, was developed that retained the anti-

leukemic and immunosuppressive effect similar to Flu, but had reduced central nervous toxicity.(51, 52) Phase I-II studies conducted recently to test potential use of clofarabine in combination with Bu, Flu in HSCT recipients have shown promising results of sufficient engraftment with moderate toxicity profile.(53)

1.1.3.2 Alkylating agents

Commonly used alkylating agents in reduced intensity regimens are Cy, Bu, Mel and treosulfan. The general mechanism of alkylating agents is to react with electron-rich atoms in the biological molecules and form covalent bonds with guanine nucleotides and thereby prevent DNA replication.(54)

Cyclophosphamide is a prodrug that undergoes several enzymatic biotransformation steps to its active metabolite phosphoramide mustard (PM). Phosphoramide mustard further undergoes non-enzymatic conversion to nor-nitrogen mustard (NOR). Phosphoramide mustard and NOR alkylate the N-7 position of guanine nucleotides on DNA. The alkylation results in formation of DNA adducts G-NOR, G-NOR-OH and G-NOR-G which prevents the DNA strand separation and thereby replication. Thus Cy prevents DNA replication and thereby exerts its cytotoxicity.

Busulfan is a bi-functional alkylating agent. Similar to Cy, it exerts its cytotoxic effect by alkylating the N7 position of guanine and adenine, forming DNA strands and leading to cell apoptosis.(55) It is mainly toxic against myeloid precursors and therefore is highly effective against AML, CML, and multiple myelomas. It has limited toxic effects against mature lymphocytes and hence cannot be used as a single agent.(24)

Treosulfan is another bifunctional agent, which has been used in combination with Flu and is associated with high engraftment rates and reduced TRM. However larger clinical trials have not been conducted with treosulfan.

1.1.3.3 Total body irradiation

Total body irradiation works by enhancing immunosuppression and exerting tumoricidal effects. Lymphocytes are highly sensitive to TBI and profoundly diminish after a short period of TBI, followed by granulocytes, and platelets.(56) The observations that TBI is highly effective but may cause fatal toxicities, led to an idea of using targeted radiotherapies using monoclonal antibodies. An ideal antigen target is the one that is homogenously distributed throughout the tumor cell surface and is absent in the normal cells. CD20, CD33 and CD45 are hematopoietic antigens that are now under investigation in HSCT settings as radio-immunotherapeutic targets.(24)

1.1.4 Adverse Outcomes In Reduced Intensity Conditioning

The adverse events following HSCT with reduced intensity conditioning are described in the following subsection.

1.1.4.1 Relapse

Reduced intensity conditioning regimens have successfully demonstrated its advantage over myeloablative regimens by significant reduction of TRM. However, most studies have failed to show an improvement in overall survival, due to increase increased relapse rate (25-60%) in reduced intensity conditioning regimens as compared to myeloablative (9-40%).(25) Several factors are associated with increased risk of relapse

such as age, initial white blood cell count, cytogenetics, prior induction therapy and ability to achieve complete remission (4) A study conducted in 274 AML/MDS patients treated with reduced intensity conditioning HSCT, increased risk of relapse related death were associated with unfavorable cytogenetics, presence of minimal residual disease at transplant, HSCT within 6 months of diagnosis and patients with incomplete PBSC recoveries before HSCT. Donor type, AML stage and disease etiology were not associated with relapse.(57) Administration of donor lymphocyte infusions has found to be a successful strategy in significantly reducing relapse.(17, 58, 59) Further, hypomethylating agent azacitidine prophylaxis with donor lymphocyte infusion has also been successful in reducing relapse risk.(58, 60)

1.1.4.2 Graft Versus Host Disease

While donor lymphocytes induce the graft vs tumor effect, they are also responsible for undesirable effects leading GVHD. Graft vs host disease is a manifestation of immune response where the transplanted donor stem cells cannot differentiate between the recipient's malignant and normal cells. Normal cells of the recipient are attacked by donor lymphocytes and is possibly stimulated by tissue injury that occurs from the conditioning regimen used before transplant.(61)

Graft vs host disease is more often observed in recipients that receive HLA-mismatched unrelated donors(62).

Graft vs host disease is diagnosed and its severity is assessed using NIH criteria based on degree of organ involvement and is broadly categorized as acute or chronic. The incidence of acute GVHD and chronic GVHD after reduced intensity conditioning HSCT

ranges from 11-63% and 18-86%, respectively, and differs between the disease type, disease risk and choice of reduced intensity conditioning regimens.(25)

Acute GVHD occurs within first 100 days of transplantation, and late GVHD occurs often after 100 days, usually during withdrawal of maintenance immunosuppressants. The clinical manifestations of acute GVHD usually occur on the skin, gastrointestinal tissues, and liver. It is staged as grade 0-4 depending on maculopapular rash, persistent nausea, abdominal pain and serum bilirubin concentrations.(63) Table 1.2 gives the National Institute of Health classification of stages of acute GVHD.

Chronic GVHD usually occurs within 3 years of transplant and is likely to be preceded by a history acute GVHD. The clinical manifestations of chronic GVHD are the result of highly complex immune reactions involving both the T and B-lymphocytes, and involve many organs. Chronic GVHD is stage as mild, moderate and severe. It is scored based on global scoring and eight organ sites (skin, eyes, gastrointestinal tract, liver, lungs, joint and fasciae, and genital tract) are used in its calculation.(64)

Studies have been conducted to evaluate risk factors for acute and chronic GVHD. Major factors associated with a higher incidence of GVHD are higher HLA-mismatch between donor and recipients (mismatched and/or unrelated donors), sex mismatch between donor and recipient (female donor and male recipient have greatest risk), donor age (HSCT recipients that receive stem cells from younger donor have higher risk of GVHD) (65), high intensity conditioning regimen, prior allosensitization, prior donor lymphocyte infusion, stem cells source (stem cells obtained from peripheral blood have greater risk of GVHD, while umbilical cord have lower risk of GVHD as compared

to those from bone marrow) and disease stage.(16, 66-70) Acute GHVD is a significant predictor of higher incidence of chronic GVHD.(69) Factors associated with lower risk of acute GVHD are use of ATG during conditioning and chronic myeloid leukemia.(71)

1.1.4.2.1 GVHD prophylaxis

Effective maintenance immunosuppression therapy is needed to promote engraftment of stem cells and also prevent adverse effects of GVHD. Methotrexate (MTX) was traditionally the therapy of choice to prevent GVHD, due to its antifolate and thereby antiproliferative action towards T lymphocytes. With the discovery of calcineurin inhibitors, cyclosporine (CSA) was identified to be as beneficial as MTX(72, 73), however results were significantly improved when the two drugs were used in combination due to their synergistic activity towards T lymphocytes.(74)

Tacrolimus (TAC) is another calcineurin inhibitor, which showed superiority in randomized clinical trials conducted to compare TAC/MTX vs CSA/MTX, where the former combination was more potent against GVHD. (75-77) However, both TAC and CSA have high inter-individual variability in drug PK and response, thus drug concentrations are routinely monitored. Further higher blood concentrations of these drugs are associated with increased risk to toxicities such as nephrotoxicity, neurotoxicity, hypertension, infections, hyperglycemia.(78, 79) Mycophenolic acid (MPA) is another immunosuppressive agent that is now widely used to prevent GVHD and has nearly replaced MTX. A retrospective meta-analysis conducted in 242 allogeneic HSCT recipients compared adverse clinical outcomes in patients receiving mycophenolate mofetil (MMF)/CSA vs historical controls of MTX/CSA. Although there was no significant difference in overall survival and TRM, MMF/CSA group had

significantly lower acute GVHD (grades II-IV) events as compared to MTX/CSA group.(80) Further patients receiving MMF/CSA experienced faster engraftment but greater risk of CMV viremia.(81) Also, a higher incidence of chronic GVHD particularly with gastrointestinal involvement was observed in recipients receiving MMF/CSA for GVHD prophylaxis.(82) There has been a debate on inclusion of MMF in GVHD profile, as it is shown that MMF can inhibit the graft vs tumor effect by inhibiting NK cells, which have a major role in this process.(83) A prospective randomized multicenter trial was conducted to compare sirolimus and TAC against the traditional MMF/CSA or MMF/TAC. Sirolimus based GVHD prophylaxis was associated with less chronic GVHD, gastrointestinal acute GVHD, and a lower hazard of TRM at 2 years post-transplant (18% in sirolimus group vs 38% in CSA group). However, sirolimus use increases the risk of sinusoidal obstructive syndrome, and also transplantation related thrombotic microangiopathy when combined with CNI especially CSA.(84) Although studies indicate that new combination with sirolimus shows improvement in overall survival vs MMF/CSA, more prospective studies are needed before sirolimus becomes standard GVHD prophylaxis.(84-86)

A course of rATG is commonly given pretransplant in addition to calcineurin inhibitors and MMF. It has been shown to reduce acute and chronic GVHD without significantly hindering the graft vs tumor effect in reduced intensity conditioning regimen.(87) Although rATG has not been shown to significantly impact the overall survival(88), results have shown that it significantly reduced chronic GVHD, and thereby improves quality of life.(89) The optimal dose of rATG within reduced intensity

conditioning is 7.5-10 mg/kg, however lower doses of ~4.5 mg/kg were also found to be effective in preventing GVHD with reduced toxicity.(90)

1.1.4.3 Treatment Related Mortality

Treatment related mortality is defined as death due to any cause other than relapse, disease progression or disease recurrence after HSCT. In the CIBMTR 2012 report, TRM accounted for 43% of all the causes of death after unrelated donor transplant.(62) Although the incidence of TRM has significantly reduced with the use of reduced intensity conditioning in comparison to myeloablative conditioning, around 15-30% TRM at one-year post-transplant is still observed.(88, 91-94) The main causes of TRM are multi-organ failure, acute and chronic GVHD, bacterial and fungal infections, hepatitis, veno-occlusive disease and neurologic events.(91-93, 95-97) Comorbidities prior to HSCT are shown to be significantly associated with post-HSCT organ toxicity and TRM.(98) Recipients receiving stem cells from peripheral blood experience greater incidence of acute GVHD and TRM as compared to those who received bone marrow stem cells.(99) Differences in the drugs used in reduced intensity conditioning regimen are also associated with risk of TRM.(25) In a study conducted in 151 patients with Flu/Bu and Flu/Mel reduced intensity conditioning, Flu/Mel (40%) was found to be significantly associated with higher TRM as compared to Flu/Bu (16%) regimen.(100) However a recent report by Acute Leukemia Working Party of the European Group of Bone Marrow Transplantation, did not show a significant difference in a 2 year TRM ($p=0.08$).(101) A study was conducted in 274 patients with median age of 60 years and AML/MDS treated with 2Gy TBI with or without Flu. TRM at 1 year was 16% and the main cause of TRM was acute and chronic GVHD, infections, and grade 4 non-

hematologic toxicities, which were mainly related to pulmonary, cardiovascular and hepatic dysfunction.(57) In another study, outcomes were compared in patients who received either Flu/Mel (a reduced intensity conditioning regimen) or Flu/cytabarabine and idarubicin (a nonmyeloablative regimen). Multi-organ toxicities were noted in both groups, with grade IV toxicities that involved the neurological, pulmonary and cardiovascular systems. The risk of TRM was 30% at 1 year and was significantly higher in patients receiving a reduced intensity conditioning regimen vs nonmyeloablative regimen.(102) In multiple myeloma patients receiving reduced intensity conditioning regimen consisting of Flu (40mg/m2/day) and Bu (3.2 mg/kg/day) for 4 days, common regimen related toxicities included, mild to moderate mucositis, and liver dysfunction. The cumulative incidence of TRM at day 100, 1 year, and 3 years was 9%, 19% and 29% respectively.(103) A phase II study was conducted to assess the efficacy and toxicity profile of bortezomib in combination with Flu and Mel as reduced intensity conditioning regimen in patients with multiple myeloma. Cumulative incidence of TRM at 3 years was 25%. Non-hematologic toxicities included peripheral neuropathy, liver toxicity and pulmonary toxicity early post-transplant.(104) In a randomized controlled trial comparing Bu/Cy, and Bu/Flu reduced intensity conditioning regimen, the Bu/Cy group had significantly greater incidence of infection (grade 3 or higher) and gastrointestinal disturbances as compared to Bu/Flu group. Hepatic adverse events were similar in both groups. Two year TRM was around 18% in Bu/Cy group and 34% in Bu/Flu group.(105) Thus the above studies show that ~20-25% of TRM at one year is observed even with reduced intensity/non-myeloablative regimens. There are several factors associated with organ toxicities and infections, however systemic exposure and pharmacokinetics (PK) of

drugs used in conditioning and post-grafting immunosuppression are also important factors associated with TRM.(91, 95, 106) An important issue that is still under appreciated is that patients receiving same doses of drugs show substantial variations in clinical response.

1.1.5 Factors Associated With Pharmacokinetics (PK), Pharmacodynamics (PD) and Pharmacogenomics (PG) Variability of Drugs used in Conditioning Regimen

As described in the section 1.1.4 there are significant differences in response to different reduced intensity conditioning regimens. However, variability in response is also observed within transplant recipients receiving the same conditioning agents. Pharmacokinetic variability of the drugs influences its systemic exposure and PD variability influences the effect of drug on its target. Genetic variability in genes involved in both PK and PD may also influence both systemic exposure and response to conditioning agents.

1.1.5.1 Purine Analogues

As described in the section 1.1.3.1 purine analogues most commonly used in HSCT are Flu and clofarabine. Pharmacokinetic studies of F-ara-A conducted after intravenous administration of Flu, have shown that nearly 40-60% of the drug is renally eliminated mainly as unchanged F-ara-A.(107-110) F-ara-A demonstrates PK variability of ~25-30%(111-114), however there are limited studied that have identified sources of this variability. F-ara-A exposure is significantly higher in patients with mild to moderate

renal impairment.(108) A population PK study showed that body surface area significantly influenced F-ara-A (the active component of Flu) clearance (Cl) and volume of distribution.(114) Variability in PK also affects drug response (PD and outcomes). A study conducted by Long-Boyle et al, showed that higher F-ara-A plasma concentrations when given with Cy/TBI were associated with greater TRM.(111) Other studies conducted to associate Flu exposure and clinical outcomes following HCT have shown inconsistent results.(112, 113, 115, 116)

Clofarabine is very recently tested for its use in reduced intensity conditioning-HSCT and hence there is limited data available on factors associated with variability in clofarabine PK-PD. Clofarabine PK was studied in 62 HSCT recipients. Clofarabine Cl was significantly associated with renal function, where patients with lower GFR (calculated using MDRD equation) had lower Cl and thereby higher dose normalized area under curve (AUC). Further, higher dose normalized AUC was significantly associated with greater risk of acute kidney injury.(117) In another study conducted in 16 patients (adults and pediatrics) a 2-3 fold variability in clofarabine AUC and Cl was observed. None of the clinical covariates tested (CrCl, serum creatinine, BUN, age, body weight) significantly correlated with clofarabine Cl, AUC, Cmin and Cmax.(118) Currently no data are available for pharmacogenomics of Flu or clofarabine. Polymorphisms in genes potentially involved in bioactivation and transport such as *NT5C2*, *NT5E*, *SLC28A3*, *SLC29A1*, *SLC29A2*, *DCK*, *ABCG2*, *ABCC4* may influence PK and/or PD.

1.1.5.2 Alkylating Agents

As described in the section 1.1.3.2 the most common alkylating agents used in reduced intensity conditioning-HSCT are Cy, Bu, Mel and treosulfan. Inter-individual variability

in PK of Cy is attributed to complex biotransformation steps to form the active metabolite that is governed by highly variable cytochrome P450 (CYP) enzymes (mainly CYP2B6, CYP2C9, CYP2C19 and CYP3A4). Figure 1.3 shows the PK pathway of Cy. Cyclophosphamide Cl is explained as sum of inducible (Cy is an auto-inducer via CYP2B6) and non-inducible mechanisms.(119, 120) In a study conducted in HSCT recipients, inter-individual variability in non-inducible Cl, inducible Cl and volume of distribution was 52.2%, 200% and 18% respectively.(33) Genetic polymorphisms especially CYP2B6 have a significant influence on Cy PK. In vitro and in vivo studies have demonstrated enhanced CYP activation in *CYP2B6*6* carriers as compared to wild type.(121) However the influence of this variant is contradictory when tested at a clinical setting. Other *CYP2B6* variants tested towards Cy metabolism include *CYP2B6*4, *5, *8* and **9*, however their influence on Cy metabolism still needs confirmation.(122) Similar results are also shown by *CYP2C19*17* allele.(123) Cy undergoes Phase II metabolism and polymorphisms in glutathione-S-transferases (GSTA1, GSTP1) and aldehyde dehydrogenase (ALDH1A and ALDH3A) have also been studied, however its influence was not found significant for towards Cy variability.(124) Cy metabolism is also significantly influenced by concomitant drug administration. Thiotepa is a CYP2B6 inhibitor and its co-administration prevents activation of Cy to its active metabolite.(125) A significant interaction is also observed between Bu and Cy metabolism. In HSCT recipients randomized to receive Bu/Cy/TBI or Cy/TBI, the drug to metabolite ratio was significantly higher in recipients of Bu/Cy/TBI as compared to those who only received Cy/TBI, suggesting significant inhibition of Cy activation.(126) But this combination is not commonly used in reduced intensity regimens. Clinical factors such as age, body

weight(127) and renal impairment(128) have also influenced Cy metabolism. Cyclophosphamide itself is inactive, and thus results associating Cy exposure to clinical outcomes are inconsistent. It is still unclear as to which is the most important metabolite marker to predict outcomes associated with Cy. Metabolism of Cy was found to be highly variable and higher Cy plasma exposure was associated with increased sinusoidal obstruction syndrome, bilirubin elevation and TRM.(129) Some other studies have focused on 4-HCy and CEPM metabolites to predict outcomes, however results are not consistent or reproducible so as to use them as clinically as biomarkers.(130-133) Plasma PM concentrations have been studied in few studies, however no study is conducted to test its association to outcomes in HSCT recipients receiving reduced intensity conditioning. Similar to Cy, high inter-patient and intra-patient variability in Bu PK variability is observed.(134) Bu is extensively metabolized in liver by Phase II enzymes and only ~2% is recovered unchanged in the urine. While glutathione-S-transferase (GST) alpha 1 is a major contributor, GSTM1 and GSTP1 are minor contributors towards metabolism. The PK pathway of Bu is shown in Figure 1.4. In a population PK study conducted in patients, ~28% inter-individual variability was observed for Bu oral Cl and a 9.4% intra-individual variability. Variability in oral Cl was partly explained by phenytoin co-administration, weight and ALT.(135) Bu when administered orally shows very high variability in absorption that affects outcome.(136-140) Age was also found to impact Bu PK, where older patients had significantly higher Cl than younger.(141) Polymorphisms in *GSTA1* have shown to influence Bu Cl clinically although not routinely clinically tested.(142-144) In a population PK study conducted in 36 allogeneic HSCT patients, a 15% decrease in Cl was observed in carriers of *GST1A* variant

(rs3957356) as compared to wild type following intravenous Bu administration.(143) Variability in PK of the Bu is associated with differences in outcomes. Higher Bu exposure is associated with veno-occlusive disorders. (145) In 75 children receiving IV Bu as part of reduced intensity conditioning regimen, higher Bu steady state concentration (Css) (<600 ng/mL) was associated with higher incidence of TRM ($p<0.001$) and grades 2-4 GVHD ($p=0.04$).(146) Several studies have been conducted, and support PK-controlled Bu dosing and therapeutic drug monitoring and is practiced in many transplant centers to control Bu plasma exposure and drug toxicity.(147-150)

1.2 KIDNEY TRANSPLANTATION

Chronic kidney disease is a progressive disorder characterized by glomerular filtration rate <60 min/min/1.73m^2 for more than 3 months irrespective of presence or absence of kidney damage.(151) The end stage (stage 5) of kidney (renal) disease (ESRD) is characterized by GFR <15 ml/min and necessitating need for kidney replacement therapy i.e. either dialysis or transplantation. The number of patients in United States reported with ESRD in 2012 was ~114,000. Of these, ~66.3% were Whites, 27.3% were Blacks, 5.1% Asians and 14. 8% were Hispanics. The primary causes of ESRD are diabetes (~44%), hypertension (~28%), glomerulonephritis (~8%), cystic kidney disease (~2%) and urological disease (~0.5%).(152)

Despite advances in treatment of ESRD, kidney transplantation still remains the most optimal treatment for these patients. In comparison to dialysis, transplantation offers better quality of life and also higher survival rates.(153) In 2012, 17305 kidney transplants were performed in United States, of which 65% were from deceased donors.

Development of acute rejection, adverse effects of immunosuppressant, chronic graft dysfunction, all of which may lead to kidney graft loss are some of the major barriers that still persist despite effective transplantation. Nearly 18% of patients return to dialysis, require re-transplantation or die within one year of transplant.(153)

1.2.1 Mechanism Of Action of Immunosuppressants Used In Kidney Transplantation

Soon after the kidney transplant, the recipient's immune system recognizes the transplanted donor kidney as a foreign body and elicits an immune response against it and prolonging the survival of allografted kidney is key challenge. Kidney transplant recipients receive life-long immunosuppressive therapy to prevent rejection, and improve graft survival rates. Extensive research has been conducted to optimize immunosuppressive therapy that can provide adequate immunosuppression but also prevent toxicity from chronic and prolonged use of these drugs. Immunosuppression is currently broadly classified as either induction or maintenance therapy.

1.2.1.1 Induction Therapy

Induction therapy includes intravenous administration of high dose immunosuppressive antibodies given at the time of transplant and around time of organ perfusion to prevent acute rejection during early post-transplantation period. Adequate induction immunosuppression is essential to improve long-term graft survival. In high risk patients induction therapy, may also be started perioperatively for additional immunosuppression and is usually given for 3-14 days post-transplant.(154) The type of induction agents are divided based on if their mechanism includes lymphocyte depletion

(ATG, alemtuzumab) or not (basiliximab, daclizumab). The most recent report suggests that 62% of induction therapies used in USA, mainly comprise of T lymphocyte depleting agents.(152) The primary purpose of induction therapy is to prevent allograft loss, decrease the severity of acute graft rejection, prevent delayed graft function and thereby improve survival. Traditionally high dose corticosteroids were used for induction therapy, but over years, the therapy has advanced to targeted monoclonal and polyclonal antibodies that have shown to be more effective agents. The choice of induction therapy is guided by recipient's immunological risk, comorbidities, financial burden and choice of maintenance immunosuppression.(154)

1.2.1.1.1 Lymphocyte depleting agents

In kidney transplant, rATG is the most widely used immunosuppressive in induction therapy. rATG is a polyclonal antibody that targets a variety of T cell surface antigens such as CD+2, CD+3, CD+4, CD+8, CD+16, CD+25 and CD+45 and leads to profound depletion of T lymphocyte within 24 hours of administration and lasts for several days to weeks.(155) It can also induce cascade of events leading to B cell and plasma cell apoptosis through caspace pathways. It also has specificity for cytokine receptors, adhesion molecules and human leukocyte adhesion.(155) Outcomes observed from different randomized clinical trials suggest that in comparison with other non-depleting induction agents, rATG is more beneficial in prevention of acute rejection in high-risk patients.(156)

Alemtuzumab is a humanized monoclonal antibody specific to CD52 antigen that is present on all lymphocytes. It is a T lymphocyte depletion agent and was initially approved by FDA for treatment of chronic lymphocytic leukemia. In a large randomized

controlled trial kidney transplant recipients were followed up to 5 years. Although single dose alemtuzumab provided similar graft function and survival rates, alemtuzumab was associated with less acute rejection and long-term infection.(157) It is superior to non-depleting induction agent, basiliximab, in reducing the incidences of biopsy proven acute rejection.(158-160) However the toxicity profile is not significantly different between alemtuzumab and the non-depleting counter-parts.(160)

1.2.1.1.2 Non-Lymphocyte depleting agents:

Basiliximab is a non-depleting IgG1 monoclonal antibody and is classified as IL2 receptor (IL2R) antagonists. It is a chimeric antibody formed out of variable domains of mouse antibody and the constant region made up of human immunoglobulin. It is most active against the alpha chain of interleukin receptor. It is not a T lymphocyte depleting agent, but alters T lymphocyte response to antigens. It is second to ATG in the frequency of its use in induction therapy.(153)

1.2.1.2 Maintenance Immunosuppression

Maintenance immunosuppression is life long therapy given to all kidney transplant recipients that consist of a combination of drugs that have different mechanisms of immunosuppression. Immunosuppressants used in maintenance therapy belong to one of the four classes; calcineurin inhibitors (CSA and TAC), antimetabolites (MPA acid and azathioprine (AZT)), mTOR inhibitors (rapamycin, sirolimus) and corticosteroids (prednisone).

Cyclosporine and AZT are older immunosuppressant and are largely replaced by TAC and MPA due to its superior efficacy. Currently the most preferred combination

used in kidney transplant recipients is TAC and MPA with or without corticosteroids. (153) Corticosteroids are currently used in early post-transplant, however more and more transplant centers are considering minimization or complete avoidance due to high number of side effects associated with it. (161) Rapamycin is also not extensively used (<6% of transplant recipients) as primary immunosuppressants, and secondarily considered in patients that experience CNI related toxicities. (153, 162) The following section is focused on TAC, CSA, MPA and AZT since these drugs are most commonly considered for routine therapeutic monitoring due to its high variability in exposure of these drugs.

1.2.1.2.1 Tacrolimus

Tacrolimus is an immunosuppressant used in patients undergoing solid organ transplantation, such as kidney, liver, pancreas, lung, and heart. It is also used in other diseases such as autoimmune diseases and hematopoietic cell transplantation. According to the most recent annual report of the U.S. Organ Procurement and Transplantation Network (OPTN) and the Scientific Registry of Transplant Recipients (SRTR), 85% of all kidney and liver transplant recipients receive TAC and MPA as their initial immunosuppression agents.(153) Tacrolimus is preferred over CSA as the calcineurin inhibitor in most transplants centers, as it is associated with better allograft survival in kidney transplant recipients.(163-165) Tacrolimus inhibits calcineurin phosphatase, a serine-threonine phosphatase enzyme by forming a complex with immunophilins called FK-binding proteins. Calcineurin inhibition further prevents dephosphorylation of NFATc (nuclear factor of activated T cells), thereby suppressing the transcription of interleukin-2 and other cytokines involved in the immune response and activation of T

lymphocytes.

1.2.1.2.2 Cyclosporine

Cyclosporine is another calcineurin inhibitor used as part of maintenance immunosuppression after organ transplantation. Cyclosporine inhibits T-cell activation similar to TAC, where it binds to the immunophilin cyclophilin, forming a CSA-cyclophilin complex that inhibits calcineurin phosphatase. As mentioned earlier, CSA is largely replaced by TAC, and <2% of patients received CSA as post-transplant immunosuppressive therapy.(153)

1.2.1.2.3 Mycophenolic acid

Mycophenolic acid (mycophenolate mofetil, mycophenolate sodium) is a potent antiproliferative immunosuppressive agent used in combination with a calcineurin inhibitor with or without prednisone for maintenance immunosuppressive therapy. It has largely replaced azathioprine (AZT) in organ transplantation as it was demonstrated to be superior in randomized trials.(166) MPA blocks DNA synthesis by non-competitive and reversible inhibition of ionosine monophosphate-5′-dehydrogenase (IMPDH) types 1 and 2 and thereby inhibiting proliferation of T and B-lymphocyte.

1.2.1.2.4 Azathioprine

Azathioprine is an imidazolyl derivative of mercaptopurine and a purine antimetabolite. Although largely replaced by MPA as an immunosuppressant in transplant recipients, it is still used in patients whose contemporary immunosuppressants have failed. Azathioprine is a prodrug and gets converted to several metabolites of which 6-mercaptopurine and 6-thioguanaine are active. Both the metabolites are responsible for

inhibiting purine synthesis and thereby halting cell proliferation and differentiation.(167)

1.2.2 Adverse Outcomes In Kidney transplantation

Improvement and introduction of new immunosuppressive therapies, and use of induction therapies in high risk kidney transplant recipients has dramatically reduced short term graft rejection, however improvement in long term graft function and overall survival yet remains as a challenge.

The adverse outcomes of kidney transplant include viral infections, malignancy and renal dysfunction.(168) Due to these adverse outcomes, approach of minimization is considered that often leads to loss of efficacy. Figure 1.5 (adapted from(169)) shows timing of complications and their approximate timing after kidney transplantation.

1.2.2.1 Graft Rejection

Rejection in transplant recipients is the result of humoral and/or cell-mediated response elicited by the recipient's immune system against the foreign antigens presented on the donor tissue. Depending on the timing of the occurrence of rejection, it is classified as either hyperacute, accelerated acute, acute, or chronic rejection. Hyperacute rejection usually occurs within the first 24 hours of transplant and is caused by pre-existing antibodies already circulating in the host. Acute rejection usually begins after a week of transplantation and its risk is highest in the first 3 months of transplantation and occurs in 10% of recipients. It is usually T cell mediated. Chronic rejection is defined by slow progressive graft dysfunction. There was a significant heterogeneity in characterizing the allograft biopsies obtained to make rejection diagnosis, which led to a

standardization of rejection classification, called the Banff classification. The most current Banff 07' classification is presented in Table 1.3

There are several factors that are associated with graft rejection following kidney transplant

1. Donor type: Recipients receiving kidney from a deceased donors have poorer acute rejection free survival and graft survival at one year post-transplant than a living donor kidney.(170)

2. Race: African Americans have poorer graft survival as compared to Caucasians and Asians.(170)

3. Age: Recipients receiving kidney from older donors have greater risk of acute rejection and graft failure. Kidneys from older patients are more immunogenic, are at higher risk of ischemia-reperfusion injury and therefore are associated with higher incidence of acute rejection.(171, 172) On the other hand, due to weaker immunity in older kidney transplant recipients, risk of acute rejection is lower compared to younger recipients.(173, 174)

4. Donor specific anti-HLA antibodies: The number of individuals on waitlist for kidney transplant is increasing every year. The wait time increases further in order to find an ideal HLA and compatible donor. The presence of high levels of donor specific anti-HLA-antibodies at the time of transplant is predictive of acute ABMR.(175) Therapy with IVIG and rituximab are often used for desensitization in recipients with donor specific anti HLA antibodies. In randomized placebo controlled trials, desensitization has shown to improve transplant outcome.(176)In addition ABOi incompatibility leads to hyperacute rejection.

5. Other risk factors include dialysis time, comorbidities, high panel reactive antibody status and choice of immunosuppressants.

1.2.2.2 Adverse Outcomes

1.2.2.2.1 New onset diabetes (NODAT):

New onset diabetes is a metabolic complication, which occurs in 4-25% of kidney transplant recipients and usually occurs early post-transplant.(177) It leads to reduced graft function and increased risk of mortality and morbidity. Risk factors for NODAT include older age, certain ethnicities (Hispanic, African America, South Asians have higher risk), genetic background, family history of diabetes mellitus, underlying polycystic kidney disease, glucose intolerance, obesity and hepatitis C infections. Etiology of NODAT is multifactorial but prolonged use of immunosuppressive drugs also are closely associated with its onset.(178) Corticosteroids and calcineurin inhibitors are strongly associated with incidence of NODAT. A large retrospective analysis conducted in ~25000 patients, estimated a 16.2% overall cumulative incidence of NODAT within 3 years of transplant. In patients who were discharged with steroids, the odds of developing NODAT were 42% higher as compared to those without steroids. Prednisone has been shown to increase insulin resistance, and thereby increased insulin demand to maintain glucose tolerance. Steroid withdrawal protocols have been successful in reducing NODAT incidences.(161, 179, 180) Calcineurin inhibitors block IL2 pathway as their major immunosuppressive mechanism, however, this blockage has an impact on transcriptional regulation of insulin gene expression in the pancreatic beta cells. Cyclosporine is less diabetogenic as compared to TAC.(181, 182) Patients who received

TAC/MMF had 25% greater odds of NODAT as compared to those who receive CSA/MMF maintenance therapy.(183) Life style changes, pre and post-transplant screening of blood glucose, corticosteroid dose reduction or avoidance, and alterations in immunosuppressive therapies are necessary to decrease risk of NODAT.(178)

1.2.2.2.2 Hypertension

Hypertension post-kidney transplantation is common (85%) and varies among different populations. Factors such as pre-transplant hypertension, males, African American race, higher body weight significantly increase the risk of post-transplant hypertension.(184) Several studies have examined relationship between hypertension and graft survival rates. In a large collaborative study conducted in ~30,000 kidney transplant recipients over 7 years, increased systolic and diastolic blood pressure were significantly associated with a graded increase in subsequent graft failure.(185) Donor factors such as older age, pre-transplant hypertension, poor allograft quality have been associated with hypertension.(184) Genetic polymorphisms in genes encoding ABCB1, CYP3A5, and APOL1 in both donor and recipients have been associated with increased risk of hypertension.(186-190) Maintenance immunosuppression drugs used also increase the risk of hypertension. Use of corticosteroids in particular is strongly correlated with hypertension. Further studies have proposed early steroid withdrawal in order to decrease the risk of post-transplant cardiac complications such as hypertension.(191-194) Calcineurin inhibitors are also associated with a higher risk of hypertension, and rates of hypertension have doubled from 40% to 70-90% after the introduction of CNI into maintenance therapies.(195, 196) Multiple mechanisms such as endothelial dysfunction, increased vascular tone, sodium retention, allograft fibrosis may induce CNI associated

hypertension.(196) Randomized controlled studies have shown that TAC based maintenance therapy is associated with lower incidence of hypertension as compared to CSA.(197-199)

1.2.2.2.3 Nephrotoxicity

Studies in animals and early human have reported that CSA induces necrosis of smooth muscles in the afferent renal arterioles, and reduces glomerular filtration rate due to vasoconstriction of renal arterioles. The major mechanisms of CSA induced nephrotoxicity are hypertension, vascular endothelial dysfunction, activation of renin-angiotensin system, enhanced sympathetic tone and increase in reactive oxygen species (oxidative stress).(200) Significant research is underway to identify prospective biomarkers associated with CNI nephrotoxicity.

1.2.2.2.4 Infections

Infections due to over immunosuppression are a major cause of morbidity and mortality post-kidney transplant. In a retrospective study conducted in 80 kidney transplant recipients, frequent infectious episodes observed (78%) after kidney transplant with an average of 3 per patient associated with over immunosuppression.(201) The 2013 atlas on ESRD, reported that 14.4% of patients were hospitalized due to infections in first year of kidney transplant. The most common form of infection was urinary tract infection followed by septicemia and post-operative infections. (http://www.usrds.org/2013/pdf/v2_ch7_13.pdf) A retrospective cohort study conducted in 46,000 adults kidney transplant recipients, showed that infections due to bacteria, viral, fungal or parasites occurred at the rate of 45.0 per 100 patients followed up to 3 years

post-transplant. The most significant factors associated with 15% increase in the rate of any type of infection was patient age > 65 years, females recipient, recipient's Hispanic ethnicity, diabetes as a cause of end stage renal disease, living donor source, panel reactive antibody >10%, pretransplant time on dialysis, use of CSA and mTOR inhibitors (rapamycin) in maintenance immunosuppression, HBV and HCV serological status and pre-transplant positive donor-recipient CMV serology.(202)

1.2.2.2.5 Hematologic toxicities

Post-transplant hematologic toxicities such as anemia, leukopenia, and thrombocytopenia are frequent side effects. Less common toxicities are passenger lymphocyte syndrome, post-transplant erythrocytosis, thrombotic microangiopathy, post-transplant lymphoproliferative disease and hemophagocytic syndrome have also been described. Certain hematologic malignancies have been associated with MPA use. Higher MPA concentrations have been associated with increased risk of leukopenia and anemia; however, these observations are not consistent across studies.(203-205)

1.2.2.2.6 Other side effects

Steroid use is associated with osteoporosis, weight gain, hypertension, hyperlipidemia and hyperglycemia.(173). Sirolimus is associated with gastrointestinal discomfort, hypercholesteremia, increased proteinurea and poor wound healing.(173) Skin cancer is another major problem in kidney transplant recipients of which the most common form are squamous cell carcinoma, followed by basal cell carcinoma. (206) Chronic use of immunosuppressants induces the oncogenic properties of factors causing cancer such as UV radiation.

1.2.3 Pharmacokinetics, Pharmacodynamics and Pharmacogenomics Variability Of Maintenance Immunosuppressive Drugs Used In Kidney Transplantation

Kidney transplant recipients are on life long maintenance immunosuppressive therapy, and the most commonly used drugs (described in section 1.2.1.2) demonstrate a very high variability in systemic drug exposure and outcomes. Several studies have been conducted to identify factors associated with variability and thereby find out ways to optimize therapy. Variability in exposure of drugs used in induction agents have not been extensively studied for kidney transplant recipients, and hence not discussed in the following section.

1.2.3.1.1 Tacrolimus and cyclosporine

Tacrolimus undergoes extensive metabolism primarily in the liver and, to a lesser extent, in the small intestine with CYP3A4 and CYP3A5 playing a major role in metabolism.(207) At least 15 active and inactive TAC metabolites have been identified.(208, 209) On oral administration, around 20-30% of the drug is bioavailable with high interindividual variability (6%–89%).(210, 211) Tacrolimus also shows high interindividual variability in Cl (3–35 L/hour) and has a narrow therapeutic index, which significantly affects systemic exposure and the degree of immunosuppression.(212) Therefore tacrolimus trough concentrations are routinely therapeutically monitored and typical TAC trough blood concentration targets in the United States kidney transplants are 8–10 ng/mL in the first 3 months and 6–8 ng/mL for 3-6 months post-transplantation, depending on the indication and time post-transplantation.(213-217) Low blood

concentrations have shown to increase the risk of graft rejection, graft loss, and/ or treatment failure and high concentrations are associated with a greater risk of toxicity, including hypertension, infections, nephrotoxicity, hyperglycemia, neurotoxicity, and malignancy.(215, 218-220)

Similar to TAC, CSA also has a narrow therapeutic index and is mainly metabolized by intestinal and hepatic *CYP3A4* and *CYP3A5*(221). The mean bioavailability of the oral formulation of CSA is about 30%, with high interindividual variability. (222, 223) Therapeutic monitoring of CSA trough blood concentrations is standard of care because of the highly variable CSA Cl and the strong association between troughs and clinical outcomes.(224, 225) Figure 1.6 shows the metabolic pathway of CSA and TAC

Tacrolimus pharmacogenomics have been extensively studied in the kidney transplant population and the role of non-functional *CYP3A5*3* (rs776746) variant is very well established.(212, 226-229) The allele frequency of CYP3A5*3 variant is significantly different by race where 94% of whites; 70% of Japanese, Chinese, and Koreans; and about 18% of African Americans carry the *CYP3A5*3*.(230) Kidney transplant recipients who were *CYP3A5* expressers had 2-fold higher Cl than non-expressers, and the *CYP3A5*3* genotype explained 25% of the variability in Cl.(231) The effect of CYP3A4 variants have also been evaluated for its effect on TAC PK but their effects are small and inconsistent. *CYP3A4*1B* (rs2740574, -392A>G) is a genetic variant in the promotor region of the *CYP3A4* gene associated with higher *CYP3A4* expression.(232, 233) CYP3A4*22 (rs35599367) is another variant in intron 6 associated

with reduced CYP3A4 activity and in few studies tacrolimus concentrations significantly increased in carriers of this variant.(234-236) Another gene associated and studied towards tacrolimus metabolism is P-450 oxidoreductase (POR), and *POR*28* variant (rs1057868, 1508 C>T) is associated with increased *CYP3A* activity, thereby enhancing metabolism. (237) Kidney transplant recipients carrying *POR*28* have lower troughs and increased dose requirements. The influence of ABCB1 transporters has also shown conflicting results. Some studies have shown that an *ABCB1* haplotype consisting of three variants, rs1045642 (3435 C>T) in exon 26, rs2032582 (2677 C>T) in exon 21, and rs1128503 (1236 C>T) in exon 21, affects TAC transport.(238) Some studies have shown a reduction in TAC concentrations, whereas others have shown no effect. Based on the available pharmacogenetic literature, Clinical Pharmacogenomic Implementation Consortium (CPIC) guidelines for TAC recommend the starting dose by 1.5 to 2 times to the recommended starting dose in CYP3A5 intensive or extensive metabolizers.(239) However the guidelines do not recommend adjusting the TAC dose based on other clinical factors and/or co-administered drugs, that are also important factors influencing trough concentrations.

Effects of *CYP3A4 and CYP3A5* variants on CSA metabolism are conflicting. Some studies have shown no effect of *CYP3A5*3* variant(240, 241), whereas other data suggest lower dose-adjusted trough concentrations and higher CSA dose requirements.(242, 243) The *CYP3A4*22* variant has been recently studied towards CSA, showing that carriers may have lower CSA Cl and higher troughs, though the effect was small. (235, 244-246)

1.2.3.1.2 Mycophenolic acid

Mycophenolic acid when administered orally undergoes intestinal and hepatic first pass metabolism via uridine diphosphate-glucuronosyltransferases (*UGTs*) (*1A1, 1A7, 1A8, 1A9, 1A10,* and *2B7*).(247, 248) Mycophenolic acid-7-*O*-glucuronide (MPAG) is the major metabolite formed in liver by UGT1A9 and in the intestine by UGT1A8 and UGT1A10. The acyl form of MPAG (Ac-MPAG) is a minor and active metabolite whose formation is mediated mainly by *UGT2B7*.(248, 249) Mycophenolic PK pathway is shown in Figure 1.7.

Pharmacogenomics of MPA has been focused mainly on the influence of SNPs in enzymes (*UGT1A9, UGT1A8, UGT1A10,* and *UGT2B7*) and transporters (*MRP2, SLCO1B1 and SLCO1B3*) on PK. (250) *UGT1A9* -275T>A (rs6714486) and -2152C>T (rs17868320) are promoter region variants that have been most studied with respect to MPA PK.(251, 252) Although *UGT1A9* variants have been evaluated in several studies, their effects have not been consistently replicated. The association between MPA exposure and clinical outcomes is weak or absent and hence therapeutic drug monitoring of MPA is not performed in all centers(248) Variants in the pharmacodynamic markers such as *IMPDH1 and IMPDH2* have been also evaluated toward rejection and toxicities.(253)

1.2.3.1.3 Azathioprine

Azathioprine is a prodrug non-enzymatically reduced to mercaptopurine and further activated to thioinosine monophosphate (TIMP) by HGPRT (hypoxanthine-guanine-phosphoribosyl transferases). Thioinosine monophosphate is then converted to

6-thioguanine nucleotides (6-TGNs) and 6-methyl mercaptonucleotides (6-MeMPNs). 6-thioguanine nucleotides gets incorporated into the growing DNA strand, and 6-MEMPNs inhibits purine synthesis. Mercaptopurine is inactivated by two major pathways, one mediated by TPMT, where mercaptopurine is inactivated to 6-methyl mercaptopurine (6-MeMP), and the other route is mediated by xanthine oxidase, which converts mercaptopurine to 6-TU (6-thiouric acid).(254) Figure 1.8 shows the AZT metabolic pathway. Higher AZT exposure is associated with several adverse effects, including bone marrow depression and gastrointestinal issues (nausea, vomiting, and hepatotoxicity). Dose-dependent toxicities such as leukopenia and thrombocytopenia are also observed and are reversed by dose-reduction or temporary cessation of therapy. Some individuals develop severe, life-threatening hematologic toxicity and require discontinuation of therapy. Variants in the gene-encoding enzyme *TPMT* lead to varying functional activity of the enzyme and are the main factors of variability in AZT exposure. Several variants such as *TPMT*2* (238G>C), **3A* (460G>A and 719A>G), **3B* (460G>A), **3C* (719A>G), and **4* (626-1G>A) have been identified that responsible for reduced or severely deficient *TPMT* enzyme activity.(255) CPIC guidelines for AZT recommend considering an extreme dose reduction or alternate immunosuppressant in kidney transplant recipients with low or deficient TPMT activity.(256)

1.3 DISSERATION MOTIVATION AND OBJECTIVE

Based on the literature review for chemotherapy used in HSCT and immunosuppressants in kidney transplant in sections 1 and 2, it is evident that wide inter-patient PK-PD variability are major barriers in drug efficacy and toxicity. Reduced

intensity conditioning/nonmyeloablative regimens used in HSCT have significantly contributed towards decreasing treatment related toxicities and improved survival after HSCT. However, TRM still accounts for ~20% of deaths at 1 year, and nearly ~50% of recipients develop acute GVHD by 6 months. Significant progress has been made in improving Bu therapy in myeloablative conditioning through individualized dosing. However there are limited examples for other agents such as Flu and Cy, which are widely used in reduced intensity conditioning regimens.

In kidney transplantation there is a persistent ongoing effort to balance immunosuppression so as to prevent both graft rejection and drug toxicity in each recipients of kidney transplant. Routine therapeutic drug monitoring is conducted for CSA, TAC, however in some patients, a number of dosage changes are required to achieve the therapeutic blood concentration range. This method of trial and error indicates that a large part of variability in exposure and response still remains unexplained. Thus there may be additional genetic (SNPs, gene expression) and clinical factors that further explain variability. Robust models need to be built to achieve the correct first dose in patients, taking into account PK, PD and PG factors that could influence an individual's drug exposure and response *apriori*.

The objective of my thesis was to develop models and identify biomarkers that could be used to personalize drug therapies in transplantation using population pharmacokinetic, pharmacodynamic and pharmacogenomic approaches.

Chapter 2 in the dissertation describes a population PK study of Flu conducted in HSCT patients to develop a F-ara-A Cl model and develop a personalized dosing equation using clinical factors. We further tested if model-predicted Cl and AUC are

associated towards adverse clinical outcomes in an independent population.

Chapter 3 in the dissertation describes a PK study conducted to evaluate an association of PM exposure (AUC) to TRM. In order to explain variability in PM kinetics we developed a population PK model in HSCT to identify significant covariates.

Chapter 4 in the dissertation describes development of a genotype guided dosing equation of TAC in African Americans. We developed a population PK based Cl model taking into account both clinical and genetic factors, to predict an individuals TAC apparent oral Cl. Predicting the individuals Cl would then help to estimate the dose to achieve the desired therapeutic TAC trough concentrations.

Chapter 5 in the dissertation describes a modern cutting-edge approach of whole transcriptome gene expression measurements through RNA sequencing to identify novel biomarkers in kidney transplant recipients. We analyzed association of changes in gene expression over time from pretransplant baseline with MPA trough concentrations, IMPDH activity and clinical outcomes. This approach may prove to be more accurate and robust than monitoring highly variable MPA trough concentrations and IMPDH activity to predict drug exposure and response.

Chapter 6 in the dissertation is the conclusion of the thesis work and future directions.

The thesis work is an effort to promote change in clinical practice from a one-size fit all approach to precision medicine. Future prospective testing of the developed models and identified biomarkers would help confirm the importance of the current work and improve therapy in transplantation.

