DEVELOPING A LIMITED SAMPLING STRATEGY FOR CYCLOSPORINE AREA UNDER THE CURVE MONITORING IN LUNG TRANSPLANT PATIENTS

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ABSTRACT

This study developed a limited sampling strategy (LSS) to provide an estimate of cyclosporine (Neoral®) area-under-the-curve (AUC) in lung transplant recipients, a population for which a cyclosporine LSS has not yet been delineated. The predictive performance of the LSS, and other published LSS in other transplant types, was evaluated. Finally, the pharmacokinetic parameters of the lung transplant patients were calculated.

Fourteen stable lung transplant patients (n = 7 male; n = 7 female) were entered into the study. Upon administration of a steady-state morning cyclosporine dose, blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, 9, 10, and 12 hours post-dose in 12 patients (and up to 8 hours post-dose in 2 patients on a q8h regimen). Blood samples were analyzed by monoclonal fluorescence polarization immunoassay. AUC was calculated by the linear trapezoidal method, and the LSS was calculated using multiple regression analysis. Predictive performance was evaluated using methods proposed by Sheiner and Beal. Pharmacokinetic analysis was performed using WinNonlin® computer software.

Patient characteristics (mean ± SD) are as follows: age: 48 ± 12 years; weight: 69 ± 17 kg; transplant type: 6 double lung, 8 single lung; total daily cyclosporine dose: 4.3 ± 1.7 mg/kg; time post-transplant: 5.1 ± 3.4 years. Eight patients were used to determine the LSS. Analysis of all available concentration-time data revealed the following equation: $AUC = 17.24 \times C6 - 58.96 \times C8 + 23.39 \times C9 + 52.29 \times C12 - 796.07$, $r^2 = 0.999$. In order to provide a clinically feasible LSS, the remainder of the analysis was restricted to the data collected in the first 3 hours post-dose. One 4-point, four 3-point, six 2-point, and four 1-point equations were determined. On the basis of the number of samples required, the coefficient of determination, comparison of predictive performance, and the percent prediction error in AUC estimation (%pe), we selected

the following equation for analysis of predictive performance: $AUC = 1.46 \times C1 + 5.36 \times C3 + 274.49$; $r^2 = 0.975$; %pe (range) = -4.47 – 8.47%. For this LSS, mean prediction error (ME, bias) was 195 ng×hr/mL, and mean absolute error (MAE, precision) was 299 ng×hr/mL. There was no significant difference in predictive performance between the LSS for lung transplant patients and other published LSS in other transplant types, with 5 exceptions. This 2-concentration LSS for lung transplant patients was significantly more biased than a 3-concentration LSS developed for renal transplant patients, and was significantly less biased and significantly more precise than 2 other LSS that were developed for renal transplant patients.

The best clinically feasible LSS for cyclosporine AUC estimation requires 2 concentrations drawn at 1 and 3 hours post-dose.

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LIST OF ABBREVIATIONS

AUC	area under the concentration versus time curve
$AUC_{0-\tau}$	area under the concentration versus time curve for one dosing interval
C	concentration
CI	confidence interval
C_{max}	maximum concentration observed during the dosing interval
CSA	cyclosporine A
CV	coefficient of variation
EMIT	enzyme multiplied immunotechnique
F	bioavailability
FPIA	fluorescence polarization immunoassay
HPLC	high performance liquid chromatography
LSS	limited sampling strategy
MAE	mean absolute error, or precision
MDAE	mean difference in absolute error
MDPE	mean difference in prediction error
ME	mean prediction error, or bias
MRT	mean residence time
M RIA	monoclonal radioimmunoassay
NEO	Neoral®
NT	nephrotoxicity
pe	prediction error
%pe	percent prediction error
P RIA	polyclonal radioimmunoassay
PO	oral
RIA	radioimmunoassay
RJ	rejection
SD	standard deviation
SIM	Sandimmune®
$t_{1/2}$	terminal elimination half-life
T_{max}	time of the maximum concentration observed during the dosing interval
TLM	trough level monitoring
Vd	volume of distribution
VHHSC	Vancouver Hospital and Health Sciences Centre
WB	whole blood

λz terminal elimination rate constant

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DEDICATION

This work is dedicated to those who were always there throughout the years to help me get where I've gotten: my family. To my parents, John and Joanne, who gave instilled in me the drive to succeed, as well as the large amount of support (read: dollars) required to get me there. To my brothers, Aaron and Carey, with whom I've shared many battles. To my love Allison, who is the sun in my sky, and my biggest supporter. And finally to my two boys, Bishop and Merlin. I will always strive to be the kind of person that both of you think I am.

CHAPTER 1

INTRODUCTION

A Chinese myth, circa 300 B.C.:

One day, two men, Lu and Chao, called on the surgeon, Pien Ch'iao. He gave them a toxic drink, and they were unconscious for three days. Pien Ch'iao operated and opened their stomachs and explored the heart; after removing and interchanging their organs, he gave a wonderful drug and the two men went home recovered.

(From Bergan 1997)

Transplantation

1.1 The History Of Transplantation

Transplantation, which, simply defined, is the transfer of tissue from one person or animal to another, or from one part of the body to another, began many centuries ago. Cave paintings are the earliest depiction of transplantation (Bergan 1997). Having surpassed many historical and ethical developments during its evolution, modern organ transplantation now offers recipients improved quality of life, often prolonged life, and for transplant physicians, a viable therapeutic option for the treatment of end-stage organ disease.

The field of transplantation is many-faceted, and it is evident in the literature that while individual institutions often follow consensus guidelines, each institution has its own preferences. For example, assay methods, immunosuppressive protocols, and target immunosuppressive concentrations are not the same at all institutions.

Transplantation can be described by the genetic relationship between the donor and recipient, the site of grafting, and the identity of the tissue being transplanted (Bergan 1997). There are 4 possible types of genetic relationships between donor and recipient: 1) autograft, which is where the donor and the recipient are the same individual; 2) isograft, also called syngeneic graft, which is when the donor and recipient are identically or nearly identically related (i.e., monozygotic twins or in highly inbred animals); 3) allograft, also referred to as homograft, which is when the donor and recipient are genetically unrelated individuals of the same species; and 4) xenograft, also referred to as heterograft, which is when the donor and recipient are individuals of different species (Bergan 1997).

