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POTENCIALNE INTERAKCIJE MED MIKOFENOLATOM IN DRUGIMI UČINKOVINAMI PRI BOLNIKIH PO PRESADITVI KRVOTVORNIH MATIČNIH CELIC

POTENTIAL OF MYCOPHENOLATE-DRUG INTERACTIONS IN HEMATOPOIETIC CELL TRANSPLANT PATIENTS

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This Master's Thesis was performed at the School of Pharmacy, University of Washington, and Faculty of Pharmacy, University of Ljubljana. I worked under the mentorship of Prof. Dr. Aleš Mrhar, PharmD, and co-mentorship of Prof. Jeannine S McCune, PharmD.

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STATEMENT

I hereby declare that I have performed and written this Master's Thesis solely by myself under the mentorship of Prof. Dr. Aleš Mrhar, PharmD, and co-mentorship of Prof. Jeannine S McCune, PharmD.

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ABSTRACT

Postgrafting pharmacotherapy in nonmyeloablative hematopoietic cell transplantation (HCT) setting is complex with numerous medications prescribed. A recipient of the nonmyeloablative HCT is expected to be at risk for drug interactions and subsequently at increased risk of adverse drug events.

Mycophenolate is an immunosuppressive agent with a narrow therapeutic index. In this Master's Thesis, we sought to identify and evaluate potential pharmacokinetic mycophenolate-drug interactions in 74 nonmyeloablative allogeneic HCT recipients who were taking mycophenolate as a part of immunosuppressive regimen. Based on the available literature and the current understanding of the underlying drug interaction mechanisms, we prepared a comprehensive list of medications that could potentially interact with mycophenolate. Then, we prepared a protocol for identifying the occurrence of a potential drug interaction in the study on days 2, 7 and 21 after allogeneic graft infusion. On these days, we performed a retrospective analysis of the patient data. Potential pharmacokinetic interactions were identified between mycophenolate and HCT as well as non-HCT medications. HCT medications comprised of broad-spectrum antibiotics, cyclosporine, fluconazole and corticosteroids. Non-HCT medications comprised of amitriptyline, lorazepam, proton-pump inhibitors and valproate. Mostly, these medications interfered with the absorption and metabolic process of mycophenolate pharmacokinetics. To each medication level of scientific evidence and appropriate management were assigned.

Every patient was taking at least 1 medication that may have a pharmacokinetic drug interaction with mycophenolate. Also, each patient was taking a median of 4 interacting medications during the first 21 days. In this time, the number of concomitant medications did not change, however the number of potential drug interactions did due to the increased use of corticosteroids on day 21.

Further statistic analysis revealed that the patient's age, HCT comorbidity index and number of concomitant medications do not correlate with the number of potential drug interactions. Findings of this Master's Thesis will be incorporated into the population pharmacokinetic analysis and serve as an invaluable tool to clinicians in optimizing nonmyeloablative post-HCT immunosuppressive therapy.

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RAZŠIRJENI POVZETEK

Nemieloablativna alogenična presaditev krvotvornih matičnih celic (PKMC) se je uveljavila kot oblika zdravljenja malignih in nemalignih bolezni krvi in krvotvornih organov. Terapija po opravljeni presaditvi je kompleksne narave. Bolniku je potrebno uvesti zdravila, ki olajšajo neželene učinke preparativnega zdravljenja pred PKMC ali same primarne bolezni, imunosupresivna zdravila, hkrati pa bolnik jemlje tudi zdravila za zdravljenje ostalih komorbidnosti. Z naraščajočim številom zdravil so ti bolniki izpostavljeni višjemu tveganju za interakcije zdravil in posledično tudi za neželene učinke zdravil oz. zavrnitev presadka.

Mikofenolat je imunosupresivno zdravilo, ki ga bolniki jemljejo po opravljeni PKMC. Ima ozko terapevtsko okno, zato že majhne spremembe v farmakokinetičnih parametrih, kot je površina pod krivuljo (AUC), bistveno vplivajo na klinični izid. Z diplomskim delom smo želeli raziskati in oceniti pomen možnih farmakokinetičnih interakcij med mikofenolatom in ostalimi učinkovinami, ki so jih bolniki prejemali v naši študiji. Interakcij med mikofenolatom in učinkovinami nismo povezali z dejansko manifestacijo neželenih učinkov, saj je to v raziskovanem kliničnem okolju praktično nemogoče. Neželeni učinki mikofenolata so namreč nevtropenija, gastrointestinalna toksičnost in povečano tveganje za pojav infekcij. Vsi našteti se prekrivajo tudi z neželenimi učinki nekaterih spremljajočih zdravil oz. samega preparativnega zdravljenja. V takšnih primerih je zato optimalna odločitev identifikacija možne interakcije in s tem določitev preventivnih ukrepov za optimalno vodenje bolnikove terapije.

Na podlagi primarne literature in poznavanja mehanizma interakcij smo najprej pripravili seznam zdravil, ki bi lahko povzročila klinično signifikantne interakcije z mikofenolatom. Klinično signifikantno interakcijo smo določili kot interakcijo, ki povzroči najmanj 20% spremembo v AUC mikofenole kisline. Tako smo pridobili informacije o 14 učinkovinah, ki dokazano povzročijo farmakokinetično interakcijo. Primarna literatura se je izkazala kot pomanjkljiva, zato smo na podlagi poznavanja mehanizmov interakcij v seznam vključili tudi učinkovine, ki znano (*in vitro* ali *in vivo*) vplivajo na metabolne poti mikofenolata.

Potencialne interakcije z mikofenolatom smo retrospektivno ugotavljali 2., 7. in 21. dan po opravljeni PKMC. Pred začetkom analize smo pripravili protokol o nastanku možne interakcije. Označili smo prisotnost možne interakcije, če je bolnik poleg mikofenolata jemal še učinkovino na opazovani dan oz. vključno do tri dni pred opazovanim dnem.

V študijo je bilo vključenih 74 bolnikov, ki so med 23.11. 2008 in 10.11.2011 prejeli nemieloablativno PKMC. Bolniki so jemali 184 različnih zdravil. Za lažje razumevanje smo zdravila razdelili v dva razreda, in sicer "HCT" ter "non-HCT". "HCT" razred je obsegal zdravila, ki se standardno uporabljajo v imunosupresivnem zdravljenju. "Non-HCT" razred je obsegal vsa ostala zdravila, ki so jih bolniki jemali kot podporno terapijo, za lajšanje bolečin nastalih zaradi primarne bolezni ter za zdravljenje ostalih komorbidnosti.

V prvih 21 dneh po opravljeni PKMC so bolniki jemali 14 zdravil, od tega 4 zdravila, ki potencialno povzročijo interakcijo z mikofenolatom (mediane vrednosti se razlikujejo po dnevih). Vredno je poudariti, da je vsak bolnik jemal najmanj eno zdravilo, ki lahko povzroči interakcijo. Identificirane interakcije so bile izražene predvsem na ravni absorpcije in metabolizma. Izrazile so se med mikofenolatom in naslednjimi učinkovinami iz "HCT" razreda: širokospektralnimi antibiotiki (amoksicilinom in klavulansko kislino, ciprofloksacinom, levofloksacinom, moksifloksacinom), ciklosporinom, flukonazolom in kortikosteroidi (metilprednizolonom in prednizonom). Možne interakcije so nastale tudi med mikofenolatom in učinkovinami iz "non-HCT" razreda: amitriptilinom, lorazepamom, inhibitorji protonske črpalke (esomeprazolom, lansoprazolom, pantoprazolom, omeprazolom) in valproatom.

Glede na identificirane možne interakcije smo predlagali tudi ustrezno nadaljnjo obravnavo terapije. Pri več kot 60% vseh interakcij bi bila možna zamenjava zdravila z ustreznim alternativnim zdravilom. Pri ostalih 40% spremembe ne bi bile možne, saj te interakcije povzročajo zdravila, ki so za optimalni klinični izid po opravljeni PKMC bistvenega pomena in za njih ne obstajajo ustrezne alternative.

Identificirane možne interakcije smo ovrednotili tudi z oceno zanesljivosti znanstvene literature. Vsaj 40% interakcij je podkrepljenih z visokim znanstvenim dokazom, medtem ko ostalih 60% predstavlja možne interakcije, za katere je priporočljivo, da bi bile podkrepljene z višjo stopnjo dokaza.

V prvih 21 dneh po opravljeni PKMC se število zdravil, ki jih je posamezni bolnik jemal, ni spreminjalo. Po drugi strani pa se je v istem obdobju statistično spremenilo število možnih interakcij na bolnika, kar lahko razložimo s povečanim številom uporabe kortikosteroidov (prednizona) pri bolnikih na 21. dan po opravljeni PKMC.

Pri naših bolnikih smo poskušali tudi oceniti, ali obstaja korelacija med številom možnih interakcij in bolnikovo starostjo, indeksom komorbidnosti ali celotnim številom zdravil, ki

jih je bolnik jemal. Korelacije med možnimi interakcijami in naštetimi faktorji nismo potrdili.

To diplomsko delo je prva analiza možnih interakcij med mikofenolatom in drugimi učinkovinami pri bolnikih po opravljeni nemieloablativni PKMC. Rezultati bodo nadalje uporabljeni v prospektivni študiji biomarkerjev, kjer bodo na podlagi metod populacijske farmakokinetike poskušali določiti, ali potencialna interakcija kot kovariata bistveno vpliva na AUC mikofenolne kisline. Hkrati so rezultati diplome, podkrepljenimi z mehanizmi interakcij, predlaganimi spremembami in identificiranimi faktorji, osnova za razumevanje interakcij z mikofenolatom kot zdravilom z ozkim terapevtskim oknom.

LIST OF ABBREVIATIONS

AcMPAG	Mycophenolic acid acyl-glucoronide
ADE	Adverse drug event
AID	Autoimmune disease
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATC	Anatomic-therapeutic-chemical classification
AUC	Area under the concentration-time curve
BBB	Blood-brain-barrier
BID	Two times per day dosing
BOV	Between-occasion variability
BSV	Between-subject variability
Cl	Clearance
CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
СҮР	Cytochrome
DI	Drug interaction
DIPS	Drug Interaction Probability Scale
DM-MPA	6-O-desmethyl mycophenolic acid
EHC	Enterohepatic recycling
FHCRC	Fred Hutchinson Cancer Research Center
GI	Gastrointestinal
GIT	Gastrointestinal tract
GDP	Guanosine diphosphate
GMP	Guanosine monophosphate
GVHD	Graft-versus-host-disease
GVT	Graft-versus-tumor
НСТ	Hematopoietic cell transplantation
HCT-CI	Hematopoietic cell transplant-specific comorbidity index
HLA	Human lymphocyte antigen

HV	Healthy volunteers
IMP	Inosine monophosphate
IMPDH	Inosine monophosphate dehydrogenase
LADME	Liberation, absorption, distribution, metabolism, excretion
MPA	Mycophenolic acid
MPAG	Mycophenolic acid glucoronide
MMF	Mycophenolate mofetil
MRP-2	Multi-drug resistant protein 2
NAD+	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
NMT	Nonmyeloablative transplantation
NTI	Narrow therapeutic index
ORCA	Operational Classification of Drug Interactions
P-gp	P-glycoprotein
PD	Pharmacodynamic
РК	Pharmacokinetic
PPi	Pyrophosphate
PPI	Proton-pump inhibitor
PRPP	Phosphoribosyl pyrophosphate
RA	Rheumatoid arthritis
RCT	Randomized clinical trial
SD	Single dose
SOT	Solid organ transplantation
TID	Three times per day dosing
UGT	Uridine 5'-diphosphate glucuronosyltransferase
XMP	Xanthosine monophosphate