Table 1.1: Common reduced intensity conditioning regimens used in United States

Conditioning Regimen	No. of HSCT recipients (%)
Flu/Bu ± other	631 (33)
Flu/Mel ± other	509 (27%)
Flu/Cy/TBI ± other	260 (11%)
Flu/TBI ± other	170 (9)
Flu/CY ± other	155 (8)
TLI/ATG	69 (3)
Other regimens	119 (6)

Table 1.2: Organ staging of acute GVHD

Stage of acute GVHD	Skin	Liver	Gastrointestinal Tract
0	No rash	Bilirubin <2 mg/ dl	None
1	Maculopapular rash <25% of body surface without associated symptoms	Bilirubin from 2 to <3 mg/dl	Diarrhea 500-1000 ml /day, nausea, emesis
2	Maculopapular rash or erythema with pruritus or other associated symptoms >25% of body surface area or localized desquamation	Bilirubin from 3 to <6 mg/dL	Diarrhea >1000–1500 ml/day
3	Generalized erythroderma; symptomatic macular, papular or vesicular eruption with bullous formation or desquamation covering > 50% of body surface area	Bilirubin 6 to <15 mg/dL	Diarrhea >1500 ml/day, nausea and emesis
	Generalized exfoliative dermatitis or bullous eruption	Bilirubin >15 mg/dL	Diarrhea >1500 ml/day, nausea and emesis, abdominal pain, or ileus

Table adapted from (257)

Table 1.3: Banff 07' classification of rejection after kidney transplant

Rejection Type	Characteristics
Hyperacute	Acute antibody mediated rejection (ABMR) Type I: C4d+, acute tubular necrosis (ATN), minimum inflammation Type II: C4d+, leukocytes in peritubular capillaries Type III: C4d+, transmural arteritis Chronic active ABMR
Accelerated Acute	Borderline to mild tubulitis: No mononuclear cells in tubules or 1–4 cells/tubular cross section interstitial inflammation: 10–50% of parenchyma inflamed
Acute rejection	T-cell-mediated rejection (TCR) Acute TCR Type IA: 26–50% or >50% of parenchyma inflamed & 5–10 cells/tubular cross section Type IB: severe tubulitis >10 cells/tubular cross section; or tubular basement membrane destruction with > 50% inflammation Type IIA: mild-moderate intimal arteritis Type IIB: severe intimal arteritis Type III: transmural arteritis Chronic active TCR
Chronic rejection	Interstitial fibrosis and tubular atrophy (IFTA) Grade I: mild Grade II: moderate Grade III: severe

Figure 1.1: Development of chimerism stages after conditioning regimen and transplant in allogeneic HSCT recipient

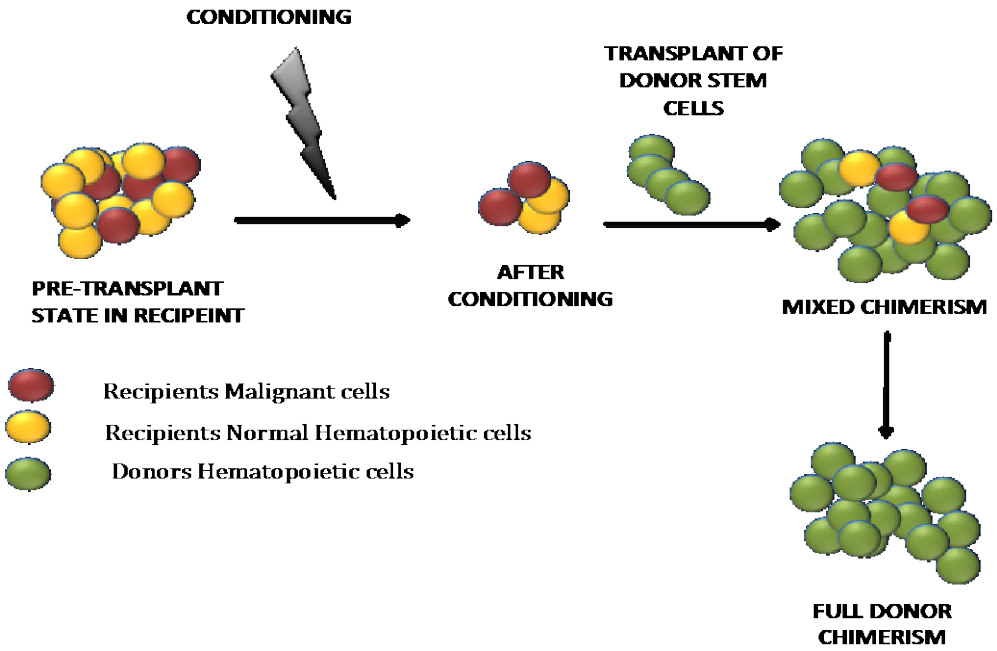
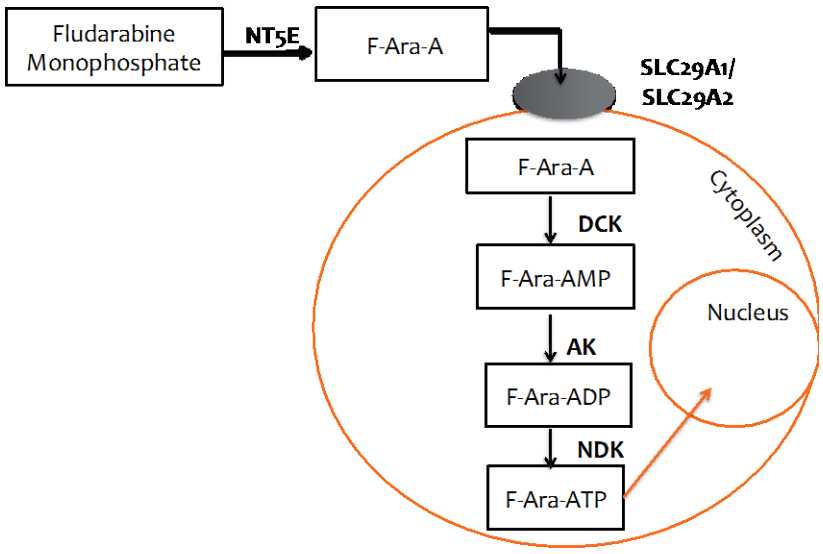
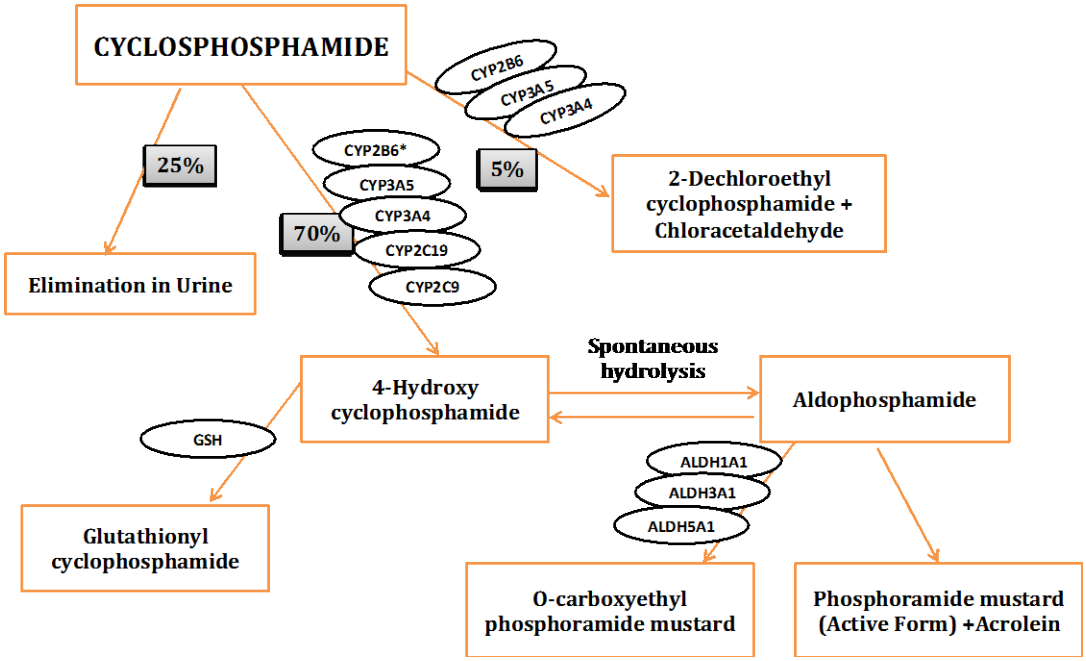


Figure 1.2: Activation pathway of Flu phosphate to F-ara-ATP



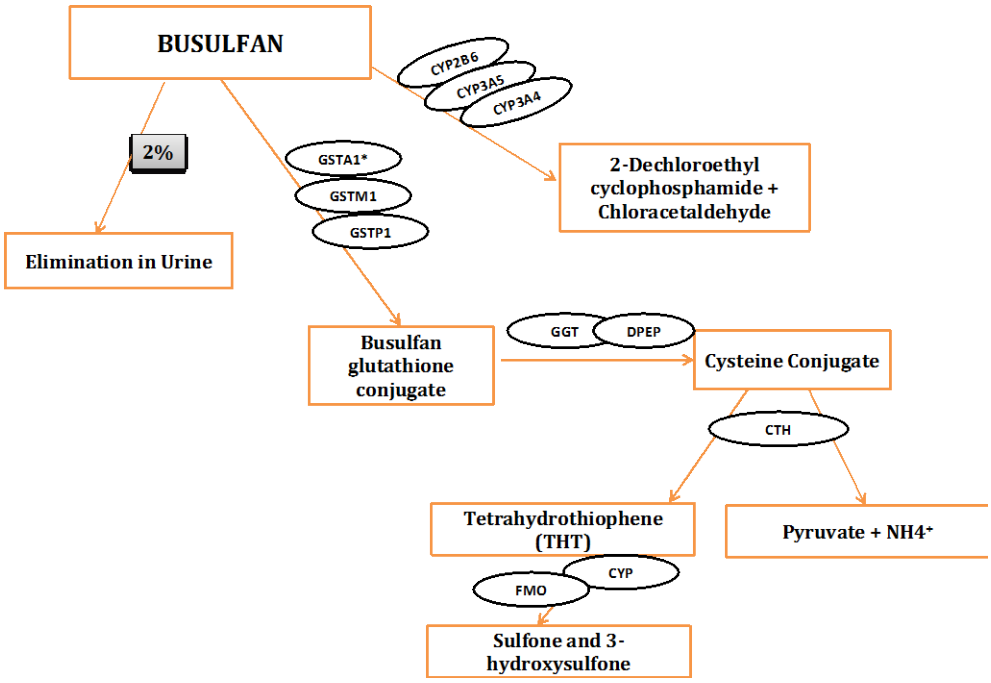
NT5E: Ecto 5' nucleotidase; SLC29A: Solute carrier family 29; DCK: Deoxycytidine kinase; AK:Adenylate kinase; NDK: Nucleoside diphosphate kinase

Figure 1.3: Pharmacokinetic pathway of cyclophosphamide



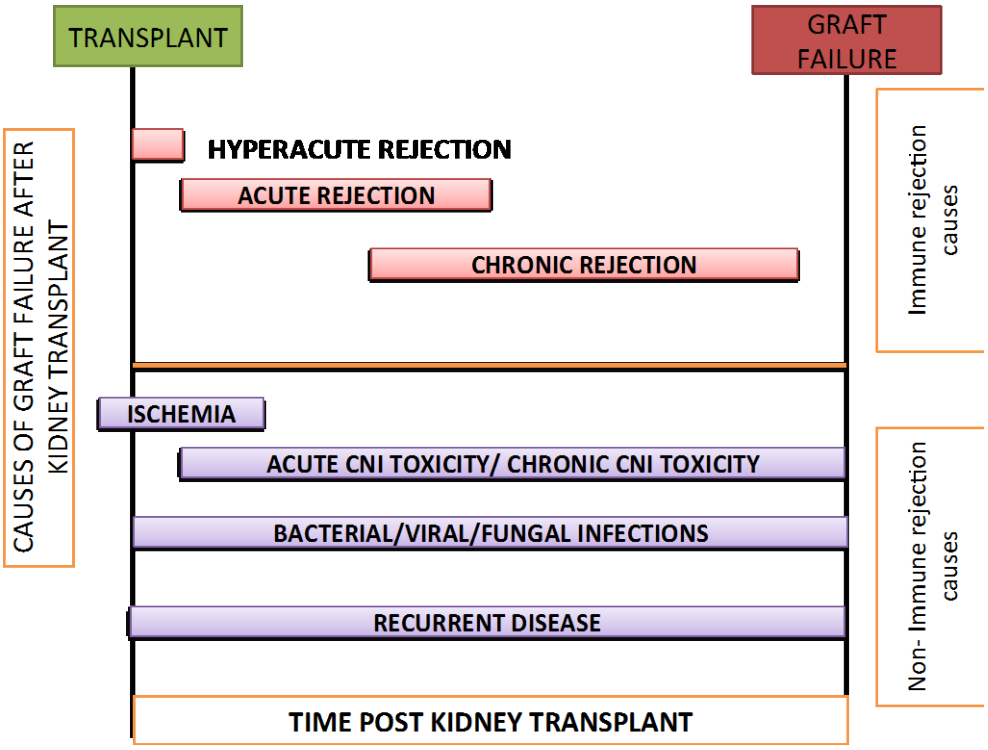
ALDH= aldehyde dehydrogenase, CYP: Cytochrome P450, GSH=Glutathione-S-transferases. * represents the major enzyme involved in metabolism.

Figure 1.4: Busulfan pharmacokinetic pathway



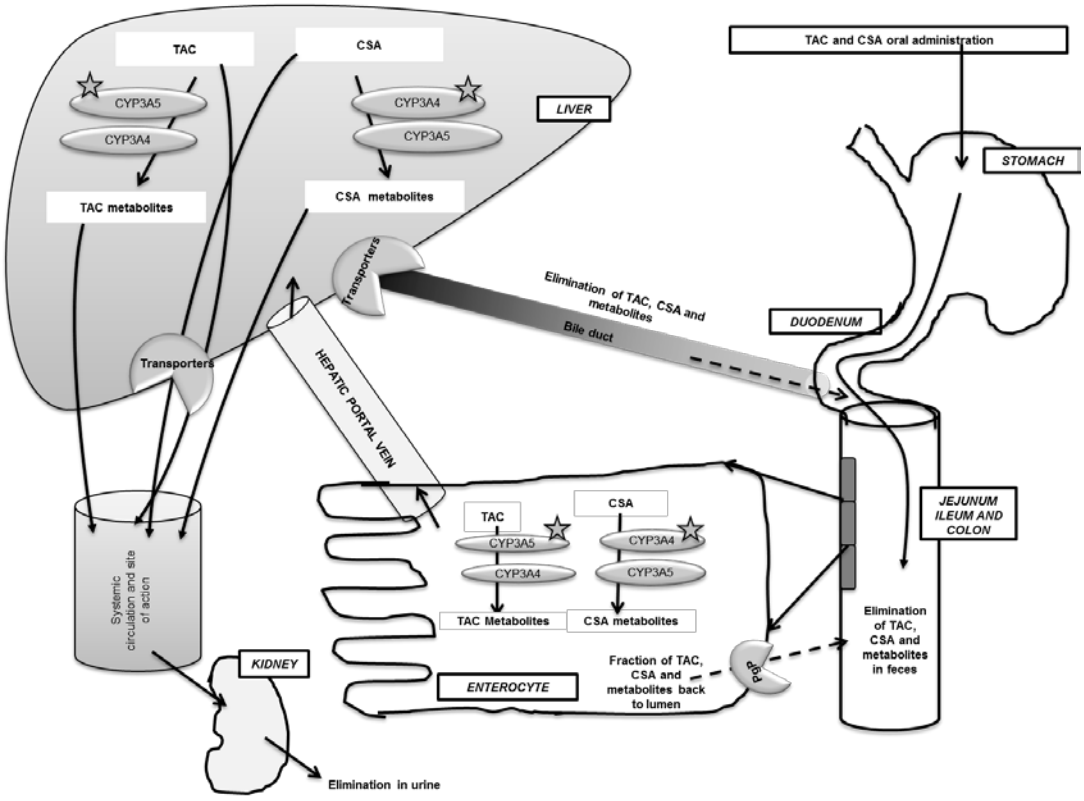
CTH= cystathionine gamma lyase, CYP=cytochrome P450, DPEP= dipeptidate/cysteinylglycinase, GSH=Glutathione-S-transferases, GGH=glutamyl transferase, FMO=flavin containing mono-oxygenase. * represents the major enzyme involved in metabolism.

Figure 1.5: Complications after kidney transplantation



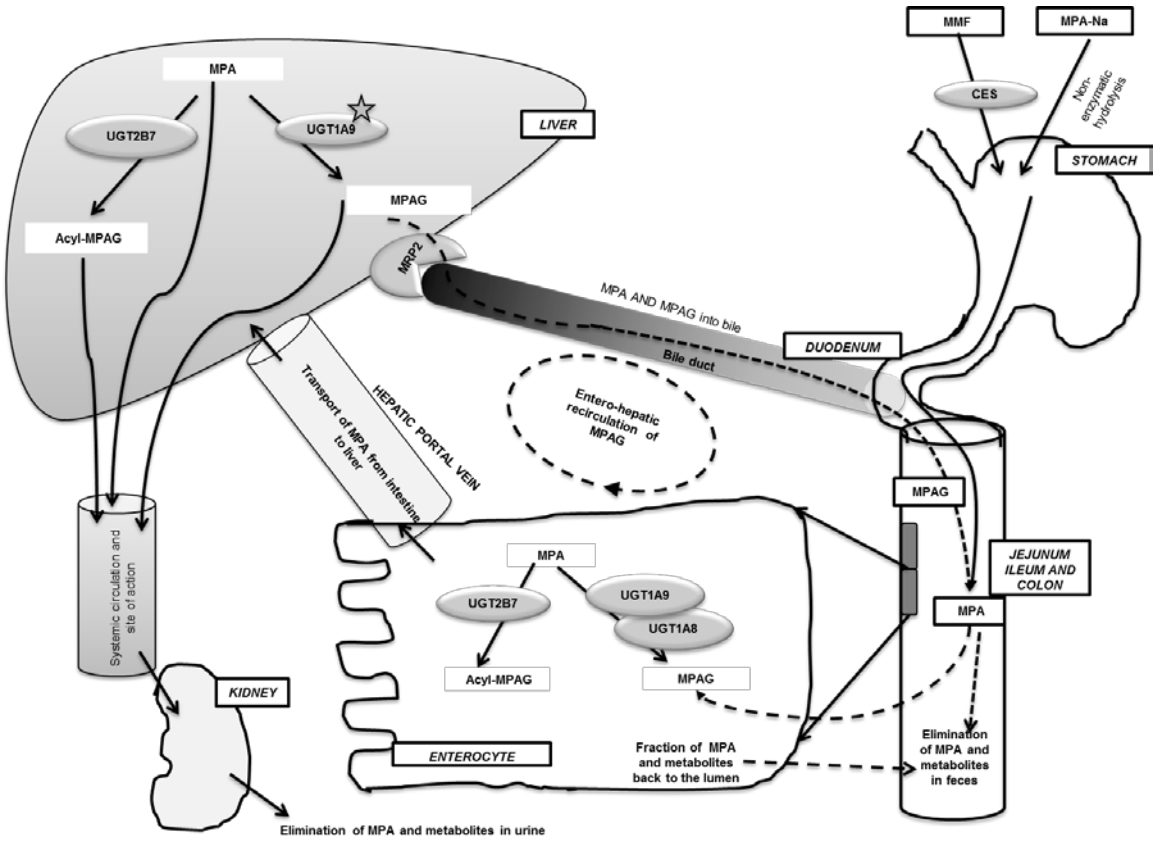
The time post-transplant (x axis) describes time from transplant up to the time of graft rejection that is variable among kidney transplant recipients (can take several months or years). In general, major complications early post-transplant include hyperacute rejection, tubular injury and ischemia. Then weeks following the transplant, complications of acute rejection (cell mediated or acute antibody mediated), and acute drug toxicity and infections are most dominant. The complications most often observed up to 1 year of transplant are chronic drug toxicity, infection and recurrent disease. After the first year major problem is graft dysfunction mainly caused due to chronic antibody mediated rejection, chronic CNI toxicities, recurrent glomerular disease. (169)

Figure 1.6: Pharmacokinetic pathway of tacrolimus and cyclosporine



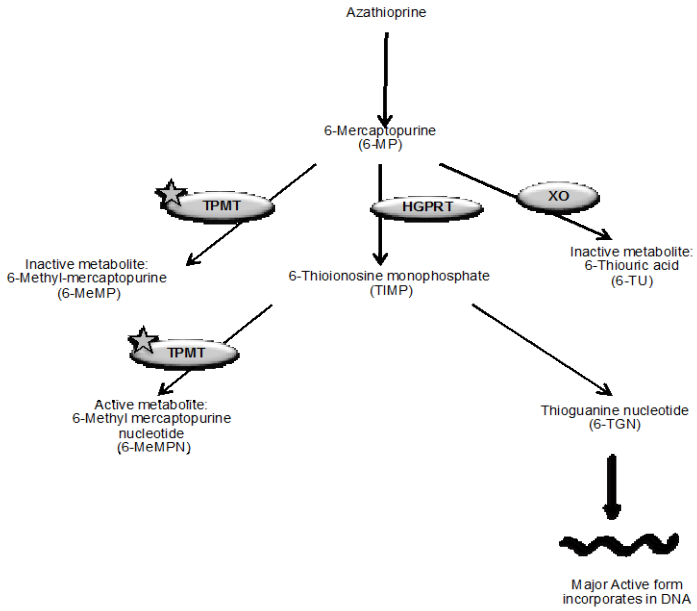
CYP=CytochromeP540, PgP = P-glycoprotein. * represents the major enzyme involved in metabolism.
Figure adapted from (258)

Figure 1.7: Pharmacokinetic pathway of mycophenolic acid



MMF = mycophenolate mofetil; MPA = mycophenolic acid; MPA-Na = mycophenolic acid sodium; CES = carboxylesterase; MPAG = MPA-7-O-methyl glucuronide; acyl MPAG = acyl-7-O-methyl glucuronide; UGT = uridine diphosphate glucuronosyltransferase; MRP2 = multidrug resistance protein 2. * represents the major enzyme involved in metabolism. (259)

Figure 1.8: Pharmacokinetic pathway of azathioprine



HGPRT = hypoxanthine-guanine-phosphoribosyl transferase;

TPMT = thiopurine S-methyltransferase; XO = xanthine oxidase. * represents the major enzyme involved in metabolism. Figure adapted from (260)

CHAPTER II

2 PERSONALIZED FLUDARABINE DOSING TO REDUCE TREATMENT RELATED MORTALITY IN HSCT RECEIVING REDUCED INTENSITY CONDITIONING

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2.1 INTRODUCTION

Reduced intensity conditioning and allogeneic hematopoietic cell transplantation (HSCT) is commonly used in patients with preexisting comorbidities who do not qualify for myeloablative conditioning. Over the last decade, reduced intensity conditioning regimens have allowed successful transplantation of patients who are heavily pretreated, older or have complicating comorbidities.(261-265) Fludarabine phosphate is an antitumor and immunosuppressive agent, and is a critical component of most reduced intensity conditioning regimens in combination with other chemotherapeutics and/or total body irradiation (TBI).

Fludarabine has dose-dependent toxicities.(48, 266-269) However, studies associating fludarabine dose and PK with toxicity and clinical outcomes are limited. (110-113, 116, 270-272) Data in HSCT suggest that higher F-ara-A (the active component of fludarabine in the plasma) concentrations may be associated with greater mortality.(111) F-ara-A PK variability has also been demonstrated in HSCT recipients although factors leading to variability are poorly understood.(107, 112-115)

Body size in m^2 is the primary determinant of fludarabine doses in HSCT despite there being a paucity of data and lack of understanding if body size alters PK disposition or contributes to variability. The ASBMT guideline for chemotherapy dose adjustment in obese HSCT patients concluded that adjustments for weight have been mostly empiric or extrapolated from non-HSCT populations and was not able to provide guidelines for fludarabine.(273) Approximately 35-60% of the fludarabine dose is recovered in the urine as F-ara-A or as fludarabine hypoxanthine within 24 hours after

administration.(107, 108, 110, 274, 275) There is a high correlation between creatinine clearance (CrCl) and F-ara-A total body clearance.(108, 269) Therefore, renal function is a source of F-ara-A PK variability. Despite this dosing guidelines for renal dysfunction are limited. The recent ASBMT dosing guideline in patients with chronic kidney disease concluded that there are no clear dosing standards for renal impairment and that the available literature is insufficient.(276) Genetic variants may also contribute to PK variability. Important effects of variants have been observed towards cytarabine and gemcitabine but no data are available for fludarabine.(277-281) Our interests have centered on improving the safety and efficacy of conditioning regimens, through personalizing fludarabine dosing by accounting for clinical factors known to affect PK variability and to develop evidence based and validated models to guide dosing and reduce TRM.

2.2 SUBJECTS AND METHODS

2.2.1 Patients and Pharmacokinetic Data for Model Development

Data for PK model development were obtained from 87 adult patients previously enrolled in a single center, prospective, observational, PK study undergoing reduced intensity conditioning allogeneic HSCT.(111) Subject characteristics are shown in Table 2.1. The research was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and informed consent was obtained from each patient. The study was approved by the Institutional Review Board and the Cancer Protocol Review Committee. The preparative regimen was i.v. cyclophosphamide (50 mg/kg/day) on day-6, i.v. fludarabine 40 mg/m^2/day on days -6 to -2 and TBI 200cGy

single fraction on day -1. An empiric dose reduction of fludarabine to 30-35 mg/m^2 was given to 9 patients due to preexisting renal impairment per physician discretion. Patients who had not received intensive chemotherapy within last 6 months were administered equine antithymocyte globulin for 3 days. Post-HSCT immunosuppression was mycophenolate and cyclosporine. Fludarabine phosphate was administered i.v. over 1 hour. Pharmacokinetic blood samples were collected with the first dose beginning immediately pre-dose and at 100 minutes, 2, 3, 4, 6, 8, 12 and 24 hours after start of infusion. F-ara-A detection and quantification in the plasma was performed with HPLC-UV as previously described.(111) The lower limit of quantification was 10 ng/mL with an assay accuracy of 93.5-100.1%. Age, gender, actual body weight, height, disease risk, serum creatinine, total bilirubin (obtained +/-48 hours of the first dose) and serum albumin data (obtained +/-48 hours of the first dose) were collected.

2.2.2 DNA Collection, Variant Selection and Genotyping

Recipient genomic DNA was obtained pre-HSCT from peripheral blood lymphocytes. DNA was quantified by measuring the absorbance at 260 nm. Genes potentially involved in fludarabine bioactivation and transport such as *NT5C2*, *NT5E*, *SLC28A3*, *SLC29A1*, *SLC29A2*, *DCK*, *ABCG2*, *ABCC4* were considered in the analysis. National Center for Biotechnology Information was searched for coding and promoter region variants. Our population was predominantly Caucasian therefore variants were identified from the Caucasian CEU population in the HapMap project (HapMart; schema: rel23a_NCBI_Build36, database: HapMap_rel23a). A total of 77 variants were identified. The Genetic Services Department, Sequenom, Inc., San Diego, CA, performed assay design and genotyping. All variants were in Hardy-Weinberg equilibrium. Sixty-six

variants were monomorphic or had a minor allele frequency of less than 5% after genotyping and were eliminated. Eleven variants were analyzed (Table 2.2).

2.2.3 Population Pharmacokinetic Model Building and Identification of Covariates Effecting Pharmacokinetics

F-ara-A plasma concentrations (n=768) were previously analyzed and reported. (111) The mean (standard deviation) of plasma concentrations at time 100 minutes, 2, 3, 4, 8, 12 and 24 hours after the start of infusion were 711 (163), 625 (145), 460 (113), 364 (82.5), 254 (61.7), 192 (50.0), 121 (33.3) and 57.3 (23.3) ng/mL, respectively. An equivalent weight of F-ara-A (MW 285) to that of fludarabine phosphate (MW 365) was used as an initial dose with the assumption of instantaneous and rapid conversion of the monophosphate form to F-ara-A in the plasma.

Data analysis was conducted using population PK with nonlinear mixed effects modeling (NONMEM) (version 7.2, ICON Development Solutions, Hanover, MD, USA). Inspection of the PK data, model diagnostics, and covariate testing, bootstrapping and visual predictive check were performed using Perl Speaks Nonmem (PSN) and Xpose version (version 4.3.2) through Pirana workbench (2.7.2), Amsterdam, The Netherlands. First-order conditional estimation with interaction (FOCEI) was utilized for model development. Pharmacokinetic parameters estimated were typical values of clearance (referred to as Clpop in this paper) and volume of distribution (referred to as Vpop in this paper).

Between-subject variability (BSV) was modeled exponentially to PK parameters as shown in equation 1.

$$Pj = TVP \times \exp(\eta j) \qquad \textit{(equation 1)}$$

where, Pj is the parameter estimate for jth individual, TVP is the typical value of the parameter in a population. ηj is the estimate of deviation of individual j from the TVP and is assumed to be normally distributed with the mean of zero and variance of omega^2 (population variability). Residual unexplained variability (RUV) is the unexplained variability between the observed and the predicted value. A combined proportional and additive error model was chosen to describe the RUV (equation 2).

Cobs,ij=Cpred,ij x (1+εij) + εij (proportional and additive RUV model) (equation 2)

where, Cobs,ij is the jth observed concentration in the ith individual, Cpred,ij is the jth predicted concentration in the ith individual and εij is the residual error that is assumed to be independent and normally distributed with a mean zero and variance of σ_ε^2.

Base model and covariate selection was based upon inspection of the diagnostic plots, a significant decrease in the objective function value (OFV) and a physiological plausible relationship to PK parameter relative to competing models.

Empirical Bayes estimates (ebe) for parameters were plotted against each covariate to identify the relationships between the parameters and the covariates. Covariates were tested for significant effects on F-ara-A Clpop using a forward inclusion and backward elimination procedure. In NONMEM, minimization of -2 log likelihood is used as a model statistic and is given by the objective function value (OFV); a measure of goodness of fit similar to sum of squares. Covariates were deemed statistically significant if their inclusion in a nested model resulted in OFV decrease $\geq$ 3.84 (X^2, df=1, $p<0.05$) and their exclusion from the full model resulted in an OFV increase $\geq$ 6.63 (X^2, df=1, $p<0.01$). The effect of continuous covariates; age, CrCl calculated using Cockcroft and

Gault equation using ideal body weight (IBW) (282), height, actual body weight, IBW calculated using Devine formula (283), adjusted body weight calculated as IBW+0.4 (actual body weight-IBW), body surface area (BSA) calculated using actual body weight, serum albumin and bilirubin were tested towards their effect on F-ara-A Clpop and volume in the central compartment (V1pop). Gender and genotypes were evaluated as categorical covariates. Genotypes were tested as 3 categories (homozygous for major allele, heterozygotes and homozygous for the minor allele). If the number of individuals homozygous for the minor allele was less than 5% then it was combined with the heterozygous group. We did not study the influence of coadministered drugs since there were no known drug interactions occurring with fludarabine in our protocol. All subjects presented with normal aspartate aminotransferase and therefore, it was not studied. The final model was then evaluated using a non-parametric bootstrap approach that evaluated the precision of the final estimated parameters. This approach used random sampling with replacement from the original dataset to generate new datasets (n=1000). The final model was fit to each of these datasets and estimates of parameters were obtained. Bootstrap parameter estimates and their 95% confidence interval were compared to parameter values obtained from the original dataset. Predictive performance of the model was also assessed using visual predictive checks. One thousand datasets were simulated from the final model using a design similar to the original dataset. The 5th, 50th and 95th percentile bands of the simulated predictions along with their 95% prediction intervals were then plotted with superimposed observed concentrations.

2.2.4 Validation of the Utility of the Pharmacokinetic Clearance Model in an Independent Cohort

We tested the utility of our model in an independent cohort by examining the association between model predicted first dose F-ara-A clearance (Clpred) and predicted $AUC_{0\rightarrow\infty}$ (AUCpred) towards clinical outcomes. Two hundred and forty patients who underwent allogeneic reduced intensity conditioning HSCT at University of Minnesota from 2008-2014 who received i.v. fludarabine (25-40 mg/m2/day) on days -6 to day -2, i.v. cyclophosphamide 50 mg/kg/day on day -6 and total body irradiation on day -1 were studied. Subject characteristics are shown in Table 2.1. Approval by the Institutional Review Board and the Cancer Protocol Review Committee was obtained. Patients who had received prior autologous HSCT more than a year prior to allogeneic HSCT and had not received intensive chemotherapy within the past 3-6 months were administered equine antithymocyte globulin for 3 days. Mycophenolate and cyclosporine were given as maintenance immunosuppression. The administered fludarabine dose, actual body weight, height and serum creatinine on day of admission, demographic data were obtained on each subject. For each patient, F-ara-A Clpred was calculated using the developed model (using equation 5 described later in results section) and then using the administered fludarabine dose in F-ara-A equivalents, the AUCpred was determined (using equation 6 described later in results section). Treatment-related mortality was defined as death due to any cause other than relapse or disease progression. Acute GVHD to month 6 was staged and graded according to the standard acute GVHD criteria based on clinical and pathological criteria. Day of neutrophil engraftment was the first of 3 consecutive days of an absolute neutrophil count >500 cells/uL by day 42.

Recursive partitioning regression analysis was performed in the independent cohort to select optimal cut points for model predicted F-ara-A Clpred and F-ara-A AUCpred towards TRM and acute GVHD. Once optimal cut points were identified the cumulative incidence of TRM and acute GVHD (grades II-IV) above and below each of the cut points was calculated using death prior to event as a competing risk. An *apriori* two sided log rank test at an alpha level of 0.05 was conducted and a sample size of 240 subjects would detect a 10% or more difference in hazard of TRM in patients above and below F-ara-A Cl cut point with a power of 0.98-1.00

Recipient gender, age, use of ATG in preparative regimen, recipient CMV status, donor source, recipient HLA type (match/mismatch), disease risk, Karnofsky score, BMI, comorbidity score were univariately tested for their influence on TRM (day 100, months 6 and12), acute GVHD (grades II-IV) (month 6). Additionally acute GVHD (grades II-IV) was tested as a time-dependent covariate towards TRM. Allele or antigen mismatch at one (7/8) of the loci (HLA-A, -B, -C and DRB1) was defined as HLA mismatch. HLA mismatch was identified under low resolution for 235 patients and on high resolution for 5 patients. Standard disease risk was defined as acute leukemia in first or second remission, CML in chronic phase, NHL and other malignancies in first and second remission and non-malignant diseases; all other malignancies were classified as high risk. Comorbidity score was defined as described in Sorror et al.(19)

Fine and Gray regression was used to estimate the effect of model predicted F-ara-A Clpred and F-ara-A AUCpred towards time to TRM at day 100, months 6 and 12, time to acute GVHD (grades II-IV) at month 6, and time to neutrophil engraftment at day 42 adjusting for clinical covariates that were significant in the univariate analysis (full

models). (284) Reduced models for each endpoint was then created using backward selection method by eliminating covariates from the full model with a p-value of >0.20. An a priori p-value of 0.025 was set to identify significant covariates in the reduced model accounting for multiple testing. Statistical analysis was performed with SAS 9.3 (SAS Institute, Cary, NC) system and R Statistical Software (Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org).

2.3 RESULTS

2.3.1 Development of F-Ara-A Clearance Model (Clpop) and Covariates Influencing Clearance

A two-compartment model with i.v. administration best described F-ara-A PK. Typical value of PK parameters, between subject variability and residual unexplained variability estimates are provided in Table 2.3. The NONMEM code for the final model is shown in Appendix 8.1

Creatinine clearance significantly influenced F-ara-A Clpop. We calculated Clpop as a sum of the renal and nonrenal Cl. Body size measures (actual body weight, IBW, adjusted body weight and BSA) also significantly influenced Clpop. We conservatively chose IBW for further scaling with a power of 0.75 (equation 3) and weight standardization (equation 4). Our previous work showed that a high AUC was associated with more treatment related mortality, and using total body weight in obese patients increases the dose administered thereby placing patients at higher risk of high AUC.(15) Age, as a continuous covariate on Clpop, significantly reduced the OFV during forward inclusion, but was not significant during backward elimination. None of the other

tested covariates were significant towards Clpop including the genetic variants. The final model for F-ara-A Clpop is shown in equation 5.

$$F\text{-}ara\text{-}A\ Clpop = (Cl_{nr} + Cl_{slope} \times RenFunc_{std}) \times (IBW/70)^{0.75} \quad \textit{(equation 3)}$$

$$RenFunc_{std} = (CrCl/85) \times (70/IBW) \quad \textit{(equation 4)}$$

$$F\text{-}ara\text{-}A\ Clpop\ (L/hr) = [7.04 + 3.90 \times \{(CrCl/85) \times (70/IBW)\}] \times (IBW/70)^{0.75} \quad \textit{(equation 5)}$$

Cl_{nr} is the nonrenal clearance of F-ara-A; Cl_{slope} is the change in renal clearance with a unit change in standardized renal function ($RenFunc_{std}$). $RenFunc_{std}$ is the CrCl as calculated by Cockcroft and Gault using IBW, and then standardized by IBW (equation 4). The Cockcroft and Gault equation included IBW and therefore CrCl and IBW were highly correlated in the model. To effectively eliminate this correlation, renal clearance was first IBW-standardized as described by Mould et al.(285) The renal function was centered using the mean CrCl (85 ml/min) observed in the study population. Clpop was scaled using IBW (equation 3).

The estimates of Cl_{slope} and Cl_{nr} were 3.90 L/hr per standardized CrCl (CrCl/85 ml/min x 70kg/IBW) per 70 kg IBW and 7.04 L/hr per 70 kg IBW, respectively. Using these estimates the Clpop for a standard 70 kg IBW subject with a CrCl of 85 mL/min was 10.9 L/hr (3.90 L/hr + 7.04 L/hr). For this standard subject, renal clearance accounts for 35.6% of Clpop and for every 10 unit decrease in CrCl, total F-ara-A Clpop decreases by 0.46 L/hr.

The diagnostic plots (Figure 2.1) were used to examine the goodness of fit of the model and demonstrated that the model adequately explained the observed data and there was no evidence of model misspecification.

The visual predictive check (Figure 2.2) shows that the model reasonably describes the data and that no systematic deviation between observed and simulated data was observed. Our final model was also evaluated for its reliability by non-parametric bootstrap. Out of 1000 datasets generated, 930 minimized successfully. Table 2.1shows the median of each estimate with 95% confidence intervals obtained from the bootstrap datasets. Estimates for PK parameters, inter- and intraindividual variability are similar and lie within 5% of the estimates obtained from the final model, indicating that the model is robust and reproducible.

2.3.2 The Relationships between F-ara-A Clpred and AUCpred with Clinical Outcomes in an Independent Cohort

For each individual in the independent cohort, CrCl and IBW obtained on day -7 pre-HSCT were used in equation 5 to predict F-ara-A Clpred. The F-ara-A AUCpred was then estimated for each individual by using equation 6:

F-ara-A AUCpred = F-ara-A equivalent dose (mg) / F-ara-A Clpred *(equation 6)*

F-ara-A equivalent dose was calculated as administered fludarabine phosphate dose /1.28. Creatinine clearances were capped at 150 ml/min since values greater than those seemed implausible. The median (range) F-ara-A Clpred, F-ara-A AUCpred and the administered fludarabine dose were 10.9 (7.51-15.4) L/hr, 4.85 (2.82-7.52) µg*hr/mL and 67 (42-100) mg in the independent cohort.

The cumulative incidence of TRM was 8, 13 and 19% at day 100, 6 and 12 months, respectively, in the independent cohort. The median (range) time to TRM was

165 (18-1518) days. Optimal cut points towards TRM for F-ara-A Clpred and AUCpred were 8.50 L/hr and 6.00 μg*hr/mL, respectively.

More rapid F-ara-A Clpred was associated with less TRM. In univariate analysis, the cumulative incidence (95% CI) of TRM at day 100 in patients with F-ara-A Clpred <8.50 L/hr was 25% (6-43) as compared to 6% (3-10) in patients with F-ara-A Clpred ≥8.50 L/hr ($p<0.01$) (Figure 2.3). In univariate analysis, donor source, HLA mismatch, high-risk disease, comorbidity score ≥3 and acute GVHD (grades II-IV) before TRM were each significant towards higher TRM and were chosen for adjustment in the full model. Fludarabine dose was not associated with TRM (25-40mg/m^2/day). The multivariate regression reduced models after backward elimination are shown in Table 2.4 and Table 2.5. At day 100 the hazard ratio (HR) of TRM in patients remained significantly lower in patients with F-ara-A Clpred ≥8.50 L/hr as compared to <8.50 L/hr [HR (95% CI) 0.1 (0.02-0.42), $p<0.01$], after adjusting for donor source, disease risk, comorbidity score, and acute GVHD (grades II-IV) before TRM. A lower F-ara-A Clpred was also associated with greater TRM at month 6 (Table 2.4). Cumulative incidence of TRM (95% CI) at day 100 was significantly higher in patients with F-ara-A AUCpred ≥6.00 μg *hr/mL as compared to <6.00 μg*hr/mL [22% (8-37) vs 6% (3-9)($p<0.01$)] (Figure 2.4). Results of multivariate regression of F-ara-A AUCpred and TRM at day 100, months 6 and 12, adjusted for clinical covariates are shown in Table 2.5. The total number and percent of patients with TRM events at day 100, months 6 and 12 in each covariate group are shown in supplementary Table 2.7 and Table 2.8.

The cumulative incidence of acute GVHD (grades II-IV) at month 6 was 43% in the independent cohort. F-ara-A Clpred and AUCpred optimal cut points were 13.0 L/hr

and 6.00 µg*hr/mL towards acute GVHD, respectively. In univariate analysis, F-ara-A Clpred ≥13.0 L/hr was associated with lower cumulative incidence (95% CI) of acute GVHD (grades II-IV) at month 6 as compared to F-ara-A Clpred <13.0 L/hr [23% (7-39) vs [45% (38-52), p=0.04] (Figure 2.5). In multivariate analysis, F-ara-A Clpred ≥13.0 L/hr also had a lower the hazard of acute GVHD at month 6 after adjusting for clinical factors but was not significant [HR (95% CI) 0.44 (0.19-1.02), p=0.05] (Table 2.6). F-ara-A AUCpred was not associated with acute GVHD (grades II-IV) in univariate analysis (p=0.05). The total number and percent of patients with acute GVHD events at month 6 in each covariate group are shown in supplementary Table 2.9.

Ninety seven percent of patients engrafted within day 42 and therefore, none of the F-ara-A PK or clinical variables were significant towards engraftment given the small event rate (data not shown).

2.4 DISCUSSION

Reduced intensity conditioning for allo-HSCT is increasingly common. These patients often present with comorbid conditions such as compromised renal function and obesity. Comorbid conditions may affect drug clearance leading to over or under dosing of chemotherapy and poor outcomes. Treatment-related mortality remains high in reduced intensity conditioning HSCT (15, 16, 286-289), which may in part be due to our inability to predict an individual's capacity for chemotherapy clearance. An understanding of the clinical factors associated with drug clearance and conditioning regimen intensity is critical in improving outcomes. In this study we identified factors affecting fludarabine clearance and developed an individualized dosing model from 87 adult patients

undergoing reduced intensity conditioning HSCT that accounts for these factors. We then evaluated the utility of our model and identified F-Ara-A clearance and AUCs associated with poor clinical outcomes in a large independent cohort.

We found that CrCl and body weight significantly influenced F-ara-A clearance. In chronic lymphocytic leukemia patients who received fludarabine (25 mg/m^2 for 5 days every 28 days) with a CrCl less than 80 ml/min had a significantly greater probability of toxicity as compared to those greater than 80 ml/min ($p<0.001$).(109) The fludarabine package insert recommends a dose reduction of 20% for a CrCl of 30-70 ml/min and avoidance if CrCl <30 ml/min.(290) These dose reductions for renal function are important but unfortunately are quite imprecise since a patient with a CrCl of 70 ml/min would receive the same dose reduction as an individual with a CrCl of 30 ml/min. Our data showed that renal clearance accounts for over one third of total clearance and that for every 10 unit decrease in CrCl in the typical patient the total F-ara-A clearance decreases by ~5% therefore small changes in CrCl are relevant towards elimination. Because we modeled CrCl as a continuous variable, precise dose reductions for any CrCl are possible.

Dosing of chemotherapy in obese patients is a growing clinical problem given the increasing number of overweight and obese patients presenting for HSCT. The 2014 ASBMT guidelines on chemotherapy dose adjustments in HSCT found insufficient data to support level 1 or 2 recommendations in overweight individuals.(273) A review of fludarabine studies in the guideline found that trials mainly used total body weight to estimate BSA and fludarabine doses; however, evidence for the basis of using total body weight was lacking. A recent ASCO guidance recommended for solid tumors that actual

body weight be used for chemotherapy dose calculation.(291) In our study, 33.3% of subjects were overweight (BMI 25.0-29.9), 23.0% obese (BMI 30.0-34.9) and 11.5% morbidly obese (BMI>35.0), therefore, weight is an problem for many patients. In clinical practice the use of total body weight in patients with obesity results in higher chemotherapy doses than IBW. In our analyses all body size measures were associated with F-ara-A clearance and since many of our patients presenting for HSCT are obese we chose a conservative approach and used IBW to develop the final model. We previously found that high F-ara-A concentrations in our reduced intensity conditioning regimen protocol were associated with greater treatment related mortality and our goal is to improve the safety.(111) We found that as IBW increased, F-ara-A clearance also increases thereby increasing dose requirements. Our data are consistent with a previous population PK analysis in HSCT recipients, which also found that all tested body size measures (BSA using adjusted IBW, height, actual body weight, adjusted IBW) were associated with PK parameters.(114) Our developed model adequately explained the observed data as shown in the diagnostic plots and visual predictive checks, with robust parameter estimates obtained though bootstrap model evaluations.

We also evaluated our PK clearance model in an independent cohort of 240 reduced intensity conditioning HSCT recipients and determined if model predicted F-ara-A clearance and predicted AUC were associated with clinical outcomes. In multivariate analysis patients with a predicted F-ara-A clearance <8.50 L/hr had a 10 times higher hazard of TRM as compared to clearance ≥8.50 L/hr at day 100. F-ara-A predicted clearance remained associated with TRM at month 6 (Table 2.4). In addition, F-ara-A predicted AUC >6.00 µg*hr/mL had a 5.30 times greater hazard of TRM at day 100

(Table 2.5). We also observed a higher hazard of acute GVHD (grades II-IV) in those with high predicted F-ara-A clearance in univariate analysis but it was not significant.

These data are consistent with our previous study where HSCT recipients receiving reduced intensity conditioning with fludarabine (40mg/m^2 x 5 days), cyclophosphamide and TBI with an F-ara-A AUC >6.50 µg*hr/mL had a 4.56 greater risk of TRM.(111) In a small study of 16 patients receiving fludarabine 50mg/m^2/day x 5 days with PK guided busulfan and rATG, an F-ara-A AUC above the mean (24.8 µM*hr or 7.07 µg*hr/mL) trended towards a higher hazard for treatment-related mortality (HR=5.2, p=0.10) and overall mortality (HR=3.4, p=0.12). Unfortunately, the study was closed early due to high toxicity.(113) In a small study of 42 subjects receiving fludarabine 30mg/m^2 days -6 to -3 and concentration-controlled busulfan dosing, no association was observed between mean F-ara-A AUC (19.1 µM/hr or 5.44 µg*hr/mL) and engraftment or T-cell chimerism but TRM was not evaluated.(116) A recent study by same group in 102 patients receiving fludarabine 30mg/m^2/day for 4 consecutive days followed by TBI on day of HSCT, found no association between F-ara-A AUC and TRM and acute GVHD (grades II-IV).(112) A letter to the editor reported on 166 HSCT recipients receiving fludarabine 50mg/m^2 days -6 to -2 and busulfan with or without TBI.(270) F-ara-A concentrations on day of HSCT were not associated with risk of acute GVHD, CMV reactivation, risk of relapse, or death due to any cause. These data may suggest that when fludarabine is combined with busulfan or given in a conditioning regimen using four or fewer doses of fludarabine the exposure response relationships may be modest. However, when fludarabine (30-40 mg/m2/day) is given as 5 consecutive days in combination with cyclophosphamide and TBI there may be a strong concentration dependent effect on outcomes.

Central to individualizing fludarabine doses is an understanding of the therapeutic blood target range for reduced intensity conditioning. Considering our results, a first dose F-ara-A AUC >6.00 µg*hr/mL carries an unacceptable risk of mortality. Therefore, it is likely that an upper limit AUC is between 4.50-5.50 µg*hr/mL for 5 days when combined with cyclophosphamide and TBI. Future studies should be directed at defining the lowest plasma exposure required to minimize toxicity without compromising efficacy.

An example of how our model can be applied to the clinical setting in a patient with a low CrCl is as follows. Consider an adult patient with an IBW of 53 kg, height of 161 cm, CrCl of 45 ml/min and a BSA of $2m^2$ calculated using actual body weight. Assume a desired F-ara-A AUC of 5.0 µg*hr/mL. The fludarabine dose would be estimated as follows:

Step 1: Determine F-ara-A clearance using equation 5:

F-ara-A Clpred (L/hr) = [7.04 + 3.90 x {(CrCl/85) x (70/IBW)}] x (IBW/70)$^{0.75}$

F-ara-A Clpred for the example = [7.04 +3.90 x {(45/85) x (70/53)}] x (53/70)$^{0.75}$ = 7.90 L/hr

Step 2: Determine F-ara-A predicted dose using equation 7:

Once the F-ara-A Clpred is estimated for an individual and a target F-ara-A AUC is selected by the clinician, the optimal dose to achieve the AUC target for any individual can be estimated.