When describing transplantation according to the site of grafting, orthotopic is when the donor tissue is surrounded by native tissue of the same type or it is transplanted in the

anatomically correct position. All other sites of grafting are heterotopic, which is when the tissue has been relocated in the recipient (Bergan 1997). Currently, tissues, whole organs, or partial organs can be transplanted. Bone, heart valves, cartilage, veins, corneas, heart, liver, lung, pancreas, and sections of the gastrointestinal tract comprise the tissues and organs that can be transplanted (Bergan 1997).

The oldest record of transplant-related surgery comes from skulls dated back to the Bronze Age. Trephination, an archaic surgical technique used to relieve intracranial pressure, involved the removal of a circular disc of bone from the calvarium. The disc of bone was later replaced as an orthotopic autograft (Bergan 1997).

Myths and legends from history provide written accounts of other examples of transplantation. While likely fictitious, the myth of Saints Cosmas and Damian from 6th century Rome is often mentioned. The legend describes a man with cancer in his leg. He spends the night in the church of the two saints. During the night, the saints examine his leg. One removes the diseased limb while the other goes to the local cemetery, exhumes the body of a recently deceased Ethiopian man, amputates and gathers the replacement limb, and returns to the church. The limb is attached, and the man awakens the next morning fully cured (Bergan 1997). A painting of the legend shows that the wrong leg was grafted to the man (Muller-Ruchholtz 1999).

There are other examples of historical transplantation from records originating in different countries. Nasal reconstruction, involving grafting of skin from the cheek of a patient, was performed in India around 700 B.C. (Bergan 1997). Nasal reconstruction was performed in 15th century Italy as well. This technique involved a skin autograft from the arm of the patient. The Italian Method, as it is now referred to, is still used today (Bergan 1997).

By the 19th century, grafts of skin, tendons, nerves, cartilage, adipose tissue, corneas, adrenal and thyroid glands, ovaries, partial digestive and urinary tracts, and muscle were documented, although the grafts were more successful in animals than in humans (Bergan 1997). By 1880, stable corneal transplants in humans were documented.

Once successful surgical techniques were established, the era of modern transplantation began. The first renal transplant was performed in 1936 in Russia by Dr. Voronoy (Bergan 1997). Unfortunately, the recipient died 2 days later. In 1947, a successful, but transient renal transplant was performed in the United States. The kidney of a dying patient was transplanted into a pregnant woman with a uterine infection. The transplanted kidney helped the woman recover from her infection, and was removed after a few days before rejection could occur. In 1950, another patient received a kidney from another patient who was not a relative but was the same age and blood type. The graft was lost after 11 months (Bergan 1997).

During World War II, it was discovered that skin grafts performed for burn patients were successful only when the donor was an identical twin. This information was applied to renal transplantation when the first renal transplant in which the donor and recipient were identical twins was performed in Boston in 1954 (Bergan 1997, Muller-Ruchholtz 1999). There was no rejection, but the patient died of cardiac complications 8 years after receiving the transplant. Another renal transplant of this type was performed in 1956, and the recipient lived into the 1990's (Bergan 1997).

1.2 Brief Overview Of Rejection

The cascade of events collectively known as rejection is a biphasic process involving host ("self") recognition of foreign antigens ("non-self") followed by a cascade of biochemical events

ultimately resulting in damage to the transplanted tissue or organ. Host recognition depends on cells that are capable of recognizing antigens expressed on the transplanted organ as foreign. These antigen presenting cells, which include macrophages, dendritic reticulum cells, endothelial cells, and some parenchymal cells, are capable of triggering a specific and coordinated host immune response that, if unmodified, results in damage to the graft, and if severe and prolonged, graft loss (Philip and Gerson 1998). The major components of the immune system involved in the immune response are T cells and macrophages, although all components are involved to some degree (Philip and Gerson 1998).

T cell sensitization involves the presentation of antigens bound to human lymphocyte antigen (commonly referred to as HLA) cell surface molecules by antigen presenting cells (Philip and Gerson 1998). Once sensitized, T cells become activated and undergo a series of changes resulting in proliferation, activation of various effector functions, and immunologic memory (Philip and Gerson 1998). Helper T cells are stimulated first. These cells secrete lymphokines that cause the proliferation of cytotoxic T cells and B cells, which secrete antibodies. More than 70 molecules are involved in the biochemical cascade resulting in activation and proliferation of the cells of the immune system (Philip and Gerson 1998). The most important of these is interleukin-2, which has regulatory functions over the whole process. Because of this, interleukin-2 is often the target of attempts to attenuate the immune response (Philip and Gerson 1998).

Graft rejection is classified as hyperacute, acute, or chronic. Hyperacute rejection is caused by pre-existing antibodies that rapidly bind to vascular endothelium in the donor organ, activate complement, and cause rapid thrombosis of vessels (Trulock 1997). When present, such antibodies are usually the result of prior exposure to alloantigens through blood transfusion,

pregnancy, or previous transplantation. Hyperacute rejection has virtually been eliminated by prescreening the recipient's serum for antibodies against a standard panel of cells (referred to as panel reactive antibodies), but it remains a major barrier to xenotransplantation (Trulock 1997).

Acute rejection is primarily caused by T cells, but it is not known if a humoral response plays a role. In lung transplant patients, perivascular mononuclear infiltrates, with or without an accompanying lymphocytic bronchitis or bronchiolitis, are the hallmark of acute rejection (Trulock 1997). Acute rejection is rarely fatal. It is usually reversible by intravenous therapy with a corticosteroid, and does not result in significant permanent damage to the graft.

Chronic rejection is an important obstacle to long-term survival of transplant patients. The defining characteristic of acute rejection is fibrous obliteration of endothelialized or epithelialized luminal structures (Trulock 1997). The exact mechanism is not known, but research has suggested both alloantigen-dependent and alloantigen-independent mechanisms are important (Trulock 1997).

The lung is particularly prone to rejection, for several reasons. The lung is one of the largest transplantable organs, and it has an extensive vasculature that is perfused by the entire cardiac output and circulating immune system (Burke *et al* 1987). Also, the lung has a vast intrinsic immune apparatus, and the respiratory tract is constantly exposed to extrinsic infectious agents and other inhaled antigens that can cause inflammatory reactions, upregulation of alloantigen expression on bronchial epithelium, and activation of T cells (Burke *et al* 1987).

1.3 Primary Immunosuppression

Early attempts at transplantation met with little success due to rejection of foreign tissue. Before advances in the field of immunology, it was thought that rejection was due to

malnutrition of the grafted tissue (Muller-Ruchholtz 1999). Peter B. Medawar, who was awarded the Nobel Prize for his pioneering work, discovered that graft rejection is immunologic in nature (Muller-Ruchholtz 1999).