INTRODUCTION

1. Drug interactions

1.1 Definition and incidence

A drug interaction is defined as "the possibility that one drug (*precipitant* drug) may alter the intensity of pharmacological effects of another drug (*object* drug) given concurrently".(1,2) A "potential drug interaction" arises when two drugs with the aforementioned characteristic are concomitantly administered, regardless of whether adverse drug events (ADEs) occur.(2) Drug interactions can produce synergistic, antagonistic, or even unanticipated responses. Synergistic drug interactions could be beneficial, if they are well understood and appropriately managed. On the other hand, drug interactions which are antagonistic or lead to ADEs represent an important challenge for pharmacotherapy.(2,3) Such ADEs may often remain unrecognized, and their clinical relevance may be underestimated by the prescribing physicians.(3) With the current understanding of the underlying mechanisms and availability of the literature, ADEs resulting from drug interactions are avoidable and are suitable targets for preventive measures.(3)

Drug interactions are of particular concern among patients taking more than 5 drugs concurrently due to the increased risk of morbidity and mortality, which may lead to hospital admission.(4) It has been estimated that drug interactions comprise 12–26% of ADEs, which seriously harm or kill over 700,000 patients in the US each year.(5)

1.2 Pharmacokinetic drug interactions

Altered gastrointestinal (GI) absorption

The process of absorption is relevant to all administration routes except intravenous and intra-arterial administration. However, pharmacokinetic drug interactions causing altered drug absorption often relate only to GI absorption and thus, the latter is the focus of this section. GI absorption of the *object drug* can be affected by altered blood flow, formation of a non-absorbable complex, or by changes in GI motility, pH, flora or mucosa (Table I).(6)

Table I. Effect on serum concentration of the object drug by various drug interaction mechanisms causing altered drug absorption.(1,6)

Mechanism	Serum concentration of the object drug
Altered blood flow	\downarrow or \uparrow
Formation of a non-absorbable complex	\rightarrow
Change in GI motility	\downarrow or \uparrow
Change in GI pH	\rightarrow
Changes in GI flora/mucosa	\rightarrow

Altered rate of absorption due to these changes tends to have minimal clinical effect. On the other hand, drug interactions altering the extent of absorption should be closely monitored.(1) Drug interactions that are avoidable by administering the medications at different times are binding interactions (avoiding the formation of a non-absorbable complex) and, in many cases, interactions with GI pH. However, separating the doses of interacting drugs may not circumvent drug interactions, especially if the precipitant drug affects GI motility (this effect is a result of a systemic response to the precipitant drug), or if the precipitant drug affects GI flora (gradual onset and dissipation of the effect). Drug interactions causing altered GI absorption are easily avoidable by using alternative administration routes (e.g. intravenous, intramuscular or subcutaneous administration).(6)

Altered drug distribution

The amount of drug available to bind to the receptor site is determined by many factors including its extent of protein binding. When a highly protein-bound (99%) drug is displaced from its inactive site by a drug competing for the same binding site, an enhanced pharmacologic action or toxicity may result. However, the resultant increased unbound fraction of the drug is also more readily excreted. Drug interactions causing changes in protein binding tend to have little clinical relevance and will usually not influence the clinical exposure of a patient to a therapeutic agent.(6,7) As a consequence, no adjustments in dosing regimens will be necessary except in rare cases (e.g. a drug with a high extraction ratio and narrow therapeutic index (NTI) that is given parenterally, or a drug with a NTI that is given orally and has a rapid pharmacokinetic-dynamic equilibration time).(7)

Altered metabolism

Metabolic drug interactions are the most common and the best known drug interactions.(1) Understanding which drugs are substrates, inhibitors and inducers of drug metabolizing enzymes is of crucial importance in predicting the risk of drug interactions.(1,5) When the precipitant drug inhibits the enzyme(s) eliminating the object drug, increased plasma concentrations and increased pharmacologic response to object drug can result which consequently increases the potential for ADEs.(5,6) On the other hand, enzyme induction occurs when the precipitant drug increases the activity of the enzyme(s) which eliminate(s) the object drug. This can result in lower plasma concentration, diminished pharmacologic response and lower effectiveness of the object drug.(6)

The metabolism of drugs occurs via phase I and/or phase II reactions. Inhibition and induction primarily affect Phase I metabolism, although some Phase II reactions may also be affected.(6) The cytochrome P450 (CYP P450) enzymes metabolize numerous medications and are well-recognized for their potential drug interactions.(5) In contrast, there is little known about the uridine 5'-diphosphate glucuronosyltransferase's (UGT's) and other enzymes' potential for drug interactions. At the moment, this is a growing area that still requires abundant research.(8,9)

Altered excretion

Drug interactions occurring due to altered excretion predominantly occur in the kidney but can also occur in the liver and gastrointestinal tract.(1) In general, drugs appear to interact with drugs of similar acid-base nature and are of competitive type. Clinically significant interactions are more likely to occur when they involve drugs excreted unchanged, having NTI and dosed to relatively high plasma concentrations.(6)

Altered drug transport

There has been increasing attention to drug transport proteins as the site of drug interactions. Drug transporting proteins are either influx or efflux pumps that are involved in the drug uptake into the hepatocytes, tubular secretion in the kidneys or limiting transport across blood-brain barrier (BBB) or placenta (Table II). Thus, they play a major role in drug uptake, distribution, and clearance (collectively affecting the extent of absorption).(5,10,11)

Table II. Description of the 3 most common drug transporting proteins as potential sites of drug interactions.(10)

Symbol	Tissue/Site	Function
P-gp	Liver, BBB,	Efflux transporter located on the apical membranes;
	kidney, intestine,	responsible for \downarrow drug accumulation and development
	placenta	of the resistance to anticancer drugs.
MRP-1	Lung, testes,	Multi-specific organic anion transporter located on
	progenitor blood	the lateral membranes; involved in multi-drug
	marrow cells	resistance.
MRP-2	Liver, kidney,	Efflux transporter located on the apical membranes;
	intestine	responsible mainly for the biliary transport of
		metabolized drugs/substrates.

It is a characteristic of drug-resistant tumors to have drug transporters (i.e. P-gp) overexpressed. Lately, it has been an increasing attention into turning these efflux characteristics into therapeutic benefits by blocking their activities and thus establishing an intentional drug-drug interaction. However, to date a P-gp inhibitor with the desired effect on tumors (and without intolerable systemic effects) was not successfully developed.(10)

1.3 The time course of pharmacokinetic drug interactions

The time course of pharmacokinetic drug interactions can vary tremendously; some drug interactions occur in seconds or minutes, while others develop over several weeks. When considering the time course of drug interactions, several time points should be taken into account: time of onset of when the drug interaction becomes detectable, time for maximal pharmacokinetic and pharmacodynamic effect of the drug interaction, time that the patient experiences an ADE because of the drug interaction, and time required for the dissipation of the drug interaction (Figure 1).(6)



Figure 1. Clinically important time points of a particular drug interaction.

Although the time that the patient will experience an ADE from a drug interaction is more difficult to predict, one can often estimate the time of maximal risk and consequently: (i) minimize the likelihood of an ADE from the drug interactions, and (ii) reduce the costs of monitoring for the interaction.(6)

1.3.1 Determinants of time course

In general, time course of the drug interaction depends largely on the interaction mechanism and the pharmacokinetics of object drug. It is often necessary to consider the following factors when making an estimate about the time course of one interaction in a particular patient (6,12):

Plasma half-lives of drugs involved (6,12)

The *plasma half-life of the precipitant drug* dictates the time course of the precipitant drug's accumulation to steady state. If it takes a long time for the precipitant drug to reach the plateau level, the drug interaction may be delayed.

The *plasma half-life of the object drug* is also important. Drugs with short plasma half-lives will relatively quickly reach new steady state concentration. After the discontinuation of the precipitant drug, one should estimate when the object drug will re-establish its former steady state. Here, it is the new plasma half-life of the object drug that must be considered.

Drug dosage (6,13)

The *dosage of the object drug* can be an influential determinant when estimating the time course of an interaction. If a patient is receiving a large dose of an object drug and its serum concentration is at the upper end of the therapeutic range, it may take only a short period of time for the serum concentration to reach toxic concentrations following administration of a precipitant drug that inhibits the metabolism and/or excretion of the object drug.

Larger doses of a precipitant drug could result in a more rapid onset of the drug interaction since the serum concentration necessary to produce the interaction may be achieved more quickly. Similarly, it may take longer for the drug interaction to dissipate after discontinuation of large doses of the precipitant drug.

Administration (6,13)

Routes of administration that rapidly achieve therapeutic serum concentrations of interacting drugs will tend to result in a more rapid development of drug interactions. Here, all parenteral administrations should be considered as an example. Additionally, the

sequence of administration of both drugs is important when the object drug is titrated to achieve the optimal therapeutic response. This is particularly the case when the precipitant drug is given to a patient already stabilized on the object drug. In contrast, if a patient is taking a stable dose of the precipitant drug, and the object drug is later initiated (and titrated), the risk of resultant ADEs is usually lower.

Drug metabolites (6)

A drug interaction could have a delayed onset if a *metabolite*, not the parent, of the precipitant drug causes the drug interaction. The drug interaction may reflect the time required for the metabolite to be produced and accumulated to a sufficient concentration to affect the concentration of the object drug.

It is crucial to know whether it is the object drug that causes the effect or its active metabolite. In the latter case, one should take into account the metabolite's plasma half-life when estimating the time course of the maximal effect of drug interaction. The metabolites of object drugs may also affect the time course of drug interactions particularly if active metabolites are involved.

1.3.2 Effects of drug interaction mechanisms on time course

Absorption interactions (6,13)

When a precipitant drug inhibits the GI absorption (e.g. by forming a non-absorbable complex), the serum concentration of the object drug usually will begin to decrease within hours of concurrent use of both drugs. This situation is similar to lowering the dose of the object drug. However, the rate of decline depends upon the object's drug plasma half-life. If a precipitant drug interferes with the enterohepatic recycling of the object drug, the latter is then excreted into feces rather than reabsorbed, and thus its excretion is more rapid and its plasma half-life is shortened.

Plasma protein-binding interactions (1,4,5)

The increased unbound fraction resulting from the drug being displaced from the protein only transiently causes increases in efficacy or toxicity. These drug interactions tend to be self-correcting with time. If an adverse effect does not occur within one week of concomitant therapy, it is very unlikely that it will manifest at all.

Enzyme-induction interactions (6)

The initial effect of an enzyme inducer may be detected within the first 2 days of concurrent therapy, however it generally takes over 1 week before the effects of maximal enzyme induction are manifested. The onset of the drug interaction also depends on the plasma half-life of the precipitant drug. After discontinuing the latter, the dissipation of an enzyme induction will occur gradually because of: (i) the discontinuation of inducing agent from the body, and (ii) the gradual decay of the enhanced enzymatic activity in the liver and/or other metabolizing sites.

Enzyme-inhibition interactions (6)

This type of drug interaction can be detected as soon as there is a sufficient concentration of the inhibitor at the metabolizing site, usually within hours. The maximal effect of the enzyme inhibitor usually occurs within the first 24 hours after the administration. Thus, the effect of enzyme inhibitors begins quickly. In contrast, the time required to reach a new steady-state serum concentration (or toxicity) of the object drug will tend to be longer.

Renal excretion interactions (6)

These drug interactions as well tend to begin when sufficient concentrations of both drugs are present in the kidney (usually within hours of administration of the second drug). Because of the nature of this interaction, discontinuation of one of the drugs results in fairly rapid dissipation of the interaction. The effect on the excretion of the object drug is usually minimal after 2 or 3 plasma half-lives of the precipitant drug have passed.