*Predicted daily F-ara-A dose (mg)=Desired AUC (µg*hr/mL) x F-ara-A Clpred (L/hr) (equation 7)*

Predicted daily F-ara-A dose (mg) = 5 µg*hr/mL x 7.90 L/hr = 39.5 mg/day

Step 3: Determine fludarabine phosphate dose using the following:

Since the drug is administered as fludarabine phosphate, the F-Ara-A estimate (MW 285.23) must be converted to an equivalent of fludarabine phosphate (MW 365.2).

*Final daily fludarabine phosphate dose mg = Predicted F-ara-A dose (mg) * 1.28*

Fludarabine phosphate dose for the example = 39.5 x 1.28 = 50.5 mg/day i.v.

For this individual, traditional dosing based on BSA alone at 35mg/m^2/day would give a dose of 70 mg. Due to the patients renal dysfunction a dose reduction of 20% may be made, if manufacturer's recommendations were followed, and the final dose would be 56 mg/day. Our model estimated a dose of 50.5 mg/day, which is lower and better accounts for reduced renal function.

One of the limitations of our study is that no patient had a CrCl<45 ml/min in our model development cohort and it is not known if the model is sufficient for lower CrCls; however, patients with CrCl lower than 45 ml/min are generally excluded from HSCT. Due to the low frequency of some of our genetic variants our samples size may have been too small to detect changes in the PK especially given the strong effect of renal function and weight. Therefore, future larger studies should reevaluate these and other genetic variants. Most of our patients engrafted by day 42 and we were unable to assess its relationship to F-ara-A PK. The minimum F-ara-A target AUC required to maintain efficacy towards engraftment will require a larger analyses and consideration of the immunosuppressive drugs comprising the conditioning regimens to sustained immunosuppression.

We provide evidence that body size and CrCl significantly influences F-ara-A PK and have developed an individualized fludarabine dosing equation to personalize fludarabine dose using IBW and accounting for CrCl. This equation would be most useful in overweight individuals and in those with renal dysfunction where traditional BSA dosing may overestimate their dosing requirements. Finally we evaluated the model in an independent cohort that found that predicted F-ara-A clearance and AUC are highly significant towards TRM even after adjusting for clinical variables. This model offers a method to personalize fludarabine dosing and control systemic exposure to reduce adverse clinical outcomes. Future studies using the equation should focus on refining the model prospectively, considering the effect of cyclophosphamide exposure on outcomes, and creating pediatric models. It is time to reconsider our long standing practice in HSCT of one size fits all dosing.

Table 2.1: Subject demographics

	Development Cohort Median (range)/N (%)	**Confirmatory Cohort Median (range)/N (%)**
Number of Patients	87	240
Administered single day dose (mg)	75 (46-100)	67 (42-100)
Age (years) median (range)	55 (20-69)	59 (19-75)
Males N (%)	56 (64.36)	139 (57.91)
Actual body weight (kg) median (range)	82.5 (41.5-139.5)	84.35 (46.60-183.40)
Ideal body weight (kg) median (range)	65.9 (40.6-81.0)	67.75 (97.72-41.97)
Body surface area (m2) median (range)	1.95 (1.3-2.5)	1.98 (1.38-3.12)
BMI (kg/m^2)		
<25	28 (32.2%)	64(26.67%
25-29.9	29(33.3%)	81(33.75%)
30-34.9	20 (23.0%)	58(24.17%)
>35	10(11.5%)	37(15.41%)
Serum creatinine (mg/dL) median (range)	0.90 (0.4-1.5)	0.82 (1.96-0.32)
Creatinine clearance (mL/min) median (range)	82.1 (45-153)	87.95 (29.25-206.23)
Total bilirubin (mg/dL) median (range)	0.40 (0.1-1.2)	Not collected
Recipient CMV positive N (%)	45 (51.72)	135 (56.25)
Disease N (%)		
Acute lymphoid leukemia	6 (7%)	22 (9.16%)
Acute myeloid leukemia	26 (30%)	71 (29.58%)
Chronic myeloid leukemia	1 (1%)	6 (2.5%)
Other leukemias	6 (7%)	21 (8.75%)
Myelodysplastic syndrome	14 (16%)	40 (16.67%)
Non-Hodgkin's lymphoma	17(20%)	36 (15.00%)
Hodgkin's lymphoma	8 (9%)	15 (6.25%)
Other	9 (10%)	29 (12.08%)
Donor Source N (%)		
Cord blood	64 (73.30%)	104 (43.34%)
Related	22 (25.29%)	35 (14.58%)

Unrelated	1 (0.01%)	101 (42.08%)

Table 2.2: Candidate fludarabine genes and variants evaluated in development cohort

Variant	Gene Abbreviation	Gene Name	Minor allele frequency (%) in our population	Variant Function	Allele Change
rs10883841	*NT5C2*	5'-nucleotidase, cytosolic II	11.3	Missense	G>A
rs3740387	*NT5C2*	5'-nucleotidase, cytosolic II	40.5	Synonymous	T>C
rs2229523	*NT5E*	5'-nucleotidase, ecto	32.0	Missense	G>A
rs2229524	*NT5E*	5'-nucleotidase, ecto	6.38	Missense	T>C
rs2231142	*ABCG2*	ATP-binding cassette, sub-family G, member 2	11.5	Missense	C>A
rs2274405	*ABCC4*	ATP-binding cassette, sub-family C, member 4	40.0	Synonymous	A>G
rs2274406	*ABCC4*	ATP-binding cassette, sub-family C, member 4	41.3	Synonymous	A>G
rs2274407	*ABCC4*	ATP-binding cassette, sub-family C, member 4	5.63	Missense	G>T
rs7867504	*SLC28A3*	Solute Carrier Family 28	27.7	Synonymous	A>G
rs7853758	*SLC28A3*	Solute Carrier Family 28	13.5	Synonymous	C>T
rs4525938	*DCK*	Deoxycytidine kinase	8.11	3'-UTR	A>T

Table 2.3: F-ara-A pharmacokinetic parameter estimates of model estimated parameters and bootstrap estimates in the development cohort

Parameters	Original Dataset (%RSE)	Bootstrap Estimates (95% C.I.)
Final Pharmacokinetic Parameters		
Cl_{nr} (L/hr) [a]	7.04 (14.1%)	6.95(5.01-9.01)
Cl_{slope} (L/hr) [a]	3.90 (25.2%)	4.02(1.99-5.98)
V1pop (L)	65.9 (2.90%)	65.9(62.2- 70.1)
Qpop (L/hr)	9.52 (6.20%)	9.58 (8.41-10.9)
V2pop (L)	67.2 (6.70%)	66.7 (56.7-77.5)
Between Subject Variability (BSV)		
BSV on Cl	0.07 CV% =26.5	0.07 (0.04-0.09) CV% =26.4 (20.0-30.0%)
BSV on V1	0.06 CV% = 24.5	0.06 (0.03-0.09) CV%=24.5 (17.3-30.0%)
Residual unexplained variability (RUV)		
RUV proportional	0.05 (15.4%) CV% =22.3	0.05 (0.03-0.07) CV% = 22.3 (17.3-26.5%)
RUV additive	11.2 ng/ml (27.9%)	10.5 ng/mL (3.73-16.5)

[a]F-ara-A Clpop is 10.94 L/hr which is a sum of estimate of Cl_{nr} (7.09 L/hr) for 70 kg IBW individual and Cl_{slope} (3.90 L/hr) for 70 kg IBW individual with CrCl of 85 ml/min.

$Cl_{nr:}$ is an estimate of non-renal clearance;

Cl_{slope} is an estimate of the change in renal clearance with a unit change in standardized renal function ($RenFunc_{std}$);

V1pop: Estimate of typical volume of distribution in central compartment;

Qpop: Estimate of typical inter-compartmental clearance;

V2pop: Estimate of typical volume of distribution in peripheral compartment.

% RSE is relative standard error

Table 2.4: Multiple regression analysis of TRM at day 100, months 6 and 12 with predicted F-ara-A clearance (Clpred) in the independent cohort

Variable	Hazard Ratio (95% CI) at day 100	p-value	Hazard Ratio (95% CI) at month 6	p-value	Hazard Ratio (95% CI) at month 12	p-value
F-ara-A Clpred						
<8.50 L/hr	1.00		1.00		1.00	
≥8.50 L/hr	0.10 (0.02-0.42)	<0.01	0.19 (0.05-0.70)	0.01	0.41 (0.17-1.00)	0.05
Donor source						
Related	1.00		1.00		1.00	
Unrelated (UR)	4.13 (1.03-16.6)	0.05	2.84 (1.05-7.69)	0.04	3.24 (1.53-6.99)	<0.01
UR cord blood	3.92 (1.28-12.0)	0.02	2.30 (0.93-5.69)	0.07	1.60 (0.75-3.39)	0.22
Disease risk						
Standard	1.00		1.00			
High	3.98 (0.80-19.7)	0.09	2.94 (0.95-9.12)	0.06	NA	NA
Comorbidity score						
0	1.00		1.00		1.00	
1-2	3.12 (0.93-10.5)	0.07	2.77 (1.19-6.48)	0.02	2.08(1.02-4.25)	0.04
≥3	2.20 (0.70-6.96)	0.18	1.48 (0.58-3.80)	0.41	1.66 (0.82-3.36)	0.16
Acute GVHD (grades II-IV) before TRM						
No	1.00		1.00		1.00	
Yes	2.40 (0.92-6.30)	0.07	2.62 (1.22-5.60)	0.01	3.23 (1.69-6.18)	<0.01

NA is not applicable and indicates that the covariate was not significant in the multivariate full model ($p > 0.20$) and was eliminated in the final reduced model.

Table 2.5: Multiple regression analysis of TRM at day 100, months 6 and 12 with predicted F-ara-A AUCpred in the independent cohort

Parameter	Hazard Ratio (95% CI) at 100 days	p-value	Hazard Ratio (95% CI) at 6 months	P value	Hazard Ratio (95% CI) at 12 months	p-value
F-ara-A AUCpred						
<6.00 (µg*hr/mL)	1.00		1.00		1.00	
≥6.00 (µg*hr/mL)	5.30 (1.59-17.7)	0.01	2.42 (0.87-6.77)	0.09	2.67 (1.31-5.43)	0.01
Donor source						
Related	1.00		1.00		1.00	
Unrelated (UR)	4.32 (1.16-16.06)	0.03	2.79 (1.06-7.31)	0.04	3.35 (1.59-7.05)	<0.01
UR cord blood	1.91(0.55-6.67)	0.31	1.71 (0.70-4.13)	0.24	1.33 (0.61-2.86)	0.47
Disease risk						
Standard	1.00		1.00			
High	2.84 (0.87-9.33)	0.08	2.27 (0.91-5.65)	0.08	NA	NA
Comorbidity score						
0						
1-2	1		1		1	
≥3	2.27 (0.72-7.13)	0.16	2.42 (1.04-5.62)	0.04	1.93 (0.93-4.01)	0.08
	2.83 (0.83-9.57)	0.10	1.72 (0.66-4.48)	0.27	1.80 (0.88-3.68)	0.11
Acute GVHD (grades II-IV) before TRM						
No	1.00		1.00		1.00	
Yes	1.89 (0.71-5.00)	0.20	2.35 (1.07-5.15)	0.03	3.04 (1.62-5.73)	<0.01

NA is not applicable and indicates that the covariate was not significant in the multivariate full model (p >0.20) and was eliminated in the final reduced model.

Table 2.6: Multiple regression analysis of acute GVHD (grades II-IV) at month 6 with predicted F-ara-A clearance (Clpred) in the independent cohort

Variable	Hazard Ratio (95% CI) of acute GVHD (grade II-IV) at 180 days	p-value
F-ara-A Clpred		
<13.0 L/hr	1.00	
≥13.0 L/hr	0.44 (0.19-1.02)	0.05
Stem Cell Source		
Related	1.00	
Unrelated (UR)	1.76 (1.08-2.87)	0.02
UR Cord Blood	0.75 (0.69-1.66)	0.75

Table 2.7: Number of patients in each group of F-ara-A Clpred and other covariates chosen for multiple regression analysis. Treatment-related mortality event rate at day 100, month 6 and 12

	TRM at Day 100			TRM at Month 6			TRM at Month 12		
Variable	**N in group**	**No. of Events**	**Event rate**	**N in group**	**No. of events**	**Event rate**	**N in group**	**No. of events**	**Event rate**
F-ara-A Clpred									
<8.50 L/hr	21	5	24%	21	5	24%	21	6	29%
≥ 8.50 L/hr	219	14	6%	219	25	11%	219	36	16%
Donor Source									
Related	101	4	4%	101	8	8%	101	12	12%
Unrelated (UR)	35	5	14%	35	8	23%	35	13	37%
UR cord blood	104	10	10%	104	14	13%	104	17	16%
Disease Risk									
Standard	100	5	5%	100	8	8%	100	15	15%
High	140	14	10%	140	22	16%	140	27	19%
Comorbidity Score									
0	103	5	5%	103	9	9%	103	14	14%
1-2	60	7	12%	60	12	20%	60	14	23%
≥3	66	7	11%	66	8	12%	66	13	20%

Acute GVHD (grades II-IV) before TRM									
No	142	8	6%	139	11	8%	139	14	10%
Yes	98	11	11%	101	19	19%	101	28	28%

Table 2.8 : Number of patients in in each group of F-ara-A AUCpred and other covariates chosen for multiple regression analysis. Treatment-related Mortality event rate at day 100, month 6 and 12

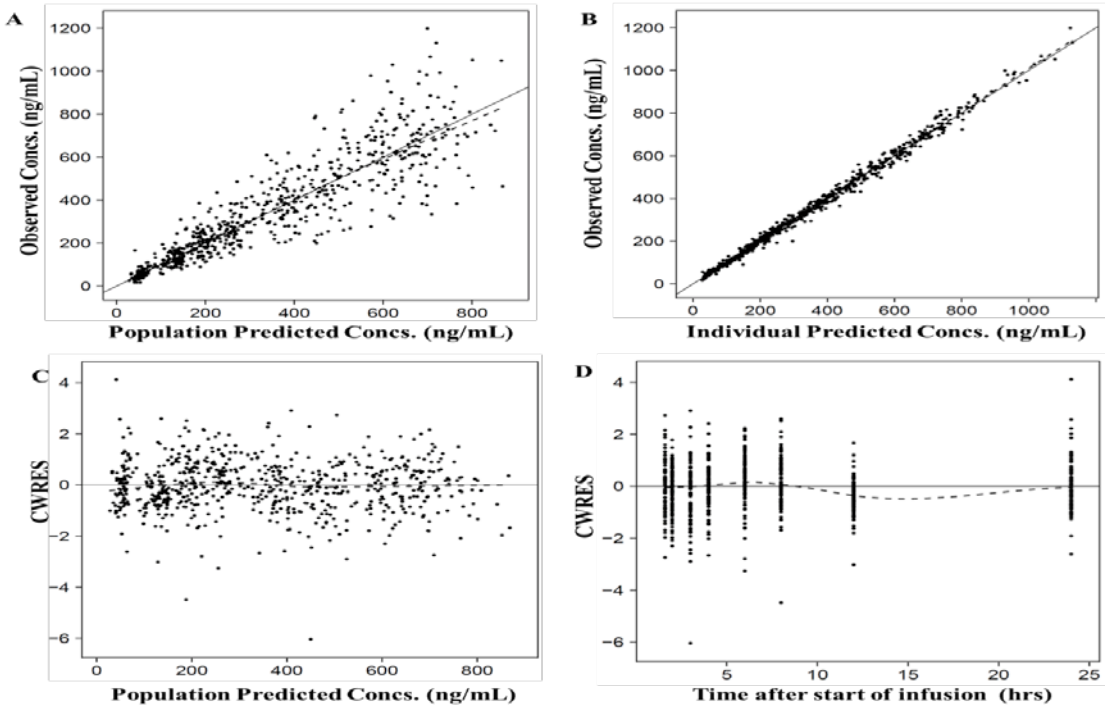
	TRM at Day 100			TRM at Month 6			TRM at Month 12		
Variable	**N in group**	**No. of events**	**Event rate**	**N in group**	**No. of events**	**Event rate**	**N in group**	**No. of events**	**Event rate**
F-ara-A AUCpred									
<6.00 µg*hr/mL	208	12	6%	208	23	11%	208	31	15%
≥ 6.00 µg*hr/mL	32	7	22%	32	7	22%	32	11	34%
Donor Source									
Related	101	4	4%	101	8	8%	101	12	2%
Unrelated (UR)	35	5	14%	35	8	23%	35	13	37%
UR cord blood	104	10	10%	104	14	13%	104	17	16%
Disease Risk									
Standard	100	5	5%	100	8	8%	100	15	15%
High	140	14	10%	140	22	16%	140	27	19%
Comorbidity Score									
0	103	5	5%	103	9	9%	103	14	14%

1-2	60	7	12%	60	12	20%	60	14	23%
≥3	66	7	11%	66	8	12%	66	13	20%
Acute GVHD (grades II-IV) before TRM									
No	142	8	6%	139	11	8%	139	14	10%
Yes	98	11	11%	101	19	19%	101	28	28%

Table 2.9: Number of patients in each group of Clpred and other covariate chosen for multiple regression analysis of acute GVHD (grade II-IV) at month 6

Variable	N in group	No. of events	Event rate
F-ara-A Clpred			
< 13.0 L/hr	214	96	45%
≥ 13.0 L/hr	26	6	23%
Donor Source			
Related	75	33	44%
Unrelated (UR)	165	69	42%
UR cord blood	103	45	44%

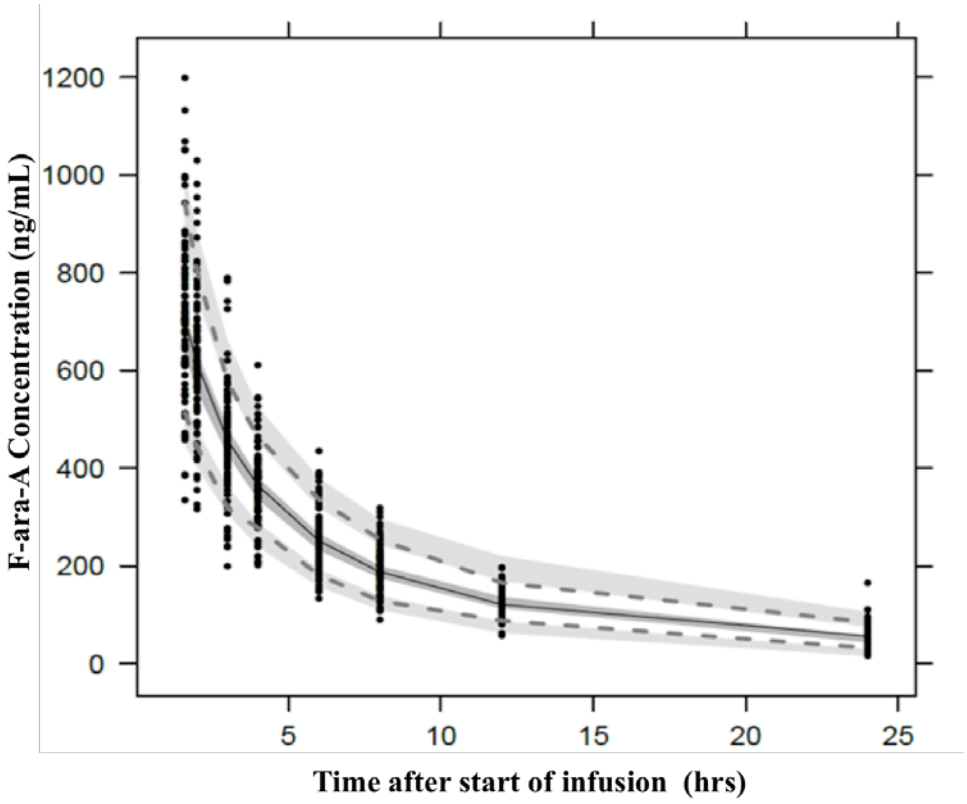
Figure 2.1 Goodness of fit plots of the final model



1A) Observed F-ara-A concentrations (ng/mL) vs Population predicted concentrations (ng/mL) and 1B) Observed F-ara-A conc. (ng/mL) vs Individual predicted concentrations (ng/mL). The black dots represent the observed F-ara-A concentrations, the solid line represents the line of unity and the dashed line represents the loess smooth. Since the observed data (black dots) are evenly scattered around the line of identity (solid line) it suggests that the model sufficiently explains the observed data.

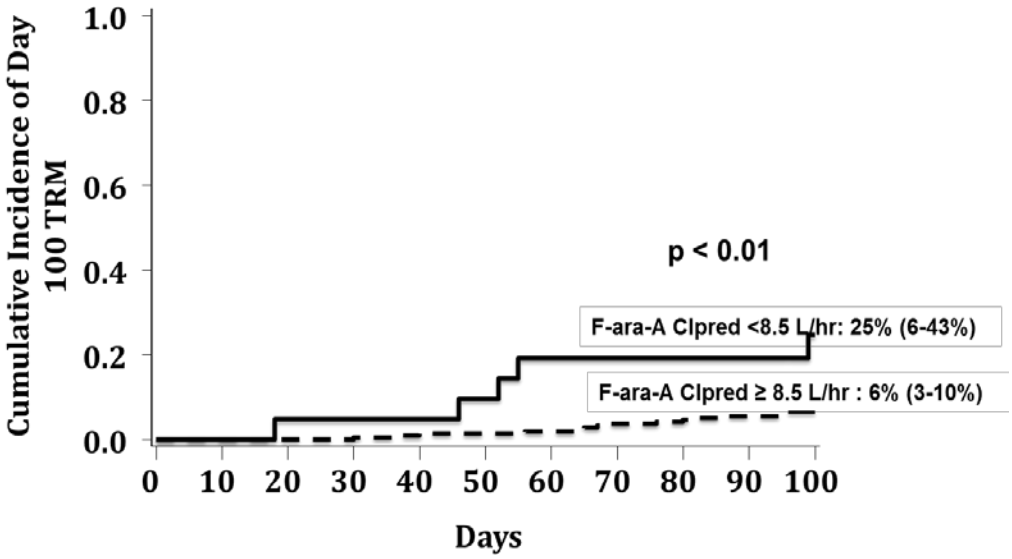
1C) Conditional weighted residuals (CWRES) vs population predicted concentrations (ng/mL) and 1D) CWRES vs time after the start of infusion (hrs). The dots represent the observed F-ara-A concentrations, the solid line is the line at y=0 and the dashed line represents the loess smooth. The plots show lack of any specific trends and thus provide evidence of no model misspecification.

Figure 2.2: Visual predictive check of the final model



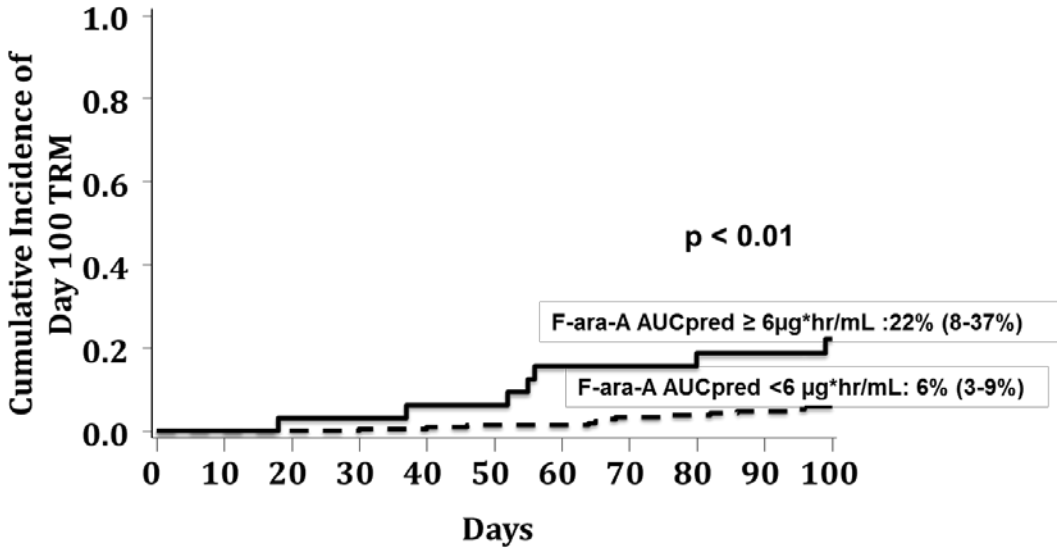
The solid black line represents the median of the observed F-ara-A plasma concentrations obtained from 87 subjects. The grey area around the solid black line is the 95% confidence interval for the median F-ara-A plasma concentrations, obtained from the simulation-based prediction. The 5^{th} and 95^{th} percentiles of the observed F-ara-A plasma concentrations are represented by dashed lines below and above respectively. The grey shaded areas around dashed lines represent 95% confidence intervals for corresponding 5^{th} and 95^{th} prediction intervals obtained from the simulations. Finally the black filled circles represent the observed F-ara-A plasma concentrations from the 87 subjects.

Figure 2.3: Cumulative incidence of TRM at day 100 above and below F-ara-A Cl cutpoint (8.5 L/hr)



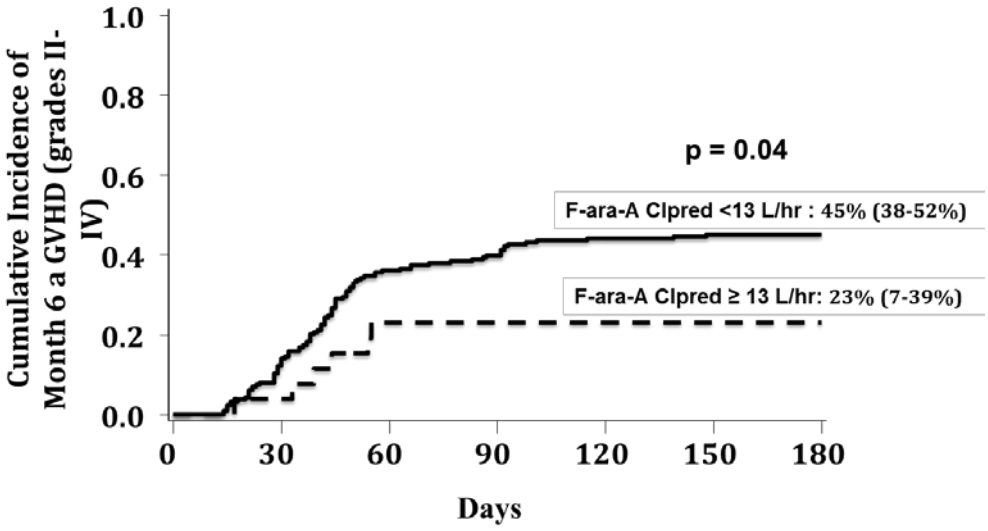
Cumulative incidence of TRM day 100 after reduced intensity conditioning HSCT in patients with first dose F-ara-A Clpred <8.50L/hr (cumulative incidence [95%CI] 25% [6-43%]) compared to patients with F-ara-A Clpred ≥ 8.50 L/hr (cumulative incidence [95%CI]: 6% [3-10%]).

Figure 2.4: Cumulative incidence of TRM at day 100 above and below F-ara-A AUC cutpoint (6 µg*hr/mL)



Cumulative incidence of TRM day 100 after reduced intensity conditioning HSCT in patients with first dose F-ara-A AUCpred ≥ 6.00 µg*hr/mL (cumulative incidence [95%CI] 22% [8-37%]) compared to patients with F-ara-A AUCpred <6.00 µg*hr/mL (cumulative incidence [95%CI] 6% [3-9%]).

Figure 2.5: Cumulative incidence of 6 month acute GVHD above and below F-ara-A Cl cutpoint (13 L/hr)



Cumulative incidence of acute GVHD (grades II-IV) at month 6 after reduced intensity conditioning HSCT in patients with first dose F-ara-A Clpred <13.0 L/hr (cumulative incidence [95%CI] 45% [38-52%]) compared to patients with F-ara-A Clpred ≥13.0 L/hr (cumulative incidence [95%CI] 23% [7-39%]).

CHAPTER III

3 PROSPECTIVE PHARMACOKINETIC STUDY TO EVALUATE FACTORS INFLUENCING VARIABILITY IN PHOSPHORAMIDE MUSTARD EXPOSURE AND RESPONSE IN HSCT RECIPIENTS UNDERGOING REDUCED INTENSITY CONDITIONING

3.1 INTRODUCTION

Cyclophosphamide (Cy) is an anti-tumor alkylating agent widely used in reduced intensity conditioning regimens with other chemotherapeutic agents and/or radiation. Cyclophosphamide is a prodrug that undergoes several enzymatic biotransformation steps to its active metabolite, phosphoramide mustard (PM). On administration, nearly 70-80% of Cy is converted to 4-hydroxy cyclophosphamide (4-HCy) primarily by hepatic CYP2B6. 4-hydroxy cyclophosphamide is highly unstable and undergoes non-enzymatic conversion to acrolein and PM. 4-hydroxycyclophosphamide is also inactivated to ketocyclophosphamide and o-carboxycyclophosphamide (CEPM) by aldehyde dehydrogenase (ALDH1). A minor route (~5%) of Cy elimination is oxidation to deschloroethyl cyclophosphamide (DCECP) mediated by CYP3A4. Around 20% of the Cy (unchanged and metabolites) elimination is through renal mechanisms. Phosphoramide mustard further undergoes non-enzymatic conversion to nor-nitrogen mustard (NOR) and both PM and NOR alkylate the N-7 position of guanine nucleotides on DNA. The immunosuppressive and anti-tumor activity of Cy is attributed to formation of G-NOR, G-NOR-OH and G-NOR-G DNA adducts that prevent DNA strand separation and replication leading to cell death. (129, 292, 293) Pharmacokinetic pathway of Cy is shown in Figure 1.3.

Cyclophosphamide exhibits high PK variability. In an extensive review published on Cy clinical PK, clearance (Cl) in adults reported by different studies ranged from 2.5 L/hr to 12.6 L/hr and volume of distribution (V) ranged from 25.2 L to 73.5L.(292) Cyclophosphamide is a substrate for CYP2B6, and it is known to induce the enzyme (auto-inducer). When administered as multiple dose therapy, Cy Cl is estimated as a sum

of inducible and non-inducible Cl. In a population PK study conducted in breast cancer recipients receiving Cy, inter-individual variability in non-inducible, inducible clearance and 4-HCy Cl was found to be ~23, 27% and 31%.(124) In patients receiving Cy chemotherapy for treatment of ovarian cancer, variability in Cy clearance was found to be 34% and metabolite exposures (PM, 4-HCY and 2-DCECP) varied by 9 fold.(294) In studies conducted in HSCT recipients, variability in non-inducible, inducible Cl and V was estimated to be 52.8-112%, 45-200% and 18%.(33, 120) In other studies in HSCT recipients, fold variability in plasma AUC of Cy, 4-HCy and CEPM was 1.5-3, 7.8 and 4-16 fold variable in a HSCT recipients receiving myeloablative dose of Cy.(115, 129) Studies conducted to explain factors influencing variability of Cy exposure are extensive. Single nucleotide variants associated with Cy metabolism, transport and elimination are important factors influencing variability in some studies.(122-124, 295-307) Other clinical factors such as body weight, age, serum albumin and creatinine clearance (CrCl) have also been found to significantly influence Cy and metabolite PK.(127, 301, 308) Co-administration of drugs such as fluconazole, itraconazole, busulfan, thiotepa and others that are substrates, inducers and/or inhibitors of CYP enzymes are also responsible for altered Cy metabolism and disposition.(119, 126, 309, 310)

Variability in exposure leads to variability in response to the drug. Toxicities such as cardiotoxicity, liver toxicity such as veno-occlusive disease (VOD) and hemorrhagic cystitis are observed in patients treated with Cy.(129, 311, 312) However due to the complex metabolic pathway of Cy, there is no clear understanding of which metabolite(s) is responsible for drug toxicity. In an *in vitro* study conducted in bovine artery pulmonary endothelial cells cultured with hepatic microsomal enzyme system, 4-HCy and acrolein

caused significant cell injury in a concentration dependent manner, indicating mechanism of Cy induced lung toxicity.(313) Another study postulated that profound depletion of glutathione in sinusoidal cells due to 4-hydroxypercyclophosphamide and acrolein might be the mechanism of VOD that often occurs with high Cy dose.(314) Clinical PK pharmacodynamic studies have been conducted to explain the relationship between drug and/or metabolite exposure and toxicity. In breast cancer patients, higher plasma 4-HCy AUC was associated with VOD, but PM AUC was not.(130) The association of 4-HCy and VOD was also shown in other study.(131) An inverse correlation was observed between Cy AUC and heart failure in patients treated with Cy for breast cancer. The authors speculate faster Cl of Cy to 4-HCy in patients with heart failure.(132) In 147 HSCT recipients receiving high dose Cy (120 mg/kg), an increased exposure to CEPM was associated with higher risk of developing liver toxicity.(129) Low Cy Cl to the active metabolite was associated with high recurrence of disease in children with non-Hodgkin lymphoma receiving HSCT.(133)

Many studies have attributed toxicity of Cy to 4-HCy or CEPM, although mechanism of toxicity due to these metabolites is unclear. 4-hydroxy cyclophosphamide is rapidly converted to PM both in plasma and intracellulary. Plasma PM cannot cross the biological membranes due to its high hydrophilic nature, thus plasma 4-HCy is may be a marker of efficacy and toxicity caused due to PM concentrations intracellularly. A phase I clinical PK study was conducted to evaluate the combination of a chemosensitizing agent, SR-2508, with Cy. It was found that PM was a major circulating metabolite in plasma with a half –life of ~15 hours with 30 fold higher AUC than 4-HCy.(315) Further PM exhibited the highest alkylating activity of all the other metabolites measured in

plasma. Hence the authors disagree with previous reports and argued that plasma PM may be an important biomarker of efficacy and toxicity.

Pharmacokinetics of plasma PM could serve as an important biomarker for Cy related clinical outcomes and to our knowledge no study has been conducted to evaluate this in patients undergoing reduced intensity conditioning prior to allogeneic HSCT. Therefore we have undertaken a study to identify the relationship between plasma PM PK and outcomes after HSCT in patients receiving reduced intensity conditioning. Understanding clinical factors associated with variability in PM exposure after reduced intensity conditioning is necessary to implement strategies to control Cy exposure and improve outcomes.

3.2 METHODS

3.2.1 Patients

This is an ongoing single center prospective observational PK study of PM conducted in allogeneic HSCT recipients. All patients received HSCT with reduced intensity conditioning regimen. Institutional Review Board and Cancer Protocol Review Committee approved the study and all patients provided informed consent. The conditioning regimen was i.v. Cy (50 mg/kg/day for 1 day on day -6), i.v. Flu (25-40 mg/m2/day for 5 days on days -6 to -2) and TBI (200 cGy as a single fraction on day -1). Post-transplant GVHD prophylaxis included immunosuppressant combination of either cyclosporine /mycophenolate or sirolimus/mycophenolate. Data from the first 70 patients enrolled from March 2013 to December 2015 were included in this analysis. Subject characteristics are shown in

Table 3.1. Cyclophosphamide was administered intravenously over 2hr at a constant rate and PK sampling was conducted at 2, 4, 6, 21, 24 and 45 hrs after the end of infusion.

3.2.2 Bioanalysis

Blood was collected in 8 mL heparinized green top BD Vacutainer tube at each sampling point for PM PK. Samples were centrifuged to collect plasma within 30 minutes of collection at 3400 rpm for 10 min at 4 degree C and frozen until further use. Phosphoramide mustard in plasma was stable for an hour at room temperature, no degradation was observed. The extraction and detection assay was based on previously described method. (316) Phosphoramide mustard was detected and quantified using HPLC (Agilent 1200 Series, Santa Clara CA) with UV detection at 276 nm. Plasma samples were thawed, pretreated with diethyldithiocarbamate (DDTC) for derivatization at 70 degrees C for 10 min. The derivatized PM was extracted from plasma using protein precipitation method with acetonitrile as solvent. Internal standard used was 3-isobutyl-1-methylxanthine. The HPLC separation was done using a mobile phase mixture of 68% acetonitrile and 32% 10mM potassium phosphate at pH 8.0. A Phenomenex Luna C18 column (150 mm X 4.6 mm, 5 micron particle size) was used for HPLC separation. The chromatographic conditions included a flow rate of 1 ml/min with a total run time of 10 min. The assay was linear in the range of 50 to 10000 ng/ml. The average assay accuracy was 100.4% and the total assay variability was 5.9%.

3.2.3 Pharmacokinetic Analysis

The first objective of the study was to identify the relationships between plasma PM exposure and clinical outcomes and was conducted in the first 40 patients. Plasma PM concentrations were analyzed using non-compartmental methods (Phoenix^{TM} Winonlin) for the first 40 patients. Area under the curve $AUC_{(0-\infty)}$ was calculated using linear up/log down trapezoidal method as AUC (0-t) + C (t)/ke where t is the last observed concentration and Ke is the terminal first order elimination rate constant. Partial areas such as $AUC_{(0-6)}$, $AUC_{(0-26)}$ and $AUC_{(0-47)}$ were also calculated.

3.2.4 Statistical Analysis For Evaluating Relationship Between PM Exposure (AUC) And Outcomes

Phosphoramide mustard AUCs calculated by NCA were associated with TRM, acute GVHD and engraftment. Data for time to TRM, acute GVHD and engraftment was obtained through transplant database. Treatment related mortality was defined as death due to any cause other than relapse or disease progression. Acute GVHD to month 6 was staged and graded according to the standard GVHD criteria based on clinical and pathological manifestations. Day of neutrophil engraftment was the first of 3 consecutive days of an absolute neutrophil count of >500 cell/uL by day 42.

Recursive partitioning regression analysis was conducted to identify optimal cut points of PM AUCs towards TRM (at day 100 and month 6) and acute GVHD (II-IV) at month 6 and engraftment at day 42. We tested AUCs ($AUC_{(0-6)}$, $AUC_{(0-26)}$, $AUC_{(0-47)}$ and $AUC_{(0-\infty)}$) for its association towards outcomes. Once the optimal cutpoints were chosen, cumulative incidence of TRM, acute GVHD and engraftment above and below

each cut point were calculated using death prior to event as competing risk by using proportional Fine and Grays method.(284)

3.2.5 Population Pharmacokinetic Model Building and Identification Of Covariates Influencing PM Pharmacokinetics

3.2.5.1 Model development

The objective of the population PK analysis was to understand PM PK, evaluate inter-individual variability in PM PK parameters and identify important clinical factors explaining the variability. Population PK analysis was conducted using 381 concentrations obtained from 70 subjects. The mean (standard deviation) of plasma PM concentrations at time 2, 4, 6, 21, 24 and 45 hours after the end of infusion were 5140 (1723), 5086 (1588), 4638 (1285), 1490 (667.6), 984.0 (490.1) and 106.1 (49.29) ng/mL respectively. Phosphoramide concentrations in 30 patients at time 45 hours after the end of infusion were below the limit of quantification and hence were not included in the analysis.

Nonlinear mixed effect modeling using NONMEM (version 7.2 ICON Development Solutions, Hanover, MD, USA) was used to perform the population PK analysis. Inspection of concentration time profile, model diagnostics and model evaluation were performed using PSN and Xpose (version 4.3.2) packages through Pirana workbench (2.7.2 Amsterdam, Netherlands). Several models were tested to explain the observed PM concentration time data. Initially, a transit compartment model was considered to explain the sequential conversion from the parent Cy to the metabolite PM. However on exploring the concentration time profile we observed that very few data

points were available to evaluate the rise in PM concentrations from Cy. To avoid problems with overparameterization, simpler models were considered. A model similar to that used for first order absorption can explain the metabolite PK of an intravenously administered parent drug. One and two compartment models were tested to identify the model that best explained the observed data. Base model selection was based on diagnostic plots, OFV for nested models and Akaike information criteria for non-nested models. Between-subject variability was modeled exponentially to the PK parameters as shown in equation 1.

$Pj = TVP \times \exp(\eta_j)$ *(equation 1)*

Where Pj is the parameter estimate of the jth individual, TVP is the typical value of the parameter in a population. η_j is the estimate of the deviation of individual j from the TVP and is assumed normally distributed with mean of zero and variance of omega2. A proportional error model was used to explain the residual unexplained variability (RUV) as shown in equation 2.

$Cobs_{,ij} = Cpred_{,ij} \times (1 + \varepsilon_{ij})$ *(equation 2)*

where, $Cobs_{,ij}$ is the observed concentration in the ith individual, $Cpred_{,ij}$ is the jth predicted concentration in the ith individual and εij is the residual error assumed to be independent and normally distributed with a mean zero and variance of σ^2. First order conditional estimation with interaction was used for model development.

Age, gender, creatinine clearance (CrCl) calculated by Cockroft and Gault equation using actual body weight (WT), total bilirubin, serum albumin, serum creatinine, total protein, alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase were tested to explain the observed variability in PM PK

parameters. All the covariates were tested as continuous variable, except gender that was evaluated as categorical covariate. A step-wise covariate model building strategy of forward inclusion and backward elimination was used to identify the effect of clinical covariates on PM PK. An objective function decrease of ≥ 3.84 (X2, df=1, $p<0.05$) was chosen for forward selection and an increase of ≥6.63 (X2, df=1, $p<0.01$) was chosen for backward elimination during the covariate analysis step.

3.2.5.2 Model evaluation

Visual predictive check was used to evaluate if the final model adequately described the observed data. One thousand simulations were generated using the final model, and the 5^{th}, 50^{th} and 95^{th} percentile bands of the simulated predictions along with their 95% prediction intervals were plotted. The final model was also evaluated using a non-parametric bootstrap approach. A method of sampling with replacement was used to generate 1000 datasets and the model applied to each dataset to evaluate the robustness and reliability of the estimated PK parameters.

3.3 RESULTS

3.3.1 The Relationship Between PM AUC With Clinical Outcomes

The median (range) of PM $AUC_{(0-6)}$, $AUC_{(0-26)}$, $AUC_{(0-47)}$, $AUC_{(0-\infty)}$, and $t_{1/2}$, in 40 patients were 21.6 (9.13-50.9) μg-hr/mL, 72.8 (35.2-123) μg-hr/mL, 85.7 (42.7-129) μg-hr/mL, 85.3 (43.8-140) μg-hr/mL and 6.45 (2.82-13.3) hr.

The cumulative incidence of TRM was 13% at day 100 and 20% at month 6. The median (range) time to TRM was 115 (37-319) days. Optimal cut-points for $AUC_{(0-6)}$, $AUC_{(0-26)}$, $AUC_{(0-47)}$, $AUC_{(0-\infty)}$ towards TRM at day 100 and month 6 were 20 μg-hr/mL,

85 μg-hr/mL, 90 μg-hr/mL and 90 μg-hr/mL. High PM exposure was associated with higher incidence of TRM. In univariate analysis, the cumulative incidence [estimate (95% CI)] of TRM at day 100 was higher in HSCT recipients with PM $AUC_{(0-6)} \geq 20$ μg*hr/mL compared to those with PM $AUC_{(0-6)}<20$ μg*hr/mL [estimate (95% CI): 22 (5-38%) vs 0%, p =0.05]. Similarly high PM $AUC_{(0-26)} \geq 85$ μg*hr/mL was also associated with higher cumulative incidence of TRM [estimate (95% CI): 36(9-64%) vs 4 (0-11%), p <0.01). Figure 3.1 A shows the results of cumulative incidence of TRM at day 100 above and below $AUC_{(0-26)}$ cutpoints. $AUC_{(0-47)}$, $AUC_{(0-\infty)}$ were not associated with TRM at day 100. At 6 months, the cumulative incidence of TRM was higher in patients with $AUC_{(0-26)} \geq 85$ μg*hr/mL [47 (17-77 %) vs 14 (0-28%), p= 0.02)]. Figure 3.2 shows the results of cumulative incidence of TRM at day 100 above and below $AUC_{(0-26)}$ cutpoints. $AUC_{(0-6)}$, $AUC_{(0-47)}$, $AUC_{(0-\infty)}$ were not associated with TRM at month 6. Table 3.2 and Table 3.3 show the number of HSCT recipients in each AUC group and the estimate of cumulative incidence of TRM at day 100 and month 6 respectively.

In univariate analysis the cumulative incidence of acute GVHD grade (II-IV) was 38% at month 6. The median (range) time of acute GVHD (II-IV) in patients who experienced the event was 37 (14-161) days. None of the PM AUC markers were associated with cumulative incidence of acute GVHD. Table 3.4 shows the number of HSCT recipients in each AUC group and estimate of cumulative incidence of acute GVHD at month 6 respectively.

Neutrophil engraftment was high and achieved in 93% of patients by day 42, and hence was not evaluated due to very few events. The median (range) time of engraftment observed in patients in the study was 11 (6-43) days.

3.3.2 Development And Evaluation Of PM Population Pharmacokinetics Model And Influence Of Covariates on PM Pharmacokinetics

3.3.2.1 Model development

The population PK model was developed in 70 HSCT recipients. One-compartment (ADVAN2 TRANS2) and two-compartment (ADVAN4 TRANS4) models with conversion rate from Cy to PM were tested to explain the observed PM concentrations over the sampling time. The exploration of the log of observed plasma PM concentrations vs time after the end of infusion suggested a one-compartment model with would best fit the observed data. The two-compartment model did not improve the fit, and hence one-compartment model with an exponential BSV and a proportional RUV variability best described the observed data. Since the dose of the metabolite and the percentage conversion from parent Cy to metabolite PM is unknown, the total clearance obtained from the model is apparent (ratio of systemic clearance over fraction of the metabolite formed from the parent (fm)) and is designated as Cl/fm. Similarly volume of distribution in the central compartment is described as apparent and designated as V/fm. The rate constant of conversion from parent to the metabolite is designated as kfm.

Creatinine clearance significantly influenced TVCl/fm and gender significantly influenced TVV/fm and hence were included in the final full model. We allometrically scaled TVCl/fm and TVV/fm to actual body weight (WT) with an exponent of 0.75 and 1, respectively and used it as base model for covariate analysis. No other covariates were important towards TVCl/fm and TVV/fm. Also we did not find any covariate significant towards kfm. The final model parameters with their relative standard error (%RSE) are shown in Table 3.5. The NONMEM code for the final model is shown in Appendix 8.2.

The final equations obtained from the model are described in the equations 3, 4, 5, 6 and 7.

TVkfm is the typical population value of the rate constant of conversion from Cy to PM and was estimated by THETA(1) (equation 3).

TVkfm = THETA(1) *(equation 3)*

The typical value of the total apparent clearance (TVCl/fm) was modeled as a sum of apparent non-renal Cl (CL_{nr}) and apparent renal Cl (Cl_{slope} x standardized renal function [RF_{std}]) clearance that changed with changes in CrCl (equation 4). The RF_{std} is weight (WT) standardized to eliminate its correlation with CrCl (Cockroft Gault equation includes weight in calculation of CrCl) (equation 5). Further this standardization method gives us the true estimate of renal function

$TVCL/fm = (Cl_{nr} + Cl_{slope} \times RF_{std}) \times (WT/83.5)^{0.75}$ *(equation 4)*

$RF_{std} = (CrCl/104.2) \times (83.5/WT)$ *(equation 5)*

Typical value of apparent volume of distribution TVV/fm was estimated from THETA (4) and was allometrically scaled using WT (equation 5).

TVV/fm = THETA (4) x WT/83.5 *(equation 6)*

THETA (6) is the parameter estimate for proportional change in TVV/fm if the patient was female (eq. 4).

IF (Gender= Female) TVV/fm= TVV/fm * THETA(6) *(equation 7)*

The final estimate of TVKfm was 0.14 hr^{-1}. The estimate of CL_{nr} was 23.9 L/hr for a typical person with a median weight of 83.5 kg and that of renal clearance was 21.9 L/hr for a typical person with weight of 83.5 and CrCl of 104.2 ml/min. Hence TVCl/fm

for a typical person is 45.8 L/hr as per equation 4 and renal function accounts for 47% of the total apparent clearance. For this typical person, every 10 unit decrease in CrCl, total PM TVCl/fm decreases by 2.1 L/hr.