Following World War II, researchers in the transplantation area began to look for methods that would eliminate hostile immune reactions. Initially, it was believed that over time, a donor organ would completely integrate itself into the host. Thus, it was only necessary to control rejection for a short time following surgery (Bergan 1997). It became quickly evident that immunosuppression was required throughout the life of the recipient.

The success of modern transplantation is due largely to immunosuppressive therapy. Immunosuppressive strategies can be divided into 4 classes: 1) chemical (also referred to as pharmaceutical; e.g., corticosteroids, azathiprine, mizoribine, cyclosporine A (CSA), tacrolimus, gusperimus, mycophenolate mofetil, and sirolimus, formerly known as rapamycin); 2) biological (also referred to as immunological; e.g., anti-lymphocyte globulin or ALG, anti-thymocyte globulin or ATG, monoclonal antibody against surface molecules of T cells, and blood transfusions); 3) physical (also referred to as radiological; e.g., local or total body irradiation, total lymphoid tissue irradiation); 4) surgical (e.g., thymectomy, splenectomy, lymphapheresis, plasmapheresis) (Oka and Yoshimura 1996).

Tissue typing, a process which attempts to match the antigens of donor and recipient as closely as possible, helps to minimize the immune response without suppressing the recipient's immune system. Histocompatibility includes ABO blood matching and the identification of both donor and recipient antigen profiles (Bergan 1997).

Total body irradiation, which was introduced in 1959, was the first attempt at inducing immunosuppression in the recipient. It was originally employed to suppress rejection in renal

transplant patients (Bergan 1997, Muller-Ruchholtz 1999). While effective in reducing the lymphocyte population, risk/benefit analysis revealed that the often severe side effects (most notably a tremendous susceptibility to infection and disease) were not worth the immunosuppression achieved (Bergan 1997, Muller-Ruchholtz 1999). It became clear that in order to improve postoperative prognosis in graft recipients, immunosuppressive drugs that were less toxic than radiation would need to be developed.

1.3.1 Corticosteroids

Cortisone, an endogenous corticosteroid produced by the adrenal gland, was discovered in 1936. By 1964, it was documented that prednisone, a more potent synthetic derivative of cortisone, was effective in reversing rejection in renal allograft recipients (Bergan 1997). It was eventually determined that corticosteroids cross cell membranes, form complexes with specific binding proteins, and are then transported across the nuclear membrane to sites near genes involved in transcription and control of cytokines (Philip and Gerson 1998). The corticosteroid-binding protein complex inhibits gene transcription. The end result in T cells is that the interleukin-2 receptors are not expressed, thus preventing sensitization, activation, and proliferation of T cells (Philip and Gerson 1998). Many leukocytes are affected by corticosteroids. They not only reduce the number of monocytes and macrophages but also impair their functions, such as responses to lymphokines, phagocytosis, and interleukin-1 secretion (Kaplan *et al*, 1983). Besides the previously mentioned effects on T cell interleukin-2 receptor expression, corticosteroids can also inhibit killer T cell activity by blocking the production of interleukin-2 by helper T cells (Oka and Yoshimura 1996). The anti-inflammatory action of corticosteroids is well known. Their potent anti-inflammatory action is due to

inhibition of prostaglandin synthesis by inhibition of cycloxygenase activity (Oka and Yoshimura 1996). Although B cells are resistant to corticosteroids, they seem to indirectly inhibit antibody production due to their effects on macrophages and helper T cells (Oka and Yoshimura 1996). Corticosteroids have other effects, including suppression of febrile responses, release of macrophage-mediated cytokines, and inhibition of neutrophil chemoattractants and inflammatory mediators involved in cell-mediated cytotoxicity in tissues (Philip and Gerson 1998).

The most desirable effect of corticosteroids as immunosuppressive agents is their immediate and selective ability to destroy cells that affect the immune system (Bergan 1997). When compared with other immunosuppressive agents, corticosteroids have a much broader spectrum of activity. However, their immunosuppressive effects are generally less potent, and their efficacy decreases or becomes non-existent with chronic usage (Oka and Yoshimura 1996). In addition, chronic use can permanently alter normal immune function and potentially cause a myriad of toxicities including: acne, hair growth, hypothalamus-pituitary-adrenal axis suppression resulting in cessation of production of endogenous corticosteroids, which could have serious deleterious consequences in times of stress (e.g., surgery or mechanical injury), psychiatric disturbances, and elevation of blood sugar due to the glucocorticoid activity of corticosteroids. More serious toxicities include iatrogenic Cushing's syndrome (which is the redistribution of body fat resulting in characteristic central obesity and a "buffalo hump"), hypertension, gastrointestinal tract toxicity, cataracts, and potential thinning and weakening of bone which can lead to hip fractures and aseptic necrosis (Bergan 1997, Philip and Gerson 1998).

1.3.2 Azathioprine

In 1959, it was discovered that 6-mercaptopurine was able to lessen the immune response in rabbits challenged with a foreign protein antigen (Bergan 1997, Philip and Gerson 1998). Azathioprine, a purine analog of 6-mercaptopurine, was found to have greater immunosuppressive potency than 6-mercaptopurine. RNA synthesis and function are altered when azathioprine incorporates into cellular DNA, resulting in inhibition of purine synthesis and metabolism (Philip and Gerson 1998). In large doses, it inhibits B cell function and therefore, the humoral (antibody) response of the immune system (Oka and Yoshimura 1996). Azathioprine was first used clinically for immunosuppression in 1963.

The primary toxicity of azathioprine is in the bone marrow. Azathioprine causes bone marrow suppression, which results in leukocytopenia, thrombocytopenia, and anemia (Philip and Gerson 1998, Oka and Yoshimura 1996). Azathioprine is also suspected to play at least a partial role in causing lymphoma in chemically immunosuppressed transplant recipients (Philip and Gerson 1998). Azathioprine is also noted for its gastrointestinal toxicity, which manifests as nausea, vomiting, and pancreatitis. Hepatotoxicity is also encountered (Philip and Gerson 1998, Oka and Yoshimura 1996). Transient alopecia and compromised renal function are seen. Finally, skin lesions and uterine and cervical dysplasia may also manifest (Bergan 1997).