2. Mycophenolate use in hematopoietic cell transplantation

2.1 Hematopoietic cell transplantation background

Allogeneic HCT was developed with the intent to cure patients suffering from malignant and nonmalignant hematologic diseases. Due to intense cytotoxic conditioning regimens which cause organ toxicities, it was at first only offered to younger patients and to patients in good medical condition.(14) However, the majority of patients who could benefit from allogeneic HCT are older and/or have other comorbidities.(14,15) To overcome this ageand medical status-related restriction, reduced intensity conditioning regimens for allogeneic HCT were developed. Of the reduced intensity conditioning regimens, nonmyeloablative allogeneic HCT is the lowest dose and least toxic conditioning regimen that allows engraftment of donor cells.(14) With these decreased doses of conditioning, however, the need for graft-versus-tumor effect (GVT) increases, since GVT contributes to the elimination of remained malignant cells (Figure 2).(16,17)

The optimal postgrafting therapy after nonmyeloablative HCT is currently being studied. Almost all patients receive mycophenolate (MMF or enteric-coated MPA) in combination with a calcineurin inhibitor (cyclosporine or tacrolimus).



Figure 2. Process of nonmyeloablative HCT transplantation.(18) Following reducedintensity conditioning regimen, a small range of malignant (blue) and nonmalignant (green) cells co-exist. After donor HC cells (orange) are infused, a state of chimerism between recipient and donor T-cells is established. The engraftment is further on supported by an additional infusion of donor lymphocytes.

Mycophenolate thus is used to support engraftment of donor cells by preventing graft rejection and preventing or treating graft-versus-host disease (GVHD). Nonmyeloablative HCT recipients receive mycophenolate dose according to the body weight, which leads to high inter-patient variability in the area under the concentration-time curve (AUC) of its active metabolite mycophenolic acid (MPA).(19)

Mechanism of action

Mycophenolate mofetil is a prodrug, and MPA is the active form. Enteric-coated formulation contains the active substance in the form of mycophenolate sodium. MPA is a potent, reversible, noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH) type II, and thus blocks the *de novo* purine synthesis (Figure 3) in T and B lymphocytes.(19) Since T and B lymphocytes cannot synthesize guanine nucleotides by any other salvage pathway, inhibition of *de novo synthesis* causes immunosuppression, leading to prevention of graft rejection or onset of GVHD. (20,21)



Figure 3. De novo synthesis of guanine nucleotides. T and B cells lack the guanine salvage pathway (see guanine), which other cells use if the IMPDH enzyme is inhibited.

IMP indicates inosine monophosphate; XMP: xanthosine monophosphate; GMP: guanosine monophosphate; GDP: guanosine diphosphate; NAD+: nicotinamide adenine dinucleotide (oxidized form), NADH: nicotinamide adenine dinucleotide (reduced form) PRPP: phosphoribosyl pyrophosphate, PPi: pyrophosphate.

2.2 Mycophenolic acid pharmacokinetics

The pharmacokinetics of MPA has so far extensively been studied in healthy volunteers, solid organ transplant patients and patients with autoimmune diseases¹.(22) Few data have been published displaying pharmacokinetic characteristics of the drug in HCT patients. A simplified description of all LADME processes can be found in Figure 4.



Figure 4. Pharmacokinetics of MMF and MPA.(23)

Absorption

After administration, the prodrug MMF is rapidly hydrolyzed to MPA by enzyme carboxyesterase, found in stomach, small intestine, liver and tissues. Once as the active form, MPA is also rapidly absorbed.(19,22)

¹ The following chapters (2.2 to 2.4) include only data derived from studies conducted with MMF, since the data available for enteric-coated MPA is sparse.

Distribution

MPA is highly bound to human serum albumin in the order of 97-99% in patients with normal renal and liver function.(22) The unbound fraction is the pharmacologically active form. Recent studies in renal transplant patients have suggested that hematologic toxicity was more closely associated with the unbound fraction of MPA than with total MPA. Thus, both the total and unbound MPA concentrations should be considered in HCT clinical setting.(19)

The binding of MPA to plasma proteins is influenced by the availability of serum albumin binding sites and competition for these sites by MPA metabolites and urea. The former also display high serum albumin binding (app. 82% in stable renal transplant patients).(22)

Metabolism

MPA is metabolized in the GI tract, kidney and liver, with the latter being the major metabolizing site. It has 4 main metabolites: MPA glucoronide (MPAG), MPA acyl-glucoronide (AcMPAG), catalysed by uridine 5'-diphosphate glucuronosyltransferase (UGTs), 7-O-MPA glucoside as well through UGT, and trace amounts of 6-O-desmethyl-MPA (DM-MPA) via CYP P450. The main metabolite, MPAG, is pharmacologically inactive, but plays an important role in enterohepatic recycling (EHC). AcMPAG is a minor metabolite, and there is an ongoing debate about its activity *in vitro*.(24) Due to its electrophilic nature, AcMPAG can covalently bind to proteins, lipids and nucleic acids, and thus may contribute to hypersensitivity, drug toxicity and immune response in patients.(22,24)

The specific role of different UGT isoforms in the metabolism of MPA is not completely known, but several in vitro studies have suggested UGT1A9 and UGT2B7 as the predominant isoforms, followed by UGT1A8, UGT1A7 and UGT1A10.(22,24) UGT2B7 is the only isoform reported to produce AcMPAG in significant amounts.(22)

Once metabolized, MPA glucoronides can be renally eliminated or excreted into the bile via the MRP-2 transporter. MPA metabolites are converted back to MPA by bacterial β -glucuronidase (which displays large between-subject variability in its activity) between the proximal and distal intestinal regions.(22)

Enterohepatic recycling

Enterohepatic recycling (EHC) of MPA leads to a secondary peak in MPA plasma profile 6-12 hours after MMF administration.(25) In healthy volunteers, solid organ transplant patients and in patients with autoimmune diseases, the EHC comprises up to 60% (range 10-60%) of total MPA AUC.(19) In contrast, allogeneic HCT recipients have a lower prevalence of a delayed second peak. In a recent study conducted by Li et al, only 8 HCT recipients of 77 had a secondary peak.(20) The reduced EHC of MPAG in HCT setting may result from mucosal damage caused by radiation or high-dose chemotherapy doses of myeloablative conditioning, reduction in the bacterial flora of the GI tract or concomitant use of immunosuppressive agents that inhibit the activity of MRP-2.(20)

Excretion

Following oral administration of radiolabelled MMF to four healthy, fasting male volunteers, 93% of MPA metabolites were excreted in urine, with 87% accounting to MPAG. Only small amount of MPA metabolites were excreted in faeces (6%). In the kidney, MPAG and AcMPAG are suggested to be mainly excreted via active tubular secretion, possibly involving MRP-2 mediated transport.(22)

2.3 Mycophenolic acid pharmacokinetic parameters in HCT

It is accepted to demonstrate MPA's pharmacokinetic parameters rather than the MMF's, since the latter undergoes rapid and complete extensive pre-systemic metabolism to the active form.(26) Following MMF administration, MPA expresses linear pharmacokinetics over the normal dosing range (MMF 2-3g/day).(20) The relationship between the dose, plasma concentrations and exposure (AUC) is difficult to predict, with up to 10-fold range in MPA dose-normalized AUC between patients.(19,24) In general, the large between-subject (BSV) and between-occasion (BOV) variability have been associated with differences in albumin concentrations, change of renal and hepatic function, bilirubin and haemoglobin concentrations, bodyweight, sex, race, and concomitant medications.(24) The available pharmacokinetic data in allogeneic HCT recipients suggest that MPA pharmacokinetics after IV or oral MMF administration do not differ based on the conditioning regimen and/or graft source.(20,27)

Area under the concentration-time curve (AUC), concentration at steady state (c_{ss}) and minimum concentration before the next administration (C_{trough})

In HCT setting, 2 recent studies investigated the impact of the frequency (BID vs. TID) of the MMF 15mg/kg dosing on the pharmacokinetic values. With TID dosing, the value of MPA $c_{ss, av}$ (average plasma concentration in steady state, which is MPA AUC divided by dosing interval) was consisted with a therapeutic range described for solid organ transplantation.(28) Furthermore, MPA AUC is also influenced by the serum albumin levels; one study reported that there was an increase in total MPA c_{ss} for 1 unit accompanied by an increase in serum albumin level of 1,07 units.(19)

Since MPA c_{ss} (or MPA AUC) is cumbersome to predict, a recent study investigated if trough concentrations could closely predict MPA c_{ss} . There was no correlation observed between these two parameters and thus authors concluded that monitoring MMF trough concentrations is not useful in nonmyeloablative HCT recipients (Figure 5).(19)



Figure 5. Correlation between total MPA C_{trough} and total MPA c_{ss} for 2 different dosing protocols. Empty squares represent values from the patients taking MMF BID, black circles represent values from the patients taking MMF TID (r=0,70; P<0,01).(19)

Plasma half-life $(t_{(1/2)})$ and time to reach maximum concentration (t_{max})

Disregarding the type of HCT conditioning regimen, data suggest that the MPA plasma half-life ranges from 1,5 to 3,5 hours following oral or intravenous administration, which is shorter than that reported in solid organ transplant patients (9-17 hours). Plasma half-life remains similar in BID vs. TID MMF regimen in HCT recipients.(19,22,24)

Due to rapid pre-systemic metabolism and absorption, the maximum plasma concentration occurs at a mean of 2 hours after the administration (range 1-10 hours).(19)

Clearance (Cl)

Median MPA clearance in HCT patients was reported to be 45,6L/h, and the value is higher than that for renal transplant patients and patients with autoimmune diseases (30,2 and 10,7L/h, respectively). HCT patients have low albumin concentrations, and are taking higher doses of cyclosporine, resulting in higher MPA clearance. When albumin concentration increases, MPA protein binding increases, resulting in a smaller MPA free-fraction and consequently, less MPA available to be cleared.(29)

2.4 Mycophenolic acid pharmacodynamics

To date, investigations have shown the existence of relationship between AUC and efficacy, toxicity.(19,28) Targeting of MPA exposure is feasible early after HCT in order to achieve optimal clinical outcomes and minimize the risk of ADEs.(19)

2.4.1 Efficacy

Donor T-cell chimerism and graft rejection

In HCT patients receiving nonmyeloablative conditioning and an unrelated donor graft, it has been suggested that low total and unbound MPA exposure, expressed as average concentration at steady state, is related to low donor T-cell chimerism (less than 50%), leading patients to a higher risk of graft rejection. Only patients with a total MPA c_{ss} below $3\mu g/ml$ had donor T-cell values below 50% after HCT and only those with total MPA c_{ss} less than 2,5 $\mu g/ml$ had graft rejection.(19) However, further pharmacodynamics studies are needed because more recent analyses did not find an association between MPA c_{ss} and donor T-cell chimerism in patients receiving NMT with HLA-matched related or unrelated donor graft.(20,27)

The impact of HCT conditioning regimen and graft source

The relationship between MPA concentration and clinical outcomes may differ based on the conditioning regimen and/or graft source.(20) In patients undergoing nonmyeloablative conditioning with an unrelated donor graft, one study has demonstrated that less frequent (i.e., Q12 hr) MMF dosing and low total MPA plasma AUC is related to a higher risk of graft rejection. Thus, in this subset of allogeneic HCT recipients, MMF is administered every 8 hr to achieve AUCs similar to those in solid organ transplant patients.(30) A recent analysis also suggests that the influence of the donor graft type on MPA pharmacodynamics is a result of different immunologic barriers receiving an unrelated donor graft.(31)

In a study conducted by Giaccone et al, no significant association was observed between total or unbound MPA c_{ss} and relapse.(19,32) Furthermore, an association was found between low total MPA c_{ss} and increased non-relapse mortality and overall mortality in this clinical setting.(19)

2.4.2 Adverse drug events (toxicity)

In a small population of myeloablative HCT recipients, Nash et al reported that administration of MMF (15mg/kg) every 6 hours leads to increased overall toxicity without improved efficacy.(28)

The onset of Graft-Versus-Host Disease (GVHD)

Acute GVHD contributes significantly to the morbidity and mortality associated with allogeneic HCT.(28) If the percent of donor T-cell chimerism overcomes 90%, the patient is at increased risk of GVHD.(33) This statement was supported by Jacobson's study, where low unbound MPA AUC was associated with more frequent acute GVHD.(34)

Neutropenia

Neutropenia has been associated with total and free MPA AUC in renal transplant patients (29,35), however, to date no data are available for HCT patients. It has been difficult to investigate this association, since the preparatory conditioning itself as well causes neutropenia within the same time frame.(19)

Cytomegalovirus reactivation

In nonmyeloablative HCT patients and an unrelated donor graft, it has been suggested that unbound MPA c_{ss} is related to cytomegalovirus (CMV) reactivation.(19)

Gastrointestinal (GI) toxicity

It is difficult to predict if nausea and vomiting are directly associated with MMF administration and not with the conditioning regimen and the onset of acute GVHD. Giaccone et al reported no statistically significant differences in total MPA c_{ss} values between patients who did or did not report GI toxicity.(19)

HYPOTHESIS AND AIMS

Hypothesis

Pharmacokinetic drug interactions with mycophenolate in nonmyeloablative HCT recipients could potentially affect a patient's clinical outcome. Identifying the potential and the factors associated with the increased risk of drug interactions can optimize outcomes post nonmyeloablative HCT.