The estimate of TVV/fm was 240 L for males. Females had 25% (estimate of 0.75) lower TVV/fm (180 L). The inter-individual variability obtained after inclusion of covariates was 24.6% for kfm, 22.5% for Cl/fm and 41.9% on V/fm. The residual unexplained variability was ~14%. The diagnostic plots were used to examine the goodness of fit of the model. Figure 3.3 shows the diagnostic plots from the final model and model adequately explains the observed data.

3.3.2.2 Model evaluation

3.3.2.2.1 Non-parametric bootstrap

Final population PK model was evaluated for its reliability with non-parametric bootstrap. Out of 1000 datasets generated, 905 minimized successfully (Table 3.5). The fixed effect estimates obtained from the final model were included in the confidence intervals obtained from bootstrap and hence the model is reproducible. The median of the bootstrap estimate of CL_{nr} was higher whereas of that Cl_{slope} was lower than that obtained by model-derived estimates. Estimates of TVkfm and TVV/fm and random effects were comparable to the median of bootstrap estimates.

3.3.2.2.2 Visual predictive check

Figure 3.4 shows that the median of the observed data (solid and dashed black line) lays within the prediction intervals (grey shaded areas). Therefore the model adequately explains the observed data.

3.4 DISCUSSION

The complex metabolic pathway along with, high inter-individual PK variability in parent and metabolites and the formation of active and inactive metabolites makes dosing and therapeutic monitoring challenging. Cyclophosphamide is itself inactive, and hence its efficacy and toxicity is attributed to metabolites. Most of the previous studies have evaluated the role of 4-HCy exposure towards efficacy and toxicity. (130, 132, 317) However, the metabolite is highly unstable and is rapidly converted to PM and acrolein. Therefore accurate measurement of the metabolite is difficult and cannot be easily used in clinical practice. We therefore chose to evaluate plasma PM profile since it is also a primary active metabolite and substantially more stable.

We found that higher plasma PM exposure $AUC_{(0-6)}$ and $AUC_{(0-26)}$ was associated with higher incidence of TRM at day 100 and that $AUC_{(0-26)}$ was significant towards TRM at month 6. No other PK studies have identified the relationship between plasma PM concentrations and TRM. Infact, most studies argue that inability of plasma PM to cross the cell membranes makes it a futile marker to predict Cy related outcomes. A study conducted by Chan et al, however counters this opinion and their study showed that PM was a major circulating metabolite in plasma with its AUC 30 fold higher than 4-HCy. Further the study also demonstrated that only high plasma PM AUC correlated with high 4-(p-nitrobenzyl) pyridine activity, which measures the over alkylating index of the drug (Cy). Although PM itself cannot cross cell membranes, it is unknown if it is a substrate of uptake transporters. However other nitrogen mustards such as bendamustine and melphalan are found to be substrates of organic anion transporters. (318, 319)

Since our data showed an association between PM PK and clinical outcomes we next conducted a population PK study to understand the factors contributing to variability observed in PM exposure that impacts outcomes. We found 24.6%, 22.5% and 41.9% between subject variability in TVkfm, TVCl/fm and TVV/fm, respectively. The formation rate constant of PM (0.14 L/hr) was greater than the elimination rate constant (0.19 hr-1, Cl/fm/V/fm). A PK study showed that PM had a parallel elimination half-life as that of Cy, indicating formation rate limited metabolite kinetics.(294) In yet another study a rapid elimination was observed for PM, and PM concentrations reached its max in 0.75 hours following an IV administration. The half-life of the PM was ~8 hours, which was similar to the half –life of Cy, again establishing that PM follows the formation rate limited metabolism.(320) This indicates that although the half-life of PM would be much shorter than the parent drug, variability in the rate and extent of conversion from Cy to PM would be an important factor that could alter PM exposure.

We allometrically scaled PM TVCl/fm and TVV/fm to actual body weight since dose reductions in obese are unclear for this drug. The ASBMT guidelines recommend dosing based on total body weight up to a dose of 200mg/kg and recommend dose adjustments in patients with a weight greater than 120% of IBW.(273) In retrospective studies conducted to study the effect of body weight and outcomes, no significant difference was observed in overall survival and event free survival in obese vs normal weight patients.(321, 322) In fact a shorter time to engraftment was observed in obese patients in comparison to normal weight patients. In a previous study conducted by our group it was observed that although obese patients received ~45% higher dose of Cy, plasma AUC was ~60% lower. Further G-NOR-G adducts normalized to Cy plasma

concentrations were found to be twice higher in obese patients as compared to lean.(323) However in another study, obese autologous HSCT recipients had a higher risk of TRM as observed in obese compared to non-obese.(324)

We also found that males had significantly higher apparent volume of distribution than females after accounting for weight in the model. This indicates that males either have a larger volume of distribution (V) or lower conversion from parent to the metabolite compared to females (fm). Expression of CYP2B6 enzyme which is involved in conversion to PM is higher in females than males, and hence males would have a lower fm compared to female thereby higher V/fm.(325)

We found CrCl to significantly influence PM Cl, where renal function accounted for 47% of the total apparent clearance. This would significantly increase PM exposure in patients with renal impairment. Few studies have evaluated PM PK in cancer patients and the influence of renal function. A PK study demonstrated a higher PM half-life in patients with renal insufficiency (CrCl <51 ml/min) than those in normal subjects [13 hrs vs 8 hrs].(326) In a patient with renal insufficiency (CrCl of 38 ml/min) who received high dose Cy (1550 mg/m2) Cy elimination was reduced and 4-HCy concentrations were increased by 11%(128) Based on our results, we expect that PM exposure is higher in patient with poor CrCl and that this exposure is associated with higher risk of TRM. Dose reductions should be considered in patients with poor CrCl.

Metabolism of Cy to PM involves several CYP enzymes and many studies have explored the influence of pharmacogenetic variants on Cy and metabolite clearance. Single nucleotide polymorphisms in *CYP2B6*, *CYP2C9*, *GST1A* were found to influence towards Cy PK(123, 327-331) SNPs are also shown to influence Cy related relapse in

HSCT recipients.(332) We have obtained pre-transplant DNA from all patients, which will be in future for its association with PM PK.

Our group has also developed an analytical assay to measure G-NOR-G adducts in DNA isolated from the buffy coat.(323) We also demonstrated higher G-NOR-G adduct concentrations in patients with Fanconi anemia who are inherently unable of DNA repair.(333) Thus measuring DNA adducts would be most physiologically relevant to measure Cy efficacy and toxicity. Currently no studies have been conducted to correlate PM plasma concentration to G-NOR-G. Cyclophosphamide exerts is cytotoxicity by forming adducts with DNA and thus halting DNA replication. Thus measuring adducts is physiologically relevant to indicate towards the cytotoxicity induced post Cy administration and thereby its efficacy and toxicity. We plan to evaluate relationship of adduct formation over time with that of plasma PM concentrations. The results from this study will further strengthen our hypothesis, that plasma PM concentrations are important and could be a promising to measure Cy related efficacy and toxicity.

Studies have attempted to propose personalized dose of Cy using metabolism based and bayesian based approaches.(334-337) In HSCT recipients, a limited sampling after the first dose of Cy was conducted to obtain maximum a posteriori estimates of individual PK parameters and subsequently used to personalize the 2^{nd} dose.(335) The study showed a significant decrease in post-transplant serum bilirubin levels and a 38% reduction in the hazard of acute kidney injury. However the study did not show an improvement in non-relapse or overall survival rates in patients who received the personalized dose. Patients in our study received only a single dose of Cy and none of

these studies have attempted to individualize the first Cy dose utilizing clinical and genetic factors influencing variability.

In conclusion we conducted an exploratory analysis to identify the relationship between plasma PM exposure and clinical outcomes in HSCT recipients. We identified that in HSCT recipients, high PM exposure was associated with higher cumulative incidence of TRM. We also found that inter-individual variability observed in PM PK could be partially explained by renal function and gender. In the future we plan to evaluate additional covariates to better explain more of the observed variability in PM PK. Our current findings are in agreement with the previous studies that plasma PM is a major circulating metabolite and could serve to be good biomarkers for chemotherapy function in HSCT.

Table 3.1: Subject characteristics

	PM Exposure Response Cohort (Subset) Median (range)/N (%)	**PM Population PK Cohort (All Subjects) Median (range)/N (%)**
Number of Patients	40	70
Administered single day Cy dose (mg), median (range)	3977.5 (2395-6300)	3942.5 (2395-6300)
Age (years), median (range)	62 (21-72)	62.5 (21-73)
Males, N (%)	22 (55%)	38 (54.2%)
Actual body weight (kg), median (range)	83.7 (47.9-117)	83.5 (47.9-177.8)
Serum creatinine (mg/dL), median (range)	0.81 (0.30-1.58)	0.81 (0.32-1.58)
Creatinine clearance (ml/min), median (range)	109 (65-309.5)	104.2 (46.2-309.5)
Total bilirubin (mg/dL), median (range)	0.50 (0.2-2.30)	0.50 (0.20-2.30)
Total protein (g/dL), median (range)	6.7 (5.3-8.3)	6.7 (5.4-8.3)
Total albumin (g/dL), median (range)	3.8 (3-4.4)	3.7 (2.7-4.8)
Alkaline phosphatase (units/L), median (range)	81.5 (42-132)	82 (18-132)
ALT (units/L), median (range)	32.5 (20-68)	32 (12-88)
AST (units/L), median (range)	29 (14-57)	28 (10-97)
Donor Source, N (%)		
Cord blood	13 (32.5%)	27 (38.4%)
Peripheral Blood Stem cell	22 (55%)	30 (43.0%)
Bone Marrow	5 (12.5%)	13 (18.6%)

Table 3.2: Number of patients and estimates of relative risk of TRM at day 100 in each group of PM AUC chosen for univariate regression analysis

Variable	N in group	No. of Events	Event rate	Relative Risk Estimate (95% CI)	p-value
AUC 0-inf					
<90 μg-hr/mL	23	1	4%	5% (0-14%)	0.08
≥ 90 μg-hr/mL	17	4	24%	24% (4-43%)	
AUC 0-45					
<90 μg-hr/mL	25	1	4%	4% (0-13%)	
≥ 90 μg-hr/mL	15	4	27%	27%(5-49%)	0.05
AUC 0-24					
<85 μg-hr/mL	29	1	3%	4% (0-11%)	
≥ 85 μg-hr/mL	11	4	36%	36% (9-64%)	<0.01
AUC 0-6					
<20 μg-hr/mL	16	0	0%	0%	
≥ 20 μg-hr/mL	24	5	21%	22% (5-38%)	0.05

Table 3.3: Number of patients and estimates of relative risk of TRM at 6 months in each group of PM AUC chosen for univariate regression analysis

Variable	N in group	No. of Events	Event rate	Relative Risk Estimate (95% CI)	p-value
AUC 0-inf					
<90 μg-hr/mL	23	3	13%	18% (0-36%)	0.22
≥ 90 μg-hr/mL	17	5	29%	31% (9-52%)	
AUC 0-45					
<90 μg-hr/mL	25	3	12%	16% (0-32%)	
≥ 90 μg-hr/mL	15	5	33%	35% (10-59%)	0.13
AUC 0-24					
<85 μg-hr/mL	29	3	10%	14% (0-28%)	
≥ 85 μg-hr/mL	11	5	45%	47% (17-77%)	0.02
AUC 0-6					
<20 μg-hr/mL	16	0	0%	0%	
≥ 20 μg-hr/mL	24	8	33%	37% (16-59%)	<0.01

Table 3.4: Number of patients and estimates of relative risk of acute GVHD (II-IV) at month 6 in each group of PM AUC chosen for univariate regression analysis towards

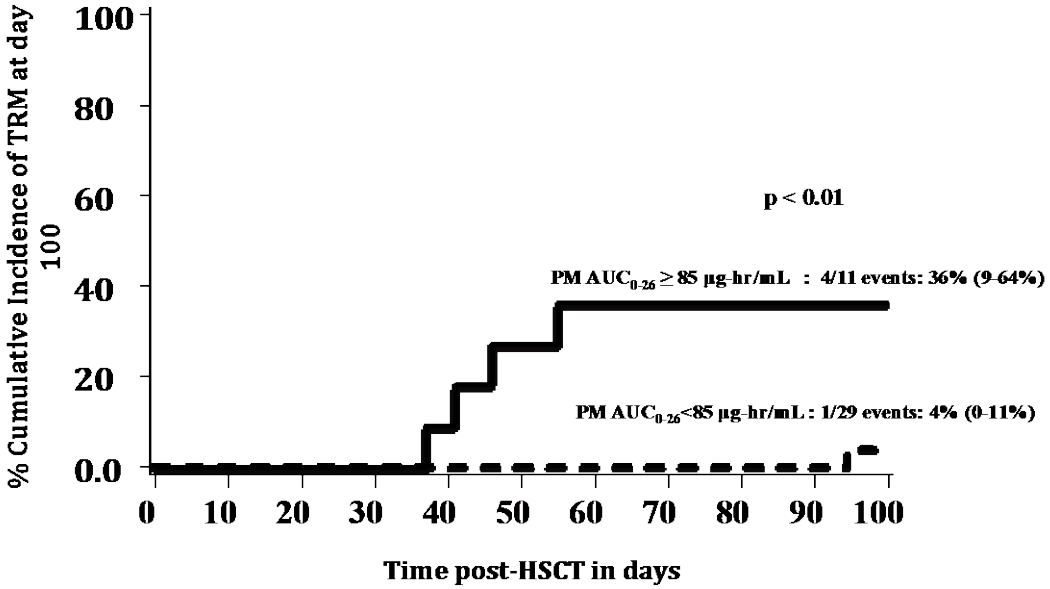
Variable	N in group	No. of Events	Event rate	Relative Risk Estimate (95% CI)	p-value
AUC 0-inf					
<100 μg-hr/mL	29	9	31%	33% (15-50%)	
≥ 100 μg-hr/mL	11	11	55%	55% (26-83%)	0.07
AUC 0-45					
<85 μg-hr/mL	19	5	26%	26% (7-46%)	0.09
≥ 85 μg-hr/mL	21	10	48%	50% (27-73%)	
AUC 0-24					
<70 μg-hr/mL	16	5	31%	34% (10-58%)	
≥ 70 μg-hr/mL	24	10	42%	42% (22-62%)	0.36
AUC 0-6					
<18 μg-hr/mL	12	3	25%	29% (2-55%)	
≥ 18 μg-hr/mL	18	12	43%	43% (24-62%)	0.12

Table 3.5: Phosphoramide mustard pharmacokinetic parameter estimates of the final model and bootstrap estimates in the development cohort

Parameters	Original Dataset (%RSE)	Bootstrap Estimates (95% C.I.)
Final Pharmacokinetic Parameters		
TVKfm	0.14 (4.7%)	0.13 (0.11-0.15)
Cl/fm_{nr} (L/hr) [a]	23.9 (21.4%)	28.0 (14.2-38.4)
Cl/fm_{slope} (L/hr) [a]	21.9 (24.4%)	15.7 (5.67-30.1)
TVV/fm (L)	240 (7.1%)	232 (179-290)
Effect of female gender on TVV/fm	0.75 (12%)	0.65 (0.43-0.91)
Between Subject Variability (BSV)		
BSV on Kfm	CV%=24.6%	CV%= 22.6% (10.0-32.4%)
BSV on Cl	CV%= 22.5%	CV%= 20.2% (14.2-28.8%)
BSV on V1	CV=41.9%	CV%= 40.2% (20.2-54.4%)
Residual unexplained variability (RUV)		
RUV proportional	CV% =14.0%	14.2% (10.0%-20.2%)

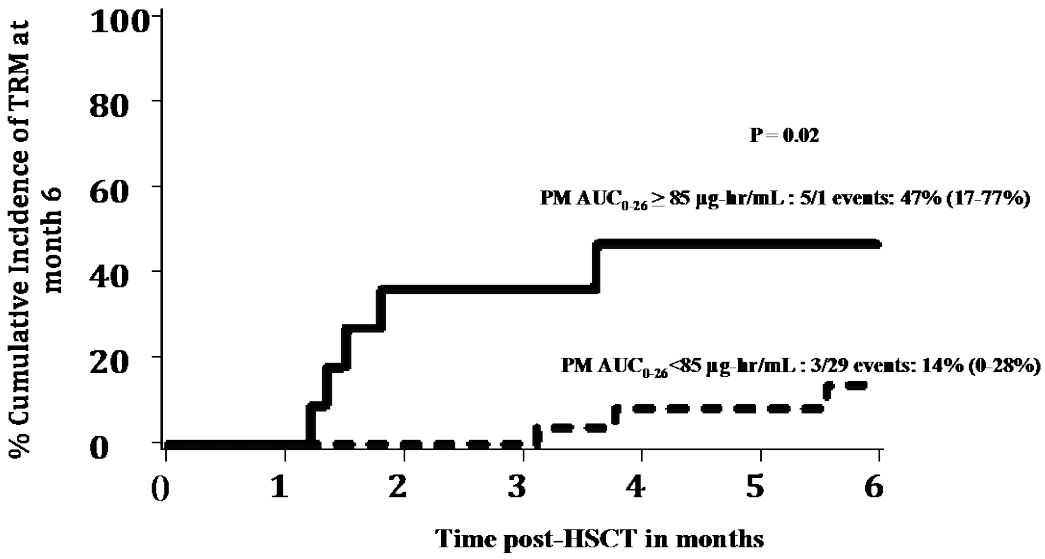
% RSE is relative standard error. [a]TVCl/fm is 45.8 L/hr which is a sum of estimate of Cl_{nr} (23.9 L/hr) for 83.5 kg actual body weight individual and Cl_{slope} (21.9 L/hr). $Cl_{nr:}$ is an estimate of non-renal clearance; Cl_{slope} is an estimate of the change in renal clearance with a unit change in standardized renal function ($RenFunc_{std}$)

Figure 3.1: Cumulative incidence of TRM at day 100 above and below PM AUC $_{(0-24)}$ cutpoint (85 µg*hr/mL)



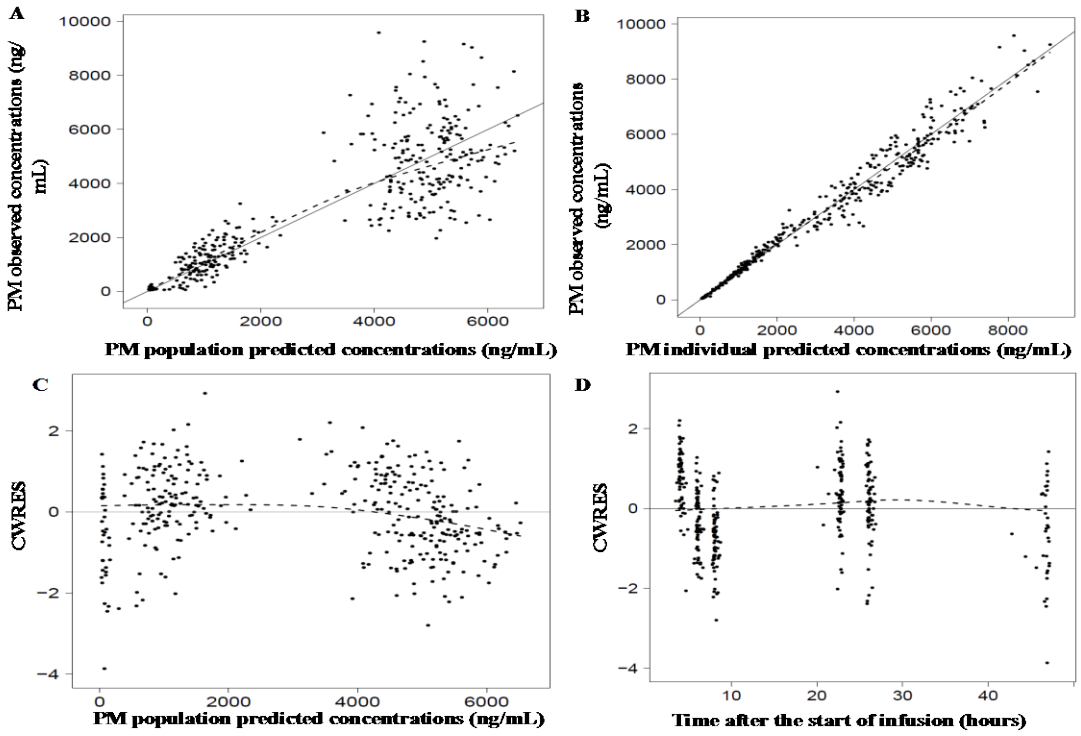
Cumulative incidence of TRM at day 100 after reduced intensity HSCT in patients with PM AUC $_{(0-26)} \geq$85 µg*hr/mL (cumulative incidence [95% CI] 36 % [9-64%]) compared AUC $_{(0-26)} <$ 85 µg*hr/mL to (cumulative incidence [95% CI] 4% (0-11%), p <0.01)

Figure 3.2: Cumulative incidence of TRM at month 6 above and below PM AUC $_{(0-24)}$ cutpoint (85 µg*hr/mL)



Cumulative incidence of TRM at month 6 after reduced intensity HSCT in patients with PM AUC $_{(0-26)}$ ≥85 µg*hr/mL (cumulative incidence [95% CI] 47 % [17-77%]) compared AUC $_{(0-26)}$ < 85 µg*hr/mL to (cumulative incidence [95% CI] 14% (0-28%), p =0.02)

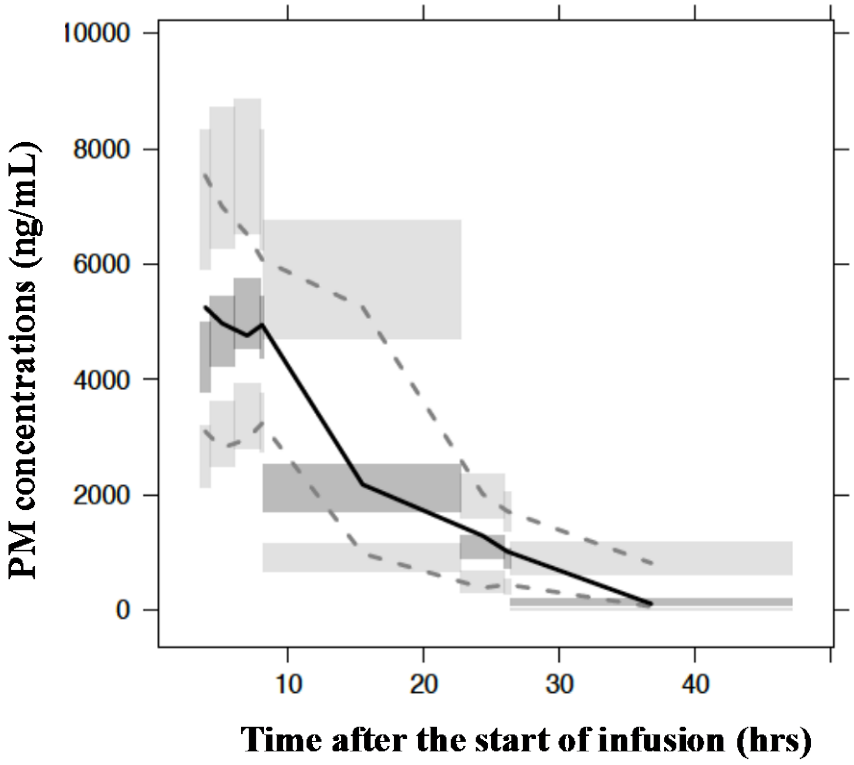
Figure 3.3: Goodness of fit plots for the final PM population pharmacokinetic Model



A) Observed PM concentration (ng/mL) (DV) vs Population predicted concentration (PRED) (ng/mL), B) Observed PM concentration (ng/mL) vs Individual predicted concentration (ng/mL). The black dots represent the observed PM concentrations, the solid line represents the line of unity and the dashed line represents the loess smooth. Since the observed data (black dots) in plots A and B are evenly scattered around the line of identity (solid line) it suggests that model sufficiently explains the observed data. C) Conditional weighted residuals (CWRES) vs Time after the end of infusion and D) CWRES vs Population Predicted Concentrations (ng/mL). The dots represent the observed PM concentrations, the solid line is the line at y=0 and the dashed line represents

the loess smooth. The plots C and D lack any specific trends and thus provide no evidence of model misspecification.

Figure 3.4: Visual predictive check of the final model



The solid black line represents the median of the observed PM concentrations obtained from 70 HSCT recipients. The grey area around the solid black line is the 95% confidence interval for the median obtained from the simulation-based prediction. The 5th and the 95th percentiles of the observed PM plasma concentrations are presented by the dashed lines below and above respectively. The light grey shaded areas around dashed lines represent 95% confidence intervals for the corresponding 5th and 95th prediction intervals obtained from simulations.

CHAPTER IV

4 GENOTYPE GUIDED TACROLIMUS DOSING IN AFRICAN AMERICAN KIDNEY TRANSPLANT RECIPIENTS

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for DEKAF Investigators

4.1 INTRODUCTION

Kidney transplantation is a common and effective treatment for end stage renal disease. African Americans (AA) represents around 34% of the candidates on the kidney transplant waiting list.(153, 338) Long-term graft survival rates are lower and all-cause mortality rates are higher in AA than in Caucasians or Asians.(339-342) There are several reasons cited for poor outcomes including greater variation in HLA, immunological differences, higher medical non-adherence, socio-economic barriers and PK differences of the immunosuppressive agents including tacrolimus.(343, 344)

Tacrolimus has a narrow therapeutic index (215, 216, 345-347) with wide interindividual variability in PK resulting in unpredictable blood concentrations.(207, 211, 348) This necessitates therapeutic drug monitoring to avoid sub-therapeutic and supra-therapeutic concentrations, which places the recipient at risk of rejection and toxicity, respectively.(349, 350) There is a significant difference in tacrolimus PK by race where AAs have 20-50% lower bioavailability, higher clearance and lower blood concentrations as compared to Caucasians.(227, 351-354) To achieve target tacrolimus trough concentrations some AA require ~1.5 to 2 times higher doses than Caucasians.(228, 355-359) However, not all AA will require a higher dose and these individuals may have nonfunctional genetic variants that lead to reduced metabolic capacity similar to Caucasians.

Tacrolimus is metabolized by hepatic and intestinal CYP3A4 and CYP3A5 enzymes.(207, 360) CYP3A5 is a more efficient catalyst of tacrolimus metabolism as compared to CYP3A4.(361) Tacrolimus is also a substrate of P-glycoprotein which is an efflux transporter expressed on enterocytes.(362, 363) Genetic variants associated with CYP3A5, CYP3A4, P450 (cytochrome) oxidoreductase (POR) and P-glycoprotein have been studied for their influence on tacrolimus clearance, although only CYP3A5 variants have demonstrated major clinical relevance.(226, 227, 229, 237, 238, 244, 360, 364-369)

*CYP3A5*3* is an intronic variant which generates a cryptic splice site resulting in a non-functional enzyme.(370-372) The presence of the *CYP3A5*3* allele is associated with lower oral tacrolimus clearance (Cl/F) whereas the *CYP3A5*1* allele is associated with high Cl/F (*CYP3A5*1/*1* individuals ~1 L/hr/kg, *CYP3A5*1/*3* ~ 0.8 L/hr/kg vs *CYP3A5*3/*3* ~ 0.5 L/hr/kg).(207, 373, 374) Therefore, the dose requirements for *CYP3A5*1/*1* or **1/*3* carriers are about 1.5-1.7 fold higher than *CYP3A5*3/*3* carriers. (227, 367, 368, 375, 376) These genotypes are also associated with delays in achieving therapeutic concentrations.(229, 377)

*CYP3A5*6* is a missense mutation that codes for a splicing defect, deleting exon 7 resulting in absence of CYP3A5 enzyme and activity.(372) *CYP3A5*7* is a frame shift mutation due to an insertion within codon 346 and termination of protein synthesis.(371, 372, 378) Few studies have evaluated the association between *CYP3A5*6* and **7* alleles and tacrolimus PK. (379-384) Brazilian transplant recipients carrying two CYP3A5 variant alleles (**3*, **6* or **7*) had higher tacrolimus trough concentrations compared to

those who did not ($p<0.0001$).(382) However no clearance models with dosing algorithms have been developed to account for these common AA variants. Algorithms that do not account for these alleles may incorrectly approximate clearance and dosing requirements. The objective of this study was to develop an AA dosing model, which comprehensively includes the common AA specific CYP3A5 variants.

4.2 METHODS

4.2.1 Subjects

The data for this analysis was obtained from our multicenter observational trial (DEKAF Genomics, clinicaltrials.gov NCT00270712). The study was approved by Institutional Review Board and an informed consent was obtained from each subject prior to the study. African American kidney transplant recipients (n=354) ≥18 years who received tacrolimus maintenance immunosuppression from 6 centers in the United States and Canada were studied. Tacrolimus was administered orally once or twice daily. The initial dose was based on weight and doses adjusted to achieve each institution's target trough concentrations. Trough blood concentrations (n=6037) were measured at each center and, in general, concentrations of 8-12 ng/mL were targeted for the first 3 months and 6-10 ng/mL for 3-6 months posttransplant. A median (range) of 18 (1-24) concentrations were obtained from each subject in the first 6 months posttransplant, and if available, concentrations were obtained twice each week for the first 2 months, and then twice in each month up to 6 months. The concentrations were quantified in each center by their standard analysis technique. The majority (92.9%) of concentrations were measured by liquid chromatography with mass spectroscopy in CLIA certified labs.

4.2.2 Genotypes

Genotyping was performed on recipient DNA isolated from peripheral blood. Single nucleotide polymorphisms CYP3A5*3(rs776746, g.6986A>G), CYP3A5*6 (rs10264272, g.14690 G>A) and CYP3A5*7 (rs41303343, g.27131-27132insT) were found to be significant in our previous GWAS analysis and therefore were chosen for this analysis.(385) In addition POR*28 *(*rs1057868, g.1058C>T) and CYP3A4*22 (rs35599367, g.15389 C>T) were also evaluated based on data from our previous analyses in a mixed race populations suggesting their importance.(386) Genotypes were determined using a custom exome-plus Affymetrix TxArray SNP chip described elsewhere. (387) The allele frequency of *CYP3A5*3* (G allele), *CYP3A5*6* (T allele), *CYP3A5*7* (A allele), *POR*28* (T allele) and *CYP3A4*22* (A allele) were 29.0%, 12.3%, 8.8%, 19.0%, 2.4%, respectively.

4.2.3 Population Modeling Of Trough Concentrations

The 354 subjects were randomly divided into a development (60%) and a validation cohort (40%). The data from the development cohort (212 subjects with 3704 troughs) was used to build the apparent oral tacrolimus clearance (Cl/F) model and subsequent dosing equation. The validation cohort (142 subjects with 2333 troughs) was used to evaluate the developed model. To assess differences in demographics, clinical and genotype distributions a two-sample t-test (for continuous factors) and sample proportion test (for categorical factors) were performed using R software package. Nonlinear mixed effect modeling was used to develop the Cl/F model with NONMEM (version 7.2, ICON development solutions, Maryland, USA) software on a Visual Fortran compiler (90/95). The NONMEM execution, model diagnostics, covariate testing and bootstrapping were

conducted with Perl Speaks NONMEM (PsN) toolkit and the Xpose4 package through Pirana workbench (version 2.7.2). R studio 3.0.3 was used for predictive performance checks. A plot of observed concentration vs time posttransplant is shown in Figure 4.1. A steady-state infusion model was used to develop the PK base model using $PRED library in NONMEM. In absence of intravenous data for the tacrolimus, it was not possible to calculate oral bioavailability. Therefore tacrolimus apparent oral clearance (Cl/F), which is the ratio of total clearance (Cl) to the bioavailability (F), was used to regress steady state tacrolimus concentrations (Css,av) to the administered dose. Cl/F was related to tacrolimus trough concentrations by the following equation

$$Css = \text{Total daily dose} / [(Cl/F)*24] \qquad \textit{(equation 1)}$$

Due to the longer half-life of tacrolimus, steady-state trough concentrations were assumed to be approximately equivalent to average steady-state concentrations (Css). Actual apparent oral clearance may vary from this approximated Cl/F; however, this difference is negligible for drugs with longer half-lives, such as tacrolimus.

An exponential error model was used to explain the inter-individual variability in Cl/F as shown in the following equation:

$$Cl/F = \text{Typical value of Cl/F (TVCl/F)} \times \exp^{\eta_{(1)}} \qquad \textit{(equation 2)}$$

where, $\eta_{(1)}$ is the estimate of deviation of individual Cl/F from TVCl/F. $\eta_{(1)}$ is assumed to be normally distributed mean of zero and variance ω^2.

An additive error model adequately explained the residual unexplained variability.

$$C_{ij} = C_{pred,ij} + \varepsilon_{ij} \qquad \textit{(equation 3)}$$

where C_{ij} is the j^{th} observed tacrolimus trough concentrations in the i^{th} individual, $C_{pred,ij}$ is the j^{th} predicted tacrolimus trough concentrations in the i^{th} individual and ε_{ij} is the

residual unexplained variability and where $\varepsilon \sim N(0,\sigma^2)$. FOCE interaction was used as the NONMEM estimation method.

4.2.4 Covariate Analysis

Clinical factors and genotypes were tested for their influence on tacrolimus TVCl/F. Covariates tested were recipient and donor age, gender, days posttransplant, steroid use (prednisone, methylprednisolone) at each trough measurement, calcium channel blocker use at each trough measurement, ACE-inhibitor use at each trough measurement, CMV sero-status at time of transplant (antibody positive or negative), anti CMV viral drug (as prophylaxis) use at each trough measurement, diabetes diagnosis at time of transplant, glomerular filtration rate calculated by the Modification of Diet in Renal Disease equation as a time varying covariate, body mass index (kg/m^2), actual body weight (kg) at baseline (time of transplant), and actual body weight (kg) at time of trough measurement as a time varying covariate. Alleles tested were *CYP3A5*3, CYP3A5*6, CYP3A5*7, POR*28,* and *CYP3A4*22*. Recipients who did not carry any *CYP3A5*3, *6* or **7* alleles were designated as *CYP3A5*1/*1* genotype and those who carried one *CYP3A5*3, *6* or **7* allele were designated *CYP3A5*1/*3, *1/*6* or **1/*7* genotype, respectively. Recipients were classified into one of nine *CYP3A5* genotypes (*CYP3A5 *3/*3, *3/*6, *3/*7, *6/*7, *6/*6, *1*3, *1*6,* and **1*7* and **1/*1*). Recipients were also classified based on *POR* (*POR*1/*1, *1*28* or **28/*28*) and *CYP3A4* (*CYP3A4*1/*1* or **1/*22*) genotype. No subjects had the *CYP3A5*7/*7* or *CYP3A4*22/*22* genotype. Recipient age, donor age and days posttransplant were tested both as continuous (using linear, exponential and power models) and categorical covariates. All other clinical factors were tested as categorical covariates. A strategy of forward inclusion and backward

elimination was tested for inclusion of the covariates. In NONMEM, minimization of -2 log likelihood is used as a model statistic and is given by the objective function value (OFV); measure of goodness of fit similar to sum of squares. The significance of inclusion of each covariate was tested based on likelihood ratio test that follows a chi square distribution. A lower OFV is considered to be a better fit and a decrease in the OFV by 3.8 ($p<0.05$) or more was considered significant for forward inclusion and an increase in OFV by 6.6 ($p < 0.01$) was chosen for backward elimination.

4.2.5 Model Evaluation

To evaluate the precision of the parameter estimates, a non-parametric bootstrap approach was performed using the development cohort. The method used random sampling with replacement to generate 1000 bootstrapped datasets using PsN toolkit. The final model developed with NONMEM was fit to each of the bootstrapped datasets and the parameters were obtained with their 5^{th} and 95^{th} prediction intervals. The model was also validated by using subjects in the validation cohort. The final model parameters were fixed in NONMEM (the estimation method was set to MAXEVAL=0 with the POSTHOC option) and were used to predict trough concentrations in validation cohort subjects. Population predicted trough concentrations (PRED) were obtained for each observed concentration (the dependent variable, DV) given their actual administered dose, the time after transplant, significant clinical covariates and genotypes (those identified from the development model). Median prediction error (MPE) and median percentage prediction error (MPPE) was then used to calculate the bias in model

predictions and median absolute prediction error (MAPE) was used to calculate the imprecision. The following equations were used:

MPE = Median (PRED –DV)

MPPE = Median [(PRED-DV)/DV X 100]

MAPE = Median [|(PRED-DV)|]

4.3 RESULTS

4.3.1 Model Development

Characteristics of the subjects in the development and validation cohorts are shown in Table 4.1. The median (range) daily dose and trough concentrations did not differ between the cohorts. The median tacrolimus concentrations were low during the first week post transplant and slowly increased over time until month 2 (2.8, 5.3, 6, 6.3, 6.9, 6.9, 7, 7.1, ng/mL in weeks 1-8 and 7.4, 7.2, 6.9 and 7 ng/mL in months 3-6, respectively). Tacrolimus TVCl/F was 54.6 L/hr and was significantly influenced by recipient age, steroid and antiviral coadministration, days posttransplant and *CYP3A5*1/*3, *3/*3, *1/*6, *1/*7, *3/*6, *6/*6, *6/*7* and **3/*7* genotypes. All other tested covariates were not significant. The effect of genotypes and clinical covariates on tacrolimus TVCl/F and final parameter estimates in the model development cohort and in the bootstrap analysis are shown in Table 4.2. The NONMEM code for the final model is shown in Appendix 8.3.

The inter-individual variability in TVCl/F after inclusion of covariates was 48.6%. Days posttransplant was the most important covariate where TVCl/F was 33% higher in

the first 9 days posttransplant compared to after 9 days. Days post-transplant was first tested as continuous covariate however the model failed to converge and hence modeled as a categorical covariate. The plot of dose normalized trough concentrations over time showed a general increase in concentrations early posttransplant (up to day 9) and stabilized later. Several cut points were tested to understand the effect of time. There was also a break point in Cl/F at day 9 similar to that observed for concentrations. Addition of a third ordered category for days post transplant was not significant, hence only categorized as a bivariate. Tacrolimus TVCl/F increased by 23% with concomitant steroid use and reduced by 8% with concomitant antiviral use. Tacrolimus TVCl/F was 24% greater in subjects under the age of 34 years vs older subjects. Similar to days post-transplant, age as a continuous covariate, had problems with model convergence giving unrealistic parameter estimates. Hence age was categorized based on clinical definition of young (18-34 years), middle age (35-64 years) and older age (>64 years). In the current study, only 6% of AA patients were older than 64 years, and therefore we were unable to test the effect of the older age group and therefore was combined with age group 35-64 years.

In subjects with *CYP3A5*1/*3, *1/*6* or **1/*7* genotypes the tacrolimus TVCl/F decreased by 16.2%, 8.2%, and 24.1%, respectively, compared to *CYP3A5*1/*1* genotype. *For CYP3A5*3/*3, *3/*6, *3/*7* or **6/*7* the TVCl/F declined by 51%, 36.5%, 54.5% and 44.2%, respectively, relative to *CYP3A5*1/*1*. Only one subject had *6/*6 genotype in the development cohort and therefore *6/*6 was not evaluable independently. To build a parsimonious model and to improve the power, we combined

the genotypes with similar effect sizes and overlapping confidence intervals on tacrolimus TVCl/F and re-ran the model. The tacrolimus TVCl/F decreased by 47% in subjects carrying two loss of function alleles (*CYP3A5*3/*3* or **3/*6* or **3/*7* or **6/*7, or *6/*6*) and by 15% in subjects carrying one loss of function allele (*CYP3A5*1/*3, *1/*6* or **1/*7*) compared to the *CYP3A5*1/*1*. The *POR*28* and *CYP3A4*22* genotypes did not influence TVCl/F.

To examine the goodness of fit, diagnostic plots were assessed during model development. Histograms of $\eta_{(1)}$s and Cl/F satisfied conditions of normal and log-normal distribution, respectively. Figure 4.2A and Figure 4.2B shows the plots of observed concentration vs population predicted concentration, observed concentrations vs individual predicted concentrations. Figure 4.2C and Figure 4.2D show the conditional weighted residuals (CWRES) vs independent variables, population predicted concentration and time. Although the model under-predicted slightly at higher concentrations, most of the data are evenly distributed across the line of unity. Also the CWRES do not show any specific trends of model misspecification. Thus the model adequately explains the observed data. The final tacrolimus TVCl/F model with clinical factors and genotypes is as follows:

Tacrolimus TVCl/F (L/hr)=54.6 L/hr x (1.33, if days less than 9 posttransplant) x [(0.53, if *CYP3A5*3/*3* or *CYP3A5*3/*7* or *CYP3A5*3/*6* or *CYP3A5*6/*7or CYP3A5*6/*6*)] x (0.85, if *CYP3A5*1/*3* or *CYP3A5*1/*6* or *CYP3A5*1/*7*) x (1.23, if receiving a steroid) x (0.92, if receiving an anti CMV viral drug) x (1.24, if recipient age 18-34 years)

Using the TVCl/F calculated using the model above and a desired target tacrolimus trough concentration; the daily tacrolimus dose can be calculated by:

Daily dose (mg/day) = [TVCl/F x target tacrolimus trough concentration (ng/ml) x 24hrs]/1000

4.3.2 Model Evaluation Using Bootstrap

Table 4.2 shows the median of the parameter estimates and their 95% prediction intervals obtained from 1000 bootstrap runs. Out of 1000 runs, 991 runs minimized successfully and the estimates from each bootstrap run were used to calculate the median and 95% interval. Parameter estimates for fixed and random effects obtained from the original dataset fell within the prediction interval of the estimates obtained from bootstrap therefore indicating that the model is robust and reproducible.

4.3.3 Model Evaluation Using The Validation Cohort

Table 4.3 shows the prediction performance of the tacrolimus TVCl/F model. The median prediction error with 95% CI was 0.48 (0.31-0.65) ng/mL and median percentage prediction error was 9.45% (6.44-12.45). Therefore, the model over-predicted the trough concentrations relative to the observed concentrations. Median absolute prediction error was 2.32 (2.21-2.44) ng/ml.

4.4 DISCUSSION

African Americans have poorer outcomes after transplantation and a possible contributory factor is high PK variability in immunosuppression leading to multiple dose

changes and longer periods of time out of the therapeutic range.(339, 358) On average AA require higher tacrolimus doses than Caucasians to achieve the same target blood concentration and most centers administer higher initial doses to AAs. However, not all individuals require higher doses and therefore some may have elevated concentrations which lead to temporary cessation of therapy and/or dose reductions. Whereas others may require even higher doses of tacrolimus to avoid insufficient blood concentrations. Most tacrolimus pharmacogenomic studies in AAs and Caucasians have classified CYP3A5 metabolism based on the presence or absence of the nonfunctional *CYP3A5*3* allele. The *CYP3A5*3* allele frequency has a minor allele frequency of 18-35% in AA and 88-95% in Caucasians.(226, 230, 372, 378, 388, 389) However, AAs also carry *CYP3A5*6* and/or **7* alleles which also encode for low activity or nonfunctional enzyme which have not been accounted for in most studies. *CYP3A5*6* and **7* are common in AAs with a minor allele frequency of 16-18% and 10-12%, respectively, but absent in Caucasians.(230, 372, 388, 390, 391) We found that AAs who carry two nonfunctional alleles *(*3, *6* or **7*) have a tacrolimus clearance similar to Caucasians whereas those who carry no nonfunctional alleles have high clearance. Therefore, AAs have a broad range of CYP3A5 metabolism phenotypes. To develop personalized strategies to reduce PK variability, we evaluated the effect of these variants on tacrolimus clearance and developed the first genotype-guided dosing model for AAs.

We found that tacrolimus TVCl/F in AAs was significantly influenced by *CYP3A5*1, *3, *6* and **7* alleles, days posttransplant, steroid and antiviral drug coadministration and age. The TVCl/F was 54.6 L/hr and higher than reported in non-AA

studies (~22-40 L/hr) (207, 392-395) which is consistent with AAs being more likely to carry a **1* expresser allele than Caucasians. The *CYP3A5*3, *6* and **7* alleles were each associated with a reduction in tacrolimus clearance. About 50% of our subjects carried one nonfunctional allele (*CYP3A5*3/*1, *6/*1* or **7/*1*), which decreased tacrolimus TVCl/F by 15%. Individually, the *CYP3A5*1/*3, *1/*6* and **1/*7* genotypes, decreased TVCl/F by 16.2%, 8.2%, and 24.1%, respectively. In addition, about 24% of our subjects carried two nonfunctional alleles – primarily CYP3A5**3/*3, *3/*6* and **3/*7* and **6/*6*. The effect of two variant alleles was large resulting in a decrease in tacrolimus TVCl/F by 47%. We did not observe any subject with more than two **3, *6* or **7* alleles. Based on our data and haplotype analyses by others the probability of this occurring is very low (<0.5%).(396, 397)

The *CYP3A5*6* allele is thought to encode for nonfunctional enzyme; however, there is some uncertainty about its functionality and it may express low levels of enzyme. In our study tacrolimus TVCl/F was 24% lower in CYP3A5 **1/*7* carriers but only 8.2% lower in **1/*6* carriers relative to the **1/*1* carriers, supporting that **6* may express low levels of enzyme. Others found no difference in tacrolimus concentrations between *CYP3A5*1/*1* and **1/*6* genotypes groups although the number of subjects was small.(381) In another study, *CYP3A5*1/*1, *1/*3* or **1/*6* carriers had lower tacrolimus troughs than *CYP3A5*3/*3* carriers but no difference in area under the curve although only one individual carried the *CYP3A5*1/*6* genotype.(379) The influence of *CYP3A5*6* and *CYP3A5*7* alleles has been studied towards other CYP3A5 substrates

and the effect may be substrate specific therefore our results may not be generalizable to other drugs. (397-403)

Day posttransplant was a significant covariate towards tacrolimus where TVCl/F is 33% higher in the first nine days posttransplant compared to after day 9 which is consistent with other studies.(207, 227, 392, 393, 404, 405) The higher TVCl/F may be due to early physiological changes such as fluid status, hepatic and kidney function and/or decreased bioavailability from dietary changes or concomitant medications. Concomitant steroid use was associated with a 23% higher tacrolimus TVCl/F most likely because steroids induce CYP3A enzymes.(406-409) We also found that younger subjects (18-34 years) had a 24% higher tacrolimus TVCl/F compared to older subjects. While some studies have not observed a significant association between tacrolimus Cl/F and age we previously showed in 1967 kidney recipients that age (18-34 vs 35-64 vs 65-84 years) had a highly significant effect on tacrolimus troughs.(207, 228, 392, 395, 410-412) We found that the co-administration of antivirals reduced tacrolimus TVCl/F but only by 8%. The mechanism of this effect is unknown. We did not find that calcium channel blockers were associated with TVCl/F. This is likely because amlodipine is the preferred agent at our centers and has a lower potential for an interaction than other calcium channel blockers.(413-415) Weight was not significant towards TVCl/F. Other studies have also not found weight to be significant.(416, 417)

The *POR*28* and *CYP3A4*22* variants have been previously associated with tacrolimus concentrations but we were unable to find an association in our AA

population.(234, 237, 244, 364, 368, 383, 418) One or two *POR*28* alleles were present in ~30% of subjects whereas the *CYP3A4*22* allele was infrequent (<5%). Our ability to detect an association with *CYP3A4*22* was therefore limited.

A prospective trial, in a primarily Caucasian kidney transplant recipients, evaluated the effect of genotype-guided tacrolimus dosing vs traditional weight based dosing.(419) The study tested an initial dose of 0.3 mg/kg/day PO in CYP3A5 expressors (CYP3A5*1) and 0.15 mg/kg/day PO for non-expressors (*CYP3A5*3*). The genotype-guided group had a higher proportion of patients with tacrolimus troughs within the target, fewer dose modifications, and more rapid achievement of the target concentration. Although genotype guided dosing did not reduce major clinical outcomes it was an important study as it showed the value of genetic targeting in controlling systemic exposure. Data such as ours shows that race specific variants and clinical factors is necessary in future trials and may improve achievement of major clinical endpoints. The Clinical Pharmacogenetics Implementation Consortium recently published guidelines for initial tacrolimus dosing. The guidelines recommend increasing the starting dose by 1.5-2 times in extensive metabolizers (CYP3A5*1/*1) and intermediate metabolizers (*CYP3A5*1/*3, *1/*6, *1/*7*), and standard dose in poor metabolizers (*CYP3A5*3/*3, *6/*6, *7/*7, *3/*6, *3/*7* and *6/*7*).(239) Our data supports these recommendations where *6 and *7 allele carriers require lower doses.