1.3.3 Cyclophosphamide

The combination of corticosteroids and cyclophosphamide was initially found to be more effective than either drug alone, and the combination was used in transplantation research prior to 1964 (Philip and Gerson 1998). Because azathioprine was deemed less toxic than cyclophosphamide, azathioprine and corticosteroids became the standard immunosuppressive

therapy until the early 1980's when CSA was introduced into clinical practice. Cyclophosphamide, like azathioprine, is an antineoplastic drug, one of whose side effects (immunosuppression) is useful for another indication (transplantation in this case). The use of cyclophospamide in transplantation medicine is not widely recognized or known, as it was traditionally used as a second-line agent to replace azathioprine when azathioprine was withdrawn due to hepatotoxicity (Philip and Gerson 1998).

1.3.4 Mizoribine

Mizoribine was developed in Japan in the late 1970's as a potential replacement for azathioprine. It has seen only limited use in Japan, and neglible use outside of Japan. Mizoribine is an imidazole nucleotide prodrug with antimicrobial activity, requiring phosphorylation to become active (Philip and Gerson 1998, Oka and Yoshimura 1996). Nucleic acid synthesis and cell proliferation are inhibited by mizoribine *via* disruption of the pathway from the xanthine-5′-nucleotide to the guanosine-5′-nucleotide in purine biosynthesis, resulting in guanine depletion (Philip and Gerson 1998, Oka and Yoshimura 1996). The disruption occurs because of inhibition of the enzyme inosine monophosphate dehydrogenase. Mizoribine is an effective immunosuppressant because of a key difference between immune cells and other cells in the body. Most cells use guanine generated through the salvage pathway for synthesis of DNA. Immune cells lack the enzymes active in the salvage pathway, and are dependent on the *de novo* synthesis of guanine.

The advantage of mizoribine over azathioprine is that mizoribine is less toxic to bone marrow and liver. However, mizoribine has less immunosuppressive potency than azathioprine (Oka and Yoshimura 1996). Mizoribine toxicity is primarily gastrointestinal tract-related. This

toxicity is especially evident in dogs where it manifests as hemorrhagic enteritis and erosive mucosal lesions (Morris 1993). There is some controversy with this immunosuppressant: studies that have shown mizoribine to be less bone marrow- and hepatotoxic than azathioprine were not controlled. Also, the advantages of mizoribine over standard immunosuppressants have not been clearly established in clinical studies (Morris 1993).

1.3.5 Tacrolimus

Tacrolimus, also commonly referred to as FK 506, was approved for clinical use in 1994 in the United States. It is a macrolide lactone metabolite produced by *Streptomyces tsukubaensis* (Philip and Gerson 1998, Oka and Yoshimura 1996). Although structurally distinct from CSA, the mechanism of action of tacrolimus is virtually identical to the mechanism of action of CSA, which will be discussed in a subsequent section. Briefly, tacrolimus ultimately affects helper T cells and inhibits cytotoxic T cells. The difference in the mechanism of action is that tacrolimus binds to FK binding protein, whereas CSA binds to cyclophilin. Tacrolimus is more potent than CSA in inhibiting T cell proliferation, B cell activation, and the production of other cytokines such as interleukin-3, interleukin-4, interferon-γ, and granulocyte colony stimulating factor. The toxicity of tacrolimus is virtually identical to the toxicity of CSA, with differing incidences of various toxicities between the two. (The toxicity of CSA will be described in a subsequent section). As with CSA, nephrotoxicity is a frequently encountered adverse effect. Interestingly, it was originally hoped that tacrolimus would be less nephrotoxic than CSA, especially since they are structurally unrelated. However, tacrolimus is now regarded to be as nephrotoxic as CSA. In a study comparing CSA and tacrolimus as the primary immunosuppressant, recipients

treated with tacrolimus had fewer rejection episodes, but tacrolimus was found to be significantly more nephrotoxic (Morris 1993).

1.3.6 Gusperimus

Gusperimus, also referred to as 15-deoxyspergualin, is a metabolite produced by *Bacillus laterosporrus*, which has antineoplastic and antimicrobial activity. It is thought that gusperimus acts on macrophages, resulting in inhibition of oxidative metabolism, lysosomal enzyme synthesis, interleukin-1 production, and cell surface expression of major histocompatibility complex II antigens (Philip and Gerson 1998, Oka and Yoshimura 1996). Gusperimus also prevents antibody production by acting on B cells. Some data available show that gusperimus is effective as rescue therapy in rejection episodes (Suzuki *et al* 1990). Minor gastrointestinal disturbance has been reported, as well as bone marrow suppression. However, the myelotoxic effects are cytostatic in nature, and thus, bone marrow toxicity is rapidly reversible following discontinuation of therapy (Philip and Gerson 1998, Oka and Yoshimura 1996). Gusperimus is currently not available for clinical use in Canada.

1.3.7 Mycophenolate Mofetil

RS-61443, or mycophenolate mofetil as it is now most commonly referred to, was originally isolated from *Penicillium glaucum* (Philip and Gerson 1998). Mycophenolate mofetil is a morpholinoethyl ester of mycophenolic acid. Mycophenolic acid is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (Hale *et al* 1998). Thus, it has properties in common with the much less widely used immunosuppressive agent, mizoribine. After oral administration, mycophenolate mofetil is rapidly and extensively

absorbed and is presystemically hydrolyzed to mycophenolic acid. The conversion to mycophenolic acid is so rapid that mycophenolate mofetil is not detected following oral administration. Intravenous administration is required to gather concentration-time data for the prodrug. Because of its enhanced effectiveness and decreased toxicity when compared to azathioprine, mycophenolate mofetil is now beginning to replace azathioprine as the secondary immunosuppressant in polytherapy. The lung transplant program at Vancouver Hospital and Health Sciences Centre (VHHSC; Vancouver, BC, Canada) is currently in the process of converting lung transplant recipients over to mycophenolate mofetil therapy. Patients at this center are now maintained on CSA or tacrolimus (only if CSA is not tolerated), mycophenolate mofetil, and prednisone. Mycophenolate mofetil is usually well tolerated, and is notable in its lack of renal, hepatic, and bone marrow toxicity. The most frequently encountered adverse effects are gastrointestinal tract-related, manifesting as nausea and diarrhea. While these symptoms usually improve with dose reduction, they can be severe enough to necessitate discontinuation of therapy (Hale *et al* 1998).