In this Master's Thesis, we will first focus into drug interaction research based on the available information from the literature (*specific aim 1*). Then, we will apply this information to the patients enrolled in our study (*specific aim 2 and 3*).

Specific aim 1: Selection of potential mycophenolate-drug interactions

- a) Review of the literature
- b) Recognition of clinical significance
- c) Inclusion of theoretical drug interactions

Specific aim 2: Study data collection

- a) Preparation of the study protocol
- b) Incorporation of the patient data into the worksheet

Specific aim 3: Study data analysis

a) Identification of the study characteristics

b) Drug interaction management proposal and scientific level of evidence ranking

c) Changes in number of concomitant medications and potential drug interactions over the observed period

d) Identification of factors associated with potential drug interactions

METHODS

At first, an expert panel was formed to prepare a comprehensive list of the potential mycophenolate-drug interactions for the study purpose. It consisted of PharmD, MD, and Master of pharmacy candidate. Of the 3 members of the panel, 2 were assigned a specific task. After the completion of the task, a collaborative meeting was organized to reach the consensus on the findings. In case of disagreements, the third member of the panel, initially not involved in the work, made the final decision.

The information was incorporated into Microsoft Excel 2007 spreadsheet.

Specific aim 1: Selection of potential mycophenolate-drug interactions

a) Review of the literature

Between July 2011 and November 2011, PharmD and Master of Pharmacy candidate independently conducted a review of the reported mycophenolate-drug interactions in the following drug interactions databases: Stockley's Drug interactions, Lexicomp, Micromedex, and one commercial online source (www.drugs.com). Also, a Pubmed search was conducted by the Master of Pharmacy candidate on the primary literature, published in the English language using the following queries: mycophenolate OR (mycophenolic acid) AND interactions, mycophenolate OR (mycophenolic acid) AND (*precipitant drug name*), mycophenolate OR (mycophenolic acid) AND (*precipitant drug name*) AND interactions. In the literature, the panel sought the following information: mycophenolate (MMF or enteric-coated MPA) regimen, precipitant drug, study design, type of clinical setting, number of participants, pharmacokinetic parameters measured (AUC, clearance).

b) Recognition of clinical significance

A drug interaction reported in the literature was identified as clinically significant, if there was a \geq 20% change in the MPA AUC documented when mycophenolate and the precipitant drug were given concomitantly.(36) This was based on the pharmacodynamic analyses in nonmyeloablative patients relating T-cell chimerism to MPA AUC (see Introduction 2.4).

c) Inclusion of theoretical drug interactions

To further characterize inhibitors and inducers, which would theoretically interfere in MPA transport and metabolism, MD and Master of Pharmacy candidate independently searched for the *in vitro* studies in the University of Washington Drug Interaction Database and Pubmed, respectively. In Pubmed, the following queries were used: mycophenolate OR (mycophenolic acid) AND (*precipitant drug name*), mycophenolate OR (mycophenolic acid) AND (*in vitro*), mycophenolate OR (mycophenolic acid) AND (*in vitro*). We looked for the following information: precipitant drug and mechanism of the drug interaction (inhibition/induction characteristics).

Specific aim 2: Study data collection

After completion of the *specific aim 1*, we conducted a retrospective analysis on drug interactions in a cohort of nonmyeloablative HCT recipients who participated in a prospective biomarker study (funded by NIH, RO1 HL 91744). Nonmyeloablative conditioning consisted of 2Gy total body irradiation and fludarabine 90 mg/m². Postgrafting immunosuppression consisted of a calcineurin inhibitor (cyclosporine or tacrolimus), mycophenolate (MMF or enteric-coated MPA) and, in certain cases, sirolimus. To conduct the analysis, Institutional Review Board approval was obtained from the Fred Hutchinson Cancer Research Center (FHCRC).

a) Preparation of the study protocol

The protocol for marking a drug interaction occurrence was prepared according to the determinants of the time course of drug interactions (see Introduction 1.3). The timeline of a drug interaction occurrence was defined as up to 3 days in advance to the observed day after allogeneic graft infusion, except for absorption interactions, for which the timeline was more carefully considered (Table III).

Description of the	Examples of medications	Comment
drug interaction		
Altered	Mineral supplements,	Mark as "1" only if taken on the
absorption	antacids, binding resins, bile	observed day.
constant (Ka)	acid resins	
Altered extent of	Proton-pump inhibitors, H2	Mark as »1« if taken up to 3 days
absorption	antagonists	in advance.
Altered GI	Broad-spectrum antibiotics	Mark as »1« if taken up to 3 days
bacteria activity		in advance.
Altered GI	Diphenoxylate, loperamide,	Mark as »1« if taken up to 3 days
motility	docusate, bisacodyl	in advance.

Table III. Timeline of the drug interaction occurrence for absorption interactions.

If a patient was taking intravenous MMF or enteric-coated MPA, absorption interactions affecting the primary absorption (i.e. lowering the first peak in the MPA AUC) were not considered.

b) Incorporation of the patient data into the worksheet

The timeline for enrolling patients was from 23rd November 2008 to 10th November 2011. Oral mycophenolate administration frequency and dose was specified by HCT clinical protocols (MMF 15mg/kg BID/TID or enteric-coated MPA equivalent).

From the standardized medication history worksheets, a total list of medications was generated in November 2011 (see Appendix I). Doses of concomitant medications and "pro re nata" medications were not collected. Standard practice at the treating institution (FHCRC) is that HCT recipients are instructed not to take herbal products and such potential drug interactions were not evaluated.

The data on interacting drugs was collected in Microsoft Excel 2007, for each patient on 3 days (day +2, day +7, day +21) after allogeneic graft infusion. If a patient was taking the interacting drug, the entry was defined as "1". If a patient was not taking the drug, the entry was defined as "0". If a patient withdrew from the study, the entry was defined as "w" and if the data was not available, the entry was defined as "nd" (see Appendix III). Due to the risk of human factor errors at inserting the data in the Excel sheet, the patient medication history worksheets were evaluated 3 separate times with 1 additional evaluation by an independent rater.

Specific aim 3: Study data analysis

Data analysis was performed using the program SigmaPlot 11.2 (Systat Software, Inc). In descriptive statistics, categorical data are presented as number of participants meeting stated criteria; continuous data are presented as median, with maximum and minimum range.

Shapiro-Wilk normality tests were performed to test for normal/non-normal distribution of results. Significance level was set to p < 0.05.

a) Identification of the study characteristics

We collected and analyzed demographics as well as disease- and biochemistry-related characteristics of the patients. The demographics comprised of number of patients, number of patients older than 60 years, gender, and age. We identified pre-transplant cancer diagnoses, HCT-specific comorbidity index (HCT-CI) (15), and evaluated hepatic and renal dysfunction based on biochemistry results (Table IV).

Table IV. Evaluation of the renal/hepatic dysfunction with the available patient biochemistry data. ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Renal dysfunction	Serum creatinine clearance <60 ml/min, calculated with	
	Cockroft-Gault equation, adjusted for ideal body weight.	
Hepatic dysfunction	Total bilirubin > than 2 times laboratory upper normal limits,	
	ALT or AST > than 3 times laboratory upper normal limits.	

Descriptive statistics were used to describe the frequency of concomitant medications and potential drug interactions. We identified all medications patients were taking and divided them into "HCT" class and "non-HCT" class. In both classes we identified medications, which cause potential drug interactions prior defined in *specific aim 1*. Lastly, we also described the underlying mechanisms of these potential drug interactions.

b) Drug interaction management proposal and scientific level of evidence ranking After the identification of potential drug interactions, we proposed an actionable management according to the Hansten and Horn's Operational Classification of Drug Interactions (ORCA) (37), as presented in Table V.

Table V. Drug interaction management classification.(37)

Class 1	Avoid combination (risk of combination outweighs benefit).	
Class 2	Usually avoid combination (use only under special circumstances):	
	- Interactions for which there are clearly preferable alternatives for one	
	or both drugs,	
	- Interactions to avoid by using an alternative drug or other therapy	
	unless the benefit is judged to outweigh the increased risk.	
Class 3	Minimize risk (assess risk and take one or more of the following	
	actions if needed):	
	- Consider alternatives,	
	- Circumvent,	
	- Monitor.	
Class 4	No special precautions (risk of adverse outcome appears small).	
Class 5	Ignore (evidence suggests that the drugs do not interact).	

The lower the class, the more recommended to seek for an appropriate management. If there was no suitable management available, we assigned the medications a special class "not actionable". To the identified interacting medications we also assigned the level of scientific evidence (as shown in Table VI).

Level of scientific evidence	Description	
1	Established: adverse effect confirmed by large clinical trials.	
2	Probable: adverse effect with high likelihood of occurence but without definitive randomized clinical trials.	
3	Suspect: adverse effect likely to occur; data derived from case reports.	
4	Possible: adverse effect may occur but data are scarce.	
5	Unlikely: adverse effect may theoretically ocur.	

Table VI. Scientific level of evidence ranking.(38)

Lower number indicates greater strength of evidence in support of the drug interaction. In addition, the collected case reports on drug interactions were assessed with the Hansten and Horn's Drug Interaction Probability Scale criteria (DIPS, see Appendix II for the total

list of criteria). Scores were assigned for each question/criterium (Table VII).(39) Only PharmD and Master of Pharmacy candidate were involved with the DIPS criteria assignment.

DIPS scale	Number of scores assigned
Highly probable drug interaction	>8
Probable drug interaction	5-8
Possible drug interaction	2-4
Doubtful drug interaction	<2

Table VII. Drug Interaction Probability Scale (DIPS) Assessment.

c) Changes in number of concomitant medications and potential drug interactions over the observed period

Friedman's repeated measures ANOVA on ranks for concomitant and interacting medications was conducted to test the difference in the variables between the observed days, respectively.

d) Identification of factors associated with potential drug interactions

Spearman correlation coefficient was used to test the correlation between potential drug interactions and the following variables: age, HCT-CI and number of concomitant medications. Scatter plots were drawn to graphically present correlations.

RESULTS AND DISCUSSION

HCT recipients are especially susceptible to drug interactions due, in part, to a high number of medications often administered to these patients. In addition to immunosuppressive agents and drugs to treat comorbid conditions, HCT recipients receive medications to treat both cancer-related syndromes and therapy-induced toxicity. Furthermore, the risk of drug interactions and ADEs could be increased because of the underlying pathophysiology of a patient, such as renal and/or hepatic dysfunction.