One of the limitations of our study is that albumin, hematocrit and antifungal agents status was not available and not tested in our model.(207) Our study used clinical

trough concentrations that were obtained as part of clinical care and draw times were not supervised by our study personnel but instead overseen by the clinicians. Compliance was also assessed by the clinical site and not through the study protocol.

To our knowledge this is the first study in which the effect of *CYP3A5* alleles *(*1, *3, *6, *7*) common in AAs have been collectively studied towards tacrolimus clearance. We identified one or more nonfunctional *CYP3A5* alleles (**3, *6* or **7*) in 74.5 % of our AA study population whereas 90-95% of Caucasians will carry one or more *CYP3A5*3* alleles.(378) This is considerably higher than what has been previously presumed in the AA population. If the **6* or **7* alleles had not been genotyped, 27% of our subjects would have been inappropriately categorized as carrying two *CYP3A5 *1* alleles, and 10% categorized as carrying one *CYP3A5*1* allele thereby overestimating tacrolimus Cl/F by nearly 50% in some individuals. Our data are consistent with a recent African study where only ~43% of individuals were considered CYP3A5 expressers since most carried one or more *CYP3A5*3, *6* or **7* nonfunctional alleles.(396)

This is the first study to develop and validate an AA specific genotype guided dosing model using variants common and relevant in the AA population. This study demonstrates the importance of race specific genotypes to determine drug clearance. Using dosing models which account for the genotypes and clinical factors may lead to precision dosing of tacrolimus.

Table 4.1: Subject demographics

	All subjects	Development Cohort subjects	Validation Cohort subjects	P-value[a]
No. of subjects	354	212	142	
No. of male subjects (%)	227(64)	140(63)	87(61)	0.35
Daily dose (mg)[b]	8(0.50-36)	8(0.5-36)	8(1-30)	0.17
No. of troughs	6037	3704	2333	0.09
Tacrolimus trough (ng/mL)[b]	6.50(0.10-65.60)	6.50 (0.10-65.60)	6.40(0.70-50.00)	0.34
Weight at baseline (kg)[b]	85(42-140)	85(42 -140)	83(47-137)	0.34
GFR by MDRD mL/min/1.73m^2 [b,d]	55.89(6.18-168.28)	55.88(6.18-168.28)	55.24(14.25-122.71)	0.08
No recipients in age category (%)				
18-34 years	66 (19)	36 (17)	30 (21)	0.32
35-64 years	268 (76)	163(77)	105 (74)	0.52
>64 years	20 (6)	13 (6)	7 (5)	0.63
Age at transplant[b]	48(20-73)	47 (20-73)	49 (21-72)	0.57
No. receiving dialysis at time of transplant (%)	56(16)	34(16)	22(15)	0.50
No. with diabetes at transplant (%)	129(36)	79(37)	50(35)	0.69

No. of troughs with calcium channel blocker (%)	2944(49)	1838(50)	1106(53)	0.01
No. of troughs with ACE inhibitor (%)	905(15)	522(14)	383(16)	0.01
No. of troughs with antiviral drug (%)	3441(57)	2128(57)	1313(56)	0.001
No. of troughs with steroid (%)	3283(54)	1941(52)	1342(58)	0.46
Simultaneous pancreas and kidney transplant (%)	16(5)	11(5)	5(4)	0.64
No. with living donor (%)	172(31)	108(30)	64(31)	0.27
No. with prior transplant (%)	34(10)	22(10)	12(8)	0.54
Primary cause of kidney disease (%)				
Diabetes	94(27)	58(27)	36(25)	0.67
Glomerular nephritis	50(14)	28(13)	22(15)	0.54
Hypertension	148(42)	93(44)	55(39)	0.34
Polycystic kidney disease	11(3)	4(2)	7(5)	0.1
Other	44(12)	26(12)	18(13)	0.91
Unknown	7(2)	3(1)	4(3)	0.35
No. of individuals with genotype (%)				
CYP3A5*1/*3	96 (27)	65 (31)	31 (22)	0.07
CYP3A5*3/*3	34 (10)	20 (9)	14 (10)	0.89

CYP3A5*1/*7	36 (10)	14 (7)	22 (15)	0.006
CYP3A5*7/*7	0	0	0	
CYP3A5*1/*6	47 (13)	30 (14)	17 (12)	0.55
CYP3A5*6/*6	4 (1)	1 (0.5)	3 (2)	0.15
CYP3A5*3/*6	21(6)	15 (7)	6 (4)	0.26
CYP3A5*3/*7	15 (4)	8 (4)	7 (5)	0.59
CYP3A5*6/*7	11 (3)	5 (2)	6 (4)	0.32
CYP3A5*1*1	80 (23)	49 (23)	31 (21)	0.77
CYP Not determined[c]	10	5	5	
POR*1/*1	151 (43)	91 (43)	60 (42)	0.90
POR*1/*28	86 (25)	55 (26)	31 (22)	0.37
POR*28/*28	25 (7)	15 (7)	10 (7)	0.99
CYP3A4*1/*1	229 (65)	140 (66)	89 (63)	0.52
CYP3A4*1/*22	17 (4)	12 (6)	5 (4)	0.35
CYP3A4*22/*22	0	0	0	

[a]p-value is the comparison of model development and validation cohorts

[b]data are median (range)

[c]These individuals did not have one or more of the CYP3A5 genotypes available and were excluded from the all analyses

[d]GFR is glomerular filtration rate calculated by Modification of Diet in Renal Disease (MDRD) equation

Table 4.2: The effect of genotypes and clinical covariates on tacrolimus clearance (Cl/F) and final parameters estimates

Parameter/Covariate	Model development cohort. Estimate (%RSE[a]) of the effect on TVCl/F	Bootstrap analysis. Median (95% confidence interval)
Typical Value of Cl/F (TVCl/F) in L/hr	54.60 (10.0%)	54.48 (44.51-66.63)
Two loss of function alleles (CYP3A5*3/*3 or *3/*7 or CYP3A5*3/*6 or *6/*7)	0.53 (10.9%)	0.53 (0.43-0.66)
One loss of function alleles (CYP3A5*1/*3 or CYP3A5*1/*6 or CYP3A5*1/*7)	0.85 (9.7%)	0.85 (0.70-1.04)
Less than day 9 posttransplant	1.33 (4.2%)	1.33 (1.23-1.45)
Steroid drug use	1.23 (6.9%)	1.24 (1.07-1.42)
Antiviral drug use	0.92 (2.9%)	0.91 (0.87-0.97)
Recipient age (18-34 yrs)	1.24 (7.8%)	1.24 (1.07-1.47)
Between subject variability[b]	0.21 (18.1%) [CV%=48.6%]	0.21 (0.14- 0.28) [CV%= 46.7% (38.76-56.84%]
Residual unexplained variability in trough (ng/mL)	2.76 (7.5%)	2.75 (2.55-2.96) ng/mL

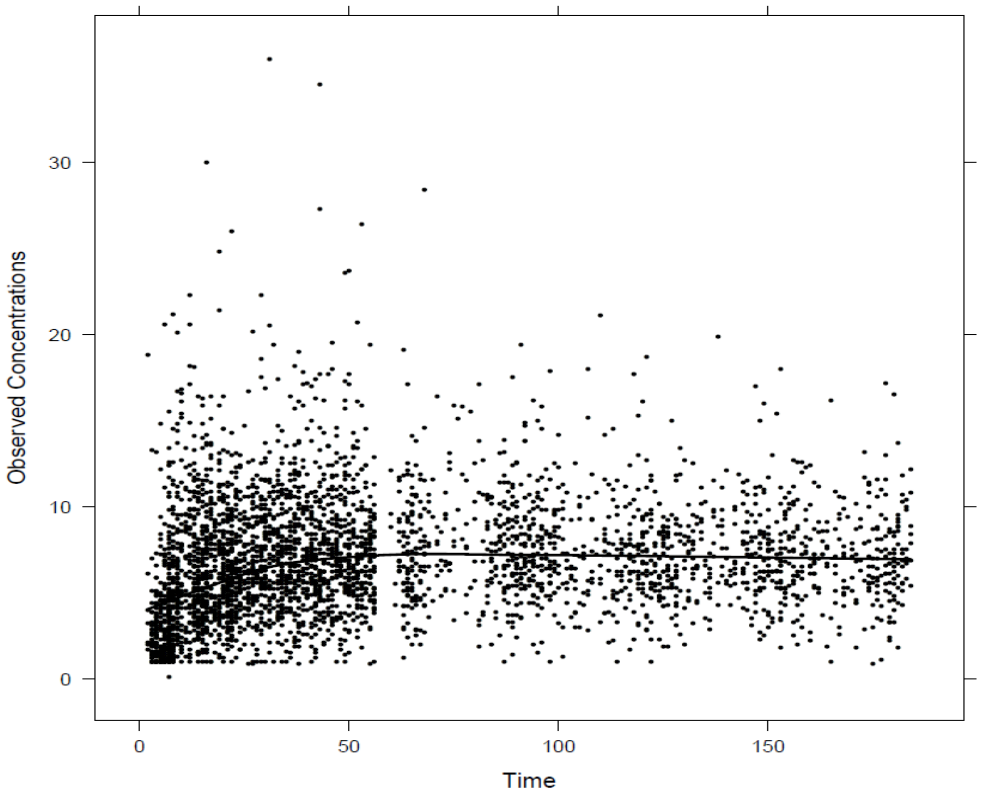
[a]RSE is relative standard error

[b]0.21 represents the estimate of the variance of individual $\eta_{(1)}$. CV% is the coefficient of variance and represents interindividual variability in the population. CV% = sqrt {[exp (variance)]-1}

Table 4.3: Predictive performance of the tacrolimus clearance model

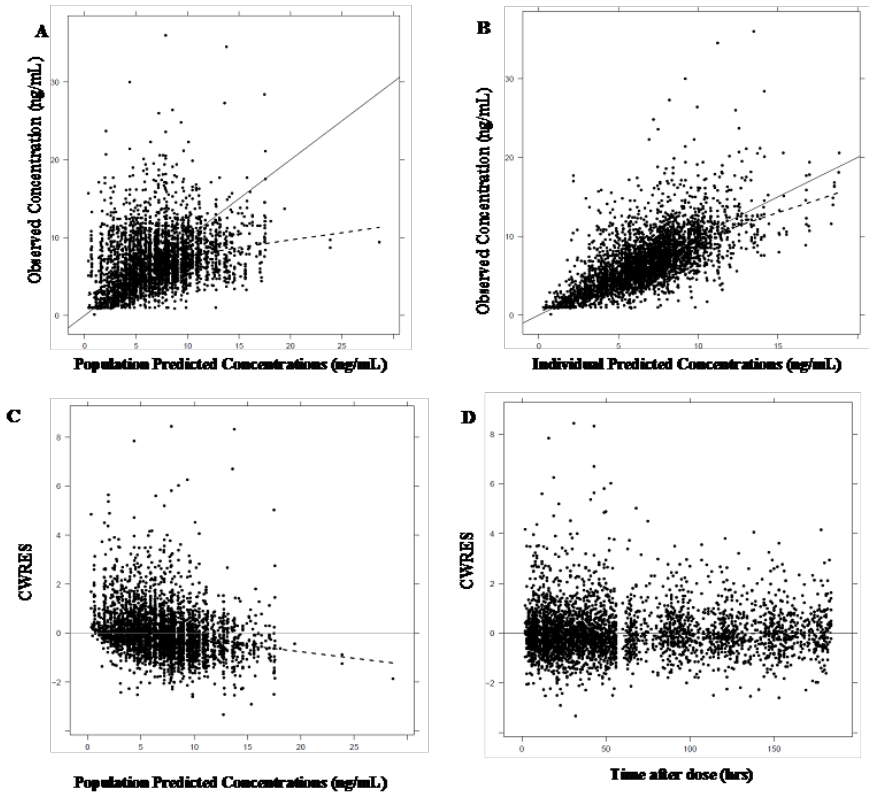
Predictive performance measure	Estimate
Median prediction error (MPE, 95% CI)	0.48(0.31-0.65)
Median percentage prediction error (MPPE, 95% CI)	9.45(6.44-12.45)
Median absolute prediction error (MAPE, 95% CI)	2.32(2.21-2.44)

Figure 4.1: Plot of observed tacrolimus trough concentration over time



The black dots are the observed tacrolimus trough concentrations and the solid black line is the loess smooth.

Figure 4.2: Goodness of fit plots for the final tacrolimus model



(A) Observed concentrations (ng/mL) vs population predicted concentrations (ng/mL) and (B) Observed conc. (ng/mL) vs individual predicted concentrations (ng/mL). The black dots represent the observed tacrolimus trough concentrations, the solid line represents the line of unity and the dashed line represents the loess smooth.

(C) Conditional weighted residuals (CWRES) vs population predicted concentrations (ng/mL) and (D) CWRES vs time after dose (hrs). The dots represent the observed tacrolimus trough concentrations, the solid line is the line at y=0 and the dashed line represents the loess smooth

CHAPTER V

5 MARKED ALTERATIONS IN GENE EXPRESSION IN PERIPHERAL BLOOD LEUKOCYTES OF KIDNEY TRANSPLANT RECIPIENTS FOLLOWING MYCOPHENOLIC ACID TREATMENT

Manuscript in Preparation

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5.1. INTRODUCTION

Kidney transplantation is the only curative treatment for end stage renal disease. Combinations of immunosuppressive agents mainly antibody induction agents, calcineurin inhibitors, mycophenolic acid (MPA) and steroids are critical in maintaining the function of the transplanted donor organ. Mycophenolic acid is one of the newer additions to the standard immunosuppressive regimen and is used in over 90% of all U.S. transplants.(153) It prevents graft rejection by inhibiting the proliferation of T and B lymphocytes through competitive and reversible inhibition of the enzyme, inosine monophosphate dehydrogenase (IMPDH).(420) Ionosine monophosphate dehydrogenase is a rate-limiting enzyme in the *de novo* DNA synthesis that catalyzes oxidation of ionosine monophosphate to xanthine monophosphate. Lymphocytes are incapable of utilizing the salvage pathway for DNA synthesis, and thus MPA exerts its immunosuppressive activity by interrupting the *de novo* DNA synthesis, cell proliferation and differentiation.(421) Two enzymes are encoded by the *IMPDH* genes; *IMPDH1* and *IMPDH2,* with 84% sequence identify and are located on chromosomes 7q31.3–q32 and 3p21.2–p24.2, respectively.(422) *IMPDH1* and *IMPDH2* have well conserved coding regions and while 3 distinct promoters control *IMPDH1*, only a single promoter controls *IMPDH2*.(423) MPA inhibits both IMPDH1 and IMPDH2 enzymes.(424) Identifying an ideal biomarker for assessing the level of immunosuppression contributed by MPA has been difficult. Although therapeutic monitoring of MPA concentrations in the plasma is used clinically to guide MPA dosing it has not been shown to be universally predictive of

rejection or toxicity.(425-432) The objective of this study was to identify possible gene(s) involved in MPA immunosuppressive mechanism in transplant recipients that might ultimately serve as a marker of immunosuppression intensity.

Studies have sought to understand if IMPDH enzyme activity is a better biomarker of MPA immunosuppressive effects than MPA plasma concentrations in transplant recipients. An *in vivo* rabbit heterotropic heart transplant model, showed an inverse correlation between MPA plasma concentrations and IMPDH activity measured in whole blood with an increase in IMPDH activity observed prior to rejection.(433) In humans, a decrease in IMPDH activity was observed following MPA administration in peripheral blood mononuclear cells (PBMC), CD4+ cells and erythrocytes.(434-438) In kidney transplant recipients, IMPDH activity decreased after MPA administration, with maximum decrease at peak MPA plasma concentrations followed by gradual return to near baseline within 3-6 hours post dose. (436, 439-441) High IMPDH activity has been associated with a higher risk of acute organ rejection, (435, 442, 443) although other studies have failed to demonstrate an association between IMPDH activity and clinical outcomes.(444, 445)

More recently gene expression has been associated with drug responsiveness. (446-450) Little data though are available regarding the relationships between immunosuppressive drugs and gene expression. MPA treatment induced a dose dependent increase in IMPDH mRNA expression in human cell lines.(451) Whole genome microarray studies using different cell lines, have found MPA to affect expression of several genes in the cell cycle and proliferation pathway.(452-454) Studies

have also demonstrated differential gene expression in other pathways, which support MPA's antiangiogenic and antifibrotic effects(455, 456), impaired stimulation of dendritic cells(457), anti-viral(458) and anti-atherosclerotic effect.(459) Limited clinical data are available related to gene expression after MPA administration but data suggest that expression changes may occur in CD25, CD71, IMPDH1 and IMPDH2 genes. (436, 444, 445, 460, 461)

Our study objectives were to identify and evaluate changes in gene expression after MPA administration in PBMCs posttransplant using whole transcriptome RNA sequencing, and to associate expression with IMPDH activity and MPA plasma concentrations. We also evaluated whether changes in gene expression, MPA plasma concentrations and IMPDH activity following MPA transplant were associated with acute rejection and toxicity. The long-term goal of this work is to identify biomarkers, easily accessible in the blood, that are predictive of response to immunosuppression, which can guide therapy.

5.2. METHODS

5.1.1 Patients

Blood samples from 44 kidney transplant recipients were obtained for RNA sequencing and measurement of IMPDH activity in PBMCs and MPA and metabolite concentrations in the plasma. Of the 44 patients, 1 patient had only pretransplant samples available and 2 patients received MPA prior to transplant therefore were excluded and the analysis was conducted in 41 patients. Patient characteristics are shown in

Table 5.1. All patients provided written informed consent and the protocol was approved by the Institutional Review Board of the University of Minnesota. All patients received induction therapy with rabbit anti-thymocyte globulin, tacrolimus or cyclosporine, mycophenolate, and short course steroids (for 5-7 days posttransplant) as their immunosuppressive therapy. Blood samples for measurement of gene expression, IMPDH activity and MPA plasma concentrations were collected simultaneously at pretransplant (before transplant surgery but no more than 2 weeks prior) and immediately prior to an MPA dose (trough) at week 1 (± 3 days), months 3 and 6 (± 2 weeks) posttransplant. Each sample was analyzed for RNA expression and IMPDH activity in PBMCs, and for MPA (total and unbound) and acylMPAG (an active metabolite) concentrations in the plasma.

5.1.2 RNA Sequencing To Measure Gene Expression

Blood was collected in BD Vacutainer® lavender top tubes with EDTA as anticoagulant. Total RNA was isolated from ~12 ml of whole blood PBMCs using a Qiagen QIAamp RNA Blood Mini kit (Germantown, MD) within 2 hours of blood draw. RNA was quantified using a Nanodrop 800 spectrophotometer. RNA sequencing libraries were built and Illumina Hi-seq 2000 sequencing was used to generate 20–40 million mapped paired-end reads per sample as previously described.(462) Quality control and paired-end reads alignment was performed using FastQC:Read and Tophat2, respectively, using iGenome human UCSC reference annotation. Transcript assembly and

abundance was determined using the Cufflinks program to determine fragments per kilobase per million reads (FPKM) for each gene transcript.

5.1.3 Bioanalysis of IMPDH Activity, MPA and MPA Metabolite

Total IMPDH enzyme (IMPDH1 and IMPDH2) activity was measured in PBMCs isolated from the buffy coat obtained after centrifugation of 8 ml of whole blood collected in BD Vacutainer® Cell Preparation Tubes (CPT™). The detection and quantification of IMPDH activity was conducted using HPLC-UV method as previously described (463) with minor modifications. Total MPA (bound and unbound), protein free (unbound) MPA and acylMPAG (an active metabolite) were measured in plasma obtained by centrifugation of 5 ml of whole blood collected in BD Vacutainer® lavender top tube containing K2EDTA as anticoagulant. The detection and quantification of total MPA, unbound MPA and acyl MPAG was performed using liquid chromatography mass spectrometry based on previously described methods.(464) Details of IMPDH activity and MPA assays are provided in the supplementary material.

5.1.4 Statistical Analysis

Log transformed, normalized FPKM values from each sample adjusted for the top two principal components computed using the surrogate variable approach (465) were used for analysis. A linear mixed effects (LME) model was used to compute associations between MPA and acylMPAG plasma concentrations, IMPDH activity, with gene expression accounting for within subject correlation. A fold change in gene expression from baseline (before MPA administration pretransplant) to various time points

posttransplant (week 1, months 3 and 6) were tested for association with MPA (unbound, total) concentrations, acylMPAG concentrations and IMPDH activity. Logistic regression analysis was used to evaluate whether pretransplant gene expression (log FPMK), fold changes in gene expressions, IMPDH activity or MPA parent and acylMPAG trough concentrations were associated with acute rejection and MPA-related leukopenia. Acute rejection was diagnosed and defined by the treating physician and >96% were biopsy confirmed. Mycophenolate related leukopenia was defined as the use of mycophenolate at least 14 days prior to a WBC count <3000 cells/mm3 that resulted in a clinical intervention. Clinical interventions included mycophenolate dose reduction lasting ≥ 2 weeks, discontinuation for ≥ 2 weeks and/or initiation of granulocyte colony stimulating factor or granulocyte macrophage colony stimulating factor therapy. To account for small sample size, Kenward-Roger approximate F-test (466) was used to test for changes in gene expression. We used a false discovery rate (FDR) of 0.1 to identify significant associations.

5.3. RESULTS

Samples were obtained at 115 time points in 41 patients and were analyzed. Of the 115 samples, 41 were collected at pretransplant, 36 at week 1, 20 at month 3 and 18 at month 6. Gene expression data was available for 38 patients at baseline (2 samples were not sent for sequencing and one failed quality control), 34 patients at week1 (one sample was not sent for sequencing), 20 patients at month 3, and 18 patients at month 6. All samples except one passed FastQC quality check and were used for TopHat alignment and Cufflink gene expression. The overall alignment rate for paired end reads was 89.9%.

There were 20983 genes with expression measurements (FPKM) greater than 0 at one time point or more. IMPDH activity was measured in 41 patients at baseline, 35 at week 1 (1 sample excluded due to interfering analytical peak), 16 patients at month 3 (1 sample was below LOQ and 3 samples had interfering analytical peaks and were excluded) and 16 patients at month 6 (1 sample was below the LOQ and 1 contained an interfering analytical peak and were excluded).

Mycophenolic acid was administered as mycophenolate mofetil or mycophenolic acid delayed release. The median (range) daily dose of mycophenolate mofetil was 2000 mg (1000-3000) and that of mycophenolate sodium was 720 mg (360-1800). MPA plasma trough concentrations were measured in 34 patients at week 1 (1 sample was not processed correctly and excluded and 1 sample was not collected), 20 patients in month 3, and 18 samples in month 6. None of the patients were receiving MPA at the time of transplant and the MPA concentrations were assumed to be zero. All MPA and acylMPAG concentrations were above the limit of quantification (25 ng/ml for total MPA, 1 ng/ml for unbound MPA and 25 ng/ml for acylMPAG). **Table 5.2** shows the median (range) IMPDH activity, and MPA and acylMPAG concentrations over time.Higher total MPA concentrations were associated with greater fold reduction in sideroflexin (*SFXN4*) expression ($p=5.87 \text{ X } 10^{-6}$, FDR=0.06) (**Figure 5.1**) at week 1, relative to pretransplant baseline. Also at week1 relative to pretransplant baseline, higher acylMPAG concentrations were associated with greater fold reduction in chromosome 1 open reading frame 123 (*C1orf123)* expression ($p=9.15 \text{ X}10^{-6}$, FDR=0.09)(Figure 5.2). At month 3, higher unbound MPA concentrations were associated with greater fold

increase in solute carrier family 22 member 14 (*SLC22A14)* expression compared to baseline ($p=4.93X10^{-6}$, FDR=0.09) (Figure 5.3). IMPDH activity was not associated with fold changes in expression of any gene from baseline to all times posttransplant.

Acute allograft rejection was observed in 29.2% (12 of 41) of transplant recipients. The median (range) time to acute rejection in the patients was 30.5 (8-332) days posttransplant. We only tested pretransplant gene expressions and fold changes at week 1 and month 3 relative to baseline since most of the rejection events occurred before month 6. MPA-related leukopenia occurred in 34.1% (14 of 41) of recipients. The median (range) time to leukopenia was 65.5 (20-82) days posttransplant. We only tested pretransplant gene expressions and fold changes at week 1 from pretransplant baseline since most of the leukopenia events occurred before month 3. MPA trough concentrations, IMPDH activity and fold changes in genes expression were not associated with acute rejection or leukopenia. Increasing log of total MPA trough concentrations [estimate (95% CI): -0.40 (-0.70-(-0.11), p=0.0072] were associated with a decrease in log IMPDH activity. Similarly, an increase in log unbound MPA trough concentrations [estimate (95% CI): -0.33 (-0.62-(-0.04), p=0.02] was also associated with decrease in IMPDH activity. Increase in acylMPAG concentrations was not associated with decrease in IMPDH activity. Plot of log IMPDH activity and plasma trough concentrations of total MPA, unbound MPA are shown in Figure 5.4 and Figure 5.5 respectively. Since IMPDH is a target of MPA, we specifically chose to analyze time trends in IMPDH gene expression. *IMPDH1* expression was increased ($p=7.35 \times 10^{-12}$) at week 1 posttransplant after beginning MPA administration relative to baseline, and then decreased and

stabilized by months 3 and 6 (The black dots represent log observed IMPDH activity of each sample and the corresponding log of unbound MPA trough concentrations over the entire post-transplant period. The solid black line is fit of linear regression. The p-value is obtained from fitting a linear mixed effect model. **Figure 5.6**) In contrast, *IMPDH2* expression decreased ($p=2.30 \times 10^{-10}$) at week 1 post-transplant compared to baseline and then increased and remained stable at months 3 and 6 post-transplant but did not return to baseline expression (Figure 5.7). The gene expression was highly variable between recipients at all time points for both IMPDH1 (CV% 24.2-28.2) and IMPDH2 (CV% 34.9-42.2%). IMPDH activity was stable over time and did not show any trend towards increase or decrease at week 1, month 3 and 6 post-transplant (Figure 5.8)

We analyzed trends in the genes in GO cell cycle pathway to identify patterns similar to that seen in *IMPDH1* and *IMPDH2* expression. The cell cycle process pathway chosen for pathway analysis comprised of 193 genes and our data had expressions for 186 of these genes. No statistical analysis was performed. In addition, among the GO cell cycle pathway genes, *ANLN, ARAP1, CCNA2, CCP110, CDC25C, CDCA5, CDK13, CDK2AP1, CDKN2D, CHFR, CHMP1A, CLIP1, CUL3, DCTN2, DCTN3, E2F1, EGF, EREF, ESPL1, FOXN3, FOXO4, GFI1B, KIF11, KIF15, KIF23, KRT7, LATS2, MAD2L2, MAP3K11, NBN, NDE1, NEK2, NEK6, NUSAP1, PAFAH1B1, PDS5B, PKMYT1, PML, POLE, PPP6C, PRMT5, PTPRC, RAD21, RAD51D, RB1, TGFA, TGFB1, TOP3A, TPX2, TTK, UBE2C,* showed an expression trend over time similar to IMPDH1, ie an initial increase at week 1 posttransplant from baseline pretransplant and

return to near baseline at later time points. Among the GO cell cycle pathway genes, *ABL1, ANAPC5, APBB1, CD28, CDC25B, CDK10, CDK4, CDK6, CEP250, CETN3, CKAP5, CUL1, CUL5, DBF4, FBXO5, GSTP1, LEPREL4, MAD2L1, MPHOSPH9, MSH5, NOLC1, NPM1, PAPD7, PCBP4, POLA1, POLD1, PPP5C, RAD1, RAD50, RAD54B, RAN, RCC1, RINT1, TBRG4, TRIAP1, TUBE1 and ZW10* showed trend similar to IMPDH2, ie an initial decrease at week 1 posttransplant from baseline pretransplant, and return to near baseline values at later time points.

An advantage of RNA sequencing over microarrays is that it allows for identification and measurement of gene transcript isoforms. We further studied the specific isoforms of IMPDH1 and IMPDH2. IMPDH2 was expressed as single transcript. However, 7 different IMPDH1 isoforms were identified. Expression of IMPDH1 isoforms over time is shown in **Figure 5.9**. The highest IMPDH1 transcript expressed in all patients was NM_001142573 (14 exons), followed by NM_001102605 (16 exons) and NM_183243 (15 exons). IMPDH1 gene expressions was not measurable for the remaining 4 transcripts with FPKM values of ~ 0 [NM_001142575 (13 exons), NM_000883 (17 exons), NM_001142576 (16 exons), NM_001142574 (14 exons)].

5.4. DISCUSSION

This is the first study to analyze gene expression changes across the whole transcriptome in PBMCs in relation to IMPDH activity and plasma concentrations of MPA in transplant recipients receiving MPA therapy. MPA is a commonly used immunosuppressive agent following kidney transplant. Insufficient immunosuppression

increases the risk of acute allograft rejection however the associations between MPA plasma concentrations and IMPDH activity, and rejection are inconsistent in the literature.(425, 426, 428, 432, 442, 444, 467-469) Hematologic toxicities such as leukopenia, anemia, gastrointestinal disturbances and infections, are common problems following prolonged exposure to MPA(470) and the relationships between MPA plasma concentrations and IMPDH activity with these toxicities are also inconsistent.(227, 425, 427, 471-476) and better biomarkers to assess MPA immunosuppression are needed. We undertook this study to identify possible gene(s) involved in MPA immunosuppressive mechanism in transplant recipients that might ultimately be helpful in quantifying immunosuppression intensity.

We identified 20893 genes expressed at pretransplant, week 1 and months 3 and 6 posttransplant and sought to understand if known pharmacodynamic and PK markers of MPA (IMPDH activity and total MPA, unbound MPA, acyl MPAG) were related to expression changes. Several studies have evaluated gene expression changes *in vitro* and *in vivo* with MPA. (433, 452-454, 477-479) A whole genome microarray was used to explore gene expression changes after MPA exposure in gastric cancer cell lines. Among the genes most affected, an upregulation in expression was observed in cyclin (*CCND1*, *CCNE2*) and cyclin dependent kinase inhibitor (*CDKN1A*) genes, whereas a downregulation in cyclin dependent kinases (*CDK4, CDK5*), cell division and cell cycle related genes (*CDC20, CDC25B, CDC25C, MCM2, CENPE, PSRC1*), genes involved in chromosomal segregation (*BUB1, BUB1B, BOP1, AURKA, AURKB and FOXM1*) was observed.(452, 453) Similar to studies discussed above, we observed an upregulation of

CDKN1A and downregulation of *CDK4, CDC25B* and *BOP1* after MPA administration at week 1 posttransplant, however these changes were transient and trended towards baseline at later follow up times. However, for all the other genes, we did not observe similar changes in expression posttransplant in our study. One reason could be the differences in gene expression in our PBMCs compared to gastric cancer cell lines. Expression of these reported genes were not associated with IMPDH activity or MPA or acylMPAG concentrations in the plasma in our study. Therefore, the effect may be due induction therapy or other factors related to the transplant. In lymphoblastoid cell lines, expression of *C17orf108, CYBRD1, NASP* and *RRM2* genes have been associated with MPA cytotoxicity.(454) An increase in *C17orf108* and *CYPBRD1* gene expression increased the resistance of cells to MPA whereas as increase in *NASP* and *RRM2* gene expressions increased the sensitivity of cells towards MPA. However, in our study none of these reported genes were associated with acute rejection. We observed a decrease in *C17orf108* at week 1 and increase towards baseline at month 3 and 6. *CYBRD1* increased at week 1 and then decreased towards baseline at months 3 and 6. *NASP* and RRM2 gene remained constant throughout 6-month follow up period. Naïve mononuclear cells from 10 healthy volunteers were treated with acylMPAG, to identify its effect on expression of genes other than IMPDH. The expression of *IL2* was significantly downregulated and that of *nucleobindin 1* was upregulated with acylMPAG treatment. In our study, *IL2* expression fell posttransplant to very low levels in most samples and was not associated with IMPDH activity or MPA and acylMPAG plasma concentrations. *Nucleobindin 1* gene expression increased at week1 posttransplant and then decreased towards baseline at

months 3 and 6 but was not associated with IMPDH activity or MPA and acylMPAG plasma concentrations.

In our analysis we identified that changes in *SFXN4* gene expression were significantly associated with total MPA concentrations at week 1 (**Figure 5.1**). *SFXN4* is mitochondrial protein expressed in all tissues.(480) Children with mutations leading to decreased SFXN4 function have a higher incidence of macrocytic anemia.(480) This was confirmed by experiments *in vivo* where SFXN4 knockdown demonstrated mitochondrial respiratory defects.(481) In our study we found that higher MPA concentrations were significantly associated with greater decrease in *SFXN4* expression and therefore might have implications in hematologic toxicities associated with MPA such as anemia. It was not associated with leukopenia in our analysis. We also found that acylMPAG concentrations were associated with *C1orf123* gene expression at week 1 (Figure 5.2). *C1orf123* is a protein coding open reading frame. An *in vivo* study in *Torpedo californica* showed that C1orf123 protein had high sequence similarity to proteins that play an important role in acetylcholine receptor clustering and signal transduction and thus might have important role at neuromuscular junction.(482) From a protein-protein interaction network study, C1orf123 protein is proposed to be in direct association with cyclinB1 which is important in progression of cell cycle especially in G2 exit and mitotic phase.(483) Downregulation of *C1orf123* with increasing acylMPAG concentrations may be indicative of MPA associated immunosuppression. However, invitro and invivo studies are required to validate the function role of this gene. Changes in *SLC22A14* expression were associated with unbound MPA concentrations (Figure 5.3). *SLC22A14*

encodes an organic cationic transporter like (ORCTL4) protein and belongs to a family of SLC22 transporter genes mainly involved in uptake of small molecules into the cells.(484, 485) The mRNA transcripts of *SLC22A14* are expressed in all tissues, but some tissue specific transcript variants are exclusively expressed in kidney, colon and intestine.(486) However, its functional role has yet to be determined.(485) It may be involved in the PK of MPA by altering the transport of MPA in the intestine or bile.

We found that *IMPDH1* expression initially increased and *IMPDH2* gene expression decreased at week 1 posttransplant. These changes were transient and expression levels of these genes returned to near baseline levels by 6 months. Expression was not associated with IMPDH activity or MPA or metabolite concentrations. Other studies have also shown transient changes in *IMPDH1* and IMPDH*2* expressions posttransplant.(444, 445, 460, 461) In stable kidney transplant recipients, *IMPDH1* expression in PBMCs was higher in the first 3 months posttransplant as compared to 6-24 months posttransplant, while *IMPDH2* expression was stable. The study also found an increase in IMPDH activity that was attributed to the increase in *IMPDH1* expression.(460) In a larger cohort of 101 renal transplant recipients, *IMPDH1* and *IMPDH2* expression measured in PBMCs both decreased at day 6 posttransplant compared to pretransplant and then increased from day 6 to 140 posttransplant.(445) Like our analysis, they observed that IMPDH activity was not significantly correlated to *IMPDH1*, *IMPDH2* or the sum of *IMPDH1* and *IMPDH2* gene expressions. Similarly, total and unbound predose MPA concentrations were also not associated with gene expression. *IMPDH1* and *IMPDH2* gene expression was measured pre- and

posttransplant in whole blood, CD4+ cells and reticulocytes in 22 kidney transplant recipients receiving mycophenolate and 8 not receiving mycophenolate.(461) Both *IMPDH1* and *IMPDH2* expression increased at day 1 posttransplant in CD4+ cells. *IMPDH1* expression remained above baseline at 2 weeks posttransplant whereas *IMPDH2* expression returned to baseline. The trends of gene expression were different when measured in whole blood and reticulocytes. They also found that gene expression were not associated with trough plasma MPA concentrations. The authors mainly attribute these initial changes in expression to glucocorticoid therapy although not statistically tested. There were no significant differences in mycophenolate and non-mycophenolate groups at initially, however after 2 weeks, patients receiving mycophenolate demonstrated an increase in *IMPDH1* and *IMPDH2* gene expression and the authors speculate an enzyme induction due to prolonged MPA therapy. In another cohort of 35 kidney transplant recipients, *IMPDH1* and *IMPDH2* expression was measured at predose and 2 hours post MMF administration. There was no significant change in predose IMPDH1 and IMPDH2 gene expression. At 2 hours post MMF administration an initial increase at week 2 was observed that later decreased at week 24 posttransplant. Like our data, MPA concentrations and IMPDH activity measured in PBMCs were not associated with the change in expressions.(444) Changes in *IMPDH* mRNA expression studied in small cohorts of healthy volunteers also show inconsistent results.(438, 487) These studies including ours indicate an initial upregulation of IMPDH1 gene expression, however results of IMPDH2 gene expression are not consistent. Also changes in expression are not associated with IMPDH activity or MPA

plasma concentrations. Thus there may be other factors that play a role in altering gene expression following MPA administration. Mycophenolic acid inhibits IMPDH activity, which in turn depletes the guanosine pools in the cells. Studies suggest that the changes in gene expression could be attributed to depletion of guanosine pool depletion in cells following MPA treatment. In an invitro study an inverse correlation was observed between guanine concentrations and IMPDH mRNA, which could explain the upregulation of IMPDH1 gene expression a week after transplantation.(451) Other studies also suggest the complex regulation of IMPDH1 and IMPDH2 expression due to depletion in cellular guanine.(445, 487) It may also be attributed to steroid co-administration given usually in the first week of transplant.(444, 445, 461) Recent data in CD4+T cells showed expression changes in PD1, CTLA-4, CD27, CD28 and CD70 genes following MPA administration suggesting alternative mechanisms.(488)

In our study we did not find association between gene expression and acute rejection or leukopenia. Higher pre-transplant IMPDH1 and/or IMPDH2 expression has been associated with acute rejection in few studies.(444, 445, 461) Studies have shown differential expression of the other genes especially cytokines that could be potentially associated with acute rejection.(489-498) However, MPA concentrations were not measured in these studies, and hence it is unknown if changes in expression could be attributed to MPA therapy alone or other changes that occur in transplant recipients. Lower IMPDH1 expression has been associated with a higher incidence of hematologic malignancies.(444)

Through RNA sequencing analysis we identified 7 transcripts of IMPDH1. The aim of this transcript analysis was to understand whether the overall IMPDH1 mRNA expression was associated due to a single transcript expression or each contributed equally. Among the 7 identified transcripts, only 3 transcripts had measurable expression, and highest expression is transcript NM_001142573. The overall change in expression over time was similar for all 3 detectable transcripts. IMPDH1 is regulated by 3 different promoters governing their expression in different cell types, however changes in IMPDH1 expression due to 3 exonic differences is yet unknown. Also, whether enzyme activity differs with different IMPDH1 isoforms is also not known.

Lack of adequate power is a limitation of our study however this is the first study that has evaluated gene expression across the genome towards (>20,000 genes) towards immunosuppression intensity. Our study indicated that expression of many genes had significant but transient changes in expression early posttransplant following MPA administration. However very few genes (*SFXN4, Corf123 and SLC22A14*) were associated with MPA plasma concentrations. These results indicate that MPA concentrations and IMPDH activity are not indicative of changes in expression especially in genes involved in cell cycle following MPA therapy, despite numerous associations found in cell-based methods.

Table 5.1: Clinical and demographic characteristics of patients included in the analysis

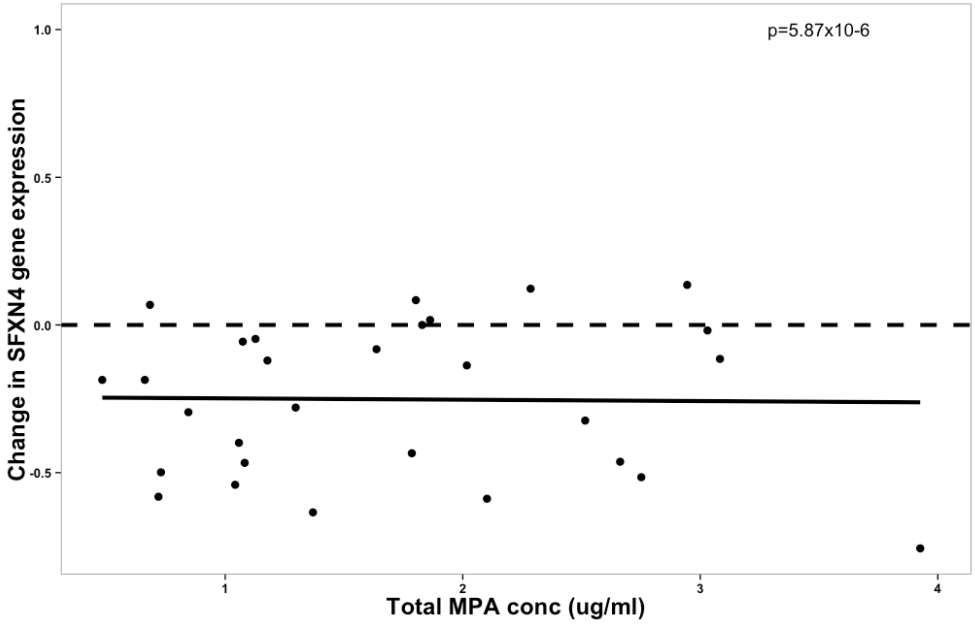
Characteristics	Median (Range) or N
Number of recipients	41
Age at transplant (years) median (range)	49 (24-76)
Recipient gender female/male	13 /28
Primary disease at transplant	
Diabetes	7
Glomerular Disease	5
Hypertension	5
Polycystic Disease	7
Nephropathy	4
Other	13
Recipient Race	
Native American or Alaskan Decent	3
African Decent	3
European Decent	35
Transplant Type	
Kidney	40
Simultaneous Pancreas and Kidney	1
Tacrolimus/Cyclosporine/none pretransplant	0/0/41
Tacrolimus/Cyclosporine/none at week 1	21/12/3
Tacrolimus/Cyclosporine/none at month 3	12 /6/2
Tacrolimus/Cyclosporine/none at month 6	11/6/1
Steroids pretransplant yes/no	78/34
Steroids at week 1 yes/no	6/30
Steroids at month 3 yes/no	4/16
Steroids at month 6 yes/no	3/15
Mycophenolate Mofetil/ Mycophenolate Sodium at baseline	0/0
Mycophenolate Mofetil/Mycophenolate Sodium at week 1	34/2
Mycophenolate Mofetil/ Mycophenolate Sodium at month 3	17/3
Mycophenolate Mofetil/ Mycophenolate Sodium at month 6	17/1
Donor Type Living/deceased	17/24
Induction therapy yes/no	40/1
Recipient CMV antibody status (positive/ negative)	22/19

Table 5.2: Summary of MPA plasma trough concentrations and IMPDH activity in PBMCs

Analyte	Pretransplant baseline	Week 1 [no. subjects]	Month 3 [no. subjects]	Month 6 [no. subjects]
IMPDH activity (umol*s^{-1}*mol^{-1} AMP)	85.8 (15.2-1310) [n=41]	65.0 (12.2-362) [n=35]	58.4(19.1-490) [n=16]	37.0(6.56-187) [n=16]
MPA, total (mcg/ml)	N.A.	1.79 (0.48-7.20) [n=34]	3.14 (0.90-7.53) [n=20]	1.87 (0.99-5.54) [n=18]
MPA, unbound (ng/ml)	N.A.	24.2 (6.11- 166) [n=34]	30.9 (9.78-87.2) [n=20]	20.10 (10.1-79.6) [n=18]
AcylMPAG (ng/ml)	N.A.	317 (67.6-1670) [n=34]	492 (235-1860) [n=20]	516 (213-1540) [n=18]

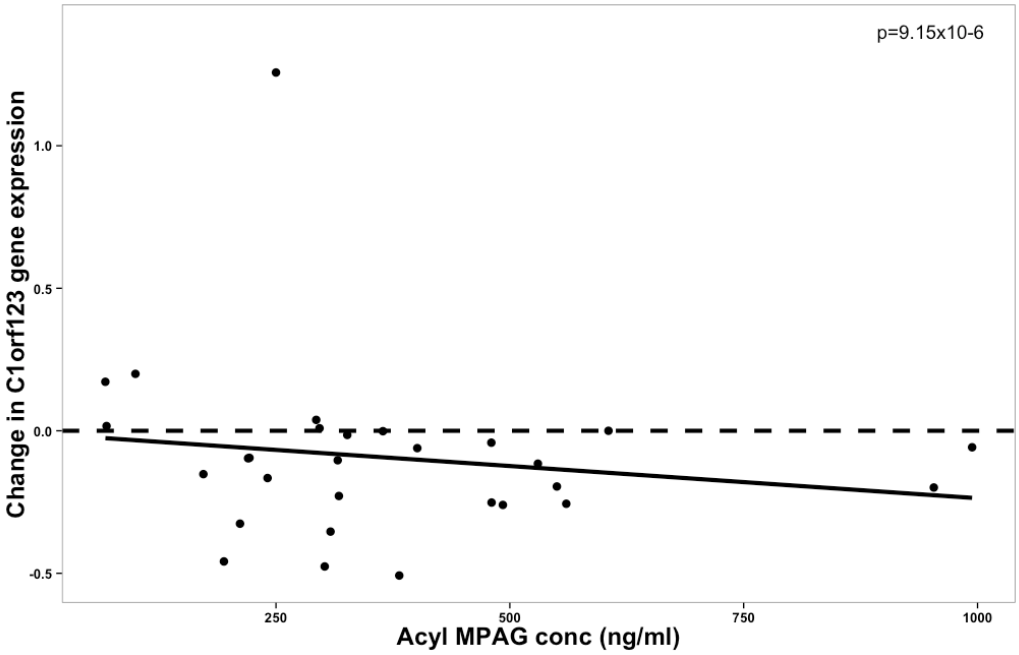
Data are median (range). N.A. is not applicable because patients were not receiving MPA pretransplant.