1.3.8 Sirolimus

Sirolimus, formerly known as rapamycin, is a macrolide product of *Streptomyces hygroscopicus*, and because of its macrolide nature, is structurally similar to tacrolimus. Like other immunosuppressive agents, it was originally discovered while screening for antimicrobial compounds. Sirolimus was reported in 1975 as having potent activity against *Candida albicans* (Martel *et al* 1975). Sirolimus affects T and B cells directly by preventing cytokines from activating them (Morris 1993). It inhibits binding of interleukin-2 to its receptor without affecting the production of interleukin-2 (Dumont *et al* 1990). Another noteworthy property is

that tacrolimus and CSA only slightly inhibit interleukin-2 and interleukin-4 induced growth of T cells, while sirolimus is very effective (Dumont *et al* 1990). Like tacrolimus, sirolimus binds to FK binding protein, but unlike tacrolimus, this sirolimus-FK binding protein complex does not result in calcineurin inhibition (Liu *et al* 1991). Experience with sirolimus in humans is limited, but in canine animal models, sirolimus causes weight loss, testicular atrophy, lethargy, and in rat models, sirolimus is diabetogenic (Morris 1993). Sirolimus is currently not available for clinical use in Canada, although it is undergoing clinical trials in North America.

1.3.9 Brequinar

Originally developed as an antineoplastic, brequinar was found to have immunosuppressive properties. It inhibits the action of dihydro-orotate dehydrogenase, an enzyme involved in the *de novo* biosynthetic pathway of pyrimidines involved in the synthesis of DNA and RNA (Allison *et al* 1993, Morris 1993). Inhibition of uridine synthesis also blocks the salvage pathway in lymphocytes (Allison *et al* 1993, Morris 1993). Brequinar does not, however, affect the synthesis of interleukin-2. In experimental models of transplantation, adverse effects of brequinar include thrombocytopenia, desquamative maculopapular dermatitis, mucositis, and gastrointestinal toxicity (Philip and Gerson 1998). These adverse effects appear to be dose-dependent and reversible upon discontinuation of therapy, and are consistently seen in all species that have been administered the drug. Preliminary data also show that there is a greater degree of toxicity with more frequent dosing (Makowka *et al* 1993). Brequinar is not currently available for clinical use in Canada.

1.3.10 Leflunomide

A77-1726, the active metabolite of leflunomide, was developed specifically as an immunosuppressant, but it was found to cause severe gastrointestinal disturbances. The prodrug, leflunomide, helps to ameliorate the gastrointestinal disturbances, as it is converted to A77-1726 following absorption (Philip and Gerson 1998). Leflunomide suppresses the immune system by inhibiting tyrosine kinase-associated growth factors (Morris 1993). Leflunomide also blocks the production and action of interleukin-2 as well as the mitogenic activity of stimulated T cells (Morris 1993).

Experience with leflunomide in clinical transplantation is limited. However, data are available regarding its use in rheumatoid arthritis patients in Europe (Morris 1993). It appears to be well tolerated. More importantly, unlike other immunosuppressive drugs, it does not cause myelotoxicity or nephrotoxicity (Morris 1993).

Lung Transplantation

1.1 The History Of Lung Transplantation

The first human lung transplant was performed in 1963 by Dr. James Hardy at the University of Mississippi (Blumenstock *et al* 1993, Grover *et al* 1997, Hardy *et al* 1963). The recipient died on the 18th postoperative day from renal failure (Grover *et al* 1997). Between 1963 and 1974, 36 lung transplants were performed worldwide, but only 2 recipients lived longer than 1 month (Veith *et al* 1974). Because of this, the area of lung transplantation was largely inactive until CSA was introduced clinically in the early 1980's (Trulock 1997). In 1981, heart-lung transplantation was utilized to treat pulmonary vascular disease (Reitz *et al* 1982). Pulmonary fibrosis was managed by single lung transplantation beginning in 1983 (Toronto

Lung Transplant Group 1986), and in 1986, double lung transplantation for obstructive lung disease was started (Cooper *et al* 1989).

Dr. Frank Veith made many contributions to the development of lung transplantation beginning in 1969 and extending through 1983 (Grover *et al* 1997). During that time, he developed techniques for pulmonary artery and pulmonary venous anastomoses, as well as a telescoping anastomosis (Grover *et al* 1997). In 1979, Dr. Veith made the extremely important discovery regarding the effect of donor bronchial length on healing of the bronchial anastomosis (Grover *et al* 1997). He noted that the shorter the bronchus, the better the healing. In 1983, Dr. Veith reported improved experimental results with lung transplantation using CSA as the primary immunosuppressant (Grover *et al* 1997).

Dr. Joel Cooper, from the University of Toronto, performed Toronto's first lung transplant in 1978. Unfortunately, the recipient survived only for 17 days, but he was considered high risk prior to the transplant (Nelems *et al* 1980). In 1981, Dr. Cooper and colleagues discovered that corticosteroids used in the immunosuppressive regimen decreased bronchial healing, while azathioprine and CSA did not (Lima *et al* 1981). In 1983, Dr. Cooper's group reported that immunosuppressive agents increased bronchial disruption and necrosis (Goldberg *et al* 1983). Also in 1983, he reported on studies using the omental wrap in an experimental model with improved bronchial healing and neovascularization (Morgan *et al* 1983).

1.2 Indications For Lung Transplantation

Current indications for single lung transplantation include restrictive lung disease, emphysema, pulmonary hypertension, and other nonseptic, end-stage pulmonary disease including restrictive lung disease secondary to connective tissue disorders (Grover *et al* 1997,

Higenbottam *et al* 1990, Trulock 1997). Indications for bilateral sequential lung transplantation include cystic fibrosis and patients in whom there is chronic infection with end-stage pulmonary failure, including patients with bronchiectasis (Grover *et al* 1997, Higenbottam *et al* 1990, Trulock 1997). Bilateral sequential lung transplantation is preferred for patients with primary and secondary pulmonary hypertension if it is possible to do so, as that type of transplant appears to decrease reperfusion edema (Grover *et al* 1997). Emphysema patients with lung volumes so great that it would be very difficult to find a large enough single lung may also be selected for bilateral sequential lung transplantation (Grover *et al* 1997). The most common reasons for lung transplantation are chronic obstructive pulmonary disease, α_1-antitrypsin deficiency emphysema, idiopathic pulmonary fibrosis, cystic fibrosis, primary pulmonary hypertension, and Eisenmenger's syndrome (Hosenpud *et al* 1996). Other less common reasons include bronchiectasis, sarcoidosis, lymphangioleiomyomatosis, and eosinophilic granuloma of the lung (Trulock 1997).