Investigating and evaluating a drug interaction potential serves as a way to prevent the manifestation of ADEs. The main ADEs to mycophenolate in HCT recipients are neutropenia, increased risk of infections due to over immunosuppression, and gastrointestinal toxicity. Each of these three toxicities is multifactorial in HCT recipients with (i) neutropenia potentially being caused by the HCT conditioning regimen or ganciclovir use for treatment of cytomegalovirus infections, (ii) over-immunosuppression being caused by HCT conditioning or post-grafting immunosuppression other than mycophenolate, and (iii) gastrointestinal toxicity potentially due to conditioning regimen or acute GVHD. Because of these facts, the clinical manifestation of the pharmacokinetic drug interactions with mycophenolate cannot be evaluated in our clinical setting and was therefore not the focus of this Thesis.
1. Selection of potential mycophenolate-drug interactions

1.1 Review of the literature

Literature review aimed to generate available data on pharmacokinetic mycophenolatedrug interactions. In order to prepare a comprehensive list, we searched for the information in several databases, such as Stockley's Drug Interactions, Micromedex, Lexicomp and www.drugs.com², as well as in the primary literature on Pubmed. The pharmacokinetic parameter of interest was MPA AUC, since Giaccone et al reported MPA AUC to be associated with MPA pharmacodynamics in nonmyeloablative allogeneic HCT setting.(19) If there was no note about AUC, we collected other MPA PK parameters (C_{max} , C_{trough}), which served as rough approximations. The review of the literature is available in Table VIII.

 $^{^{2}}$ It should be noted however that this Master's Thesis was not designed to address the accuracy and applicability of different drug interaction resources.

Table VIII. Review of the primary literature. Precipitant drug indicates the drug identified to affect the MPA pharmacokinetics; N indicates the number of patients involved in the study; Study characteristics describe a type of the clinical setting and study design; CS indicates clinical significance as \geq 20% change in MPA AUC. Rf indicates reference.

Precipitant drug	Ν	Study characteristics	MMF dose	Pharmacokinetic parameters and findings	CS	Rf.
Immunosuppressive medications						
- Cyclosporine (C)	408	HCT, popPK study	/	MPA Cl \uparrow for 34% in C. group in comparison to T.	Y	27
- Tacrolimus (T)				group.		
- Sirolimus (target c _{trough} 0,01-0,015mg/L)	31	SOT, PK interaction	1g BID	MPA AUC \downarrow for 32% in C. group in comparison to	Y	40
(S)		study		S. group.		
- Cyclosporine (target c _{trough} 0,15-						
0,20mg/L) (C)						
- Sirolimus	30	SOT, PK interaction	2g/d	After 2 weeks, MPA AUC \downarrow for 47% in C. group in	Y	41
$(target c_{trough} 0,010-0,02 mg/L) (S)$		study		comparison to S.group.		
- Cyclosporine						
(target c_{trough} 150-300ng/mL) (C)						
Antibiotics						
Ciprofloxacin	1	HCT, case report	/	MPA AUC \downarrow for 65%.	Y	42
Co-amoxiclav	2	SOT, 2 case reports	1,5-4g/d	C1: MPA AUC (0-12h) \downarrow for 39%.	Y	43
		(C1, C2)		C2: MPA AUC $(0-12h)$ ↑ for 91% when co-		
				amoxiclav D/C.		
- Ciprofloxacin 500mg BID (Ci)	64	SOT, prospective	15mg/kg/d	No note about MPA AUC.	/	44
- Co-amoxiclav 375mg TID (Co)		study		Ci: MPA $c_{trough} \downarrow$ for 46%.		
				Co: MPA $c_{trough} \downarrow$ for 46%.		
Co-trimoxazole 960mg BID	12	HV, /	1,5g SD	MPA AUC \downarrow for 5%. $C_{max} \downarrow$ for 1%.	Ν	45
Mycostatin 3 million IU/d + tobramycin	6	SOT, prospective trial	1g BID	MPA AUC (6-12h) \downarrow for 31%.	Y	46
0,6 g/d + cefuroxime 6g/d						
- Norfloxacin (NOR) 400mg BID,	11	HV, prospective, 4	1g SD	NOR: MPA AUC (0-48h) \downarrow for 10%.	Y	47

- Metronidazole (MET) 500mg TID		treatment periods		MET: MPA AUC $(0-48h) \downarrow$ for 19%.		
- Combination (COMB)				COMB: MPA AUC (0-48h) \downarrow for 33%.		
Rifampin 600mg OD	1	SOT, case report	1g BID	MPA AUC \downarrow for 221%.	Y	48
Rifampin 600mg OD	8	SOT, PK interaction	0,75-1g	MPA AUC (0-12h) \downarrow for 17,5%.	Ν	49
Antifungola		study	BID			
Acyclovir 800mg	/	HV gross over study	la SD	No note about MPA_AUC	/	50
Acyclovii 800ing	/	IIV, closs-over study	Ig SD	No significant differences in MPA PK parameters.	/	50
- Acyclovir 800mg	15	HV. cross-over study	1g SD	No note about MPA AUC.	/	51
- Valaciclovir 2g		,	0	No significant differences in MPA PK parameters.		
-				MPAG AUC \downarrow for 12% by valaciclovir.		
Valaciclovir 6g/d	1	SOT, case report	1g BID	No note about MPA AUC.	/	52
				MPA c_{trough} \uparrow for 127% when valaciclovir D/C.		
Ganciclovir 5mg/kg IV	12	SOT, cross-over study	1,5g SD	No note about MPA AUC.	Ν	53
				MPA Cl \uparrow for 3%.		
Corticosteroids						
Methylprednisolone	26	SOT, PK interaction study	1-2g/d	MPA AUC \uparrow for up to 50%.	Y	54
Prednisone	52	SOT, /	1g BID	No note about MPA AUC.	/	55
				After 6 months, MPA $c_{trough} \downarrow$ for 13,4%.		
				After 9 months, MPA $c_{trough} \uparrow$ for 14,5%.		
Proton pump inhibitors						
- Lansoprazole 30mg (L)	39	SOT, PK interaction	0,5-2g/d	L: MPA AUC (0-12h) \downarrow for 25%.	Y	56
- Rabeprazole 10mg (R)		study		R: MPA AUC (0-12h) \uparrow for 22%.		
Omeprazole 20mg BID	12	HV, cross-over study	1g/ 720mg	MMF: MPA AUC \downarrow for 23%.	Y	57
			(EC-MPA) SD	EC-MPA: no change in MPA AUC.		
Pantoprazole 40mg/d	36	AID, PK interaction	1-2g/SD	MPA AUC \downarrow for 37%.	Y	58

		study		MPA $c_{max} \downarrow$ for 60%.		
Pantoprazole 40mg BID	22	HV, case-controlled study	1g SD /720mg (EC-MPA)	MMF: MPA AUC \downarrow for 27%. MPA $C_{max} \downarrow$ for 80%. No changes in EC-MPA PK.	Y	59
Antacids						
Antacids: Al(OH)3 + Mg(OH)2	10	RA, cross-over study	2g/d SD	MPA AUC \downarrow for 17%. MPA C _{max} for 37%.	N	60
Antacids (Al(OH)3 and Mg(OH)2)	41	SOT, /	MMF	MPA AUC $(0-12h) \downarrow$ for 3%.	Ν	45
Cations						
Ca, Fe, Mg, Na, Al supplements	15	SOT, /	/	No note about MPA AUC. MPA $c_{trough} \downarrow$ for 56% in Tacrolimus group. No significant differences in Cyclosporine group.	N	61
Ferrous sulfate SR 1050mg (210mg Fe)	7	HV, cross-over study	1g SD	MPA AUC (0-12h) \downarrow for 89,7% MPA C _{max} \downarrow for 93,5%.	Y	62
Ferrous sulfate SR 650mg (210mg Fe)	16	HV, cross-over study	1g SD	MPA AUC (0-24h): \uparrow for 5%. MPA C _{max} : \uparrow for 7%.	N	63
Ferrous sulfate SR 650mg (210mg Fe)	40	SOT, RCT	1g BID	Concomitant Fe: MPA AUC \downarrow for 2%. Subsequent (4hr) Fe: MPA AUC \downarrow for 7%.	N	64
Ferrous sulfate SR 105mg SD	10	SOT, /	1g	Concomitant Fe: MPA AUC (0-12h) \uparrow for 2,7%. Subsequent (4hr) Fe: MPA AUC (0-12h) \uparrow for 7,3%.	N	65
- Polysaccharide Fe complex - Ferrous sulfate SR	12	SOT, co- administration (1) and 2 hrs apart (2)	1g BID	 With polysaccharide Fe complex: 1: MPA AUC (0-12h): ↑ for 10,2%. 2: MPA AUC (0-12h): ↑ for 21,9%. With ferrous sulfate SR: 1: MPA AUC (0-12h): ↓ for 5,3%. 2: MPA AUC (0-12h): ↓ for 17,2%. 	N	66
Oral contraceptives						
Ethinylestradiol 35mcg + norethisterone	15	HV, /	SD	No changes in MPA PK.	Ν	45

1mg						
Ethinylestradiol 20-40mcg + gestodene 50-100mcg / desogestrel 150mcg /	18	HV, /	1g BID	No note about MPA AUC. No clinically relevant PK interaction.	N	45
Binding agents						
Binding agents						
Cholestyramine 4g TID	12	HV, cross-over study	1,5g SD	MPA AUC \downarrow for 37%.	Y	67
				MPA $C_{max} \downarrow$ for 6%.		
Sevelamer 1,2-1,6g BID	9	SOT, /	0,5-1g/d	MPA AUC \downarrow for 25%. C _{max} \downarrow for 30%.	Y	68
Calcium polycarbophil 2400mg	6	HV, cross-over study	1 g SD	MPA AUC (0-12h) \downarrow for 48,9%.	Y	69
				MPA $C_{max} \downarrow$ for 68%.		
Other						
Rosiglitazone	1	SOT, case report	0,5-1g BID	MPA AUC \uparrow for 110%. Unsure whether this change due to rosiglitazone.	N	70
- Telmisartan 40mg (T)	10	SOT	0,5-1,5g/d	T: MPA AUC $(0-12h) \downarrow$ for 31%.	Y	71
- Valsartan 80mg (V)			C	V, C: No significant effect on MPA PK.		
- Candesartan 8mg (C)						
St John's Wort extract 600mg	8	SOT	1-2g/d	MPA AUC \uparrow for 4,7%.	N	72
Valproate	3	SOT, case reports (C1,	1-2g/d	C1: MPA AUC \uparrow for 80,5% when valproate D/C.	Y	73
		C2, C3)		C2: MPA AUC \uparrow for 123% when valproate D/C.		
				C3: MPA AUC \downarrow for 54% with valproate.		

Percentages calculated from mean values. Missing data in the table not available in the literature.

Abbreviations under "Study characteristics": AID: autoimmune disease; HCT: hematopoietic cell transplantation; PK: pharmacokinetic; popPK: population pharmacokinetic; RA: rheumatoid arthritis; SOT: solid organ transplantation; HV: healthy volunteers; Abbreviations under "Precipitant drug" and "MMF dose": OD: once daily; BID: two times daily; TID: three times daily; SD: single dose; d: daily; Abbreviations under parameters: D/C: discontinued. Abbreviations under "clinical significance":Y: yes; N: no; /: not available.

We collected over 35 studies on mycophenolate-drug interactions. A large number of studies were performed in healthy volunteers and SOT patients (mainly renal transplant patients). Of note, there were few data available from the HCT patients, with one case report (ciprofloxacin interaction) and one population pharmacokinetic study (cyclosporine interaction). This confirms previous reports on lack of drug-interaction knowledge in this patient population.

In addition to this review, we also collected 2 population pharmacokinetics studies investigating the role of drug interactions on MPA as covariates. In a study by Le Guellec et al (74), corticosteroids were reported not to affect MPA clearance. In a study conducted by van Hest et al (75), cyclosporine was reported to affect MPA clearance.