Figure 5.1: Association of fold change in *SFXN4* gene expression at week 1 relative to pretransplant with total MPA concentrations



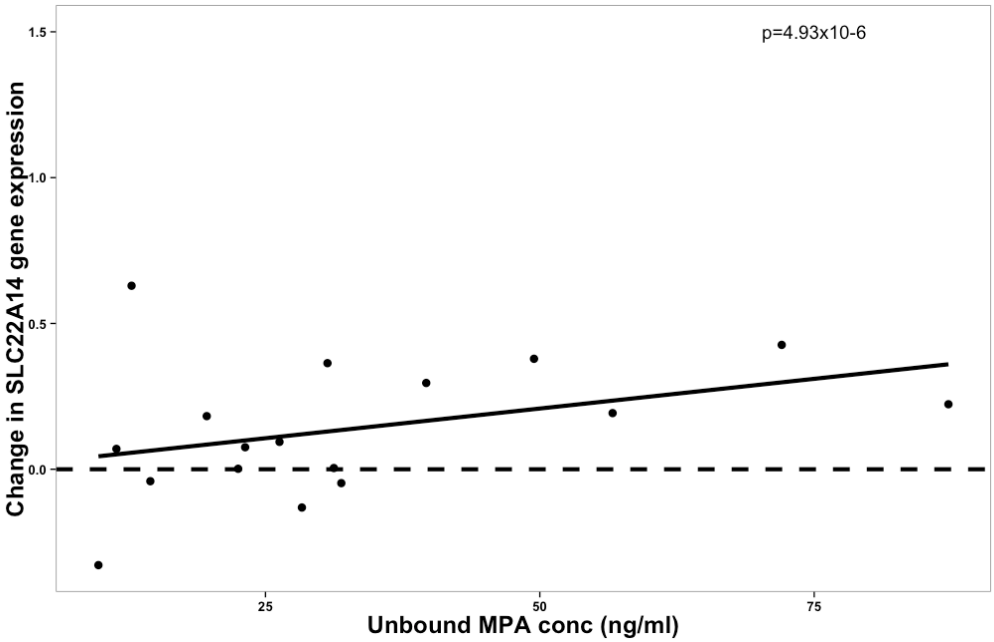
The black dots represent fold change at week 1 of *SFXN4* gene expression from baseline. The fold change was calculated as log [FPKM at week 1]- log [FPKM at baseline]. The solid black line represents linear regression fit and the dotted horizontal line at 0 represent no fold change in gene expression at week 1 from baseline. The black dots below the horizontal line indicate decrease in expression compared to pretransplant baseline, and black dots above the line indicate increase in gene expression compared to pretransplant baseline

Figure 5.2: Association of fold change in *C1orf123* gene expression at week 1 relative to pretransplant with acylMPAG concentrations



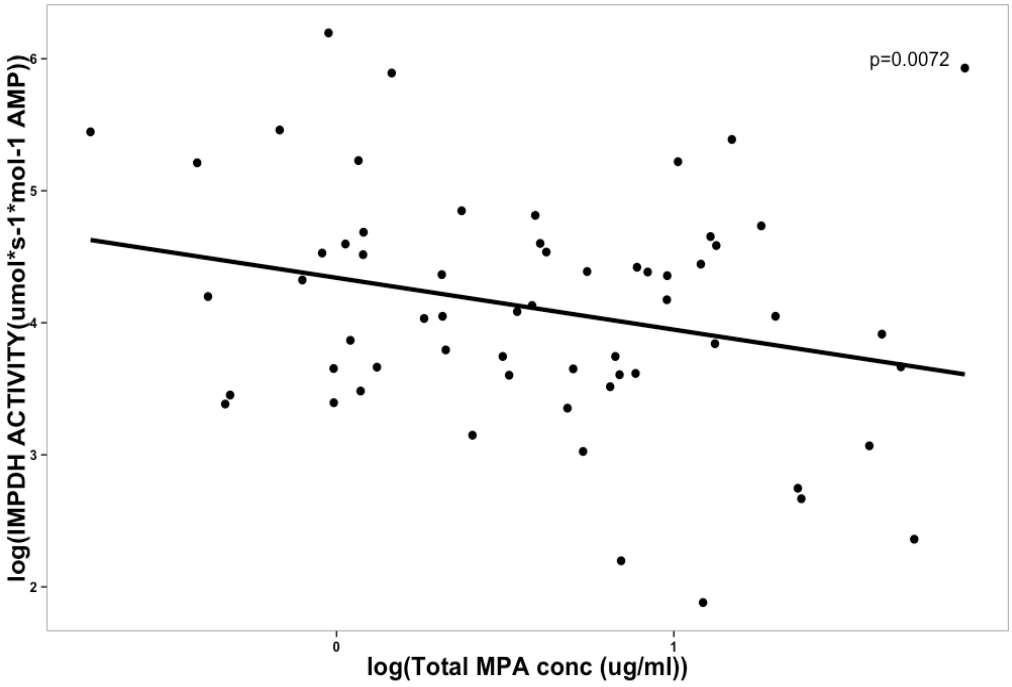
The black dots represent fold change at week 1 of *C1orf123* gene expression from baseline. The fold change was calculated as log [FPKM at week 1]- log [FPKM at baseline]. The solid black line represents linear regression fit and the dotted horizontal line at 0 represent no fold change in gene expression at week 1 from baseline. The black dots below the horizontal line indicate decrease in expression compared to pretransplant baseline, and black dots above the line indicate increase in gene expression compared to pretransplant baseline

Figure 5.3: Association of fold change in *SLC22A14* gene expression at month 3 relative to pretransplant in PBMCs with unbound MPA concentrations



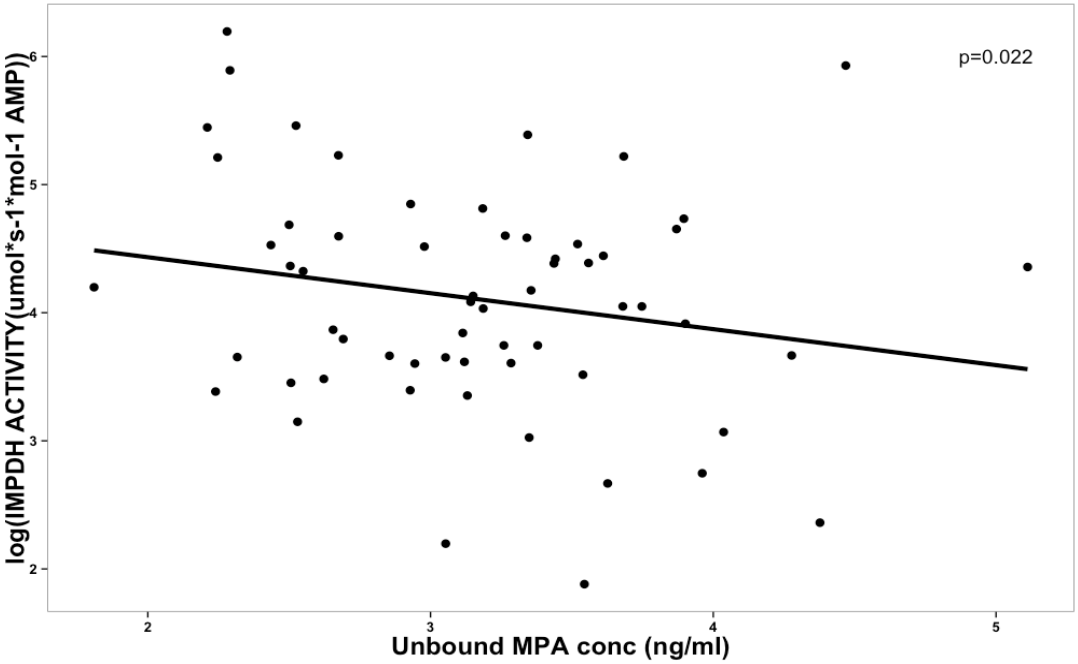
The black dots represent fold change at month 3 of SLC22A14 gene expression from baseline. The fold change was calculated as log [FPKM at week 1]- log [FPKM at baseline]. The solid black line represents linear regression fit and the dotted horizontal line at 0 represent no fold change in gene expression at month 3 from baseline. The black dots below the horizontal line indicate decrease in expression compared to pretransplant baseline, and black dots above the line indicate increase in gene expression compared to pretransplant baseline.

Figure 5.4: **Scatter plot of log (IMPDH activity) vs total MPA plasma concentrations**



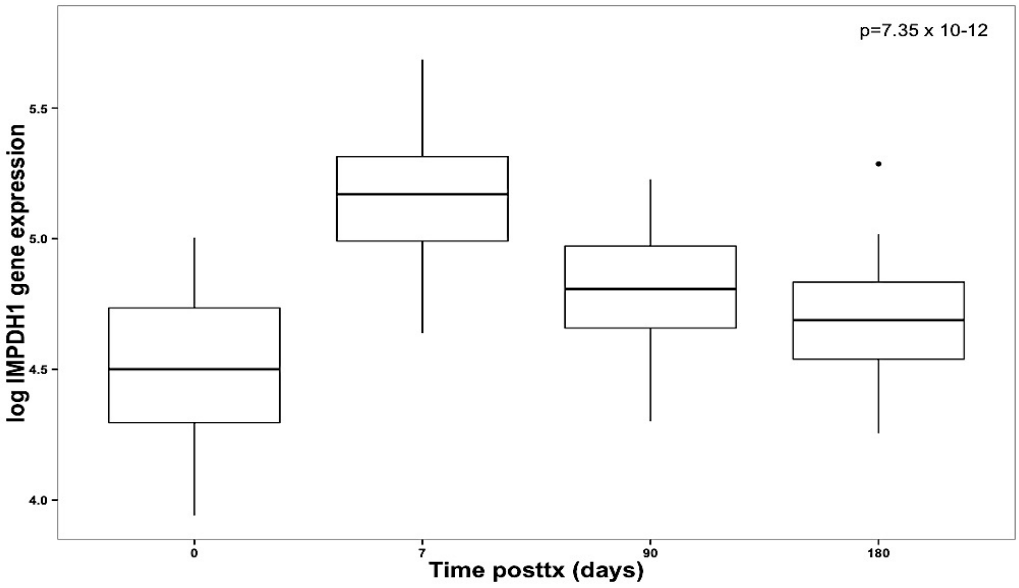
The black dots represent log observed IMPDH activity of each sample and the corresponding log of total MPA trough concentrations over the entire post-transplant period. The solid black line is fit of linear regression. The p-value is one obtained from fitting a linear mixed effect model.

Figure 5.5: **Scatterplot of log (IMPDH activity) vs unbound MPA plasma concentrations**



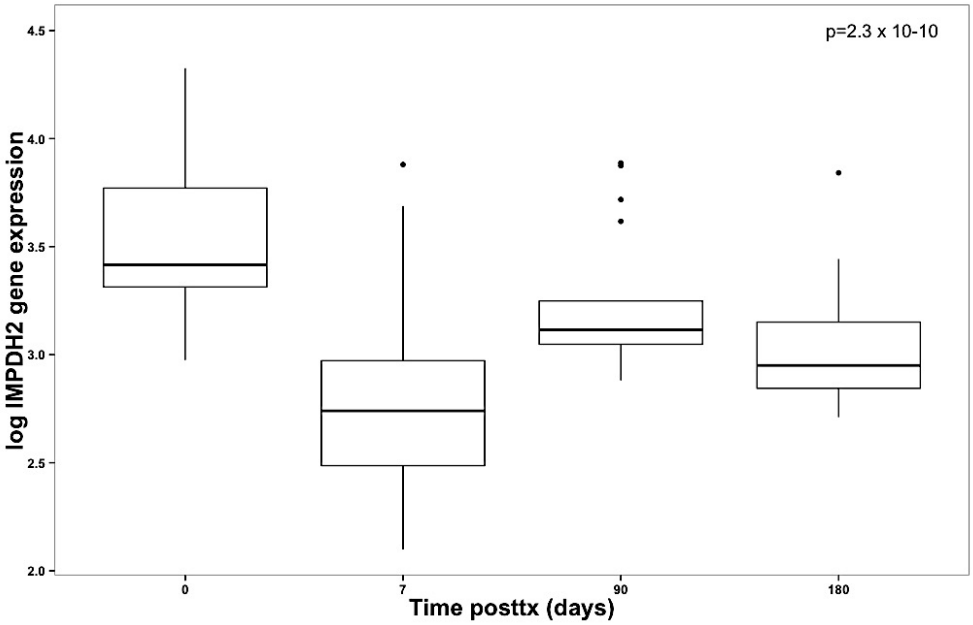
The black dots represent log observed IMPDH activity of each sample and the corresponding log of unbound MPA trough concentrations over the entire post-transplant period. The solid black line is fit of linear regression. The p-value is one obtained from fitting a linear mixed effect model.

Figure 5.6: **Change in IMPDH1 gene expression over time posttransplant**



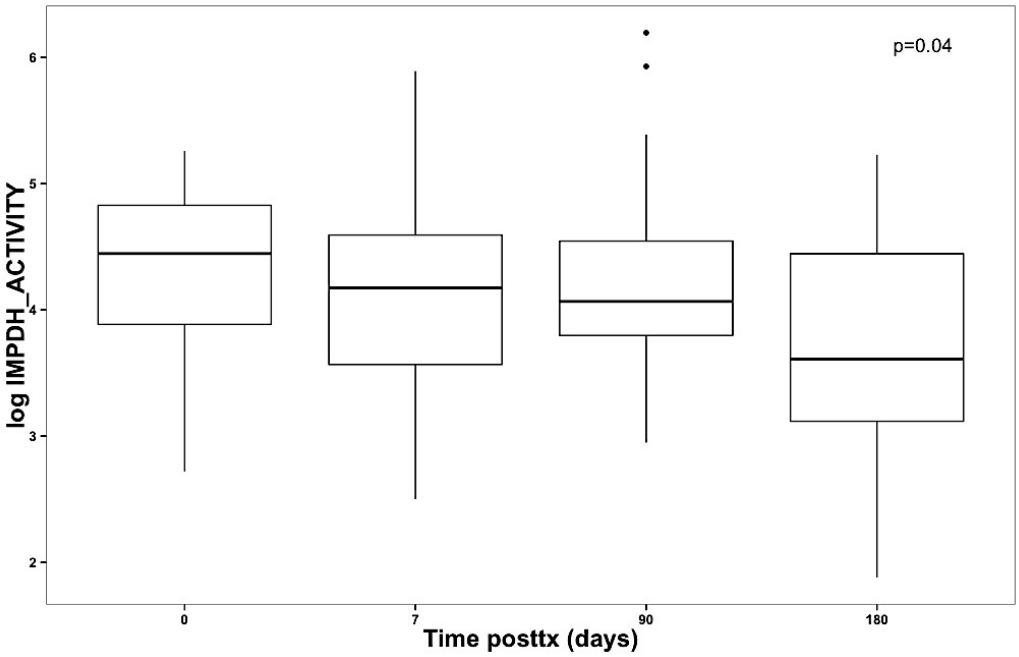
Expression is given in FKPM units. The solid black line in the box represents the median; upper and lower hinge represent the 25th and 75th quartile of the data. The whiskers extend to 1.5 times the interquartile range from the quartiles. Data points beyond the whiskers are represented by solid black dots.

Figure 5.7: Change in IMPDH2 gene expression over time posttransplant



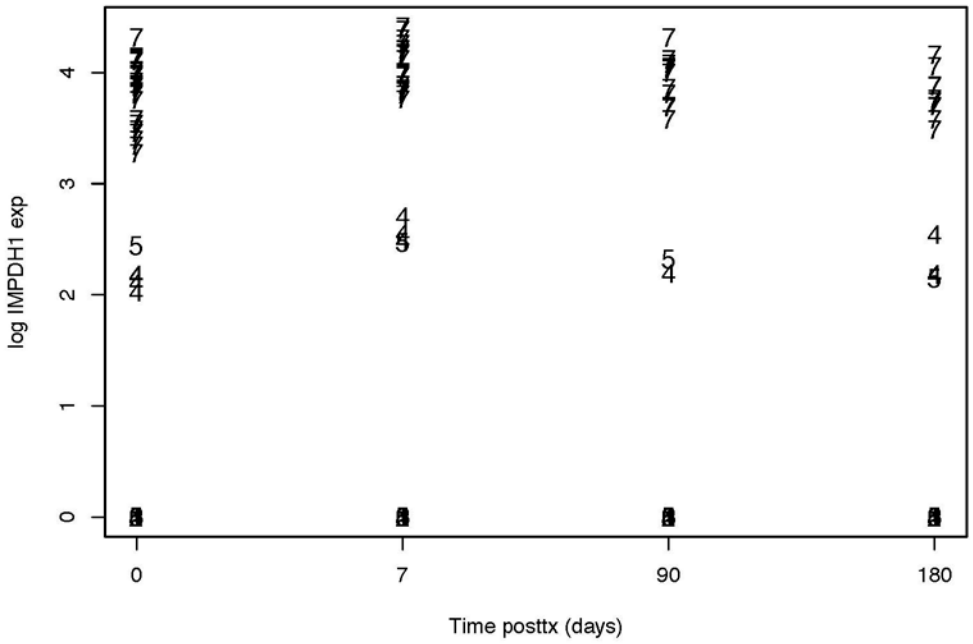
Expression is given in FKPM units. The solid black line in the box represents the median; upper and lower hinge represent the 25th and 75th quartile of the data. The whiskers extend to 1.5 times the interquartile range from the quartiles. Data points beyond the whiskers are represented by solid black dots.

Figure 5.8: Change in IMPDH activity gene expression over time posttransplant



The solid black line in the box represents the median; upper and lower hinge represent the 25th and 75th quartile of the data. The whiskers extend to 1.5 times the interquartile range from the quartiles. Data points beyond the whiskers are represented by solid black dots.

Figure 5.9: Change in expression of IMPDH1 isoforms over time



Expression is given in FKPM units. Each number represents an IMPDH1 isoform. IMPDH1 transcripts are 1=NM_001142575, 2= NM_000883, 3= NM_001142576, 4= NM_001102605, 5= NM_183243, 6= NM_001142574, 7= NM_001142573

CHAPTER VI

6 CONCLUSION AND FUTURE DIRECTIONS

Treatment approaches to most diseases are designed based on the response of a drug in a typical or an average patient and hence a standardized dosing strategy or "one size fits all" is a common practice. Although this approach works for most, a significant proportion of patients either experience a lack of drug efficacy or develop toxicities with this type of approach. Development of highly sensitive and robust assays, high throughput technology, availability of the entire human genome sequence, sophisticated statistical modeling tools have now opened up ample research opportunities to question the ongoing traditional clinical practices in selecting a dosing group. The realization among clinicians and researchers to not only cure diseases but also improve the quality of life has led us to an era of personalized medicine. The objectives of this thesis were to work towards and develop personalized dosing strategies in patients undergoing HSCT and kidney transplantation. This final chapter discusses the main findings of each chapter and proposes future studies that would help consider improvement in clinical practice.

Chapter II was to personalize chemotherapy for HSCT patients. In **Chapter II** of the thesis we identified that CrCl and IBW significantly influenced F-ara-A Cl and thereby F-ara-A AUC. We demonstrated that a lower model predicted F-ara-A Cl and higher F-ara-A AUC significantly increased the hazard of TRM and acute GHVD even after adjusting for important known clinical factors. We developed and validated a dosing model that could individualize fludarabine dose using patients CrCl, IBW and a target F-ara-A AUC. The dosing model could be most useful in obese and in patients with renal impairment where standard BSA based dosing might prove inadequate to choose an

optimal dose. The next steps to this project are to test the dosing model in a small prospective cohort of patients and validate its utility over the standardized methods.

In the next chapter **(Chapter III)** we used similar approach to Chapter II to address variability in the Cy metabolite; PM in HSCT recipients. In an interim analysis we identified that HSCT recipients with higher PM exposure had a significantly higher cumulative incidence of TRM at day 100 and month 6. This triggered the next research question as to what are the factors influencing PM PK and we conducted a population PK study in larger cohort of HSCT recipients. A one-compartment model with first order conversion from parent to metabolite, and first order elimination best explained the observed data. Creatinine clearance significantly influenced PM apparent clearance and gender significantly influenced apparent volume of distribution. This study is ongoing, and a additional clinical factors will be studied as well as genetic factors as we have collected DNA on all subjects. Additionally evaluating correlation between PM concentrations and DNA adducts formed over time, will further strengthen the biological relevance of measuring plasma PM as a marker of efficacy.

In kidney transplant recipients, tacrolimus and mycophenolic acid are presented in this study. **Chapter IV** describes a dosing model developed for tacrolimus in African American kidney transplant recipients. The Clinical Pharmacogenetics Implementation Consortium guidelines developed to recommend tacrolimus dosing in African American kidney transplant recipients are only based on *CYP3A5*3* genotype. However we identified that loss/reduced function *CYP3A5*6* and *CYP3A5*7* variants exclusively found in African Americans also significantly influence tacrolimus PK. We developed an

African American dosing model that includes days post-transplant, age, steroid and anti-CMV drug coadministration and *CYP3A5* variant to predict tacrolimus Cl and allowing for preemptive dose selection. A prospective randomized control trial is under development to demonstrate superiority of the individualized dosing model over standard of care dosing.

In **Chapter V** we analyzed changes in expression of ~20000 genes towards MPA related IMPDH activity and expression in kidney transplant recipients. We found transient changes in expression of many genes at week1 after kidney transplant compared to baseline. Out of the ~20000 genes, expression changes in 3 genes (*SFXN4*, *SLC22A14* and *C1orf123*) were significantly associated to MPA trough concentrations. None of the genes were associated with IMPDH activity. We did not find association of gene expression towards acute rejection or mycophenolate related leukopenia although the number of events were small. Transient changes in gene expression now warrant additional studies to identify biological pathways and mechanisms that play an important role post-transplant, and to help optimize treatment in each patient.

CHAPTER VII

7 BIBILOGRAPHY

1. Majhail NS, Mau LW, Chitphakdithai P, Payton T, Eckrich M, Joffe S, et al. National Survey of Hematopoietic Cell Transplantation Center Personnel, Infrastructure, and Models of Care Delivery. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015;21:1308-1314.
2. Pasquini M, Wang Z, Horowitz MM, Gale RP. 2013 report from the Center for International Blood and Marrow Transplant Research (CIBMTR): current uses and outcomes of hematopoietic cell transplants for blood and bone marrow disorders. Clinical transplants 2013:187-197.
3. Park M, Seo JJ. Role of HLA in Hematopoietic Stem Cell Transplantation. Bone marrow research 2012;2012:680841.
4. Yanada M. Allogeneic hematopoietic cell transplantation for acute myeloid leukemia during first complete remission: a clinical perspective. International journal of hematology 2015;101:243-254.
5. Walter RB, Pagel JM, Gooley TA, Petersdorf EW, Sorror ML, Woolfrey AE, et al. Comparison of matched unrelated and matched related donor myeloablative hematopoietic cell transplantation for adults with acute myeloid leukemia in first remission. Leukemia 2010;24:1276-1282.
6. Schlenk RF, Dohner K, Mack S, Stoppel M, Kiraly F, Gotze K, et al. Prospective evaluation of allogeneic hematopoietic stem-cell transplantation from matched related and matched unrelated donors in younger adults with high-risk acute myeloid leukemia: German-Austrian trial AMLHD98A. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:4642-4648.
7. Gupta V, Tallman MS, He W, Logan BR, Copelan E, Gale RP, et al. Comparable survival after HLA-well-matched unrelated or matched sibling donor transplantation for acute myeloid leukemia in first remission with unfavorable cytogenetics at diagnosis. Blood 2010;116:1839-1848.
8. Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. The New England journal of medicine 2014;371:339-348.
9. Eapen M, O'Donnell P, Brunstein CG, Wu J, Barowski K, Mendizabal A, et al. Mismatched related and unrelated donors for allogeneic hematopoietic cell transplantation for adults with hematologic malignancies. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2014;20:1485-1492.
10. Bachanova V, Burns LJ, Wang T, Carreras J, Gale RP, Wiernik PH, et al. Alternative donors extend transplantation for patients with lymphoma who lack an HLA matched donor. Bone marrow transplantation 2015;50:197-203.
11. Brunstein CG, Petersdorf EW, DeFor TE, Noreen H, Maurer D, MacMillan ML, et al. Impact of Allele-Level HLA Mismatch on Outcomes in Recipients of Double Umbilical Cord Blood Transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015.

12. Marks DI, Woo KA, Zhong X, Appelbaum FR, Bachanova V, Barker JN, et al. Unrelated umbilical cord blood transplant for adult acute lymphoblastic leukemia in first and second complete remission: a comparison with allografts from adult unrelated donors. Haematologica 2014;99:322-328.
13. Peffault de Latour R, Brunstein CG, Porcher R, Chevallier P, Robin M, Warlick E, et al. Similar overall survival using sibling, unrelated donor, and cord blood grafts after reduced-intensity conditioning for older patients with acute myelogenous leukemia. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2013;19:1355-1360.
14. Rodrigues CA, Rocha V, Dreger P, Brunstein C, Sengeloev H, Finke J, et al. Alternative donor hematopoietic stem cell transplantation for mature lymphoid malignancies after reduced-intensity conditioning regimen: similar outcomes with umbilical cord blood and unrelated donor peripheral blood. Haematologica 2014;99:370-377.
15. Warlick ED, Peffault de Latour R, Shanley R, Robin M, Bejanyan N, Xhaard A, et al. Allogeneic hematopoietic cell transplantation outcomes in acute myeloid leukemia: similar outcomes regardless of donor type. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015;21:357-363.
16. Weisdorf D, Eapen M, Ruggeri A, Zhang MJ, Zhong X, Brunstein C, et al. Alternative donor transplantation for older patients with acute myeloid leukemia in first complete remission: a center for international blood and marrow transplant research-eurocord analysis. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2014;20:816-822.
17. Vyas P, Appelbaum FR, Craddock C. Reprint of: Allogeneic hematopoietic cell transplantation for acute myeloid leukemia. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015;21:S3-10.
18. Parimon T, Au DH, Martin PJ, Chien JW. A risk score for mortality after allogeneic hematopoietic cell transplantation. Annals of internal medicine 2006;144:407-414.
19. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood 2005;106:2912-2919.
20. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens: working definitions. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2009;15:1628-1633.
21. Santos GW, Tutschka PJ, Brookmeyer R, Saral R, Beschorner WE, Bias WB, et al. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. The New England journal of medicine 1983;309:1347-1353.

22. Thomas ED, Buckner CD, Banaji M, Clift RA, Fefer A, Flournoy N, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. Blood 1977;49:511-533.
23. Clift RA, Buckner CD, Appelbaum FR, Bearman SI, Petersen FB, Fisher LD, et al. Allogeneic marrow transplantation in patients with acute myeloid leukemia in first remission: a randomized trial of two irradiation regimens. Blood 1990;76:1867-1871.
24. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. 2014;124:344-353.
25. Pingali SR, Champlin RE. Pushing the envelope-nonmyeloablative and reduced intensity preparative regimens for allogeneic hematopoietic transplantation. Bone marrow transplantation 2015;50:1157-1167.
26. Battiwalla M, Barrett J. Allogeneic transplantation using non-myeloablative transplant regimens. Best practice & research Clinical haematology 2001;14:701-722.
27. Maris M, Woolfrey A, McSweeney PA, Sandmaier BM, Nash RA, Georges G, et al. Nonmyeloablative hematopoietic stem cell transplantation: transplantation for the 21st century. Frontiers in bioscience : a journal and virtual library 2001;6:G13-16.
28. Carella AM, Champlin R, Slavin S, McSweeney P, Storb R. Mini-allografts: ongoing trials in humans. Bone marrow transplantation 2000;25:345-350.
29. McSweeney PA, Storb R. Mixed chimerism: preclinical studies and clinical applications. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 1999;5:192-203.
30. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. The New England journal of medicine 1981;304:1529-1533.
31. Bacigalupo A. Second EBMT Workshop on reduced intensity allogeneic hemopoietic stem cell transplants (RI-HSCT). Bone marrow transplantation 2002;29:191-195.
32. Ballen KK, Colvin G, Porter D, Quesenberry PJ. Low dose total body irradiation followed by allogeneic lymphocyte infusion for refractory hematologic malignancy--an updated review. Leukemia & lymphoma 2004;45:905-910.
33. Kim IW, Yun HY, Choi B, Han N, Kim MG, Park S, et al. Population pharmacokinetics analysis of cyclophosphamide with genetic effects in patients undergoing hematopoietic stem cell transplantation. European journal of clinical pharmacology 2013;69:1543-1551.
34. Nagler A, Slavin S, Varadi G, Naparstek E, Samuel S, Or R. Allogeneic peripheral blood stem cell transplantation using a fludarabine-based low intensity conditioning regimen for malignant lymphoma. Bone marrow transplantation 2000;25:1021-1028.
35. Mohty M, Fegueux N, Exbrayat C, Lu ZY, Legouffe E, Quittet P, et al. Reduced intensity conditioning: enhanced graft-versus-tumor effect following dose-reduced conditioning and allogeneic transplantation for refractory lymphoid malignancies after high-dose therapy. Bone marrow transplantation 2001;28:335-339.

36. Niiya H, Kanda Y, Saito T, Ohnishi T, Kanai S, Kawano Y, et al. Early full donor myeloid chimerism after reduced-intensity stem cell transplantation using a combination of fludarabine and busulfan. Haematologica 2001;86:1071-1074.
37. Wasch R, Reisser S, Hahn J, Bertz H, Engelhardt M, Kunzmann R, et al. Rapid achievement of complete donor chimerism and low regimen-related toxicity after reduced conditioning with fludarabine, carmustine, melphalan and allogeneic transplantation. Bone marrow transplantation 2000;26:243-250.
38. Khouri IF, Keating M, Korbling M, Przepiorka D, Anderlini P, O'Brien S, et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1998;16:2817-2824.
39. Weissinger F, Sandmaier BM, Maloney DG, Bensinger WI, Gooley T, Storb R. Decreased transfusion requirements for patients receiving nonmyeloablative compared with conventional peripheral blood stem cell transplants from HLA-identical siblings. Blood 2001;98:3584-3588.
40. Bornhauser M, Thiede C, Platzbecker U, Jenke A, Helwig A, Plettig R, et al. Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. Clinical cancer research : an official journal of the American Association for Cancer Research 2001;7:2254-2262.
41. Schlenk RF, Hartmann F, Hensel M, Jung W, Weber-Nordt R, Gabler A, et al. Less intense conditioning with fludarabine, cyclophosphamide, idarubicin and etoposide (FCIE) followed by allogeneic unselected peripheral blood stem cell transplantation in elderly patients with leukemia. Leukemia 2002;16:581-586.
42. Giralt S, Estey E, Albitar M, van Besien K, Rondon G, Anderlini P, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. Blood 1997;89:4531-4536.
43. Escalon MP, Champlin RE, Saliba RM, Acholonu SA, Hosing C, Fayad L, et al. Nonmyeloablative allogeneic hematopoietic transplantation: a promising salvage therapy for patients with non-Hodgkin's lymphoma whose disease has failed a prior autologous transplantation. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2004;22:2419-2423.
44. Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2009;15:367-369.
45. Reshef R, Porter DL. Reduced-intensity conditioned allogeneic SCT in adults with AML. Bone marrow transplantation 2015;50:759-769.
46. Yamauchi T, Nowak BJ, Keating MJ, Plunkett W. DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by

fludarabine and clofarabine. Clinical cancer research : an official journal of the American Association for Cancer Research 2001;7:3580-3589.
47. Annaloro C, Costa A, Fracchiolla NS, Mometto G, Artuso S, Saporiti G, et al. Severe fludarabine neurotoxicity after reduced intensity conditioning regimen to allogeneic hematopoietic stem cell transplantation: a case report. Clinical case reports 2015;3:650-655.
48. Beitinjaneh A, McKinney AM, Cao Q, Weisdorf DJ. Toxic leukoencephalopathy following fludarabine-associated hematopoietic cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2011;17:300-308.
49. Saumoy M, Castells G, Escoda L, Mares R, Richart C, Ugarriza A. Progressive multifocal leukoencephalopathy in chronic lymphocytic leukemia after treatment with fludarabine. Leukemia & lymphoma 2002;43:433-436.
50. Sioka C, Kyritsis AP. Central and peripheral nervous system toxicity of common chemotherapeutic agents. Cancer chemotherapy and pharmacology 2009;63:761-767.
51. Bonate PL, Arthaud L, Cantrell WR, Jr., Stephenson K, Secrist JA, 3rd, Weitman S. Discovery and development of clofarabine: a nucleoside analogue for treating cancer. Nature reviews Drug discovery 2006;5:855-863.
52. Chevallier P, Labopin M, Buchholz S, Ganser A, Ciceri F, Lioure B, et al. Clofarabine-containing conditioning regimen for allo-SCT in AML/ALL patients: a survey from the Acute Leukemia Working Party of EBMT. European journal of haematology 2012;89:214-219.
53. Fozza C. The role of Clofarabine in the treatment of adults with acute myeloid leukemia. Critical reviews in oncology/hematology 2015;93:237-245.
54. Hall AG, Tilby MJ. Mechanisms of action of, and modes of resistance to, alkylating agents used in the treatment of haematological malignancies. Blood reviews 1992;6:163-173.
55. Ciurea SO, Andersson BS. Busulfan in hematopoietic stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2009;15:523-536.
56. Lawton C. Total-body irradiation for bone marrow transplantation. Oncology (Williston Park, NY) 1999;13:972, 975-978, 981-972.
57. Gyurkocza B, Storb R, Storer BE, Chauncey TR, Lange T, Shizuru JA, et al. Nonmyeloablative allogeneic hematopoietic cell transplantation in patients with acute myeloid leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:2859-2867.
58. Magenau J, Couriel DR. Hematopoietic stem cell transplantation for acute myeloid leukemia: to whom, when, and how. Current oncology reports 2013;15:436-444.
59. Warlick ED, DeFor T, Blazar BR, Burns L, Verneris MR, Ustun C, et al. Successful remission rates and survival after lymphodepleting chemotherapy and donor lymphocyte infusion for relapsed hematologic malignancies postallogeneic hematopoietic cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2012;18:480-486.

60. Schroeder T, Czibere A, Platzbecker U, Bug G, Uharek L, Luft T, et al. Azacitidine and donor lymphocyte infusions as first salvage therapy for relapse of AML or MDS after allogeneic stem cell transplantation. Leukemia 2013;27:1229-1235.
61. Copelan EA. Hematopoietic stem-cell transplantation. The New England journal of medicine 2006;354:1813-1826.
62. Paczesny S. Discovery and validation of graft-versus-host disease biomarkers. Blood 2013;121:585-594.
63. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2005;11:945-956.
64. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015;21:389-401.e381.
65. Lim AB, Storek J, Beligaswatte A, Collins M, Tacey M, Williamson T, et al. Older recipient age is paradoxically associated with a lower incidence of chronic GVHD in thymoglobulin recipients: a retrospective study exploring risk factors for GVHD in allogeneic transplantation with thymoglobulin GVHD prophylaxis. Bone marrow transplantation 2015;50:566-572.
66. Eisner MD, August CS. Impact of donor and recipient characteristics on the development of acute and chronic graft-versus-host disease following pediatric bone marrow transplantation. Bone marrow transplantation 1995;15:663-668.
67. Flowers ME, Inamoto Y, Carpenter PA, Lee SJ, Kiem HP, Petersdorf EW, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. Blood 2011;117:3214-3219.
68. Gale RP, Bortin MM, van Bekkum DW, Biggs JC, Dicke KA, Gluckman E, et al. Risk factors for acute graft-versus-host disease. British journal of haematology 1987;67:397-406.
69. Koreth J, Antin JH. Current and future approaches for control of graft-versus-host disease. Expert review of hematology 2008;1:111.
70. Nash RA, Pepe MS, Storb R, Longton G, Pettinger M, Anasetti C, et al. Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. Blood 1992;80:1838-1845.
71. Pavletic SZ, Fowler DH. Are we making progress in GVHD prophylaxis and treatment? Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program 2012;2012:251-264.
72. Deeg HJ, Storb R, Thomas ED, Flournoy N, Kennedy MS, Banaji M, et al. Cyclosporine as prophylaxis for graft-versus-host disease: a randomized study in patients

undergoing marrow transplantation for acute nonlymphoblastic leukemia. Blood 1985;65:1325-1334.
73. Storb R, Deeg HJ, Thomas ED, Appelbaum FR, Buckner CD, Cheever MA, et al. Marrow transplantation for chronic myelocytic leukemia: a controlled trial of cyclosporine versus methotrexate for prophylaxis of graft-versus-host disease. Blood 1985;66:698-702.
74. Storb R, Deeg HJ, Pepe M, Appelbaum F, Anasetti C, Beatty P, et al. Methotrexate and cyclosporine versus cyclosporine alone for prophylaxis of graft-versus-host disease in patients given HLA-identical marrow grafts for leukemia: long-term follow-up of a controlled trial. Blood 1989;73:1729-1734.
75. Nash RA, Antin JH, Karanes C, Fay JW, Avalos BR, Yeager AM, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood 2000;96:2062-2068.
76. Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood 1998;92:2303-2314.
77. Yanada M, Emi N, Naoe T, Sakamaki H, Takahashi S, Hirabayashi N, et al. Tacrolimus instead of cyclosporine used for prophylaxis against graft-versus-host disease improves outcome after hematopoietic stem cell transplantation from unrelated donors, but not from HLA-identical sibling donors: a nationwide survey conducted in Japan. Bone marrow transplantation 2004;34:331-337.
78. Devine SM, Geller RB, Lin LB, Dix SP, Holland HK, Maurer D, et al. The outcome of unrelated donor bone marrow transplantation in patients with hematologic malignancies using tacrolimus (FK506) and low dose methotrexate for graft-versus-host disease prophylaxis. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 1997;3:25-33.
79. Nash RA, Pineiro LA, Storb R, Deeg HJ, Fitzsimmons WE, Furlong T, et al. FK506 in combination with methotrexate for the prevention of graft-versus-host disease after marrow transplantation from matched unrelated donors. Blood 1996;88:3634-3641.
80. Kharfan-Dabaja M, Mhaskar R, Reljic T, Pidala J, Perkins JB, Djulbegovic B, et al. Mycophenolate mofetil versus methotrexate for prevention of graft-versus-host disease in people receiving allogeneic hematopoietic stem cell transplantation. The Cochrane database of systematic reviews 2014;7:Cd010280.
81. Hamad N, Shanavas M, Michelis FV, Uhm J, Gupta V, Seftel M, et al. Mycophenolate-based graft versus host disease prophylaxis is not inferior to methotrexate in myeloablative-related donor stem cell transplantation. American journal of hematology 2015;90:392-399.
82. Perez-Simon JA, Martino R, Parody R, Cabrero M, Lopez-Corral L, Valcarcel D, et al. The combination of sirolimus plus tacrolimus improves outcome after reduced-intensity conditioning, unrelated donor hematopoietic stem cell transplantation compared with cyclosporine plus mycofenolate. Haematologica 2013;98:526-532.

83. Ohata K, Espinoza JL, Lu X, Kondo Y, Nakao S. Mycophenolic acid inhibits natural killer cell proliferation and cytotoxic function: a possible disadvantage of including mycophenolate mofetil in the graft-versus-host disease prophylaxis regimen. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2011;17:205-213.
84. Abouelnasr A, Roy J, Cohen S, Kiss T, Lachance S. Defining the role of sirolimus in the management of graft-versus-host disease: from prophylaxis to treatment. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2013;19:12-21.
85. Perez-Simon JA, Caballero D, Diez-Campelo M, Lopez-Perez R, Mateos G, Canizo C, et al. Chimerism and minimal residual disease monitoring after reduced intensity conditioning (RIC) allogeneic transplantation. Leukemia 2002;16:1423-1431.
86. Ziakas PD, Zervou FN, Zacharioudakis IM, Mylonakis E. Graft-versus-host disease prophylaxis after transplantation: a network meta-analysis. PloS one 2014;9:e114735.
87. Dulery R, Mohty M, Duhamel A, Robin M, Beguin Y, Michallet M, et al. Antithymocyte globulin before allogeneic stem cell transplantation for progressive myelodysplastic syndrome: a study from the French Society of Bone Marrow Transplantation and Cellular Therapy. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2014;20:646-654.
88. Baron F, Labopin M, Niederwieser D, Vigouroux S, Cornelissen JJ, Malm C, et al. Impact of graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation for acute myeloid leukemia: a report from the Acute Leukemia Working Party of the European group for blood and marrow transplantation. Leukemia 2012;26:2462-2468.
89. Sanacore M, Zhang X, Brown SL, Connor K, Hilton S, Morris LE, et al. Preferential depletion of host over donor T cells through in vivo decay of active rabbit-anti-thymocyte globulin levels during reduced intensity conditioning. Bone marrow transplantation 2015;50:829-833.
90. Mohty M, Bacigalupo A, Saliba F, Zuckermann A, Morelon E, Lebranchu Y. New directions for rabbit antithymocyte globulin (Thymoglobulin((R))) in solid organ transplants, stem cell transplants and autoimmunity. Drugs 2014;74:1605-1634.
91. Bertz H, Illerhaus G, Veelken H, Finke J. Allogeneic hematopoetic stem-cell transplantation for patients with relapsed or refractory lymphomas: comparison of high-dose conventional conditioning versus fludarabine-based reduced-intensity regimens. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2002;13:135-139.
92. Dreger P, Brand R, Hansz J, Milligan D, Corradini P, Finke J, et al. Treatment-related mortality and graft-versus-leukemia activity after allogeneic stem cell transplantation for chronic lymphocytic leukemia using intensity-reduced conditioning. Leukemia 2003;17:841-848.

93. Eom KS, Shin SH, Yoon JH, Yahng SA, Lee SE, Cho BS, et al. Comparable long-term outcomes after reduced-intensity conditioning versus myeloablative conditioning allogeneic stem cell transplantation for adult high-risk acute lymphoblastic leukemia in complete remission. American journal of hematology 2013;88:634-641.
94. Sureda A, Canals C, Arranz R, Caballero D, Ribera JM, Brune M, et al. Allogeneic stem cell transplantation after reduced intensity conditioning in patients with relapsed or refractory Hodgkin's lymphoma. Results of the HDR-ALLO study - a prospective clinical trial by the Grupo Espanol de Linfomas/Trasplante de Medula Osea (GEL/TAMO) and the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. Haematologica 2012;97:310-317.
95. Barba P, Pinana JL, Valcarcel D, Querol L, Martino R, Sureda A, et al. Early and late neurological complications after reduced-intensity conditioning allogeneic stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2009;15:1439-1446.
96. Koh LP, Chen CS, Tai BC, Hwang WY, Tan LK, Ng HY, et al. Impact of postgrafting immunosuppressive regimens on nonrelapse mortality and survival after nonmyeloablative allogeneic hematopoietic stem cell transplant using the fludarabine and low-dose total-body irradiation 200-cGy. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2007;13:790-805.
97. Vigouroux S, Michallet M, Porcher R, Attal M, Ades L, Bernard M, et al. Long-term outcomes after reduced-intensity conditioning allogeneic stem cell transplantation for low-grade lymphoma: a survey by the French Society of Bone Marrow Graft Transplantation and Cellular Therapy (SFGM-TC). Haematologica 2007;92:627-634.
98. Sorror ML. Comorbidities and hematopoietic cell transplantation outcomes. Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program 2010;2010:237-247.
99. Nagler A, Labopin M, Shimoni A, Niederwieser D, Mufti GJ, Zander AR, et al. Mobilized peripheral blood stem cells compared with bone marrow as the stem cell source for unrelated donor allogeneic transplantation with reduced-intensity conditioning in patients with acute myeloid leukemia in complete remission: an analysis from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2012;18:1422-1429.
100. Shimoni A, Hardan I, Shem-Tov N, Rand A, Herscovici C, Yerushalmi R, et al. Comparison between two fludarabine-based reduced-intensity conditioning regimens before allogeneic hematopoietic stem-cell transplantation: fludarabine/melphalan is associated with higher incidence of acute graft-versus-host disease and non-relapse mortality and lower incidence of relapse than fludarabine/busulfan. Leukemia 2007;21:2109-2116.
101. Baron F, Labopin M, Peniket A, Jindra P, Afanasyev B, Sanz MA, et al. Reduced-intensity conditioning with fludarabine and busulfan versus fludarabine and melphalan for patients with acute myeloid leukemia: a report from the Acute Leukemia Working

Party of the European Group for Blood and Marrow Transplantation. Cancer 2015;121:1048-1055.
102. de Lima M, Anagnostopoulos A, Munsell M, Shahjahan M, Ueno N, Ippoliti C, et al. Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. Blood 2004;104:865-872.
103. Pawarode A, Mineishi S, Reddy P, Braun TM, Khaled YA, Choi SW, et al. Reducing Treatment-Related Mortality Did Not Improve Outcomes of Allogeneic Myeloablative Hematopoietic Cell Transplantation for High-Risk Multiple Myeloma: A University of Michigan Prospective Series. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015.
104. Caballero-Velazquez T, Lopez-Corral L, Encinas C, Castilla-Llorente C, Martino R, Rosinol L, et al. Phase II clinical trial for the evaluation of bortezomib within the reduced intensity conditioning regimen (RIC) and post-allogeneic transplantation for high-risk myeloma patients. British journal of haematology 2013;162:474-482.
105. Lee JH, Joo YD, Kim H, Ryoo HM, Kim MK, Lee GW, et al. Randomized trial of myeloablative conditioning regimens: busulfan plus cyclophosphamide versus busulfan plus fludarabine. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31:701-709.
106. Bornhauser M, Kienast J, Trenschel R, Burchert A, Hegenbart U, Stadler M, et al. Reduced-intensity conditioning versus standard conditioning before allogeneic haemopoietic cell transplantation in patients with acute myeloid leukaemia in first complete remission: a prospective, open-label randomised phase 3 trial. The Lancet Oncology 2012;13:1035-1044.
107. Bonin M, Pursche S, Bergeman T, Leopold T, Illmer T, Ehninger G, et al. F-ara-A pharmacokinetics during reduced-intensity conditioning therapy with fludarabine and busulfan. Bone marrow transplantation 2007;39:201-206.
108. Lichtman SM, Etcubanas E, Budman DR, Eisenberg P, Zervos G, D'Amico P, et al. The pharmacokinetics and pharmacodynamics of fludarabine phosphate in patients with renal impairment: a prospective dose adjustment study. Cancer investigation 2002;20:904-913.
109. Martell RE, Peterson BL, Cohen HJ, Petros WP, Rai KR, Morrison VA, et al. Analysis of age, estimated creatinine clearance and pretreatment hematologic parameters as predictors of fludarabine toxicity in patients treated for chronic lymphocytic leukemia: a CALGB (9011) coordinated intergroup study. Cancer chemotherapy and pharmacology 2002;50:37-45.
110. Yin W, Karyagina EV, Lundberg AS, Greenblatt DJ, Lister-James J. Pharmacokinetics, bioavailability and effects on electrocardiographic parameters of oral fludarabine phosphate. Biopharmaceutics & drug disposition 2010;31:72-81.
111. Long-Boyle JR, Green KG, Brunstein CG, Cao Q, Rogosheske J, Weisdorf DJ, et al. High fludarabine exposure and relationship with treatment-related mortality after

nonmyeloablative hematopoietic cell transplantation. Bone marrow transplantation 2011;46:20-26.
112. McCune JS, Mager DE, Bemer MJ, Sandmaier BM, Storer BE, Heimfeld S. Association of fludarabine pharmacokinetic/dynamic biomarkers with donor chimerism in nonmyeloablative HCT recipients. Cancer chemotherapy and pharmacology 2015;76:85-96.
113. McCune JS, Woodahl EL, Furlong T, Storer B, Wang J, Heimfeld S, et al. A pilot pharmacologic biomarker study of busulfan and fludarabine in hematopoietic cell transplant recipients. Cancer chemotherapy and pharmacology 2012;69:263-272.
114. Salinger DH, Blough DK, Vicini P, Anasetti C, O'Donnell PV, Sandmaier BM, et al. A limited sampling schedule to estimate individual pharmacokinetic parameters of fludarabine in hematopoietic cell transplant patients. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15:5280-5287.
115. Bemer MJ, Sorror M, Sandmaier BM, O'Donnell PV, McCune JS. A pilot pharmacologic biomarker study in HLA-haploidentical hematopoietic cell transplant recipients. Cancer chemotherapy and pharmacology 2013;72:607-618.
116. Bornhauser M, Storer B, Slattery JT, Appelbaum FR, Deeg HJ, Hansen J, et al. Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. Blood 2003;102:820-826.
117. Petri CR, O'Donnell PH, Cao H, Artz AS, Stock W, Wickrema A, et al. Clofarabine-associated acute kidney injury in patients undergoing hematopoietic stem cell transplant. Leukemia & lymphoma 2014;55:2866-2873.
118. Long-Boyle J, Huang J, Rydholm N, Smith A, Orchard P, Tolar J, et al. Pharmacokinetics of clofarabine in patients with high-risk inherited metabolic disorders undergoing brain-sparing hematopoietic cell transplantation. Journal of clinical pharmacology 2011;51:679-686.
119. Huitema AD, Mathot RA, Tibben MM, Rodenhuis S, Beijnen JH. A mechanism-based pharmacokinetic model for the cytochrome P450 drug-drug interaction between cyclophosphamide and thioTEPA and the autoinduction of cyclophosphamide. Journal of pharmacokinetics and pharmacodynamics 2001;28:211-230.
120. Qiu R, Yao A, Vicini P, McDonald GB, Batchelder AL, Bouvier ME, et al. Diminishing the risk of nonrelapse mortality in hematopoietic stem cell transplantation: Prediction of exposure to the cyclophosphamide metabolite carboxyethylphosphoramide mustard. Clinical pharmacology and therapeutics 2004;76:270-280.
121. Shu WY, Li JL, Wang XD, Huang M. Pharmacogenomics and personalized medicine: a review focused on their application in the Chinese population. Acta pharmacologica Sinica 2015;36:535-543.
122. Zanger UM, Klein K. Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance. Frontiers in genetics 2013;4:24.
123. Helsby NA, Hui CY, Goldthorpe MA, Coller JK, Soh MC, Gow PJ, et al. The combined impact of CYP2C19 and CYP2B6 pharmacogenetics on cyclophosphamide bioactivation. British journal of clinical pharmacology 2010;70:844-853.