There is some debate as to how to manage patients with primary pulmonary hypertension. Single, bilateral sequential, and heart-lung transplantation are all therapeutic options for this patient population. The advantages of single lung transplantation for these patients are that it conserves donor organs, is an easier procedure, is very effective in decreasing pulmonary vascular resistance which improves right ventricular ejection fraction, and provides good symptomatic relief (Mal *et al* 1989). The disadvantages are that reperfusion pulmonary edema is frequently a major problem and can be a cause of mortality, 85% of pulmonary blood flow goes to the transplanted lung which means that dysfunction in the transplanted lung is not tolerated, and rejection is more serious because of the mismatch in pulmonary blood flow (Mal *et al* 1989). With this procedure, the operative mortality is 26%, and the actuarial survival at 1 year is 66%

(Hosenpud *et al* 1994). There are those clinicians who believe that because primary pulmonary hypertension patients are often young, they will benefit more in the long term by receiving 2 lungs. An advantage of bilateral sequential lung transplantation is that it offers even distribution of pulmonary blood flow to both lungs, which results in less reperfusion edema and easier perioperative management. Because of the balanced flow, acute or chronic rejection is better tolerated. In addition, similar to single lung transplantation, pulmonary vascular resistance is reduced and right ventricular ejection fraction is increased. An advantage of bilateral sequential lung transplantation over heart-lung transplantation is that the heart is available for another recipient (Grover *et al* 1997). The disadvantages are that the procedure is more challenging than single lung transplantation, requires longer cardiopulmonary bypass time, and uses 2 lungs per patient, which is a concern given the dramatic shortage of donor lungs (Grover *et al* 1997). The operative mortality is 10% and the 1-year actuarial survival is 77% (Hosenpud *et al* 1994).

1.3 Recipient Criteria

Ideally the potential recipient has disease confined to the lungs with no other major organ dysfunction or disease (Grover *et al* 1997). In most circumstances, the patient should be under 65 years of age for a single lung transplant and under 60 for a bilateral sequential lung transplant (Grover *et al* 1997, Trulock 1997). In addition, there should be no history of alcohol or recreational drug dependency, and the recipient should be psychologically stable (Grover *et al* 1997).

Absolute contraindications for lung transplantation include multisystem disease other than lung, history of carcinoma or sarcoma with a possibility of recurrence, current infection, significant renal or hepatic dysfunction, cigarette smoking within 3 to 4 months, drug or alcohol

abuse, psychiatric instability, and poor medical compliance. Bronchiectasis and chronic or recurrent pulmonary infection are contraindications for single lung transplantation, and presence of these conditions necessitates bilateral sequential lung transplantation (Grover *et al* 1997). Relative contraindications include insulin-dependent diabetes mellitus, age above the recommended guidelines, the presence of significant coronary artery disease and/or left ventricular dysfunction, long-term ventilation support, previous thoracic surgery, use of corticosteroids greater than either 20 mg per day or 0.2 to 0.3 mg/kg/day, hemodynamic instability, extreme cachexia, morbid obesity, advanced connective tissue disease associated with other organ dysfunction, and previous lung transplantation (Grover *et al* 1997, Massard *et al* 1993, Schafers *et al* 1992).

Lung transplantation is appropriate when other therapeutic options have been exhausted and when the patient's prognosis will be improved by the procedure. The risks of aggressive medical treatment (e.g., a vasodilator for primary pulmonary hypertension or a cytotoxic drug for idiopathic pulmonary fibrosis) are generally considered to be less than those of transplantation (Trulock 1997). Medical treatment may delay the need for transplantation or serve as a bridge through the long waiting period. Quality of life is the prime motivation for transplantation for many patients, but prognosis should be the main determinant of timing (Trulock 1997).

The median waiting time for lung transplantation was 550 days for patients who entered the waiting list in 1994 (Trulock 1997). Thus, a realistic waiting time of 1 to 2 years should be incorporated into the transplantation strategy. For patients with chronic obstructive pulmonary disease and α_1-antitrypsin deficiency, lung transplantation should be undertaken when post-bronchodilator forced expiratory volume in 1 second is less than 25% predicted, there is resting hypoxia (partial pressure of oxygen less than 55 to 60 mm Hg), hypercapnia, secondary

pulmonary hypertension, and a clinical course characterized by rapid rate of decline of forced expiratory volume in 1 second or life threatening exacerbations are present (Trulock 1997). When post-bronchodilator forced expiratory volume in 1 second is less than 30% predicted, there is resting hypoxia (partial pressure of oxygen less than 55 mm Hg), hypercapnia, and a clinical course with increasing frequency and severity of exacerbations are present, lung transplantation should be undertaken for cystic fibrosis patients (Trulock 1997). For patients with idiopathic pulmonary fibrosis, lung transplantation should be undertaken when vital capacity is less than 60 to 65% predicted, and when there is resting hypoxia, secondary pulmonary hypertension, and clinical, radiographic, or physiologic progression while on medical therapy (Trulock 1997). Finally, if the patient has a New York Heart Association functional class III or IV rating, mean right atrial pressure greater than 10 mm Hg, mean pulmonary arterial pressure greater than 50 mm Hg, and a cardiac index less than 2.5 L/min/m^2, lung transplantation should be undertaken in patients with primary pulmonary hypertension (Trulock 1997).

1.4 Donor Criteria

During the mid-1960's, surgeons were performing few transplants, and there was not much of a demand for donor organs. In the case of renal transplants, most donors were living relatives of the recipients (Bergan 1997). Because instances occurred that resulted in donor abuse (e.g., psychological abuse from relatives of a potential donor who refused to donate) and death, donation was not as complication-free as it is today, and because the demand for donated organs was slowly growing, alternatives were needed (Bergan 1997).

Also, in the late 1960's, physical death was defined as the moment the heart stopped beating. Because there was no concept of brain death at that time, it was unethical for organs to

be harvested for transplantation from a patient who was brain dead but whose heart was still beating. This changed when a Boston physician, Dr. Moore, transplanted a liver from a brain dead police officer killed in the line of duty into a patient who desperately needed a liver transplant (Bergan 1997). The concept of brain death was formally defined by Harvard Medical School, and this increased the donor pool of organs and tissues for transplantation. It also allowed donated organs to be harvested from a healthy blood supply, allowing them to be preserved in optimum condition (Bergan 1997).