Ideally, this review would have clearly identified which medications would interact with mycophenolate. On one hand, substantive data was available for some potentially interacting drugs, such as ferrous sulfate, antacids, PPIs, antivirals. On the other, insufficient detail was available for antifungals and many antibiotics. Also, studies from different authors reported contradictory findings (e.g. for rifampin, iron, calcium, corticosteroids). Although the review was performed thoroughly, the interaction potential was in the end not clear for some medications.

1.2 Clinical significance of published drug interactions

At present, there is no accepted change in MPA AUC that is considered clinically significant. For the purpose of this Thesis, the expert panel chose the 20% change in MPA AUC arbitrarily. It equals the percentage of variability allowed between immunosuppressive brand-name compounds and generic to ensure bioequivalence.(36) In the literature, some authors of the mycophenolate-drug interaction studies stated 66%(65) or 29%(64) change in MPA AUC as clinically significant, however the expert panel mutually agreed to accept a more conservative range. This allowed us to identify 14 clinically significant interactions (see Table VIII, "CS" column). While defining this significance, the expert panel's opinion differed only on mycophenolate-calcium supplements interaction, which was supported by two studies displaying contradictory results.(61,69) The third member of the party (MD) gave the final remark of not considering this interaction as clinically significant.

1.3 Inclusion of in vitro drug interaction studies

The inclusion of "theoretical" drug interactions would not be possible without a comprehensive understanding of the pharmacokinetics of MMF and MPA, leading to a new aspect of the Thesis. In this part of the research, we also began to screen medication sheets from our patients to see which medications patients were actually taking to consider whether or not they could theoretically cause a drug interaction. Only three medications that were previously known as UGT enzyme inhibitors were added, specifically amitriptyline, fluconazole and lorazepam.

2. Study data collection

The protocol for marking the presence of the interaction in our study has basis in Hansten & Horn's guidelines Pitfalls in evaluating drug interaction literature.(76) Since we could not link a drug interaction with the exerted ADE in our study, it was of crucial importance to prepare a protocol, which would recognize only significant potential drug interactions. Thus, for the purpose of manually screening the patient medication history worksheet, the following considerations were taken into account:

- the time course of a drug interaction,
- the underlying mechanisms of potential drug interactions,
- in case of corticosteroids and proton-pump inhibitors, appropriate extrapolation of a drug interaction from one member of a drug class to all members of that class,
- avoidance of the false positive results by not including medications, which do not alter mycophenolate absorption by significant amount (e.g. antacids, cation supplements, GI motility agents).

The aforementioned guidelines strongly recommend considering the effects of dose when evaluating drug interactions as well. However, these could not be evaluated because of the resource intensity of collecting this detailed information.

Based on the prepared protocol, the potential drug interactions were incorporated into the worksheet (see Appendix III).

3. Study findings

3.1 Characteristics of patients in the study (Table IX)

Table IX. Patient demographic characteristics, index disease diagnosis, comorbidity evaluation by comorbidity index and presence of renal/liver dysfunction. All numbers represent absolute numbers of patients with a particular characteristic.

Number (N) of patients		74
N > 60 years		44
Demographic characteristics	Gender (male/female)	47/27
	Age, median (range)	62.1 (20.0-73.1)
Cancer types	Non-Hodgkin's lymphoma	17
	Chronic lymphocytic leukemia	14
	Acute myelogenous leukemia	12
	Myelodysplastic syndrome	7
	Multiple myeloma	5
	Diffuse large B cell lymphoma	4
	Mantle cell lymphoma	3
	Myeloproliferative syndrome	3
	Acute lymphocytic leukemia	2
	Other*	7
Comorbidity index (HCT-CI)	0	6
scores**	1-2	11
	3-4	28
	≥5	28
Presence of organ dysfunction	Renal dysfunction	8
	Liver dysfunction	1

*Other: Aplastic Anemia, Blastic plasmacytoid dendritic cell neoplasm, Composite Lymphoma-CHL + Mediastinal Lg cell, Follicular Lymphoma, NK/T-cell Lymphoma, PNH- with aplasia, T-cell Lymphoma.

** HCT-CI scores were assigned to 73 patients.

A total of 74 patients were included in this retrospective analysis within the 3-year period of time. 47 patients were male and 27 female. The median age was 62.1 years, with 44 patients older than 60 years. This number confirms the fact that nonmyeloablative conditioning truly offers cure to older population and is not limited only to young subjects. The pre-transplant cancer diagnoses of our patients differed, with more than 15 different cancer types present. The most common cancer diagnosis was Non-Hodgkin's lymphoma (N=17), which is commonly the type of cancer that requires hematopoietic cell transplantation.(77)

HCT-CI is a valuable tool for assessing the impact of comorbidities on cancer, since the sicker the patients are prior to transplantation, the worse is the outcome.(78) In our study, only 6 patients (less than 10%) had "0" score, 11 patients had "1-2" score and 28 patients (almost 40%) had scores "3-4" or " \geq 5", respectively. The maximal assigned score was "11". This is an important finding when predicting the outcome in these patients; in a recent study conducted by Sorror et al., the patients with HCT-CI scores of "1" or greater were associated with worse survival than patients with HCT-CI score "0".(15)

Few of our patients had serious organ dysfunction: 8 patients suffered from renal dysfunction, and only 1 patient suffered from hepatic dysfunction. Data on kidney and liver functions is essential in the HCT setting especially when assigning the HCT-CI scores, since the worse the organ dysfunction, the more scores assigned to a patient.

3.2 Medications prescribed per patient

In this study, patients were taking a total of 184 different medications (Appendix I). On day 2 and day 7 after allogeneic graft infusion, HCT patients were taking a median of 14 (range 9-22 on day 2 and range 9-25 on day 7) medications (Table X, Figure 6). On day 21 after the infusion, patients were taking a median of 13.5 medications (range 8-24). The types of the medications varied and comprised of all anatomic-therapeutic chemical (ATC) classification groups. For the purpose of the Thesis, we divided medications into "HCT" and "non-HCT" class. Roughly, on all 3 observed days HCT medications represented 1/3 of all medications taken by a patient; and non-HCT medications represented the other 2/3.

Table X. Number of medications taken per patient on 3 observed days after allogeneic graft infusion. Values represent median (range) of medications. All medications were divided into HCT medications and non-HCT medications.

Type of medications	Day 2	Day 7	Day 21
All	14 (9-22)	14 (9-25)	13.5 (8-24)
HCT medications	4 (3-6)	4 (3-5)	4 (2-6)
Non-HCT medications	10 (5-17)	9 (5-19)	9 (4-19)



Figure 6. Box-and-whisker plot representing distribution in number of medications per patient on 3 observed days after allogeneic graft infusion.

HCT medications were considered those medications, which are generally part of the post-grafting therapy:

- (i) immunosuppressants (mycophenolate mofetil, enteric-coated MPA, cyclosporine, tacrolimus, sirolimus),
- (ii) broad-spectrum antibiotics (amoxicillin & clavulanic acid, ciprofloxacin, levofloxacin, moxifloxacin, metronidazole),
- (iii) antifungals (fluconazole, voriconazole),
- (iv) antivirals (acyclovir, valaciclovir, ganciclovir),
- (v) corticosteroids (methylprednisolone, prednisone).

In addition to mycophenolate (MMF or enteric-coated MPA), postgrafting immunosuppression in the study comprised of either cyclosporine or tacrolimus and furthermore, app. 10% of patients were also given sirolimus (Table XI). The majority of patients were taking MMF and only 2 patients were taking enteric-coated MPA. Once immuosuppressives were prescribed, no discontinuation or replacement of these medications occurred for the duration of the post-transplant therapy.

Table XI. Immunosuppressive agents taken by our patients. All patients were taking mycophenolate (MMF or enteric-coated MPA) and either cyclosporine or tacrolimus. In addition to these, some patients were as well taking a third medication, sirolimus.

Immunosuppressant	Nr. of patients
Mycophenolate mofetil	72
Enteric-coated MPA	2
Cyclosporine	49
Tacrolimus	25
Sirolimus & calcineurin inhibitor	10

"Non-HCT" class comprised of all other medications; medications to alleviate toxicities as well as medications to treat other patient comorbidities.

3.3 Potential drug interactions

To our knowledge, this is the first study investigating the potential of harmful mycophenolate-drug interactions in nonymeloablative subset of HCT patients. On day 2 after the allogeneic graft infusion, patients were taking a median of 3 potentially interacting medications (range 1-6), whereas on day 7 and day 21, patients were taking a median of 4 interacting medications (range 1-6). (Table XII). It is noteworthy that literally every patient was taking at least 1 potentially interacting drug, with the resultant higher risk of mycophenolate toxicities or graft rejection.

Table XII. Number of potential drug interactions per patient. For the purpose of this study, all potential drug interactions were divided into (i) mycophenolate-HCT drug interactions and (ii) mycophenolate-non-HCT drug interactions.

Type of medications	Day 2	Day 7	Day 21
All	3 (1-6)	4 (1-6)	4 (1-6)
HCT medications	2 (0-4)	2 (0-4)	2 (0-4)
Non-HCT medications	1 (0-2)	1 (0-3)	1 (0-3)

Each patient's pharmacotherapy consisted of 2/3 of interacting medications from the "HCT" class, and the rest 1/3 interacting from the "non-HCT" class (Figure 7).

Day 2

Day 7

Day 21



Figure 7. The incidence of interacting medications on 3 observed days.

As assumed in advance, patients were not exclusively taking all medications that were previously reported to interact (see Table VIII). This is manly due to the fact that the available literature comprises of reported interactions from clinical settings other than HCT, requiring different pharmacotherapies. A list of interacting medications with a description of the interaction mechanism is available in Table XIII.

Table XIII. Interacting medications identified in our study. Description indicates the effect on MPA parameters and identification of the underlying interaction mechanism. N indicates number of cases, "Ev." level of scientific evidence and "Mgn." proposed management.

Drug	Description	Ν	Mgn.	Ev.
Amitriptyline	Inhibition of MPA metabolism, ↑ MPA exposure, ↑ risk of toxicities.	1	3	5
Amoxicillin & clavulanic acid	Reduction of EHC and transport, ↓ MPA exposure, ↑ risk for graft rejection.	1	/	2, 3
Ciprofloxacin	Reduction of EHC and transport, ↓ MPA exposure, ↑ risk for graft rejection.	6	/	2, 3
Cyclosporine	Inhibition of transport of MPA metabolites, ↓ MPA exposure, ↑ risk for graft rejection.	49	2	2
Esomeprazole	Decreased conversion of MMF to MPA, ↓ MPA exposure, ↑ risk for graft rejection.	1	2	2
Fluconazole	Inhibition of MPA metabolism, ↑ MPA exposure, ↑ risk of toxicities.	60	/	5
Lansoprazole	Decreased conversion of MMF to MPA, ↓ MPA exposure, ↑ risk for graft rejection.	1	2	2
Levofloxacin	Reduction of EHC, ↓ MPA exposure, ↑ risk for graft rejection.	53	/	5
Lorazepam	Inhibition of MPA metabolism, ↑ MPA exposure, ↑ risk of toxicities.	73	2	5
Methyl- prednisolone	Induction of MPA metabolism, ↓ MPA exposure, ↑ risk for graft rejection.	2	/	2
Metronidazole	Reduction of EHC, ↓ MPA exposure, ↑ risk for graft rejection.	1	/	2
Moxifloxacin	Reduction of EHC, ↓ MPA exposure, ↑ risk for graft rejection.	1	/	5
Omeprazole	Decreased conversion of MMF to MPA, ↓ MPA exposure, ↑ risk for graft rejection.	23	2	2
Pantoprazole	Decreased conversion of MMF to MPA, ↓ MPA exposure, ↑ risk for graft rejection.	19	2	2
Prednisone	Induction of MPA metabolism, ↓ MPA exposure, ↑ risk for graft rejection.	15	/	2
Valproate	Inhibition of MPA metabolism, ↑ MPA exposure, ↑ risk of toxicities.	1	2	3

3.3.1 The "HCT" class interacting medications

Broad-spectrum antibiotics (alone or in combination) are used in HCT as a prophylactic treatment of bacterial infections. One of the treatment side effects is the impaired activity of the GI bacterial flora and consequently impaired or absent conversion of MPA glucoronides back to MPA by GI bacterial β -glucuronidase. At most, our patients were taking levofloxacin, which successfully eradicates Gramnegative and Gram-positive bacteria species.(79) A few patients were taking ciprofloxacin, which can lower the MPA exposure by multiple mechanisms; with the reduction of the GI flora activity and by inhibition of MRP-2 transport.(80) Only a couple of other patients were taking amoxicillin & clavulanic acid (N=1), metronidazole (N=1), and moxifloxacin (N=1).