124. Ekhart C, Doodeman VD, Rodenhuis S, Smits PH, Beijnen JH, Huitema AD. Influence of polymorphisms of drug metabolizing enzymes (CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, GSTA1, GSTP1, ALDH1A1 and ALDH3A1) on the pharmacokinetics of cyclophosphamide and 4-hydroxycyclophosphamide. Pharmacogenetics and genomics 2008;18:515-523.
125. de Jonge ME, Huitema AD, Rodenhuis S, Beijnen JH. Integrated Population Pharmacokinetic Model of both cyclophosphamide and thiotepa suggesting a mutual drug-drug interaction. Journal of pharmacokinetics and pharmacodynamics 2004;31:135-156.
126. Slattery JT, Kalhorn TF, McDonald GB, Lambert K, Buckner CD, Bensinger WI, et al. Conditioning regimen-dependent disposition of cyclophosphamide and hydroxycyclophosphamide in human marrow transplantation patients. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1996;14:1484-1494.
127. Balasubramanian P, Desire S, Panetta JC, Lakshmi KM, Mathews V, George B, et al. Population pharmacokinetics of cyclophosphamide in patients with thalassemia major undergoing HSCT. Bone marrow transplantation 2012;47:1178-1185.
128. Ekhart C, Kerst JM, Rodenhuis S, Beijnen JH, Huitema AD. Altered cyclophosphamide and thiotepa pharmacokinetics in a patient with moderate renal insufficiency. Cancer chemotherapy and pharmacology 2009;63:375-379.
129. McDonald GB, Slattery JT, Bouvier ME, Ren S, Batchelder AL, Kalhorn TF, et al. Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. Blood 2003;101:2043-2048.
130. de Jonge ME, Huitema AD, Beijnen JH, Rodenhuis S. High exposures to bioactivated cyclophosphamide are related to the occurrence of veno-occlusive disease of the liver following high-dose chemotherapy. British journal of cancer 2006;94:1226-1230.
131. Huitema AD, Spaander M, Mathjt RA, Tibben MM, Holtkamp MJ, Beijnen JH, et al. Relationship between exposure and toxicity in high-dose chemotherapy with cyclophosphamide, thiotepa and carboplatin. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2002;13:374-384.
132. Petros WP, Broadwater G, Berry D, Jones RB, Vredenburgh JJ, Gilbert CJ, et al. Association of high-dose cyclophosphamide, cisplatin, and carmustine pharmacokinetics with survival, toxicity, and dosing weight in patients with primary breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2002;8:698-705.
133. Yule SM, Price L, McMahon AD, Pearson AD, Boddy AV. Cyclophosphamide metabolism in children with non-Hodgkin's lymphoma. Clinical cancer research : an official journal of the American Association for Cancer Research 2004;10:455-460.
134. Grochow LB, Jones RJ, Brundrett RB, Braine HG, Chen TL, Saral R, et al. Pharmacokinetics of busulfan: correlation with veno-occlusive disease in patients undergoing bone marrow transplantation. Cancer chemotherapy and pharmacology 1989;25:55-61.

135. Sandstrom M, Karlsson MO, Ljungman P, Hassan Z, Jonsson EN, Nilsson C, et al. Population pharmacokinetic analysis resulting in a tool for dose individualization of busulphan in bone marrow transplantation recipients. Bone marrow transplantation 2001;28:657-664.
136. Almog S, Kurnik D, Shimoni A, Loebstein R, Hassoun E, Gopher A, et al. Linearity and stability of intravenous busulfan pharmacokinetics and the role of glutathione in busulfan elimination. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2011;17:117-123.
137. Horn B, Baxter-Lowe LA, Englert L, McMillan A, Quinn M, Desantes K, et al. Reduced intensity conditioning using intravenous busulfan, fludarabine and rabbit ATG for children with nonmalignant disorders and CML. Bone marrow transplantation 2006;37:263-269.
138. Martino R, Perez-Simon JA, Moreno E, Queralto JM, Caballero D, Mateos M, et al. Reduced-intensity conditioning allogeneic blood stem cell transplantation with fludarabine and oral busulfan with or without pharmacokinetically targeted busulfan dosing in patients with myeloid leukemia ineligible for conventional conditioning. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2005;11:437-447.
139. Shaughnessy P, Alexander W, Tran H, Ririe D, Splichal J, Pollack M, et al. Phase I and pharmacokinetic study of once-daily dosing of intravenously administered busulfan in the setting of a reduced-intensity preparative regimen and allogeneic hematopoietic stem cell transplantation as immunotherapy for renal cell carcinoma. Military medicine 2006;171:161-165.
140. Takamatsu Y, Sasaki N, Ogata K, Yukawa E, Jimi S, Hara S, et al. Population pharmacokinetic study of a test dose oral busulfan in Japanese adult patients undergoing hematopoietic stem cell transplantation. Cancer chemotherapy and pharmacology 2010;65:1203-1207.
141. Beumer JH, Owzar K, Lewis LD, Jiang C, Holleran JL, Christner SM, et al. Effect of age on the pharmacokinetics of busulfan in patients undergoing hematopoietic cell transplantation; an alliance study (CALGB 10503, 19808, and 100103). Cancer chemotherapy and pharmacology 2014;74:927-938.
142. Abbasi N, Vadnais B, Knutson JA, Blough DK, Kelly EJ, O'Donnell PV, et al. Pharmacogenetics of intravenous and oral busulfan in hematopoietic cell transplant recipients. Journal of clinical pharmacology 2011;51:1429-1438.
143. Choi B, Kim MG, Han N, Kim T, Ji E, Park S, et al. Population pharmacokinetics and pharmacodynamics of busulfan with GSTA1 polymorphisms in patients undergoing allogeneic hematopoietic stem cell transplantation. Pharmacogenomics 2015;16:1585-1594.
144. ten Brink MH, van Bavel T, Swen JJ, van der Straaten T, Bredius RG, Lankester AC, et al. Effect of genetic variants GSTA1 and CYP39A1 and age on busulfan clearance in pediatric patients undergoing hematopoietic stem cell transplantation. Pharmacogenomics 2013;14:1683-1690.

145. Grochow LB. Busulfan disposition: the role of therapeutic monitoring in bone marrow transplantation induction regimens. Seminars in oncology 1993;20:18-25; quiz 26.
146. Ansari M, Theoret Y, Rezgui MA, Peters C, Mezziani S, Desjean C, et al. Association between busulfan exposure and outcome in children receiving intravenous busulfan before hematopoietic stem cell transplantation. Therapeutic drug monitoring 2014;36:93-99.
147. Kuwatsuka Y, Kohno A, Terakura S, Saito S, Shimada K, Yasuda T, et al. Phase II study of dose-modified busulfan by real-time targeting in allogeneic hematopoietic stem cell transplantation for myeloid malignancy. Cancer science 2012;103:1688-1694.
148. Ljungman P, Hassan M, Bekassy AN, Ringden O, Oberg G. High busulfan concentrations are associated with increased transplant-related mortality in allogeneic bone marrow transplant patients. Bone marrow transplantation 1997;20:909-913.
149. Pidala J, Kim J, Anasetti C, Kharfan-Dabaja MA, Nishihori T, Field T, et al. Pharmacokinetic targeting of intravenous busulfan reduces conditioning regimen related toxicity following allogeneic hematopoietic cell transplantation for acute myelogenous leukemia. Journal of hematology & oncology 2010;3:36.
150. Zhang H, Graiser M, Hutcherson DA, Dada MO, McMillan S, Ali Z, et al. Pharmacokinetic-directed high-dose busulfan combined with cyclophosphamide and etoposide results in predictable drug levels and durable long-term survival in lymphoma patients undergoing autologous stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2012;18:1287-1294.
151. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. American journal of kidney diseases : the official journal of the National Kidney Foundation 2007;49:S12-154.
152. Smith JM, Biggins SW, Haselby DG, Kim WR, Wedd J, Lamb K, et al. Kidney, pancreas and liver allocation and distribution in the United States. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2012;12:3191-3212.
153. Matas AJ, Smith JM, Skeans MA, Thompson B, Gustafson SK, Schnitzler MA, et al. OPTN/SRTR 2012 Annual Data Report: kidney. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2014;14 Suppl 1:11-44.
154. Laftavi MR, Sharma R, Feng L, Said M, Pankewycz O. Induction therapy in renal transplant recipients: a review. Immunological investigations 2014;43:790-806.
155. Hardinger KL. Rabbit antithymocyte globulin induction therapy in adult renal transplantation. Pharmacotherapy 2006;26:1771-1783.
156. Ponticelli C. Basiliximab: efficacy and safety evaluation in kidney transplantation. Expert opinion on drug safety 2014;13:373-381.
157. Stratta RJ, Rogers J, Orlando G, Farooq U, Al-Shraideh Y, Farney AC. 5-year results of a prospective, randomized, single-center study of alemtuzumab compared with

rabbit antithymocyte globulin induction in simultaneous kidney-pancreas transplantation. Transplantation proceedings 2014;46:1928-1931.
158. Hanaway MJ, Woodle ES, Mulgaonkar S, Peddi VR, Kaufman DB, First MR, et al. Alemtuzumab induction in renal transplantation. The New England journal of medicine 2011;364:1909-1919.
159. Haynes R, Harden P, Judge P, Blackwell L, Emberson J, Landray MJ, et al. Alemtuzumab-based induction treatment versus basiliximab-based induction treatment in kidney transplantation (the 3C Study): a randomised trial. Lancet (London, England) 2014;384:1684-1690.
160. Morgan RD, O'Callaghan JM, Knight SR, Morris PJ. Alemtuzumab induction therapy in kidney transplantation: a systematic review and meta-analysis. Transplantation 2012;93:1179-1188.
161. Pascual J, Zamora J, Galeano C, Royuela A, Quereda C. Steroid avoidance or withdrawal for kidney transplant recipients. The Cochrane database of systematic reviews 2009:Cd005632.
162. Lien YH. Top 10 Things Primary Care Physicians Should Know About Maintenance Immunosuppression for Transplant Recipients. The American journal of medicine 2015.
163. Ekberg H, Bernasconi C, Tedesco-Silva H, Vitko S, Hugo C, Demirbas A, et al. Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2009;9:1876-1885.
164. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. Transplantation 1997;63:977-983.
165. Silva HT, Jr., Yang HC, Meier-Kriesche HU, Croy R, Holman J, Fitzsimmons WE, et al. Long-term follow-up of a phase III clinical trial comparing tacrolimus extended-release/MMF, tacrolimus/MMF, and cyclosporine/MMF in de novo kidney transplant recipients. Transplantation 2014;97:636-641.
166. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U.S. Renal Transplant Mycophenolate Mofetil Study Group. Transplantation 1995;60:225-232.
167. Zaza G, Cheok M, Krynetskaia N, Thorn C, Stocco G, Hebert JM, et al. Thiopurine pathway. Pharmacogenetics and genomics 2010;20:573-574.
168. Zsom L, Wagner L, Fulop T. Minimization vs tailoring: Where do we stand with personalized immunosuppression during renal transplantation in 2015? World journal of transplantation 2015;5:73-80.
169. Williams WW, Taheri D, Tolkoff-Rubin N, Colvin RB. Clinical role of the renal transplant biopsy. Nature reviews Nephrology 2012;8:110-121.

170. Harada KM, Mandia-Sampaio EL, de Sandes-Freitas TV, Felipe CR, Park SI, Pinheiro-Machado PG, et al. Risk factors associated with graft loss and patient survival after kidney transplantation. Transplantation proceedings 2009;41:3667-3670.
171. Shi YY, Hesselink DA, van Gelder T. Pharmacokinetics and pharmacodynamics of immunosuppressive drugs in elderly kidney transplant recipients. Transplantation reviews (Orlando, Fla) 2015.
172. de Fijter JW, Mallat MJ, Doxiadis, II, Ringers J, Rosendaal FR, Claas FH, et al. Increased immunogenicity and cause of graft loss of old donor kidneys. Journal of the American Society of Nephrology : JASN 2001;12:1538-1546.
173. Heldal K, Midtvedt K. Managing transplant rejection in the elderly: the benefits of less aggressive immunosuppressive regimens. Drugs & aging 2013;30:459-466.
174. Heldal K, Leivestad T, Hartmann A, Svendsen MV, Lien BH, Midtvedt K. Kidney transplantation in the elderly--the Norwegian experience. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2008;23:1026-1031.
175. Yaich S. ABO-Incompatible kidney transplantation. Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia 2013;24:463-472.
176. Vo AA, Choi J, Cisneros K, Reinsmoen N, Haas M, Ge S, et al. Benefits of rituximab combined with intravenous immunoglobulin for desensitization in kidney transplant recipients. Transplantation 2014;98:312-319.
177. Pham PT, Pham PC, Lipshutz GS, Wilkinson AH. New onset diabetes mellitus after solid organ transplantation. Endocrinology and metabolism clinics of North America 2007;36:873-890; vii.
178. Palepu S, Prasad GV. New-onset diabetes mellitus after kidney transplantation: Current status and future directions. World journal of diabetes 2015;6:445-455.
179. Guitard J, Rostaing L, Kamar N. New-onset diabetes and nephropathy after renal transplantation. Contributions to nephrology 2011;170:247-255.
180. Phelan PJ, Moran AM, O'Kelly P, Heng PY, Yussof SM, Walshe JJ, et al. Steroid withdrawal in renal transplant patients: the Irish experience. Irish journal of medical science 2011;180:429-433.
181. Borda B, Lengyel C, Varkonyi T, Kemeny E, Ottlakan A, Kubik A, et al. Side effects of the calcineurin inhibitor, such as new-onset diabetes after kidney transplantation. Acta physiologica Hungarica 2014;101:388-394.
182. Vincenti F, Friman S, Scheuermann E, Rostaing L, Jenssen T, Campistol JM, et al. Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2007;7:1506-1514.
183. Luan FL, Steffick DE, Ojo AO. New-onset diabetes mellitus in kidney transplant recipients discharged on steroid-free immunosuppression. Transplantation 2011;91:334-341.

184. Weir MR, Burgess ED, Cooper JE, Fenves AZ, Goldsmith D, McKay D, et al. Assessment and management of hypertension in transplant patients. Journal of the American Society of Nephrology : JASN 2015;26:1248-1260.
185. Opelz G, Wujciak T, Ritz E. Association of chronic kidney graft failure with recipient blood pressure. Collaborative Transplant Study. Kidney international 1998;53:217-222.
186. Babel N, Cherepnev G, Kowalenko A, Horstrup J, Volk HD, Reinke P. Nonimmunologic complications and gene polymorphisms of immunoregulatory cytokines in long-term renal transplants. Kidney international 2004;66:428-432.
187. Basset el EA, Berthoux P, Cecillon S, Deprle C, Thibaudin D, De Filippis JP, et al. Hypertension after renal transplantation and polymorphism of genes involved in essential hypertension: ACE, AGT, AT1 R and ecNOS. Clinical nephrology 2002;57:192-200.
188. Ferraresso M, Turolo S, Ghio L, Tirelli AS, Belingheri M, Villa R, et al. Association between CYP3A5 polymorphisms and blood pressure in kidney transplant recipients receiving calcineurin inhibitors. Clinical and experimental hypertension (New York, NY : 1993) 2011;33:359-365.
189. Freedman BI, Murea M. Target organ damage in African American hypertension: role of APOL1. Current hypertension reports 2012;14:21-28.
190. Moes AD, Hesselink DA, Zietse R, van Schaik RH, van Gelder T, Hoorn EJ. Calcineurin inhibitors and hypertension: a role for pharmacogenetics? Pharmacogenomics 2014;15:1243-1251.
191. Afaneh C, Cheng E, Aull MJ, Watkins AC, Kim J, Leeser DB, et al. Renal allograft outcomes following early corticosteroid withdrawal in Hispanic transplant recipients. Clinical transplantation 2013;27:E611-618.
192. Bergmann TK, Barraclough KA, Lee KJ, Staatz CE. Clinical pharmacokinetics and pharmacodynamics of prednisolone and prednisone in solid organ transplantation. Clinical pharmacokinetics 2012;51:711-741.
193. Gulhan B, Topaloglu R, Karabulut E, Ozaltin F, Aki FT, Bilginer Y, et al. Post-transplant hypertension in pediatric kidney transplant recipients. Pediatric nephrology (Berlin, Germany) 2014;29:1075-1080.
194. Wissing KM, Pipeleers L. Obesity, metabolic syndrome and diabetes mellitus after renal transplantation: prevention and treatment. Transplantation reviews (Orlando, Fla) 2014;28:37-46.
195. Campistol JM, Romero R, Paul J, Gutierrez-Dalmau A. Epidemiology of arterial hypertension in renal transplant patients: changes over the last decade. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2004;19 Suppl 3:iii62-66.
196. Thomas B, Taber DJ, Srinivas TR. Hypertension after kidney transplantation: a pathophysiologic approach. Current hypertension reports 2013;15:458-469.
197. Anil Kumar MS, Irfan Saeed M, Ranganna K, Malat G, Sustento-Reodica N, Kumar AM, et al. Comparison of four different immunosuppression protocols without

long-term steroid therapy in kidney recipients monitored by surveillance biopsy: five-year outcomes. Transplant immunology 2008;20:32-42.
198. Park JB, Kim SJ, Oh HY, Han YS, Kim DJ, Park JW, et al. Steroid withdrawal in living donor renal transplant recipients using tacrolimus and cyclosporine: a randomized prospective study. Transplant international : official journal of the European Society for Organ Transplantation 2006;19:478-484.
199. Strozecki P, Adamowicz A, Wlodarczyk Z, Manitius J. The influence of calcineurin inhibitors on pulse wave velocity in renal transplant recipients. Renal failure 2007;29:679-684.
200. Damiano S, Ciarcia R, Montagnaro S, Pagnini U, Garofano T, Capasso G, et al. Prevention of nephrotoxicity induced by cyclosporine-A: role of antioxidants. Journal of cellular biochemistry 2015;116:364-369.
201. Sanders-Pinheiro H, da Silveira ST, Carminatti M, Braga LS, Marsicano EO, Magalhaes GL, et al. Excessive immunosuppression in kidney transplant patients: prevalence and outcomes. Transplantation proceedings 2012;44:2381-2383.
202. Snyder JJ, Israni AK, Peng Y, Zhang L, Simon TA, Kasiske BL. Rates of first infection following kidney transplant in the United States. Kidney international 2009;75:317-326.
203. Kuypers DR, Claes K, Evenepoel P, Maes B, Vanrenterghem Y. Clinical efficacy and toxicity profile of tacrolimus and mycophenolic acid in relation to combined long-term pharmacokinetics in de novo renal allograft recipients. Clinical pharmacology and therapeutics 2004;75:434-447.
204. Smak Gregoor PJ, Hesse CJ, van Gelder T, van der Mast BJ, JN IJ, van Besouw NM, et al. Relation of mycophenolic acid trough levels and adverse events in kidney allograft recipients. Transplantation proceedings 1998;30:1192-1193.
205. Tredger JM, Brown NW, Adams J, Gonde CE, Dhawan A, Rela M, et al. Monitoring mycophenolate in liver transplant recipients: toward a therapeutic range. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society 2004;10:492-502.
206. Ponticelli C, Cucchiari D, Bencini P. Skin cancer in kidney transplant recipients. Journal of nephrology 2014;27:385-394.
207. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clinical pharmacokinetics 2004;43:623-653.
208. Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. Drug metabolism and pharmacokinetics 2007;22:328-335.
209. Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. Clinical pharmacokinetics 2010;49:141-175.
210. Undre NA, Stevenson P, Schafer A. Pharmacokinetics of tacrolimus: clinically relevant aspects. Transplantation proceedings 1999;31:21s-24s.
211. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clinical pharmacokinetics 1995;29:404-430.

212. Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part II. Clinical pharmacokinetics 2010;49:207-221.
213. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. The New England journal of medicine 2007;357:2562-2575.
214. Jusko WJ, Thomson AW, Fung J, McMaster P, Wong SH, Zylber-Katz E, et al. Consensus document: therapeutic monitoring of tacrolimus (FK-506). Therapeutic drug monitoring 1995;17:606-614.
215. Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. Transplantation 1996;62:920-926.
216. McMaster P, Mirza DF, Ismail T, Vennarecci G, Patapis P, Mayer AD. Therapeutic drug monitoring of tacrolimus in clinical transplantation. Therapeutic drug monitoring 1995;17:602-605.
217. Schiff J, Cole E, Cantarovich M. Therapeutic monitoring of calcineurin inhibitors for the nephrologist. Clinical journal of the American Society of Nephrology : CJASN 2007;2:374-384.
218. Hesselink DA, Bouamar R, van Gelder T. The pharmacogenetics of calcineurin inhibitor-related nephrotoxicity. Therapeutic drug monitoring 2010;32:387-393.
219. Naesens M, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. Clinical journal of the American Society of Nephrology : CJASN 2009;4:481-508.
220. Webster AC, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomised trial data. BMJ (Clinical research ed) 2005;331:810.
221. Dai Y, Iwanaga K, Lin YS, Hebert MF, Davis CL, Huang W, et al. In vitro metabolism of cyclosporine A by human kidney CYP3A5. Biochemical pharmacology 2004;68:1889-1902.
222. Akhlaghi F, Gonzalez L, Trull AK. Association between cyclosporine concentrations at 2 hours post-dose and clinical outcomes in de novo lung transplant recipients. The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation 2005;24:2120-2128.
223. Kapturczak MH, Meier-Kriesche HU, Kaplan B. Pharmacology of calcineurin antagonists. Transplantation proceedings 2004;36:25s-32s.
224. Jorga A, Holt DW, Johnston A. Therapeutic drug monitoring of cyclosporine. Transplantation proceedings 2004;36:396s-403s.
225. Thiel G, Bock A, Spondlin M, Brunner FP, Mihatsch M, Rufli T, et al. Long-term benefits and risks of cyclosporin A (sandimmun)--an analysis at 10 years. Transplantation proceedings 1994;26:2493-2498.
226. Birdwell KA, Grady B, Choi L, Xu H, Bian A, Denny JC, et al. The use of a DNA biobank linked to electronic medical records to characterize pharmacogenomic predictors

of tacrolimus dose requirement in kidney transplant recipients. Pharmacogenetics and genomics 2012;22:32-42.
227. Jacobson PA, Oetting WS, Brearley AM, Leduc R, Guan W, Schladt D, et al. Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. Transplantation 2011;91:300-308.
228. Jacobson PA, Schladt D, Oetting WS, Leduc R, Guan W, Matas AJ, et al. Lower calcineurin inhibitor doses in older compared to younger kidney transplant recipients yield similar troughs. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2012;12:3326-3336.
229. Macphee IA, Fredericks S, Mohamed M, Moreton M, Carter ND, Johnston A, et al. Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in whites and South Asians. Transplantation 2005;79:499-502.
230. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature 2012;491:56-65.
231. Benkali K, Rostaing L, Premaud A, Woillard JB, Saint-Marcoux F, Urien S, et al. Population pharmacokinetics and Bayesian estimation of tacrolimus exposure in renal transplant recipients on a new once-daily formulation. Clinical pharmacokinetics 2010;49:683-692.
232. Kurzawski M, Dabrowska J, Dziewanowski K, Domanski L, Peruzynska M, Drozdzik M. CYP3A5 and CYP3A4, but not ABCB1 polymorphisms affect tacrolimus dose-adjusted trough concentrations in kidney transplant recipients. Pharmacogenomics 2014;15:179-188.
233. Shi WL, Tang HL, Zhai SD. Effects of the CYP3A4*1B Genetic Polymorphism on the Pharmacokinetics of Tacrolimus in Adult Renal Transplant Recipients: A Meta-Analysis. PloS one 2015;10:e0127995.
234. Elens L, Capron A, van Schaik RH, De Meyer M, De Pauw L, Eddour DC, et al. Impact of CYP3A4*22 allele on tacrolimus pharmacokinetics in early period after renal transplantation: toward updated genotype-based dosage guidelines. Therapeutic drug monitoring 2013;35:608-616.
235. Elens L, van Gelder T, Hesselink DA, Haufroid V, van Schaik RH. CYP3A4*22: promising newly identified CYP3A4 variant allele for personalizing pharmacotherapy. Pharmacogenomics 2013;14:47-62.
236. Gijsen VM, van Schaik RH, Elens L, Soldin OP, Soldin SJ, Koren G, et al. CYP3A4*22 and CYP3A combined genotypes both correlate with tacrolimus disposition in pediatric heart transplant recipients. Pharmacogenomics 2013;14:1027-1036.
237. Elens L, Hesselink DA, Bouamar R, Budde K, de Fijter JW, De Meyer M, et al. Impact of POR*28 on the pharmacokinetics of tacrolimus and cyclosporine A in renal transplant patients. Therapeutic drug monitoring 2014;36:71-79.
238. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence

variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proceedings of the National Academy of Sciences of the United States of America 2000;97:3473-3478.
239. Birdwell KA, Decker B, Barbarino JM, Peterson JF, Stein CM, Sadee W, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing. Clinical pharmacology and therapeutics 2015;98:19-24.
240. Bouamar R, Hesselink DA, van Schaik RH, Weimar W, Macphee IA, de Fijter JW, et al. Polymorphisms in CYP3A5, CYP3A4, and ABCB1 are not associated with cyclosporine pharmacokinetics nor with cyclosporine clinical end points after renal transplantation. Therapeutic drug monitoring 2011;33:178-184.
241. von Ahsen N, Richter M, Grupp C, Ringe B, Oellerich M, Armstrong VW. No influence of the MDR-1 C3435T polymorphism or a CYP3A4 promoter polymorphism (CYP3A4-V allele) on dose-adjusted cyclosporin A trough concentrations or rejection incidence in stable renal transplant recipients. Clinical chemistry 2001;47:1048-1052.
242. Crettol S, Venetz JP, Fontana M, Aubert JD, Pascual M, Eap CB. CYP3A7, CYP3A5, CYP3A4, and ABCB1 genetic polymorphisms, cyclosporine concentration, and dose requirement in transplant recipients. Therapeutic drug monitoring 2008;30:689-699.
243. Zochowska D, Wyzgal J, Paczek L. Impact of CYP3A4*1B and CYP3A5*3 polymorphisms on the pharmacokinetics of cyclosporine and sirolimus in renal transplant recipients. Annals of transplantation : quarterly of the Polish Transplantation Society 2012;17:36-44.
244. Elens L, van Schaik RH, Panin N, de Meyer M, Wallemacq P, Lison D, et al. Effect of a new functional CYP3A4 polymorphism on calcineurin inhibitors' dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenomics 2011;12:1383-1396.
245. Lunde I, Bremer S, Midtvedt K, Mohebi B, Dahl M, Bergan S, et al. The influence of CYP3A, PPARA, and POR genetic variants on the pharmacokinetics of tacrolimus and cyclosporine in renal transplant recipients. European journal of clinical pharmacology 2014.
246. Moes DJ, Swen JJ, den Hartigh J, van der Straaten T, van der Heide JJ, Sanders JS, et al. Effect of CYP3A4*22, CYP3A5*3, and CYP3A Combined Genotypes on Cyclosporine, Everolimus, and Tacrolimus Pharmacokinetics in Renal Transplantation. CPT: pharmacometrics & systems pharmacology 2014;3:e100.
247. Barraclough KA, Lee KJ, Staatz CE. Pharmacogenetic influences on mycophenolate therapy. Pharmacogenomics 2010;11:369-390.
248. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of mycophenolate in solid organ transplant recipients. Clinical pharmacokinetics 2007;46:13-58.
249. Shipkova M, Armstrong VW, Wieland E, Niedmann PD, Schutz E, Brenner-Weiss G, et al. Identification of glucoside and carboxyl-linked glucuronide conjugates of

mycophenolic acid in plasma of transplant recipients treated with mycophenolate mofetil. British journal of pharmacology 1999;126:1075-1082.
250. Lamba V, Sangkuhl K, Sanghavi K, Fish A, Altman RB, Klein TE. PharmGKB summary: mycophenolic acid pathway. Pharmacogenetics and genomics 2014;24:73-79.
251. Girard H, Court MH, Bernard O, Fortier LC, Villeneuve L, Hao Q, et al. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. Pharmacogenetics 2004;14:501-515.
252. Guo D, Pang LF, Han Y, Yang H, Wang G, Tan ZR, et al. Polymorphisms of UGT1A9 and UGT2B7 influence the pharmacokinetics of mycophenolic acid after a single oral dose in healthy Chinese volunteers. European journal of clinical pharmacology 2013;69:843-849.
253. Gensburger O, Van Schaik RH, Picard N, Le Meur Y, Rousseau A, Woillard JB, et al. Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil. Pharmacogenetics and genomics 2010;20:537-543.
254. Cara CJ, Pena AS, Sans M, Rodrigo L, Guerrero-Esteo M, Hinojosa J, et al. Reviewing the mechanism of action of thiopurine drugs: towards a new paradigm in clinical practice. Medical science monitor : international medical journal of experimental and clinical research 2004;10:Ra247-254.
255. Garat A, Cauffiez C, Renault N, Lo-Guidice JM, Allorge D, Chevalier D, et al. Characterisation of novel defective thiopurine S-methyltransferase allelic variants. Biochemical pharmacology 2008;76:404-415.
256. Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui CH, Yee SW, et al. Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. Clinical pharmacology and therapeutics 2013;93:324-325.
257. Ball LM, Egeler RM. Acute GvHD: pathogenesis and classification. Bone marrow transplantation 2008;41 Suppl 2:S58-64.
258. Sanghavi K, Jacobson P. Pharmacogenomics of Immunosuppressants. In: Johnson JA, Ellingrod VL, Kroetz DL, Kuo GM, editors. Pharmacogenomics: Application To Patient Care 3ed. Kansas: ACCP; 2015. p. 178.
259. Sanghavi K, Jacobson P. Pharmacogenomics of Immunosuppressants. In: Johnson JA, Ellingrod VL, Kroetz DL, Kuo GM, editors. Pharmacogenomics: Application To Patient Care. 3 ed. Kansas: ACCP; 2015. p. 188.
260. Sanghavi K, Jacobson P. Pharmacogenomics of Immunosuppressants. In: Johnson JA, Ellingrod VL, Kroetz DL, Kuo GM, editors. Pharmacogenomics: Application To Patient Care. 3 ed. Kansas: ACCP; 2015. p. 186.
261. Abdul Wahid SF, Ismail NA, Mohd-Idris MR, Jamaluddin FW, Tumian N, Sze-Wei EY, et al. Comparison of reduced-intensity and myeloablative conditioning regimens for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia and acute lymphoblastic leukemia: a meta-analysis. Stem cells and development 2014;23:2535-2552.

262. Aoki J, Kanamori H, Tanaka M, Yamasaki S, Fukuda T, Ogawa H, et al. Impact of age on outcomes of allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning in elderly patients with acute myeloid leukemia. American journal of hematology 2015 [Epub ahead of print].
263. Oran B, Weisdorf DJ. Survival for older patients with acute myeloid leukemia: a population-based study. Haematologica 2012;97:1916-1924.
264. Schneidawind D, Federmann B, Buechele C, Helwig A, Schmohl J, Vogel W, et al. Reduced-intensity conditioning with fludarabine and busulfan for allogeneic hematopoietic cell transplantation in elderly or infirm patients with advanced myeloid malignancies. Annals of hematology 2016;95:115-124.
265. Tomblyn M, Brunstein C, Burns LJ, Miller JS, MacMillan M, DeFor TE, et al. Similar and promising outcomes in lymphoma patients treated with myeloablative or nonmyeloablative conditioning and allogeneic hematopoietic cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2008;14:538-545.
266. Avramis VI, Champagne J, Sato J, Krailo M, Ettinger LJ, Poplack DG, et al. Pharmacology of fludarabine phosphate after a phase I/II trial by a loading bolus and continuous infusion in pediatric patients. Cancer research 1990;50:7226-7231.
267. Chun HG, Leyland-Jones BR, Caryk SM, Hoth DF. Central nervous system toxicity of fludarabine phosphate. Cancer treatment reports 1986;70:1225-1228.
268. Grever M, Leiby J, Kraut E, Metz E, Neidhart J, Balcerzak S, et al. A comprehensive phase I and II clinical investigation of fludarabine phosphate. Seminars in oncology 1990;17:39-48.
269. Malspeis L, Grever MR, Staubus AE, Young D. Pharmacokinetics of 2-F-ara-A (9-beta-D-arabinofuranosyl-2-fluoroadenine) in cancer patients during the phase I clinical investigation of fludarabine phosphate. Seminars in oncology 1990;17:18-32.
270. Griffiths CD, Ng ES, Kangarloo SB, Williamson TS, Chaudhry MA, Booker R, et al. Fludarabine metabolite level on day zero does not affect outcomes of hematopoietic cell transplantation in patients with normal renal function. Bone marrow transplantation 2014;49:589-591.
271. Hersh MR, Kuhn JG, Phillips JL, Clark G, Ludden TM, Von Hoff DD. Pharmacokinetic study of fludarabine phosphate (NSC 312887). Cancer chemotherapy and pharmacology 1986;17:277-280.
272. Knebel W, Davis JC, Jr., Sanders WD, Fessler B, Yarboro C, Pucino F, et al. The pharmacokinetics and pharmacodynamics of fludarabine in rheumatoid arthritis. Pharmacotherapy 1998;18:1224-1229.
273. Bubalo J, Carpenter PA, Majhail N, Perales MA, Marks DI, Shaughnessy P, et al. Conditioning chemotherapy dose adjustment in obese patients: a review and position statement by the American Society for Blood and Marrow Transplantation practice guideline committee. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2014;20:600-616.

274. Kemena A, Fernandez M, Bauman J, Keating M, Plunkett W. A sensitive fluorescence assay for quantitation of fludarabine and metabolites in biological fluids. Clinica chimica acta; international journal of clinical chemistry 1991;200:95-106.
275. Kuo GM, Boumpas DT, Illei GG, Yarboro C, Pucino F, Burstein AH. Fludarabine pharmacokinetics after subcutaneous and intravenous administration in patients with lupus nephritis. Pharmacotherapy 2001;21:528-533.
276. Bodge MN, Reddy S, Thompson MS, Savani BN. Preparative regimen dosing for hematopoietic stem cell transplantation in patients with chronic kidney disease: analysis of the literature and recommendations. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2014;20:908-919.
277. Baker JA, Wickremsinhe ER, Li CH, Oluyedun OA, Dantzig AH, Hall SD, et al. Pharmacogenomics of gemcitabine metabolism: functional analysis of genetic variants in cytidine deaminase and deoxycytidine kinase. Drug metabolism and disposition: the biological fate of chemicals 2013;41:541-545.
278. Farrell JJ, Bae K, Wong J, Guha C, Dicker AP, Elsaleh H. Cytidine deaminase single-nucleotide polymorphism is predictive of toxicity from gemcitabine in patients with pancreatic cancer: RTOG 9704. The pharmacogenomics journal 2012;12:395-403.
279. Khatri A, Williams BW, Fisher J, Brundage RC, Gurvich VJ, Lis LG, et al. SLC28A3 genotype and gemcitabine rate of infusion affect dFdCTP metabolite disposition in patients with solid tumours. British journal of cancer 2014;110:304-312.
280. Wong AL, Yap HL, Yeo WL, Soong R, Ng SS, Wang LZ, et al. Gemcitabine and platinum pathway pharmacogenetics in Asian breast cancer patients. Cancer genomics & proteomics 2011;8:255-259.
281. Yue L, Saikawa Y, Ota K, Tanaka M, Nishimura R, Uehara T, et al. A functional single-nucleotide polymorphism in the human cytidine deaminase gene contributing to ara-C sensitivity. Pharmacogenetics 2003;13:29-38.
282. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41.
283. Pai MP, Paloucek FP. The origin of the "ideal" body weight equations. The Annals of pharmacotherapy 2000;34:1066-1069.
284. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association 1999;94:496-509.
285. Mould DR, Holford NH, Schellens JH, Beijnen JH, Hutson PR, Rosing H, et al. Population pharmacokinetic and adverse event analysis of topotecan in patients with solid tumors. Clinical pharmacology and therapeutics 2002;71:334-348.
286. Brunstein CG, Eapen M, Ahn KW, Appelbaum FR, Ballen KK, Champlin RE, et al. Reduced-intensity conditioning transplantation in acute leukemia: the effect of source of unrelated donor stem cells on outcomes. Blood 2012;119:5591-5598.
287. Raj K, Pagliuca A, Bradstock K, Noriega V, Potter V, Streetly M, et al. Peripheral blood hematopoietic stem cells for transplantation of hematological diseases from related, haploidentical donors after reduced-intensity conditioning. Biology of blood and marrow

transplantation : journal of the American Society for Blood and Marrow Transplantation 2014;20:890-895.
288. Verneris MR, Lee SJ, Ahn KW, Wang HL, Battiwalla M, Inamoto Y, et al. HLA Mismatch Is Associated with Worse Outcomes after Unrelated Donor Reduced-Intensity Conditioning Hematopoietic Cell Transplantation: An Analysis from the Center for International Blood and Marrow Transplant Research. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015;21:1783-1789.
289. Weisdorf D, Zhang MJ, Arora M, Horowitz MM, Rizzo JD, Eapen M. Graft-versus-host disease induced graft-versus-leukemia effect: greater impact on relapse and disease-free survival after reduced intensity conditioning. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2012;18:1727-1733.
290. Fludara(R). Wayne, NJ: Bayer HealthCare Pharmaceuticals Inc.; 2008. http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020038s032lbl.pdf.
291. Griggs JJ, Mangu PB, Anderson H, Balaban EP, Dignam JJ, Hryniuk WM, et al. Appropriate chemotherapy dosing for obese adult patients with cancer: American Society of Clinical Oncology clinical practice guideline. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:1553-1561.
292. de Jonge ME, Huitema AD, Rodenhuis S, Beijnen JH. Clinical pharmacokinetics of cyclophosphamide. Clinical pharmacokinetics 2005;44:1135-1164.
293. Moore MJ. Clinical pharmacokinetics of cyclophosphamide. Clinical pharmacokinetics 1991;20:194-208.
294. de Jonge ME, Huitema AD, van Dam SM, Rodenhuis S, Beijnen JH. Population pharmacokinetics of cyclophosphamide and its metabolites 4-hydroxycyclophosphamide, 2-dechloroethylcyclophosphamide, and phosphoramide mustard in a high-dose combination with Thiotepa and Carboplatin. Therapeutic drug monitoring 2005;27:756-765.
295. Chang TK, Yu L, Goldstein JA, Waxman DJ. Identification of the polymorphically expressed CYP2C19 and the wild-type CYP2C9-ILE359 allele as low-Km catalysts of cyclophosphamide and ifosfamide activation. Pharmacogenetics 1997;7:211-221.
296. Ekhart C, Rodenhuis S, Smits PH, Beijnen JH, Huitema AD. Relations between polymorphisms in drug-metabolising enzymes and toxicity of chemotherapy with cyclophosphamide, thiotepa and carboplatin. Pharmacogenetics and genomics 2008;18:1009-1015.
297. Gervot L, Rochat B, Gautier JC, Bohnenstengel F, Kroemer H, de Berardinis V, et al. Human CYP2B6: expression, inducibility and catalytic activities. Pharmacogenetics 1999;9:295-306.
298. Gor PP, Su HI, Gray RJ, Gimotty PA, Horn M, Aplenc R, et al. Cyclophosphamide-metabolizing enzyme polymorphisms and survival outcomes after adjuvant chemotherapy for node-positive breast cancer: a retrospective cohort study. Breast cancer research : BCR 2010;12:R26.

299. Haroun F, Al-Shaar L, Habib RH, El-Saghir N, Tfayli A, Bazarbachi A, et al. Effects of CYP2B6 genetic polymorphisms in patients receiving cyclophosphamide combination chemotherapy for breast cancer. Cancer chemotherapy and pharmacology 2015;75:207-214.
300. Jamieson D, Lee J, Cresti N, Jackson R, Griffin M, Sludden J, et al. Pharmacogenetics of adjuvant breast cancer treatment with cyclophosphamide, epirubicin and 5-fluorouracil. Cancer chemotherapy and pharmacology 2014;74:667-674.
301. Joy MS, La M, Wang J, Bridges AS, Hu Y, Hogan SL, et al. Cyclophosphamide and 4-hydroxycyclophosphamide pharmacokinetics in patients with glomerulonephritis secondary to lupus and small vessel vasculitis. British journal of clinical pharmacology 2012;74:445-455.
302. Nakajima M, Komagata S, Fujiki Y, Kanada Y, Ebi H, Itoh K, et al. Genetic polymorphisms of CYP2B6 affect the pharmacokinetics/pharmacodynamics of cyclophosphamide in Japanese cancer patients. Pharmacogenetics and genomics 2007;17:431-445.
303. Ngamjanyaporn P, Thakkinstian A, Verasertniyom O, Chatchaipun P, Vanichapuntu M, Nantiruj K, et al. Pharmacogenetics of cyclophosphamide and CYP2C19 polymorphism in Thai systemic lupus erythematosus. Rheumatology international 2011;31:1215-1218.
304. Pinto N, Ludeman SM, Dolan ME. Drug focus: Pharmacogenetic studies related to cyclophosphamide-based therapy. Pharmacogenomics 2009;10:1897-1903.
305. Tulsyan S, Agarwal G, Lal P, Mittal B. Significant role of CYP450 genetic variants in cyclophosphamide based breast cancer treatment outcomes: a multi-analytical strategy. Clinica chimica acta; international journal of clinical chemistry 2014;434:21-28.
306. van Schaik RH. Implications of cytochrome P450 genetic polymorphisms on the toxicity of antitumor agents. Therapeutic drug monitoring 2004;26:236-240.
307. Xie H, Griskevicius L, Stahle L, Hassan Z, Yasar U, Rane A, et al. Pharmacogenetics of cyclophosphamide in patients with hematological malignancies. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 2006;27:54-61.
308. Rocha V, Porcher R, Fernandes JF, Filion A, Bittencourt H, Silva W, Jr., et al. Association of drug metabolism gene polymorphisms with toxicities, graft-versus-host disease and survival after HLA-identical sibling hematopoietic stem cell transplantation for patients with leukemia. Leukemia 2009;23:545-556.
309. Marr KA, Leisenring W, Crippa F, Slattery JT, Corey L, Boeckh M, et al. Cyclophosphamide metabolism is affected by azole antifungals. Blood 2004;103:1557-1559.
310. Upton A, McCune JS, Kirby KA, Leisenring W, McDonald G, Batchelder A, et al. Fluconazole coadministration concurrent with cyclophosphamide conditioning may reduce regimen-related toxicity postmyeloablative hematopoietic cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2007;13:760-764.

311. Braverman AC, Antin JH, Plappert MT, Cook EF, Lee RT. Cyclophosphamide cardiotoxicity in bone marrow transplantation: a prospective evaluation of new dosing regimens. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1991;9:1215-1223.
312. Tsuboi K, Kishi K, Ohmachi K, Yasuda Y, Shimizu T, Inoue H, et al. Multivariate analysis of risk factors for hemorrhagic cystitis after hematopoietic stem cell transplantation. Bone marrow transplantation 2003;32:903-907.
313. Kachel DL, Martin WJ, 2nd. Cyclophosphamide-induced lung toxicity: mechanism of endothelial cell injury. The Journal of pharmacology and experimental therapeutics 1994;268:42-46.
314. DeLeve LD. Cellular target of cyclophosphamide toxicity in the murine liver: role of glutathione and site of metabolic activation. Hepatology (Baltimore, Md) 1996;24:830-837.
315. Chan KK, Hong PS, Tutsch K, Trump DL. Clinical pharmacokinetics of cyclophosphamide and metabolites with and without SR-2508. Cancer research 1994;54:6421-6429.
316. Huitema AD, Tibben MM, Kerbusch T, Kettenes-van den Bosch JJ, Rodenhuis S, Beijnen JH. Simple and selective determination of the cyclophosphamide metabolite phosphoramide mustard in human plasma using high-performance liquid chromatography. Journal of chromatography B, Biomedical sciences and applications 2000;745:345-355.
317. Huitema AD, Kerbusch T, Tibben MM, Rodenhuis S, Beijnen JH. Reduction of cyclophosphamide bioactivation by thioTEPA: critical sequence-dependency in high-dose chemotherapy regimens. Cancer chemotherapy and pharmacology 2000;46:119-127.
318. Hagos Y, Hundertmark P, Shnitsar V, Marada VV, Wulf G, Burckhardt G. Renal human organic anion transporter 3 increases the susceptibility of lymphoma cells to bendamustine uptake. American journal of physiology Renal physiology 2015;308:F330-338.
319. Marada VV, Florl S, Kuhne A, Muller J, Burckhardt G, Hagos Y. Interaction of human organic anion transporter 2 (OAT2) and sodium taurocholate cotransporting polypeptide (NTCP) with antineoplastic drugs. Pharmacological research 2015;91:78-87.
320. Juma FD, Rogers HJ, Trounce JR. The pharmacokinetics of cyclophosphamide, phosphoramide mustard and nor-nitrogen mustard studied by gas chromatography in patients receiving cyclophosphamide therapy. British journal of clinical pharmacology 1980;10:327-335.
321. Deeg HJ, Seidel K, Bruemmer B, Pepe MS, Appelbaum FR. Impact of patient weight on non-relapse mortality after marrow transplantation. Bone marrow transplantation 1995;15:461-468.
322. Hadjibabaie M, Tabeefar H, Alimoghaddam K, Iravani M, Eslami K, Honarmand H, et al. The relationship between body mass index and outcomes in leukemic patients undergoing allogeneic hematopoietic stem cell transplantation. Clinical transplantation 2012;26:149-155.

323. Johnson LA, Tretyakova N, Jacobson PA. Obesity effects on cyclophosphamide-induced DNA damage in hematopoietic cell transplant recipients. In vivo (Athens, Greece) 2012;26:853-857.
324. Meloni G, Proia A, Capria S, Romano A, Trape G, Trisolini SM, et al. Obesity and autologous stem cell transplantation in acute myeloid leukemia. Bone marrow transplantation 2001;28:365-367.
325. Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, et al. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. The Journal of pharmacology and experimental therapeutics 2003;307:906-922.
326. Juma FD, Rogers HJ, Trounce JR. Effect of renal insufficiency on the pharmacokinetics of cyclophosphamide and some of its metabolites. European journal of clinical pharmacology 1981;19:443-451.
327. El-Serafi I, Fares M, Abedi-Valugerdi M, Afsharian P, Moshfegh A, Terelius Y, et al. Cytochrome P450 2J2, a new key enzyme in cyclophosphamide bioactivation and a potential biomarker for hematological malignancies. 2015;15:405-413.
328. Johnson GG, Lin K, Cox TF, Oates M, Sibson DR, Eccles R, et al. CYP2B6*6 is an independent determinant of inferior response to fludarabine plus cyclophosphamide in chronic lymphocytic leukemia. Blood 2013;122:4253-4258.
329. Shu W, Guan S, Yang X, Liang L, Li J, Chen Z, et al. Genetic markers in CYP2C19 and CYP2B6 for prediction of cyclophosphamide's 4-hydroxylation, efficacy and side effects in Chinese patients with systemic lupus erythematosus. British journal of clinical pharmacology 2016;81:327-340.
330. Veal GJ, Cole M, Chinnaswamy G, Sludden J, Jamieson D, Errington J, et al. Cyclophosphamide pharmacokinetics and pharmacogenetics in children with B-cell non-Hodgkin's lymphoma. European journal of cancer (Oxford, England : 1990) 2016;55:56-64.
331. Wang HN, Zhu XY, Zhu Y, Xie QH, Lai LY, Zhao M, et al. The GSTA1 polymorphism and cyclophosphamide therapy outcomes in lupus nephritis patients. Clinical immunology (Orlando, Fla) 2015;160:342-348.
332. Bachanova V, Shanley R, Malik F, Chauhan L, Lamba V, Weisdorf DJ, et al. Cytochrome P450 2B6*5 Increases Relapse after Cyclophosphamide-Containing Conditioning and Autologous Transplantation for Lymphoma. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015;21:944-948.
333. Johnson LA, Malayappan B, Tretyakova N, Campbell C, MacMillan ML, Wagner JE, et al. Formation of cyclophosphamide specific DNA adducts in hematological diseases. Pediatric blood & cancer 2012;58:708-714.
334. Lainez JM, Orcun S, Pekny JF, Reklaitis GV, Suvannasankha A, Fausel C, et al. Comparison of an assumption-free Bayesian approach with Optimal Sampling Schedule to a maximum a posteriori Approach for Personalizing Cyclophosphamide Dosing. Pharmacotherapy 2014;34:330-335.