Potential lung donors should have a partial pressure of oxygen greater than 300 mm Hg with ventilator settings of 100% fraction of inspired oxygen, a positive end-expiratory pressure of 5 cm H_2O, and a tidal volume of 12 mL/kg/min (Grover *et al* 1997, Trulock 1997). The chest X-ray should be clear, the sputum stain should be negative for fungus and preferably negative for gram-negative rods, there should be no purulent secretions, the age of the donor should be less than 60 years, HIV and hepatitis B and C antigens should be negative, and the donor should be on a ventilator for less than 1 week (Grover *et al* 1997, Trulock 1997). The donor should have no history of lung disease including asthma, no history of cancer except for non-melanomatous skin cancer, and no history of "high risk" behavior. The circumference of the nipple line should be within 10 to 13 cm of the recipient's. Also, there should be reasonable matching of height, weight, and vertical and horizontal lung dimensions, a less than 30-pack year smoking history, and no gross purulence from lobar or segmental orifices on bronchoscopy (Grover *et al* 1997, Trulock 1997).

1.5 Current Challenges In Lung Transplantation

Some notable problems need to be overcome before lung transplantation will offer consistent and long-term relief of symptoms and improved quality and duration of life. The most important of these problems is chronic rejection, as manifested by obliterative bronchiolitis. Although there is no way to prevent the bronchiolitis obliterans syndrome, the consensus is that aggressive prophylaxis of cytomegalovirus infection, along with switching CSA to tacrolimus in patients with repeated episodes of rejection, is important (Grover *et al* 1997). Early diagnosis of rejection is also very important. If either acute or chronic rejection is suspected, fiberoptic bronchoscopy and bronchoalveolar lavage with routine, fungal, and viral cultures and transbronchial biopsy should be undertaken (Grover *et al* 1997).

Another less common but potentially fatal problem in lung transplantation is post-transplant lymphoproliferative disorder. This occurs in approximately 5% of lung transplant recipients, and is initially treated by decreasing the doses of the immunosuppressive drugs. Chemotherapy is added if this intervention is unsuccessful (Randhawa *et al* 1989).

A controversial problem is whether or not to retransplant patients who develop either early graft failure or chronic rejection as manifested by bronchiolitis obliterans. There is a marked difference in survival between first time lung transplants and reoperative lung transplants, with a 1-year survival of approximately 40% in retransplants versus greater than 70% in first time recipients. For some as yet unknown reason, the figure for retransplants had risen to slightly greater than 50% by 1995 (Novick *et al* 1994, 1995). This problem offers an ethical dilemma as well: is it ethically acceptable to deplete the already limited supply of donor lungs even further by using them for retransplantation, knowing that the survival is less than that for first time recipients (Grover *et al* 1997)?

Another challenge that continues to be present in lung transplantation is donor shortage. In 1993, there was a 2:1 demand:supply ratio for lung transplants (Grover *et al* 1997). Since that time, the number of donors has remained relatively constant, but the demand for donor lungs has increased (Trulock 1997). Because of this, many patients die while on the waiting list.

1.6 Recent Advances In Lung Transplantation

A study published in 1994 (Cohen *et al* 1994) shows that it is possible to use living related donors for cystic fibrosis patients. Seven recipients received bilateral lobes from 14 living related donors. There were no donor deaths or complications except for prolonged air leaks in 3 of the donors. These donors had also donated the middle lobe of their lung, in addition to the lower lobe. Normally, only the lower lobe of the lung is harvested for this type of procedure (Cohen *et al* 1994). There were no deaths in the recipients with cystic fibrosis, although there has been some mortality in infant and pediatric recipients who received lobes for other reasons (Starnes *et al* 1994).

Living related transplantation can offer earlier transplantation for patients who are at risk for dying while waiting for a transplant. Because donors are related, there is the potential for better organ compatibility. In addition, the donor supply is enlarged, and it is a continuation of a tradition of living related organ donation that has been long established for renal transplantation and used occasionally for liver transplantation (Grover *et al* 1997).

Current lung preservation techniques are capable of preserving harvested lungs for up to 8 hours of cold ischemia. Ongoing investigations into supplementary preservation measures, including the use of antioxidants, leukocyte depletion, nitric oxide, and cytokine manipulation, may extend this time past 8 hours (Grover *et al* 1997).

1.7 The Future Of Lung Transplantation

Concurrent transplantation of bone marrow along with the primary solid organ is currently a major area of investigation. It has been shown that in recipients of cadaveric kidneys, livers, hearts, and lungs who are given donor bone marrow at the time of their transplant, and who are given tacrolimus and prednisone for immunosuppresion, there is a trend towards donor specific non-reactivity and increasing survival (Pham *et al* 1995).

The area of xenotransplantation has been a topic of much research as well. One of the most well known examples of xenotransplantation occurred in the early 1980's. Dr. Leonard Bailey transplanted a baboon's heart into a critically ill infant termed Baby Fae (Bergan 1997). Baby Fae survived for only 20 days, but much research was performed during this short time (Bergan 1997). Hyperacute rejection and transmission of infection are major problems with xenografts of this type. It was discovered that grafts from non-human primates stimulate a greater immune response than grafts from other mammals. This stimulated research into the use of pigs as potential donors. These animals are large, plentiful, and have a short gestation period.

Finally, it is my opinion that the field of transplantation may also see the use of donor-specific organs grown *in vitro* if ethical concerns are worked out. This would eliminate the need for immunosuppressive drugs and their associated toxicities, as well as the long waits for donor organs.

Cyclosporine

1.1 The History Of Cyclosporine

The journey from discovery to market began with a soil sample collected from the Hardanger Vidda in Norway (Borel 1983, Borel *et al* 1989, Stahelin 1986). The soil contained

fungi that were of particular interest to drug discovery scientists. The cyclosporines were originally discovered in the early 1970's by scientists at Sandoz Ltd. in Basel, Switzerland (Borel 1983, Borel *et al* 1989). They were performing routine screening for compounds with antifungal activity (Borel *et al* 1989). The crude extracts of two strains of fungi, *Cylindrocarpon lucidum* Booth and *Tolypocladium inflatum* Gams, possessed antifungal activity. However, this antifungal activity was not potent enough to warrant further development (Borel 1983, Borel *et al* 1989). It was subsequently observed that the antifungal activity was coupled with an unusually low toxicity in mice. Because of this, the metabolite mixture was put through a limited pharmacological screening program (Borel *et al* 1989). In early 1972, J. F. Borel and also, H. Stahelin observed that the metabolite mixture appeared to have immunosuppressive properties in murine animal models (Borel *et al* 1989). CSA was purified in 1973, and it was observed that CSA exerted unique immunosuppressive effects while having no bone marrow toxicity (Borel *et al* 1976, Borel *et al* 1977). CSA was first studied clinically in 1978, and was approved for use in organ transplantation first in Switzerland and then the United States in 1983 (Borel *et al* 1989). Table 1 summarizes the development of CSA.