Antifungals are used in HCT as a prophylactic treatment of fungal infections. Of these, fluconazole inhibits the UGT enzyme and so potentially causes increased MPA exposure, leading to an increased risk of MPA toxicities.(81) Although the clinical significance of this interaction is unclear, we considered it relevant due to a high percentage of patients (over 80%) taking fluconazole and possible serious MPA toxicities.

Corticosteroids are used in HCT patients to treat the onset of acute or chronic GVHD. Corticosteroid use potentially results in UGT induction and subsequently lower MPA exposure.(54,55) In our study, the number of patients taking corticosteroids increased greatly from day 2 to day 21 after allogeneic graft infusion, indicating that some patients did develop GVHD, despite the immunosuppressive regimen. In all but two occasions (when methylprednisolone was used), patients were taking prednisone (N=15), which induces MPA metabolism, resulting in reduced MPA exposure.

Cyclosporine is an immunosuppressive agent, which is commonly prescribed with mycophenolate to exhibit immunosuppressive effect post nonmyeloablative HCT. Over 60% of our patients were prescribed this immunosuppressant. Cyclosporine is a known MRP-2 inhibitor, and significantly affects MPA pharmacokinetics. This is supported by McCune's et al. recent MPA population pharmacokinetic modeling after oral MMF administration suggesting that the more rapid clearance occurs in HCT

recipients due to decreased serum albumin concentrations and concomitant cyclosporine use.(27)

3.3.2 The "non-HCT" class interacting medications

Amitriptyline is a medication used to treat depression. *In vitro* it affects MPA metabolism, by inhibiting UGT1A7, 1A8, 1A9 and 2B7, which possibly results in increased MPA exposure.(80) Amitriptyline was taken only by 1 patient.

Lorazepam is used in HCT recipients as an anxiolytic. *In vitro*, it increases MPA exposure by inhibiting UGT2B7 enzyme activity.(82) In our study, almost 90% of patients were taking lorazepam (N=64), which made it the most frequently taken non-HCT medication.

Proton-pump inhibitors have a potent gastric acid secretion inhibitory effect, followed by subsequent increase in the gastric pH. This effect might decrease elution and hydrolysis of MMF and thereby decrease MPA absorption.(59) The proposed interaction mechanism is also supported by the fact that enteric-coated MPA formulation absorption is not affected by the concomitant use of PPIs.(83) In the transplant setting, PPIs are used to alleviate the acid-peptic symptoms such as heartburn, epigastric pain, and hoarseness.(84) Approximately 1/3 of the patients were taking PPIs, with one half taking omeprazole and the other pantoprazole. Only 2 patients used esomeprazole and lansoprazole.

Although the gastric pH increase is considered to be a class effect, it is known that the potency between these medications differs.(85) If there was available data on PPI doses, a link between decreased MPA AUC and this variable could be established.

Valproate is used in the treatment of epilepsy. It is a known UGT2B7 inhibitor and thus potentially inhibits MPA metabolism, resulting in increased MPA AUC.(80) In our study, only 1 patient was taking this medication.

3.4 Evaluation of potential drug interactions

3.4.1 Proposed management

The management of drug interactions was based on the Operational Classification of Drug Interactions (ORCA).(37) Due to the crucial role of HCT medications in a patient's outcome, we added another class to the ORCA classification – "*not actionable*", which indicates no management options (Table XIV).

Number of medications		Day 2		Day 7		Day	/ 21
		No.	%	No.	%	No.	%
Number of po	tentially interacting drugs	218	100	214	100	232	100
	Avoid (1)	0	0	0	0	0	0
	Usually avoid (2)	134	61	122	57	130	56
Proposed	Minimize risk (3)	0	0	1	0	1	0
management	No precautions (4)	0	0	0	0	0	0
	Ignore (5)	0	0	0	0	0	0
	Not actionable (/)	84	39	91	43	101	44

Table XIV. Proposed management for the identified potential drug interactions.

See specific aim 3 for exact definition of proposed management classes.

Of all identified drug interactions, over 40% fell into "not actionable" category and comprised only of HCT interacting medications (except for cyclosporine). Interacting HCT medications (aforementioned broad-spectrum antibiotics, fluconazole, and corticosteroids) are usually the only choice of treatment. Cyclosporine on the other hand fell under class "2", since tacrolimus as a suitable alternative could be recommended.(27)

For interacting non-HCT medications appropriate recommendations can be given by carefully considering the risk-benefit ratio associated with the co-administration. The incidence of potentially interacting drugs, for which appropriate management could be addressed can be found in Figure 8.



Figure 8. The incidence of clinically actionable (ORCA class 2 or 3) potentially interacting drugs in the study.

Almost 60% of these were considered as class "2", describing an interaction that should usually be avoided. These are interactions (i) for which there are clearly preferable alternatives for one or both drugs, and (ii) where an alternative drug or other therapy can be used unless the benefit is judged to outweigh the increased risk. From interacting non-HCT medications, all PPIs, lorazepam and valproate fell into class "2". This is a very important result, since a high number of patients was taking both PPIs and lorazepam and, with the appropriate management, the potential of drug interactions could be reduced. Especially for PPIs, it should be first investigated how many patients were actually taking them to treat peptic ulcer disease and further give recommendations to reduce the dose, or change the taken PPI with less potent or with antacids. Consideration should be given when assessing the management of valproate, since it is part of a chronic antiepileptic therapy. Amitriptyline was the only medication classified under category "3". It could be changed to other antidepressants.

3.4.2 Level of scientific evidence ranking

Approximately 40% of the drug interactions were assigned level "2", which indicates a probable drug interaction. Although these drug interactions were not supported with randomized clinical trials, there was still high likelihood of occurrence of an ADE supported by e.g. cross-over drug interaction studies performed in a cohort of healthy volunteers or patients from various clinical settings (e.g. SOT, AID). (Table XV)

Number of medications		Day 2		Da	у 7	Day 21	
		No.	%	No.	%	No.	%
Number of po	tentially interacting drugs	218	100	214	100	232	100
	Established (1)	0	0	0	0	0	0
Level of	Probable (2)	80	37	77	36	92	40
scientific	Suspect (3)	1	0	1	0	1	0
evidence	Possible (4)	0	0	0	0	0	0
	Unlikely (5)	137	63	136	64	139	60

Table XV. Level of scientific evidence for identified potential drug interactions.

See specific aim 3 for exact definitions of each category ranking.

Caution is necessary when making decisions on drug interactions that are supported by case reports, especially if these are not comprehensively written. Thus, in addition to the aforementioned ranking, we decided to evaluate case reports separately by the Drug Interaction Probability Scale (DIPS) criteria (see Appendix II). These criteria were established especially to evaluate case reports and provide a good tool to assess such information. Of our potentially interacting medications, 3 case reports confirming mycophenolate-drug interaction with amoxicillin & clavulanic acid, ciprofloxacin and valproate were evaluated (Table XVI). Amoxicillin & clavulanic acid and ciprofloxacin interactions were assigned 7 scores and were subsequently categorized as a "probable" drug interaction. Also, of these 2 mycophenolateamoxicillin & clavulanic acid interaction was already supported by a higher level of scientific evidence.

5	0	
Drug	DIPS score	Evaluation of the drug interaction
Amoxicillin&clavulanic acid	7	Probable
Ciprofloxacin	7	Probable
Valproate	3	Possible

Table XVI. Evaluation of the drug interactions case reports by the DIPS criteria.

See specific aim 3 and Appendix II for the exact DIPS criteria.

Approximately 60% of potential drug interactions were assigned the lowest level of evidence – "5", which indicates that the ADEs may only theoretically occur. When making decisions on potential drug interactions, the expert panel agreed to feel more comfortable if there were more data supported by a higher level of evidence. The findings of this thesis could also more strongly support the potential of drug interactions if there was stronger evidence available.

Notably, lorazepam and fluconazole, which were medications most frequently taken by patients, fell into this category. However, these two medications are strongly supported by the *in vivo* reports on drug interactions with other medications that have exactly the same metabolic pathway as mycophenolate (e.g. are metabolized by the same UGT isoenzymes).(81,82)

Levofloxacin and moxifloxacin were as well assigned level of evidence "5", since no pharmacokinetic studies have been conducted documenting a mycophenolate-drug interaction. On the other hand, there is clinical data supporting interaction between mycophenolate and broad-spectrum antibiotics, in particular ciprofloxacin (42,44) and norfloxacin in combination with metronidazole.(47) As levo- and moxifloxacin both exhibit a potent activity against a broad bacteria spectrum, it is very likely that this potential drug interaction exists.

Lastly, amitriptyline was as well assigned the lowest level of evidence, however it is a known *in vivo* inhibitor of UGT1A7, 1A8, 1A9 and 2B7 isoenzymes, which metabolize MPA.(80)

4. Analysis: number of concomitant medications and potential drug interactions

At first, we sought to identify the distribution of the number of concomitant medications and interacting medications, respectively. Shapiro-Wilk normality test identified non-normal distribution of both number of concomitant and interacting medications per patient on all 3 days after allogeneic graft infusion (day 2, 7 and 21) (Table XVII).

Table XVII. Identification of normal/non-normal distribution of the number of concomitant medications and interacting medications per observed day by the Shapiro-Wilk normality test (W-statistic). Positive test indicates normal distribution of the results. Failed test indicates non-normal distribution of the results.

Medications per patient	Observed day	W-statistic	P value	Result	Ν
Concomitant	D2	0,958	P=0,027	Failed	65
	D7	0,922	P=0,001	Failed	59
	D21	0,947	P=0,009	Failed	62
Interacting	D2	0,927	P<0,001	Failed	65
	D7	0,920	P<0,001	Failed	59
	D21	0,921	P<0,001	Failed	62

After this initial step, non-parametric tests were used to further analyze the results.

4.1 Number of concomitant and interacting medications per patient over the observed period

To identify the differences in numbers of medications per patient occurring over time, we used the Friedman's ANOVA test on ranks, which detects differences in treatments across multiple test attempts (Table XVIII).

Table XVIII. Identification of the difference in number of concomitant and interacting medications, respectively, between the observed days by the Friedman Repeated Measures ANOVA on Ranks. The table contains information on chi-square value (H), degrees of freedom (N) and P value.

Medications	Н	Degrees of freedom (N)	P value
Concomitant	0,373	2	0,830
Interacting	6,796	2	0,033

Over the first 21 days after allogeneic graft infusion, according to the test, the number of concomitant medications did not differ. This indicates a constant medication burden each patient was exposed to in this period of time post-transplant.

However, the number of potential drug interactions did differ over the first 21 days (p=0,033). One might propose that this statistically significant difference was observed due to the increased number of interacting medications on day 21, which can be mainly due to the increased use of corticosteroids.

4.2 Factors associated with the number of potential drug interactions

Spearman Rank Order Correlation Coefficient was used to identify any association between the number of potentially interacting medications and the following variables: age, HCT-CI and number of concomitant medications (Table XIX).