335. McCune JS, Batchelder A, Guthrie KA, Witherspoon R, Appelbaum FR, Phillips B, et al. Personalized dosing of cyclophosphamide in the total body irradiation-cyclophosphamide conditioning regimen: a phase II trial in patients with hematologic malignancy. Clinical pharmacology and therapeutics 2009;85:615-622.
336. McDonald GB, McCune JS, Batchelder A, Cole S, Phillips B, Ren AG, et al. Metabolism-based cyclophosphamide dosing for hematopoietic cell transplant. Clinical pharmacology and therapeutics 2005;78:298-308.
337. Salinger DH, McCune JS, Ren AG, Shen DD, Slattery JT, Phillips B, et al. Real-time dose adjustment of cyclophosphamide in a preparative regimen for hematopoietic cell transplant: a Bayesian pharmacokinetic approach. Clinical cancer research : an official journal of the American Association for Cancer Research 2006;12:4888-4898.
338. McCullough KP, Keith DS, Meyer KH, Stock PG, Brayman KL, Leichtman AB. Kidney and pancreas transplantation in the United States, 1998-2007: access for patients with diabetes and end-stage renal disease. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2009;9:894-906.
339. Fan PY, Ashby VB, Fuller DS, Boulware LE, Kao A, Norman SP, et al. Access and outcomes among minority transplant patients, 1999-2008, with a focus on determinants of kidney graft survival. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2010;10:1090-1107.
340. Gondos A, Dohler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. Transplantation 2013;95:267-274.
341. Press R, Carrasquillo O, Nickolas T, Radhakrishnan J, Shea S, Barr RG. Race/ethnicity, poverty status, and renal transplant outcomes. Transplantation 2005;80:917-924.
342. Young CJ, Gaston RS. Renal transplantation in black Americans. The New England journal of medicine 2000;343:1545-1552.
343. Eckhoff DE, Young CJ, Gaston RS, Fineman SW, Deierhoi MH, Foushee MT, et al. Racial disparities in renal allograft survival: a public health issue? Journal of the American College of Surgeons 2007;204:894-902; discussion 902-893.
344. Martins D, Tareen N, Norris KC. The epidemiology of end-stage renal disease among African Americans. The American journal of the medical sciences 2002;323:65-71.
345. de Jonge H, Naesens M, Kuypers DR. New insights into the pharmacokinetics and pharmacodynamics of the calcineurin inhibitors and mycophenolic acid: possible consequences for therapeutic drug monitoring in solid organ transplantation. Therapeutic drug monitoring 2009;31:416-435.
346. Kahan BD, Keown P, Levy GA, Johnston A. Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. Clinical therapeutics 2002;24:330-350; discussion 329.

347. Monchaud C, Marquet P. Pharmacokinetic optimization of immunosuppressive therapy in thoracic transplantation: part I. Clinical pharmacokinetics 2009;48:419-462.
348. Shaw LM, Holt DW, Keown P, Venkataramanan R, Yatscoff RW. Current opinions on therapeutic drug monitoring of immunosuppressive drugs. Clinical therapeutics 1999;21:1632-1652; discussion 1631.
349. Staatz C, Taylor P, Tett S. Low tacrolimus concentrations and increased risk of early acute rejection in adult renal transplantation. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2001;16:1905-1909.
350. Undre NA, van Hooff J, Christiaans M, Vanrenterghem Y, Donck J, Heeman U, et al. Low systemic exposure to tacrolimus correlates with acute rejection. Transplantation proceedings 1999;31:296-298.
351. Dirks NL, Huth B, Yates CR, Meibohm B. Pharmacokinetics of immunosuppressants: a perspective on ethnic differences. International journal of clinical pharmacology and therapeutics 2004;42:701-718.
352. Fitzsimmons WE, Bekersky I, Dressler D, Raye K, Hodosh E, Mekki Q. Demographic considerations in tacrolimus pharmacokinetics. Transplantation proceedings 1998;30:1359-1364.
353. Mancinelli LM, Frassetto L, Floren LC, Dressler D, Carrier S, Bekersky I, et al. The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. Clinical pharmacology and therapeutics 2001;69:24-31.
354. Hricik DE, Anton HA, Knauss TC, Rodriguez V, Seaman D, Siegel C, et al. Outcomes of African American kidney transplant recipients treated with sirolimus, tacrolimus, and corticosteroids. Transplantation 2002;74:189-193.
355. Andrews PA, Sen M, Chang RW. Racial variation in dosage requirements of tacrolimus. Lancet (London, England) 1996;348:1446.
356. Beermann KJ, Ellis MJ, Sudan DL, Harris MT. Tacrolimus dose requirements in African-American and Caucasian kidney transplant recipients on mycophenolate and prednisone. Clinical transplantation 2014;28:762-767.
357. Laftavi MR, Pankewycz O, Patel S, Nader N, Kohli R, Feng L, et al. African American renal transplant recipients (RTR) require higher tacrolimus doses to achieve target levels compared to white RTR: does clotrimazole help? Transplantation proceedings 2013;45:3498-3501.
358. Narayanan M, Pankewycz O, El-Ghoroury M, Shihab F, Wiland A, McCague K, et al. Outcomes in African American kidney transplant patients receiving tacrolimus and mycophenolic acid immunosuppression. Transplantation 2013;95:566-572.
359. Vadivel N, Garg A, Holt DW, Chang RW, MacPhee IA. Tacrolimus dose in black renal transplant recipients. Transplantation 2007;83:997-999.
360. Barbarino JM, Staatz CE, Venkataramanan R, Klein TE, Altman RB. PharmGKB summary: cyclosporine and tacrolimus pathways. Pharmacogenetics and genomics 2013;23:563-585.

361. Kamdem LK, Streit F, Zanger UM, Brockmoller J, Oellerich M, Armstrong VW, et al. Contribution of CYP3A5 to the in vitro hepatic clearance of tacrolimus. Clinical chemistry 2005;51:1374-1381.
362. Hebert MF. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. Advanced drug delivery reviews 1997;27:201-214.
363. Jeong H, Chiou WL. Role of P-glycoprotein in the hepatic metabolism of tacrolimus. Xenobiotica; the fate of foreign compounds in biological systems 2006;36:1-13.
364. de Jonge H, Metalidis C, Naesens M, Lambrechts D, Kuypers DR. The P450 oxidoreductase *28 SNP is associated with low initial tacrolimus exposure and increased dose requirements in CYP3A5-expressing renal recipients. Pharmacogenomics 2011;12:1281-1291.
365. Elens L, Nieuweboer AJ, Clarke SJ, Charles KA, de Graan AJ, Haufroid V, et al. Impact of POR*28 on the clinical pharmacokinetics of CYP3A phenotyping probes midazolam and erythromycin. Pharmacogenetics and genomics 2013;23:148-155.
366. Gomez-Bravo MA, Salcedo M, Fondevila C, Suarez F, Castellote J, Rufian S, et al. Impact of donor and recipient CYP3A5 and ABCB1 genetic polymorphisms on tacrolimus dosage requirements and rejection in Caucasian Spanish liver transplant patients. Journal of clinical pharmacology 2013;53:1146-1154.
367. Hesselink DA, Bouamar R, Elens L, van Schaik RH, van Gelder T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. Clinical pharmacokinetics 2014;53:123-139.
368. Kuypers DR, de Loor H, Naesens M, Coopmans T, de Jonge H. Combined effects of CYP3A5*1, POR*28, and CYP3A4*22 single nucleotide polymorphisms on early concentration-controlled tacrolimus exposure in de-novo renal recipients. Pharmacogenetics and genomics 2014;24:597-606.
369. Pallet N, Jannot AS, El Bahri M, Etienne I, Buchler M, de Ligny BH, et al. Kidney transplant recipients carrying the CYP3A4*22 allelic variant have reduced tacrolimus clearance and often reach supratherapeutic tacrolimus concentrations. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2015;15:800-805.
370. Busi F, Cresteil T. CYP3A5 mRNA degradation by nonsense-mediated mRNA decay. Molecular pharmacology 2005;68:808-815.
371. Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, et al. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 2001;11:773-779.
372. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nature genetics 2001;27:383-391.
373. de Jonge H, de Loor H, Verbeke K, Vanrenterghem Y, Kuypers DR. Impact of CYP3A5 genotype on tacrolimus versus midazolam clearance in renal transplant recipients: new insights in CYP3A5-mediated drug metabolism. Pharmacogenomics 2013;14:1467-1480.

374. Passey C, Birnbaum AK, Brundage RC, Oetting WS, Israni AK, Jacobson PA. Dosing equation for tacrolimus using genetic variants and clinical factors. British journal of clinical pharmacology 2011;72:948-957.
375. Rojas L, Neumann I, Herrero MJ, Boso V, Reig J, Poveda JL, et al. Effect of CYP3A5*3 on kidney transplant recipients treated with tacrolimus: a systematic review and meta-analysis of observational studies. The pharmacogenomics journal 2014.
376. Vannaprasaht S, Reungjui S, Supanya D, Sirivongs D, Pongskul C, Avihingsanon Y, et al. Personalized tacrolimus doses determined by CYP3A5 genotype for induction and maintenance phases of kidney transplantation. Clinical therapeutics 2013;35:1762-1769.
377. Roy JN, Barama A, Poirier C, Vinet B, Roger M. Cyp3A4, Cyp3A5, and MDR-1 genetic influences on tacrolimus pharmacokinetics in renal transplant recipients. Pharmacogenetics and genomics 2006;16:659-665.
378. Lee SJ, Usmani KA, Chanas B, Ghanayem B, Xi T, Hodgson E, et al. Genetic findings and functional studies of human CYP3A5 single nucleotide polymorphisms in different ethnic groups. Pharmacogenetics 2003;13:461-472.
379. Haufroid V, Wallemacq P, VanKerckhove V, Elens L, De Meyer M, Eddour DC, et al. CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2006;6:2706-2713.
380. Hesselink DA, van Schaik RH, van der Heiden IP, van der Werf M, Gregoor PJ, Lindemans J, et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. Clinical pharmacology and therapeutics 2003;74:245-254.
381. Lopez-Montenegro Soria MA, Kanter Berga J, Beltran Catalan S, Milara Paya J, Pallardo Mateu LM, Jimenez Torres NV. Genetic polymorphisms and individualized tacrolimus dosing. Transplantation proceedings 2010;42:3031-3033.
382. Santoro A, Felipe CR, Tedesco-Silva H, Medina-Pestana JO, Struchiner CJ, Ojopi EB, et al. Pharmacogenetics of calcineurin inhibitors in Brazilian renal transplant patients. Pharmacogenomics 2011;12:1293-1303.
383. Santoro AB, Struchiner CJ, Felipe CR, Tedesco-Silva H, Medina-Pestana JO, Suarez-Kurtz G. CYP3A5 genotype, but not CYP3A4*1b, CYP3A4*22, or hematocrit, predicts tacrolimus dose requirements in Brazilian renal transplant patients. Clinical pharmacology and therapeutics 2013;94:201-202.
384. Zheng S, Tasnif Y, Hebert MF, Davis CL, Shitara Y, Calamia JC, et al. Measurement and compartmental modeling of the effect of CYP3A5 gene variation on systemic and intrarenal tacrolimus disposition. Clinical pharmacology and therapeutics 2012;92:737-745.
385. Oetting W, Schaldt D, Guan W, Israni A, Remmel R, Dorr C, et al. Identification of Genetic Variants Associated With Variation of Tacrolimus Levels in African Americans Using GWAS. American journal of transplantation : official journal of the

American Society of Transplantation and the American Society of Transplant Surgeons 2015;15 Suppl 3, American Transplantation Congress Abstracts:259.
386. Pulk R, Schladt D, Guan W, Oetting W, A.Israni, Matas A, et al. Multi-Gene Pharmacogenomics of Tacrolimus Troughs in Kidney Transplant Recipients. doi 10.2217/PGS.15.42 [Epub ahead of print]. Pharmacogenomics 2014.
387. Li YR, van Setten J, Verma SS, Lu Y, Holmes MV, Gao H, et al. Concept and design of a genome-wide association genotyping array tailored for transplantation-specific studies. Genome medicine 2015;7:90.
388. Thompson EE, Kuttab-Boulos H, Witonsky D, Yang L, Roe BA, Di Rienzo A. CYP3A variation and the evolution of salt-sensitivity variants. American journal of human genetics 2004;75:1059-1069.
389. Zhang J, Zhang X, Liu L, Tong W. Value of CYP3A5 genotyping on determining initial dosages of tacrolimus for Chinese renal transplant recipients. Transplantation proceedings 2010;42:3459-3464.
390. Roy JN, Lajoie J, Zijenah LS, Barama A, Poirier C, Ward BJ, et al. CYP3A5 genetic polymorphisms in different ethnic populations. Drug metabolism and disposition: the biological fate of chemicals 2005;33:884-887.
391. Lee SJ, Goldstein JA. Functionally defective or altered CYP3A4 and CYP3A5 single nucleotide polymorphisms and their detection with genotyping tests. Pharmacogenomics 2005;6:357-371.
392. Bergmann TK, Hennig S, Barraclough KA, Isbel NM, Staatz CE. Population pharmacokinetics of tacrolimus in adult kidney transplant patients: impact of CYP3A5 genotype on starting dose. Therapeutic drug monitoring 2014;36:62-70.
393. Staatz CE, Willis C, Taylor PJ, Lynch SV, Tett SE. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society 2003;9:130-137.
394. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. Clinical pharmacology and therapeutics 2002;72:660-669.
395. Han N, Ha S, Yun HY, Kim MG, Min SI, Ha J, et al. Population pharmacokinetic-pharmacogenetic model of tacrolimus in the early period after kidney transplantation. Basic & clinical pharmacology & toxicology 2014;114:400-406.
396. Bains RK, Kovacevic M, Plaster CA, Tarekegn A, Bekele E, Bradman NN, et al. Molecular diversity and population structure at the Cytochrome P450 3A5 gene in Africa. BMC genetics 2013;14:34.
397. Jin Y, Wang YH, Miao J, Li L, Kovacs RJ, Marunde R, et al. Cytochrome P450 3A5 genotype is associated with verapamil response in healthy subjects. Clinical pharmacology and therapeutics 2007;82:579-585.
398. Dennison JB, Mohutsky MA, Barbuch RJ, Wrighton SA, Hall SD. Apparent high CYP3A5 expression is required for significant metabolism of vincristine by human

cryopreserved hepatocytes. The Journal of pharmacology and experimental therapeutics 2008;327:248-257.
399. Floyd MD, Gervasini G, Masica AL, Mayo G, George AL, Jr., Bhat K, et al. Genotype-phenotype associations for common CYP3A4 and CYP3A5 variants in the basal and induced metabolism of midazolam in European- and African-American men and women. Pharmacogenetics 2003;13:595-606.
400. Miao J, Jin Y, Marunde RL, Gorski CJ, Kim S, Quinney S, et al. Association of genotypes of the CYP3A cluster with midazolam disposition in vivo. The pharmacogenomics journal 2009;9:319-326.
401. Mirghani RA, Sayi J, Aklillu E, Allqvist A, Jande M, Wennerholm A, et al. CYP3A5 genotype has significant effect on quinine 3-hydroxylation in Tanzanians, who have lower total CYP3A activity than a Swedish population. Pharmacogenetics and genomics 2006;16:637-645.
402. Mukonzo JK, Waako P, Ogwal-Okeng J, Gustafsson LL, Aklillu E. Genetic variations in ABCB1 and CYP3A5 as well as sex influence quinine disposition among Ugandans. Therapeutic drug monitoring 2010;32:346-352.
403. Roberts PJ, Rollins KD, Kashuba AD, Paine MF, Nelsen AC, Williams EE, et al. The influence of CYP3A5 genotype on dexamethasone induction of CYP3A activity in African Americans. Drug metabolism and disposition: the biological fate of chemicals 2008;36:1465-1469.
404. Antignac M, Hulot JS, Boleslawski E, Hannoun L, Touitou Y, Farinotti R, et al. Population pharmacokinetics of tacrolimus in full liver transplant patients: modelling of the post-operative clearance. European journal of clinical pharmacology 2005;61:409-416.
405. Fukatsu S, Yano I, Igarashi T, Hashida T, Takayanagi K, Saito H, et al. Population pharmacokinetics of tacrolimus in adult recipients receiving living-donor liver transplantation. European journal of clinical pharmacology 2001;57:479-484.
406. Eeckhoudt SL, Horsmans Y, Verbeeck RK. Differential induction of midazolam metabolism in the small intestine and liver by oral and intravenous dexamethasone pretreatment in rat. Xenobiotica; the fate of foreign compounds in biological systems 2002;32:975-984.
407. Hukkanen J, Vaisanen T, Lassila A, Piipari R, Anttila S, Pelkonen O, et al. Regulation of CYP3A5 by glucocorticoids and cigarette smoke in human lung-derived cells. The Journal of pharmacology and experimental therapeutics 2003;304:745-752.
408. Ogg MS, Williams JM, Tarbit M, Goldfarb PS, Gray TJ, Gibson GG. A reporter gene assay to assess the molecular mechanisms of xenobiotic-dependent induction of the human CYP3A4 gene in vitro. Xenobiotica; the fate of foreign compounds in biological systems 1999;29:269-279.
409. Schuetz EG, Wrighton SA, Barwick JL, Guzelian PS. Induction of cytochrome P-450 by glucocorticoids in rat liver. I. Evidence that glucocorticoids and pregnenolone 16 alpha-carbonitrile regulate de novo synthesis of a common form of cytochrome P-450 in cultures of adult rat hepatocytes and in the liver in vivo. The Journal of biological chemistry 1984;259:1999-2006.

410. Miura M, Satoh S, Kagaya H, Saito M, Inoue T, Tsuchiya N, et al. No impact of age on dose-adjusted pharmacokinetics of tacrolimus, mycophenolic acid and prednisolone 1 month after renal transplantation. European journal of clinical pharmacology 2009;65:1047-1053.
411. Staatz CE, Tett SE. Pharmacokinetic considerations relating to tacrolimus dosing in the elderly. Drugs & aging 2005;22:541-557.
412. Stratta P, Quaglia M, Cena T, Antoniotti R, Fenoglio R, Menegotto A, et al. The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation. European journal of clinical pharmacology 2012;68:671-680.
413. Bhatnagar V, Garcia EP, O'Connor DT, Brophy VH, Alcaraz J, Richard E, et al. CYP3A4 and CYP3A5 polymorphisms and blood pressure response to amlodipine among African-American men and women with early hypertensive renal disease. American journal of nephrology 2010;31:95-103.
414. Zhu Y, Wang F, Li Q, Zhu M, Du A, Tang W, et al. Amlodipine metabolism in human liver microsomes and roles of CYP3A4/5 in the dihydropyridine dehydrogenation. Drug metabolism and disposition: the biological fate of chemicals 2014;42:245-249.
415. Zuo XC, Zhou YN, Zhang BK, Yang GP, Cheng ZN, Yuan H, et al. Effect of CYP3A5*3 polymorphism on pharmacokinetic drug interaction between tacrolimus and amlodipine. Drug metabolism and pharmacokinetics 2013;28:398-405.
416. Passey C, Birnbaum AK, Brundage RC, Schladt DP, Oetting WS, Leduc RE, et al. Validation of tacrolimus equation to predict troughs using genetic and clinical factors. Pharmacogenomics 2012;13:1141-1147.
417. Press RR, Ploeger BA, den Hartigh J, van der Straaten T, van Pelt J, Danhof M, et al. Explaining variability in tacrolimus pharmacokinetics to optimize early exposure in adult kidney transplant recipients. Therapeutic drug monitoring 2009;31:187-197.
418. de Jonge H, Elens L, de Loor H, van Schaik RH, Kuypers DR. The CYP3A4*22 C>T single nucleotide polymorphism is associated with reduced midazolam and tacrolimus clearance in stable renal allograft recipients. The pharmacogenomics journal 2015;15:144-152.
419. Thervet E, Loriot MA, Barbier S, Buchler M, Ficheux M, Choukroun G, et al. Optimization of initial tacrolimus dose using pharmacogenetic testing. Clinical pharmacology and therapeutics 2010;87:721-726.
420. Eugui EM, Almquist SJ, Muller CD, Allison AC. Lymphocyte-selective cytostatic and immunosuppressive effects of mycophenolic acid in vitro: role of deoxyguanosine nucleotide depletion. Scandinavian journal of immunology 1991;33:161-173.
421. Allison AC, Hovi T, Watts RW, Webster AD. The role of de novo purine synthesis in lymphocyte transformation. Ciba Foundation symposium 1977:207-224.
422. Gu JJ, Spychala J, Mitchell BS. Regulation of the human inosine monophosphate dehydrogenase type I gene. Utilization of alternative promoters. The Journal of biological chemistry 1997;272:4458-4466.
423. Zimmermann AG, Gu JJ, Laliberte J, Mitchell BS. Inosine-5'-monophosphate dehydrogenase: regulation of expression and role in cellular proliferation and T

lymphocyte activation. Progress in nucleic acid research and molecular biology 1998;61:181-209.
424. Jain J, Almquist SJ, Ford PJ, Shlyakhter D, Wang Y, Nimmesgern E, et al. Regulation of inosine monophosphate dehydrogenase type I and type II isoforms in human lymphocytes. Biochemical pharmacology 2004;67:767-776.
425. Borrows R, Chusney G, Loucaidou M, James A, Lee J, Tromp JV, et al. Mycophenolic acid 12-h trough level monitoring in renal transplantation: association with acute rejection and toxicity. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2006;6:121-128.
426. Daher Abdi Z, Essig M, Rizopoulos D, Le Meur Y, Premaud A, Woillard JB, et al. Impact of longitudinal exposure to mycophenolic acid on acute rejection in renal-transplant recipients using a joint modeling approach. Pharmacological research 2013;72:52-60.
427. Kaplan B. Mycophenolic acid trough level monitoring in solid organ transplant recipients treated with mycophenolate mofetil: association with clinical outcome. Current medical research and opinion 2006;22:2355-2364.
428. Kuypers DR, van Gelder T. Therapeutic drug monitoring of mycophenolates: why make simple things complicated? Transplantation reviews (Orlando, Fla) 2011;25:45-46.
429. Le Meur Y, Buchler M, Thierry A, Caillard S, Villemain F, Lavaud S, et al. Individualized mycophenolate mofetil dosing based on drug exposure significantly improves patient outcomes after renal transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2007;7:2496-2503.
430. Lu YP, Zhu YC, Liang MZ, Nan F, Yu Q, Wang L, et al. Therapeutic drug monitoring of mycophenolic acid can be used as predictor of clinical events for kidney transplant recipients treated with mycophenolate mofetil. Transplantation proceedings 2006;38:2048-2050.
431. van Gelder T, Domke I, Engelmayer J, de Fijter H, Kuypers D, Budde K, et al. Clinical utility of a new enzymatic assay for determination of mycophenolic acid in comparison with an optimized LC-MS/MS method. Therapeutic drug monitoring 2009;31:218-223.
432. van Gelder T, Silva HT, de Fijter H, Budde K, Kuypers D, Mamelok RD, et al. How delayed graft function impacts exposure to mycophenolic acid in patients after renal transplantation. Therapeutic drug monitoring 2011;33:155-164.
433. Langman LJ, Nakakura H, Thliveris JA, LeGatt DF, Yatscoff RW. Pharmacodynamic monitoring of mycophenolic acid in rabbit heterotopic heart transplant model. Therapeutic drug monitoring 1997;19:146-152.
434. Budde K, Glander P, Bauer S, Braun K, Waiser J, Fritsche L, et al. Pharmacodynamic monitoring of mycophenolate mofetil. Clinical chemistry and laboratory medicine : CCLM / FESCC 2000;38:1213-1216.
435. Glander P, Hambach P, Braun KP, Fritsche L, Giessing M, Mai I, et al. Pre-transplant inosine monophosphate dehydrogenase activity is associated with clinical

outcome after renal transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2004;4:2045-2051.
436. Kamar N, Glander P, Nolting J, Bohler T, Hambach P, Liefeldt L, et al. Effect of mycophenolate mofetil monotherapy on T-cell functions and inosine monophosphate dehydrogenase activity in patients undergoing a kidney transplantation. Transplantation proceedings 2006;38:2292-2294.
437. Mino Y, Naito T, Otsuka A, Ozono S, Kagawa Y, Kawakami J. Inosine monophosphate dehydrogenase activity depends on plasma concentrations of mycophenolic acid and its glucuronides in kidney transplant recipients. Clinica chimica acta; international journal of clinical chemistry 2009;409:56-61.
438. Vethe NT, Bremer S, Rootwelt H, Bergan S. Pharmacodynamics of mycophenolic acid in CD4+ cells: a single-dose study of IMPDH and purine nucleotide responses in healthy individuals. Therapeutic drug monitoring 2008;30:647-655.
439. Brunet M, Martorell J, Oppenheimer F, Vilardell J, Millan O, Carrillo M, et al. Pharmacokinetics and pharmacodynamics of mycophenolic acid in stable renal transplant recipients treated with low doses of mycophenolate mofetil. Transplant international : official journal of the European Society for Organ Transplantation 2000;13 Suppl 1:S301-305.
440. Thi MT, Mourad M, Capron A, Tshinanu FM, Vincent MF, Wallemacq P. Plasma and intracellular pharmacokinetic-pharmacodynamic analysis of mycophenolic acid in de novo kidney transplant patients. Clinical biochemistry 2015;48:401-405.
441. Vethe NT, Mandla R, Line PD, Midtvedt K, Hartmann A, Bergan S. Inosine monophosphate dehydrogenase activity in renal allograft recipients during mycophenolate treatment. Scandinavian journal of clinical and laboratory investigation 2006;66:31-44.
442. Chiarelli LR, Molinaro M, Libetta C, Tinelli C, Cosmai L, Valentini G, et al. Inosine monophosphate dehydrogenase variability in renal transplant patients on long-term mycophenolate mofetil therapy. British journal of clinical pharmacology 2010;69:38-50.
443. Raggi MC, Siebert SB, Steimer W, Schuster T, Stangl MJ, Abendroth DK. Customized mycophenolate dosing based on measuring inosine-monophosphate dehydrogenase activity significantly improves patients' outcomes after renal transplantation. Transplantation 2010;90:1536-1541.
444. Molinaro M, Chiarelli LR, Biancone L, Castagneto M, Boschiero L, Pisani F, et al. Monitoring of inosine monophosphate dehydrogenase activity and expression during the early period of mycophenolate mofetil therapy in de novo renal transplant patients. Drug metabolism and pharmacokinetics 2013;28:109-117.
445. Sombogaard F, Peeters AM, Baan CC, Mathot RA, Quaedackers ME, Vulto AG, et al. Inosine monophosphate dehydrogenase messenger RNA expression is correlated to clinical outcomes in mycophenolate mofetil-treated kidney transplant patients, whereas inosine monophosphate dehydrogenase activity is not. Therapeutic drug monitoring 2009;31:549-556.

446. Amadoz A, Sebastian-Leon P, Vidal E, Salavert F, Dopazo J. Using activation status of signaling pathways as mechanism-based biomarkers to predict drug sensitivity. Scientific reports 2015;5:18494.
447. Brown HR, Castellino S, Groseclose MR, Elangbam CS, Mellon-Kusibab K, Yoon LW, et al. Drug-induced Liver Fibrosis: Testing Nevirapine in a Viral-like Liver Setting Using Histopathology, MALDI IMS, and Gene Expression. Toxicologic pathology 2016;44:112-131.
448. De Groof A, Ducreux J, Humby F, Nzeusseu Toukap A, Badot V, Pitzalis C, et al. Higher expression of TNFalpha-induced genes in the synovium of patients with early rheumatoid arthritis correlates with disease activity, and predicts absence of response to first line therapy. Arthritis research & therapy 2016;18:19.
449. Liapis K, Kastritis E, Bagratouni T, Vassiliou S, Papachristidis A, Charitaki E, et al. Early tumor-cell gene expression changes may predict the response to first-line bortezomib-based therapy in patients with newly diagnosed multiple myeloma. Journal of BUON : official journal of the Balkan Union of Oncology 2015;20:1314-1321.
450. Synowiec E, Hoser G, Bialkowska-Warzecha J, Pawlowska E, Skorski T, Blasiak J. Doxorubicin Differentially Induces Apoptosis, Expression of Mitochondrial Apoptosis-Related Genes, and Mitochondrial Potential in BCR-ABL1-Expressing Cells Sensitive and Resistant to Imatinib. BioMed research international 2015;2015:673512.
451. Glesne DA, Collart FR, Huberman E. Regulation of IMP dehydrogenase gene expression by its end products, guanine nucleotides. Molecular and cellular biology 1991;11:5417-5425.
452. Dun B, Sharma A, Teng Y, Liu H, Purohit S, Xu H, et al. Mycophenolic acid inhibits migration and invasion of gastric cancer cells via multiple molecular pathways. PloS one 2013;8:e81702.
453. Dun B, Sharma A, Xu H, Liu H, Bai S, Zeng L, et al. Transcriptomic changes induced by mycophenolic acid in gastric cancer cells. American journal of translational research 2013;6:28-42.
454. Wu TY, Fridley BL, Jenkins GD, Batzler A, Wang L, Weinshilboum RM. Mycophenolic acid response biomarkers: a cell line model system-based genome-wide screen. International immunopharmacology 2011;11:1057-1064.
455. Domhan S, Muschal S, Schwager C, Morath C, Wirkner U, Ansorge W, et al. Molecular mechanisms of the antiangiogenic and antitumor effects of mycophenolic acid. Molecular cancer therapeutics 2008;7:1656-1668.
456. Roos N, Poulalhon N, Farge D, Madelaine I, Mauviel A, Verrecchia F. In vitro evidence for a direct antifibrotic role of the immunosuppressive drug mycophenolate mofetil. The Journal of pharmacology and experimental therapeutics 2007;321:583-589.
457. Geng L, Jiang G, Xie H, Fang Y, Dong S, Chen Y, et al. Mycophenolic acid upregulates B7-DC expression on dendritic cells, which is associated with impaired allostimulatory capacity of dendritic cells. Transplantation proceedings 2006;38:1622-1624.
458. Pan Q, de Ruiter PE, Metselaar HJ, Kwekkeboom J, de Jonge J, Tilanus HW, et al. Mycophenolic acid augments interferon-stimulated gene expression and inhibits

hepatitis C Virus infection in vitro and in vivo. Hepatology (Baltimore, Md) 2012;55:1673-1683.
459. Xu Y, Lai F, Xu Y, Wu Y, Liu Q, Li N, et al. Mycophenolic acid induces ATP-binding cassette transporter A1 (ABCA1) expression through the PPARgamma-LXRalpha-ABCA1 pathway. Biochemical and biophysical research communications 2011;414:779-782.
460. Sanquer S, Maison P, Tomkiewicz C, Macquin-Mavier I, Legendre C, Barouki R, et al. Expression of inosine monophosphate dehydrogenase type I and type II after mycophenolate mofetil treatment: a 2-year follow-up in kidney transplantation. Clinical pharmacology and therapeutics 2008;83:328-335.
461. Bremer S, Mandla R, Vethe NT, Rasmussen I, Rootwelt H, Line PD, et al. Expression of IMPDH1 and IMPDH2 after transplantation and initiation of immunosuppression. Transplantation 2008;85:55-61.
462. Dorr C, Wu B, Guan W, Muthusamy A, Sanghavi K, Schladt DP, et al. Differentially expressed gene transcripts using RNA sequencing from the blood of immunosuppressed kidney allograft recipients. PloS one 2015;10:e0125045.
463. Glander P, Sombogaard F, Budde K, van Gelder T, Hambach P, Liefeldt L, et al. Improved assay for the nonradioactive determination of inosine 5'-monophosphate dehydrogenase activity in peripheral blood mononuclear cells. Therapeutic drug monitoring 2009;31:351-359.
464. Streit F, Shipkova M, Armstrong VW, Oellerich M. Validation of a rapid and sensitive liquid chromatography-tandem mass spectrometry method for free and total mycophenolic acid. Clinical chemistry 2004;50:152-159.
465. Leek JT. svaseq: removing batch effects and other unwanted noise from sequencing data. Nucleic acids research 2014;42.
466. Kenward MG, Roger JH. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 1997;53:983-997.
467. Le Meur Y, Thierry A, Glowacki F, Rerolle JP, Garrigue V, Ouali N, et al. Early steroid withdrawal and optimization of mycophenolic acid exposure in kidney transplant recipients receiving mycophenolate mofetil. Transplantation 2011;92:1244-1251.
468. van Gelder T. Therapeutic drug monitoring for mycophenolic acid is value for (little) money. Clinical pharmacology and therapeutics 2011;90:203-204.
469. van Gelder T, Silva HT, de Fijter JW, Budde K, Kuypers D, Tyden G, et al. Comparing mycophenolate mofetil regimens for de novo renal transplant recipients: the fixed-dose concentration-controlled trial. Transplantation 2008;86:1043-1051.
470. Staatz CE, Tett SE. Pharmacology and toxicology of mycophenolate in organ transplant recipients: an update. Archives of toxicology 2014;88:1351-1389.
471. Atcheson BA, Taylor PJ, Mudge DW, Johnson DW, Hawley CM, Campbell SB, et al. Mycophenolic acid pharmacokinetics and related outcomes early after renal transplant. British journal of clinical pharmacology 2005;59:271-280.
472. Kiberd BA, Lawen J, Fraser AD, Keough-Ryan T, Belitsky P. Early adequate mycophenolic acid exposure is associated with less rejection in kidney transplantation.

American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2004;4:1079-1083.
473. Le Meur Y, Borrows R, Pescovitz MD, Budde K, Grinyo J, Bloom R, et al. Therapeutic drug monitoring of mycophenolates in kidney transplantation: report of The Transplantation Society consensus meeting. Transplantation reviews (Orlando, Fla) 2011;25:58-64.
474. Pazik J, Oldak M, Podgorska M, Lewandowski Z, Sitarek E, Ploski R, et al. Lymphocyte counts in kidney allograft recipients are associated with IMPDH2 3757T>C gene polymorphism. Transplantation proceedings 2011;43:2943-2945.
475. Sobiak J, Kaminska J, Glyda M, Duda G, Chrzanowska M. Effect of mycophenolate mofetil on hematological side effects incidence in renal transplant recipients. Clinical transplantation 2013;27:E407-414.
476. Vanhove T, Kuypers D, Claes KJ, Evenepoel P, Meijers B, Naesens M, et al. Reasons for dose reduction of mycophenolate mofetil during the first year after renal transplantation and its impact on graft outcome. Transplant international : official journal of the European Society for Organ Transplantation 2013;26:813-821.
477. Laliberte J, Yee A, Xiong Y, Mitchell BS. Effects of guanine nucleotide depletion on cell cycle progression in human T lymphocytes. Blood 1998;91:2896-2904.
478. Raggi MC, Siebert SB, Abendroth DK, Steimer W, Friess H, Thorban SG. Neither mycophenolate acyl-glucuronide levels nor their areas under the curve are responsible for the gastrointestinal side effects in kidney transplant recipients receiving EC-MPA: a prospective trial. Transplantation proceedings 2010;42:4049-4052.
479. Wieland E, Shipkova M, Schellhaas U, Schutz E, Niedmann PD, Armstrong VW, et al. Induction of cytokine release by the acyl glucuronide of mycophenolic acid: a link to side effects? Clinical biochemistry 2000;33:107-113.
480. Zheng H, Ji C, Zou X, Wu M, Jin Z, Yin G, et al. Molecular cloning and characterization of a novel human putative transmembrane protein homologous to mouse sideroflexin associated with sideroblastic anemia. DNA sequence : the journal of DNA sequencing and mapping 2003;14:369-373.
481. Hildick-Smith GJ, Cooney JD, Garone C, Kremer LS, Haack TB, Thon JN, et al. Macrocytic anemia and mitochondriopathy resulting from a defect in sideroflexin 4. American journal of human genetics 2013;93:906-914.
482. Mate SE, Brown KJ, Hoffman EP. Integrated genomics and proteomics of the Torpedo californica electric organ: concordance with the mammalian neuromuscular junction. Skeletal muscle 2011;1:20.
483. Mohamed-Hussein ZA, Harun S. Construction of a polycystic ovarian syndrome (PCOS) pathway based on the interactions of PCOS-related proteins retrieved from bibliomic data. Theoretical biology & medical modelling 2009;6:18.
484. Roth M, Obaidat A, Hagenbuch B. OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. British journal of pharmacology 2012;165:1260-1287.

485. VanWert AL, Gionfriddo MR, Sweet DH. Organic anion transporters: discovery, pharmacology, regulation and roles in pathophysiology. Biopharmaceutics & drug disposition 2010;31:1-71.
486. Nishiwaki T, Daigo Y, Tamari M, Fujii Y, Nakamura Y. Molecular cloning, mapping, and characterization of two novel human genes, ORCTL3 and ORCTL4, bearing homology to organic-cation transporters. Cytogenetics and cell genetics 1998;83:251-255.
487. Kim S, Lee W. Expression of IMPDH mRNA after mycophenolate administration in male volunteers. 2014;2014:870209.
488. He X, Smeets RL, Koenen HJ, Vink PM, Wagenaars J, Boots AM, et al. Mycophenolic acid-mediated suppression of human CD4+ T cells: more than mere guanine nucleotide deprivation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2011;11:439-449.
489. Gunther OP, Balshaw RF, Scherer A, Hollander Z, Mui A, Triche TJ, et al. Functional genomic analysis of peripheral blood during early acute renal allograft rejection. Transplantation 2009;88:942-951.
490. Heidt S, Vergunst M, Anholts JD, Reinders ME, de Fijter JW, Eikmans M, et al. B Cell Markers of Operational Tolerance Can Discriminate Acute Kidney Allograft Rejection From Stable Graft Function. Transplantation 2014.
491. Lee A, Jeong JC, Choi YW, Seok HY, Kim YG, Jeong KH, et al. Validation study of peripheral blood diagnostic test for acute rejection in kidney transplantation. Transplantation 2014;98:760-765.
492. Lee B, Oh CK, Kim MS, Kim JH, Kim SJ, Kim HS, et al. Cytokine gene expression in peripheral blood mononuclear cells during acute renal allograft rejection. Transplantation proceedings 2012;44:236-240.
493. Li L, Khatri P, Sigdel TK, Tran T, Ying L, Vitalone MJ, et al. A peripheral blood diagnostic test for acute rejection in renal transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2012;12:2710-2718.
494. Li L, Khush K, Hsieh SC, Ying L, Luikart H, Sigdel T, et al. Identification of common blood gene signatures for the diagnosis of renal and cardiac acute allograft rejection. PloS one 2013;8:e82153.
495. Luo Y, Shi B, Qian Y, Bai H, Chang J. Sequential monitoring of TIM-3 gene expression in peripheral blood for diagnostic and prognostic evaluation of acute rejection in renal graft recipients. Transplantation proceedings 2011;43:3669-3674.
496. Mao Y, Wang M, Zhou Q, Jin J, Wang Y, Peng W, et al. CXCL10 and CXCL13 Expression were highly up-regulated in peripheral blood mononuclear cells in acute rejection and poor response to anti-rejection therapy. Journal of clinical immunology 2011;31:414-418.
497. Shin H, Gunther O, Hollander Z, Wilson-McManus JE, Ng RT, Balshaw R, et al. Longitudinal analysis of whole blood transcriptomes to explore molecular signatures

associated with acute renal allograft rejection. Bioinformatics and biology insights 2014;8:17-33.
498. Viklicky O, Krystufkova E, Brabcova I, Sekerkova A, Wohlfahrt P, Hribova P, et al. B-cell-related biomarkers of tolerance are up-regulated in rejection-free kidney transplant recipients. Transplantation 2013;95:148-154.

8 APPENDIX

8.1 NONMEM CODE OF FINAL FLUDARABINE MODEL DEVELOPMENT

```
;; 1. Based on: run171
;; 2. Description: Final Model
;; x1. Author: user
;; 3. Label:

$PROB RUN 175
$INPUT C ID TIM=DROP TIME AMT AMOUNT=DROP DV CMT RATE EVID
MDV AGE GEN HT=DROP WT=DROP IBW BSA=DROP SCR=DROP CLCR
DIAG=DROP COND=DROP CYST=DROP EGFR=DROP BILI=DROP ALB=DROP
HTM=DROP BMI
$DATA FLU0807.CSV IGNORE=C
$SUBROUTINES ADVAN3 TRANS4
$PK
RF=(CLCR/85)*(70/IBW)
CLNRST = THETA(1)
CLRST = THETA(7)
TVCL=(CLNRST + (CLRST*RF))*(IBW/70)**0.75
CL=TVCL*EXP(ETA(1))
TVV1=THETA(2)*(IBW/70)
V1=TVV1*EXP(ETA(2))
TVQ=THETA(3)*(IBW/70)**0.75
Q=THETA(3)*EXP(ETA(3))
TVV2=THETA(4)*(IBW/70)
V2=TVV2*EXP(ETA(4))
S1=V1/1000

AUC= AMT/CL
$ERROR
W = THETA(5)*ERR(1) + THETA(6)*F*ERR(2)
Y=F+W
IPRED=F
DEL=.001
IRES=DV-IPRED
IWRES=IRES/(w+DEL)
$THETA
(0,6)     ; CLNRST
(0,50)    ; V1
(0,9)     ; Q
(0,25 )   ; V2
```

```
(0.1)     ; ERR1
(0.04)    ; ERR2
(0.1)     ; CLRST



$OMEGA BLOCK(2)
0.1          ;IIV on CL
0.01 0.1     ; CORR between CL and V1 and IIV on V1
$OMEGA 0.1   ;IIV on Q
$OMEGA 0.1   ;IIV on V2

$SIGMA
(1 FIX) ; Additive
(1 FIX) ; Proportional

$EST METHOD=1 INTER MAXEVAL=9999 NOABORT NOSIGMABOUNDTEST
SIG=3 PRINT=1

$COV PRINT=E UNCONDITIONAL

; Xpose
$TABLE ID TIME DV MDV EVID PRED IPRED WRES CWRES IWRES AUC AGE
GEN IBW CLCR BMI ONEHEADER NOPRINT FILE=sdtab175
$TABLE ID CL V1 Q V2 AUC ETA(1) ETA(2) ETA(3) ETA(4) NOPRINT
ONEHEADER FILE=patab175
$TABLE AGE IBW CLCR BMI NOPRINT ONEHEADER FILE=cotab175
$TABLE GEN NOPRINT ONEHEADER FILE=catab175
```

8.2 NONMEM CODE OF FINAL PM MODEL DEVELOPMENT

```
;; 1. Based on: run184
;; 2. Description: FINAL FORWARD MODEL
;; x1. Author: user
;; 3. Label:

$PROB RUN 185
$INPUT C PATID=DROP ID DATE=DROP TIME AMT DV RATE CMT EVID GEN
BMI AGE PROTEIN ALBUMIN AST ALT ALKPHOS BILI SCR CRCL CRCLIBW
WT IBW

$DATA cypdata.CSV IGNORE=C
$SUBROUTINES ADVAN2 TRANS2

$PK
CLCRCL= THETA(5)*(CRCL/104)*(83/WT)
;GEN
IF (GEN.EQ.0) COVGEN=1
IF (GEN.EQ.1) COVGEN= THETA(4)
TVKA=THETA(1)
KA=TVKA*EXP(ETA(1))
TVCL=(THETA(2)+CLCRCL)*(WT/83)**0.75
CL=TVCL*EXP(ETA(2))
TVV=THETA(3)*(WT/83)*COVGEN
V=TVV*EXP(ETA(3))
S2=V/1000


$ERROR
W=F
IPRED=F
DEL=.001
IRES=DV-IPRED
IWRES=IRES/(w+DEL)
Y=F+F*ERR(1)

$THETA
(0,0.2)    ;Kfm
(0,100)     ; CL/fm
(0,1000)    ; V/fm
(0.1)       ; GEN on KA
(20)       ;CRCL on CL
```

```
$OMEGA
0.1 ;Kfm
$OMEGA BLOCK(2)
0.1 ;CL
0.01 0.1 ; COV V

$SIGMA
(0.4) ; Proportional error PK


$EST METHOD=1 INTER MAXEVAL=9999 NOABORT SIG=3 PRINT=1 POSTHOC
$COV PRINT=E UNCONDITIONAL


; Xpose
$TABLE ID TIME DV MDV EVID PRED IPRED WRES CWRES IWRES
ONEHEADER NOPRINT FILE=sdtab185
$TABLE ID KA CL V ETA(1) ETA(2) NOPRINT ONEHEADER FILE=patab185
$TABLE BMI AGE PROTEIN ALBUMIN AST ALT ALKPHOS BILI Fara FLU
NOPRINT ONEHEADER FILE=cotab185
$TABLE GEN NOPRINT ONEHEADER FILE=catab185
```

8.3 NONMEM CODE OF FINAL TACROLIMUS MODEL DEVELOPMENT

```
;; 1. Based on: run142
;; 2. Description: Final Model used for validation in run 160
;; x1. Author: user
$PROBLEM run
;---------------------------------
$INPUT C ID pid=DROP TIME DOSE DV MDV RATE LN_TAC=DROP
FREQ=DROP POSTTXDAYS STEROID DIABETES GEN GEN_D DIALYSIS CCB
ACEINHI ANTIVIRAL AGEGRP_R AGEGRP_D rs1057868 rs35599367 rs41303343
rs10264272 rs776746 CMV SPK D_RACE DONORSTATUS UNIT R_AGE D_AGE
BMI WT GFRCKG GFR SCR WT_BASE STRATA DOUBLE SINGLE CATEGORY

$DATA tacnewgwas2.csv IGNORE=@
$SUBROUTINES

$PRED
TVCL =THETA(1)/1000
IF(CATEGORY.EQ.1.OR.CATEGORY.EQ.2.OR.CATEGORY.EQ.3.OR.CATEGORY.
EQ.4.OR.CATEGORY.EQ.8) TVCL=TVCL*THETA(2)
IF(CATEGORY.EQ.5.OR.CATEGORY.EQ.6.OR.CATEGORY.EQ.7)
TVCL=TVCL*THETA(3)
IF(STEROID.EQ.1) TVCL=TVCL*THETA(4)
IF(ANTIVIRAL.EQ.1) TVCL=TVCL*THETA(5)
IF(POSTTXDAYS.LE.9)TVCL=TVCL*THETA(6)
IF(AGEGRP_R.EQ.1) TVCL=THETA(7)*TVCL
CL= TVCL*EXP(ETA(1))
CLm = CL*1000
CSS = RATE/CL
F= CSS
W=F
IPRED=F
DEL=.001
IRES=DV-IPRED
IWRES=IRES/(W+DEL)
Y= F +ERR(1)

$EST METHOD=1 INTERACTION PRINT=5 MAX=9999 SIG=3

$THETA
(0, 50.3) ; TVCL
```

```
(0, 0.479) ; HOMOZYGOTE FOR VARIANT
(0, 0.839) ; HETEROZYGOTE
(0, 1.22) ; Yes Steroids
(0, 1) ;Yes Antiviral
(0, 1.33) ; Days<9
(0, 1.25) ; Age<25

$OMEGA
0.206 ;ETA1

$SIGMA
7.53 ;ERR1

$COV
;Xpose
$TABLE ID TIME DOSE DV CL WT_BASE CATEGORY ANTIVIRAL STEROID
AGEGRP_R PRED IPRED WRES CWRES rs41303343 rs10264272 rs776746 STRATA
DOUBLE SINGLE CATEGORY ONEHEADER NOPRINT FILE=sdtab157
$TABLE ID CL RATE POSTTXDAYS PRED IPRED WRES CWRES rs41303343
rs10264272 rs776746 STRATA DOUBLE SINGLE NOPRINT ONEHEADER
FILE=patab157
$TABLE POSTTXDAYS R_AGE D_AGE BMI WT GFRCKG GFR SCR NOPRINT
ONEHEADER FILE=cotab157
$TABLE STEROID DIABETES GEN GEN_D DIALYSIS CCB ACEINHI
ANTIVIRAL AGEGRP_R AGEGRP_D rs1057868 rs35599367 rs41303343 rs10264272
rs776746 CMV SPK D_RACE DONORSTATUS UNIT NOPRINT ONEHEADER
FILE=catab157
```