Table XIX. Correlation between potential drug interactions and other variables (age, HCT-CI, number of concomitant medications) per patient by Spearman Rank Order Correlation.

Variable	Observed day	Correlation	P value	Ν
		coefficient		
Age	Day 2	-0,0873	0,488	65
	Day 7	-0,0417	0,753	59
	Day 21	-0,0755	0,559	62
Comorbidity index	Day 2	-0,375	0,002	64
	Day 7	-0,184	0,169	57
	Day 21	-0,0107	0,935	60
Number of	Day 2	0,105	0,406	65
concomitant	Day 7	0,244	0,0624	59
medications	Day 21	0,200	0,119	62

As seen from the table XIX, we did not conduct the analysis for all 74 patients, but only for those with data from all 3 observed days. This Master's Thesis was part of a prospective biomarker study, in which adherence to MPA pharmacokinetic sampling was challenging. If a patient withdrew from the study, a decreased amount of drug interaction data thus became available for further analysis.

The analysis did not reveal any correlation between age and the number of potential drug interactions (an example is shown in Figure 9). Similarly, it was previously confirmed that age was not a risk factor for cyclosporine-drug interactions.(86) However, one must note that numerous epidemiologic studies indicate individuals older than 65 have up to 3 times as many drug interactions as younger people.



Figure 9. Absence of correlation between age and potential drug interactions on day 21.

There was a significant negative correlation (P=0,002) between HCT-CI and the number of potential drug interactions on day 2, but not on day 7 or day 21 after allogeneic graft infusion (see Figure 10). It has to be noted that in our study, lower HCT-CI scores were not exclusively assigned to younger patients, and the HCT-CI score was randomly distributed in patients disregarding the age (however, this is only an observation which was not statistically confirmed). This suggests that even though more than 50% of our patients were aged 60 years or over, the age *per se* did not mean that patients had a higher HCT-CI (i.e. were sicker).



Figure 10. Significant negative correlation between HCT-comorbidity index and potential drug interactions on day 2 (left). Absence of correlation on day 21 (right).

There was no significant correlation observed between the number of concomitant medications and the number of potential mycophenolate-drug interactions (the P value however was low on day 7 and day 21, see Figure 11). Although this finding seems surprising at first, we present some arguments that can be of its support. Firstly, our study investigated only mycophenolate-drug interactions and not interactions of all medications between each other. In the later case, the number of interactions is very likely to increase with the number of concomitant medications. Secondly, we only investigated the effect of medications on mycophenolate, but not vice-versa, which may lower the incidence of drug interactions. Lastly, mycophenolate is metabolized through CYP450 only in trace amounts, therefore avoiding a great number of interactions based on inhibition/induction potential of numerous drugs on the CYP450 isoenzymes.



Figure 11. Absence of correlation between concomitant medications and potential drug interactions on day 21.

5. Limitations of the study

The major limitation of this study is the lack of information about potential drug interactions resulted in ADEs. As discussed before, because of the confounders with interpreting mycophenolate ADE, it would be almost impossible to connect the ADE directly to the MPA AUC change.

6. Prospective use of the Master's Thesis findings

This Master's Thesis findings can/will be applied in several areas. First, the summary collated regarding mycophenolate-drug interactions can be used in other patient populations who frequently receive the drug. Specifically, these patient populations are solid organ transplantation and several autoimmune diseases.

Secondly, the data summarizing mycophenolate-drug interactions will be incorporated into population pharmacokinetics analysis, which seeks to identify the measurable factors causing changes the dose-concentration relationship. The aim of such analyses is to validate the current population pharmacokinetic model and to conduct pharmacodynamic analysis to evaluate if MPA AUC is associated with clinical outcomes in nonmyeloablative HCT recipients. These studies will help determine if personalizing dosing of mycophenolate can optimize clinical outcomes.

Lastly, this is the first comprehensive study of mycophenolate-drug interactions, which can significantly affect patient's outcomes. Since this Thesis describes the mechanisms of the underlying interactions, it can also be an invaluable tool for pharmacists to help them better understand these pharmacokinetic drug interactions and properly consult a patient regarding the use of concomitant medications.

CONCLUSIONS

- Every patient in the study was taking at least 1 drug that may have a pharmacokinetic interaction with mycophenolate.
- The postgrafting pharmacotherapy of this patient population was complex and comprised of 1/3 of HCT medications and 2/3 of non-HCT medications.
- During the first 21 days after allogeneic graft infusion, nonmyeloablative HCT patients were taking a median of 14 medications and a median of 4 medications potentially causing pharmacokinetic interactions with mycophenolate.
- Pharmacokinetic drug interactions were identified between mycophenolate and the following HCT medications: broad-spectrum antibiotics (amoxicillin & clavulanic acid, ciprofloxacin, levofloxacin, moxifloxacin), cyclosporine, fluconazole, corticosteroids (methylprednisolone and prednisone).
- Pharmacokinetic drug interactions were identified between mycophenolate and the following non-HCT medications: amitriptyline, lorazepam, PPIs (esomeprazole, lansoprazole, pantoprazole, omeprazole) and valproate.
- Proposed drug interaction mechanisms reveal that most medications taken by the patients in the study interfere with the absorption and metabolic process of mycophenolate pharmacokinetics.
- The number of concomitant medications did not differ between day 2, day 7, and day 21 after allogeneic graft infusion. The number of potential drug interactions differed between day 2, day 7, and day 21, possibly due to an increased use of corticosteroids on day 21.
- In general, no statistically significant correlations can be found between the number of potential mycophenolate-drug interactions and the following variables: age, HCT-CI, and number of concomitant medications.
- Our data suggests clinically significant drug interactions with mycophenolate, for which alternative medications could be recommended.
- Additional drug interactions studies should be performed to evaluate mycophenolate-drug interactions in nonmyeloablative HCT after day 21.

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APPENDIX

Appendix I: List of medications taken by patients in the study

A

Acetaminophen Acetaminophen & hydrocodone Acetylsalicylic acid Acyclovir Albuterol Allopurinol Alprazolam Amitriptyline Amlodipine Amoxicillin & clavulanic acid Atenolol Atorvastatin Atovaquone Aztreonam

В

Baclofen Beclomethasone Benzonatate Bisacodyl Bivalirudin Budesonide Bupropion

С

Calcium carbonate Carvedilol Cefazolin Cefepime Ceftazidime Ceftriaxone Chlorthalidone Cholecalciferol Cidofovir Ciprofloxacin Citalopram Clobetasol Clonazepam Clotrimazole Colchicin Cosyntropin Cyclosporine

D

Dapsone Daptomycin Deferasirox Diazepam Digoxin Diltiazem Diphenhydramine Diphenoxylate & atropine Docusate Dramamine Dronabinol Duloxetine Dutasteride

E

Enalapril Enoxaparin Epinephrine Ertapenem Escitalopram Esomeprazole Estradiol Estradiol & norethindrone Ethinylestradiol Ethinylestradiol & noregistemate

F

Fentanyl & midazolam Fexofenadine Finasteride Fluconazole Fludarabine Fluticasone Fluticasone & salmeterol Folic acid Fondaparinux Furosemide
G

G-CSF Gabapentin Ganciclovir Glucagone Glyburide Granisetron Guaifenesin

Η

Halobetasol Hydralazine Hydrochlorotiazide Hydrochlorotiazide & triamteren Hydrocortisone Hydromorphone Hydroxychloroquine Hydroxyurea

I

Ibandronat Imipenem Insulin aspart Insulin glargin Insulin humalog Insulin lantus Ipratropium Isotretinoin Itraconazole

J

К

L

Lansoprazole Letrozole Levofloxacin Levothyroxin Linezolid Lisinopril Loperamide Loratadine Lorazepam Losartan Lovastatin

Μ

Magnesium sulphate Medroxyprogesterone Meperidine Meropenem Metformin Methadone Methylphenidate Methylprednisolone Metoclopramide Metoprolol Metronidazole Micafungin Mometasone Montelukast Morphin Moxifloxacin Multivitamin Mycophenolate mofetil Mycophenolic acid

N

Naloxone Nicotine Nitroglycerin Nystatin

0

Olanzapine Omeprazole Ondansetron Oseltamivir Oxazepam Oxybutinin Oxycodone Oxycontin

P

Pantoprazole Paroxetine Polyetileneglycole Penicillin Phenazopyridine Pilocarpine Posaconazole Potassium Pravastatin Prednisone Pregabalin Premarin Prochlorperazine Promethazine Pseudoephedrine Pyridoxine

Q

R

Rituximab Rosuvastatin Roxicodone

S

Salbutamol Scopolamine Senna Simethicone Simvastatin Sirolimus Spironolactone

Т

Tacrolimus Tadalafil Tamsulozin Temazepam Trimetophrim & sulfamethoxazole Testosterone Theophylline Tobramycin Tolterodine Trazodone

U

Ursodiol

V

Valaciclovir Valproate Vancomycin Varenicline Venlafaxin Vitamin B12 Voriconazole

W

Warfarin

X

Y

Z

Zaleplon Ziprasidone Zoledronat Zolpidem Zopiclone

Questions	Yes	No	Unk/
			NA
1. Are there previous credible reports of this interaction in	+1	-1	0
humans?			
2. Is the observed interaction consistent with the known	+1	-1	0
interactive properties of precipitant drug?			
3. Is the observed interaction consistent with the known	+1	-1	0
interactive properties of object drug?			
4. Is the event consistent with the known or reasonable time	+1	-1	0
course of the interactions (onset and/or offset)?			
5. Did the interaction remit upon dechallenge of the precipitant	+1	-2	0
drug with no change in the object drug? (If no dechallenge, use			
Unk or NA and skip Question 6)			
6. Did the interaction reappear when the precipitant drug was	+2	-1	0
readministered in the presence of continued use of object drug?			
7. Are there reasonable alternative causes for the event?	-1	+1	0
8. Was the object drug detected in the blood or other fluids in	+1	0	0
concentrations consistent with the proposed interaction?			
9. Was the drug interaction confirmed by any objective evidence	+1	0	0
consistent with the effects on the object drug (other than drug			
concentrations from Question 8)?			
10. Was the interaction greater when the precipitant drug dose	+1	-1	0
was increased or less when the precipitant drug dose decreased?			

Appendix II: Drug Interactions Probability Scale (DIPS) questions

Appendix III: Data collection sheet for the first 35 patients; Levofloxacin as an example of a medication taken; Number of DI (drug interactions) as the total number of potential drug interactions on the observed day.

Nr.	Levo- floxacin day 2	Levo- floxacin day 7	Levo- floxacin day 21	Nr. of DI day 2	Nr. of DI day 7	Nr. of DI day 21
1	0	1	0	3	4	3
2	nd	1	1	nd	4	5
3	0	0	1	4	4	5
4	0	0	1	3	3	4
5	1	1	0	4	5	3
6	0	1	1	4	4	3
7	0	0	1	2	2	3
8	0	1	1	2	3	3
9	1	1	0	6	5	4
10	0	0	0	4	4	4
11	0	0	1	3	3	4
12	0	0	0	3	3	2
13	0	0	W	4	4	W
14	0	1	0	2	3	3
15	1	nd	nd	3	nd	nd
16	nd	0	1	nd	3	4
17	0	0	W	2	2	W
18	1	1	1	4	4	4
19	0	0	1	3	3	4
20	1	1	1	4	4	4
21	1	1	1	4	4	4
22	1	1	1	3	3	3
23	nd	1	1	nd	2	2
24	1	1	0	5	5	5
25	0	1	1	2	3	4
26	1	0	1	4	4	6
27	1	nd	nd	6	nd	nd
28	1	nd	0	5	nd	4
29	0	nd	1	2	nd	3
30	0	1	1	3	5	5
31	0	1	0	3	4	3
32	1	1	1	5	5	5
35	0	0	0	2	3	3
54 25	0	1	1	3	4	4
35	nd	1	1	nd	4	4