Table 1. The development of cyclsporine. (Adapted from Borel 1983 and Borel *et al* 1989.)

Year	Development
1970	Isolation of 2 new strains of fungi which produce antifungal metabolites by B. Thiele. Isolation of a metabolite mixture and characterization as novel neutral polypeptides by Z. L. Kis and colleagues.
1971	Isolation of the partially purified 2-component metabolite mixture (24-556) on a preparative scale for initial biological screening by Haerri and Ruegger.
1972	Discovery of the immunosuppressive properties of metabolite 24-556 in mice by J. F. Borel.
1973	Purification of CSA (27-400) by Ruegger. Culture and production of CSA by Dreyfus and colleagues.
1974	Animal studies of the immunosuppressive activity of CSA, *in vitro* and *in vivo* by J. F. Borel.
1975	Elucidation of the structure of CSA via X-ray studies and chemical degradation by Petcher and colleagues and Ruegger and colleagues.
1976	Toxicity studies in rats and monkeys demonstrate the selectivity of CSA for lymphocytes and a lack of effect on hematopoiesis. World-wide confirmation of specific immunosuppressive effect of CSA in experimental transplantations and other models.
1978	The first clinical trials using CSA by Calne and colleagues and Powles and colleagues.
1980	Total synthesis of CSA by Wenger.
1981	Publication of the antischistosomal and anti-malarial activity of CSA by Bueding and colleagues and Thommen.
1983	Sandimmune® first approved for clinical use in organ transplantation in Switzerland, and then the United States and some other countries later in the year.
1985	The first controlled clinical trials with CSA in autoimmune diseases.

1.2 Cyclosporine Physical-Chemical Properties

CSA ($C_{62}H_{111}N_{11}O_{12}$, Figure 1), also known as cyclosporine A, cyclosporin A, antibiotic S 7481F1, and CsA, is normally encountered as a white powder, occasionally with a faint yellow cast (Cyclosporine AHFS monograph 1998, Sigma Product Information Sheet 2000). It has a melting point range of 148 to 151 °C, a molecular weight of 1202.6, and an optical rotation of -244° (Sigma Product Information Sheet 2000). In its natural powder form, CSA is expected to

be stable at 2 to 8 °C for at least 2 years if stored sealed in the dark (Sigma Product Information Sheet 2000).

CSA has a solubility of 10 mg/mL in methylene chloride, 6 mg/mL in chloroform, 10 mg/mL in ethanol, and 50 mg/mL in DMSO (Sigma Product Information Sheet 2000). Solutions are clear and colorless to faint yellow. CSA is often reported to be slightly soluble in water and saturated hydrocarbons. It is recommended that stock solutions in ethanol or DMSO should be stored at -20°C (Sigma Product Information Sheet 2000).

Since CSA is poorly water soluble, but is often administered intravenously, admixtures must be thoroughly mixed to ensure that CSA is properly dissolved. Solutions in intravenous fluids must be shaken vigorously to assure proper dispersion. CSA is stable if solutions are protected from light, but its concentration may drop due to adsorption to container walls (Sigma Product Information Sheet 2000). If diluted in glucose 5% or glucose/amino acid solutions and stored at room temperature in the dark, CSA is stable for 72 hours (Sigma Product Information Sheet 2000). However, when diluted in sodium chloride 0.9%, CSA is stable for only 8 hours under similar storage conditions (Sigma Product Information Sheet 2000).

CSA is a hydrophobic cyclic peptide composed of 11 amino acid residues, all having the S-configuration except for the D-alanine in position 8, which has the R-configuration, and sarcosine in position 3 (Wenger 1983). Ten of the amino acids are known amino acids: α-aminobutyric acid in position 2, sarcosine in position 3, N-methylleucine in positions 4, 6, 9, and 10, valine in position 5, alanine in position 7, D-alanine in position 8, and N-methylvaline in position 11 (Wenger 1983). At the time of synthesis, the amino acid in position 1, a novel β-hydroxy, unsaturated, 9 carbon amino acid {(4R)-4-[(E)-2-butenyl]-4,N-dimethyl-L-threonine); MeBmt} (Kahan 1989) had not been previously isolated or known in free form (Wenger 1983).

1.3 Pharmacology And Therapeutic Use Of Cyclosporine

CSA has antifungal activity, but it is of no use clinically because it does not have action against human pathogens except for coccidioidomycosis (Borel *et al* 1989). CSA also has antischistosomal and antimalarial activity (Borel 1983). In addition, CSA has been used to mitigate multidrug resistance in hematological cancer patients, due to CSA's inhibition of P-glycoprotein activity (Wu *et al* 1995). However, CSA is primarily used therapeutically for its potent immunosuppressive activity. While it has been used to treat autoimmune diseases (Fathman and Myers 1992) and for the prevention and treatment of graft-versus-host-disease in bone marrow transplant patients (Pai *et al* 1994), CSA is mainly used as the cornerstone of immunosuppressive polytherapy in solid organ transplant patients (Trulock 1997, Tsunoda and Aweeka 1996). As mentioned previously, the lung transplant program at VHHSC typically uses triple immunosuppressive therapy in lung transplant patients: CSA or tacrolimus (only if CSA is not tolerated or contraindications are present), mycophenolate or azathioprine (although patients are being converted to mycophenolate), and prednisone. A standard protocol for CSA dosing and desired CSA trough levels is utilized (Table 2).

Table 2. The cyclosporine dosing protocol at Vancouver Hospital and Health Sciences Centre

Pre-operative: cyclosporine (Neoral®) 3 mg/kg	
Post-operative: cyclosporine (Neoral®) 9 mg/kg/day given in two divided doses	
Time Post-Transplant (months)	Target Cyclosporine Trough Level (μg/L)
< 1	350 to 400
1 to 3	300 to 350
3 to 6	250 to 300
6 to 12	200 to 250
> 12	150 to 200
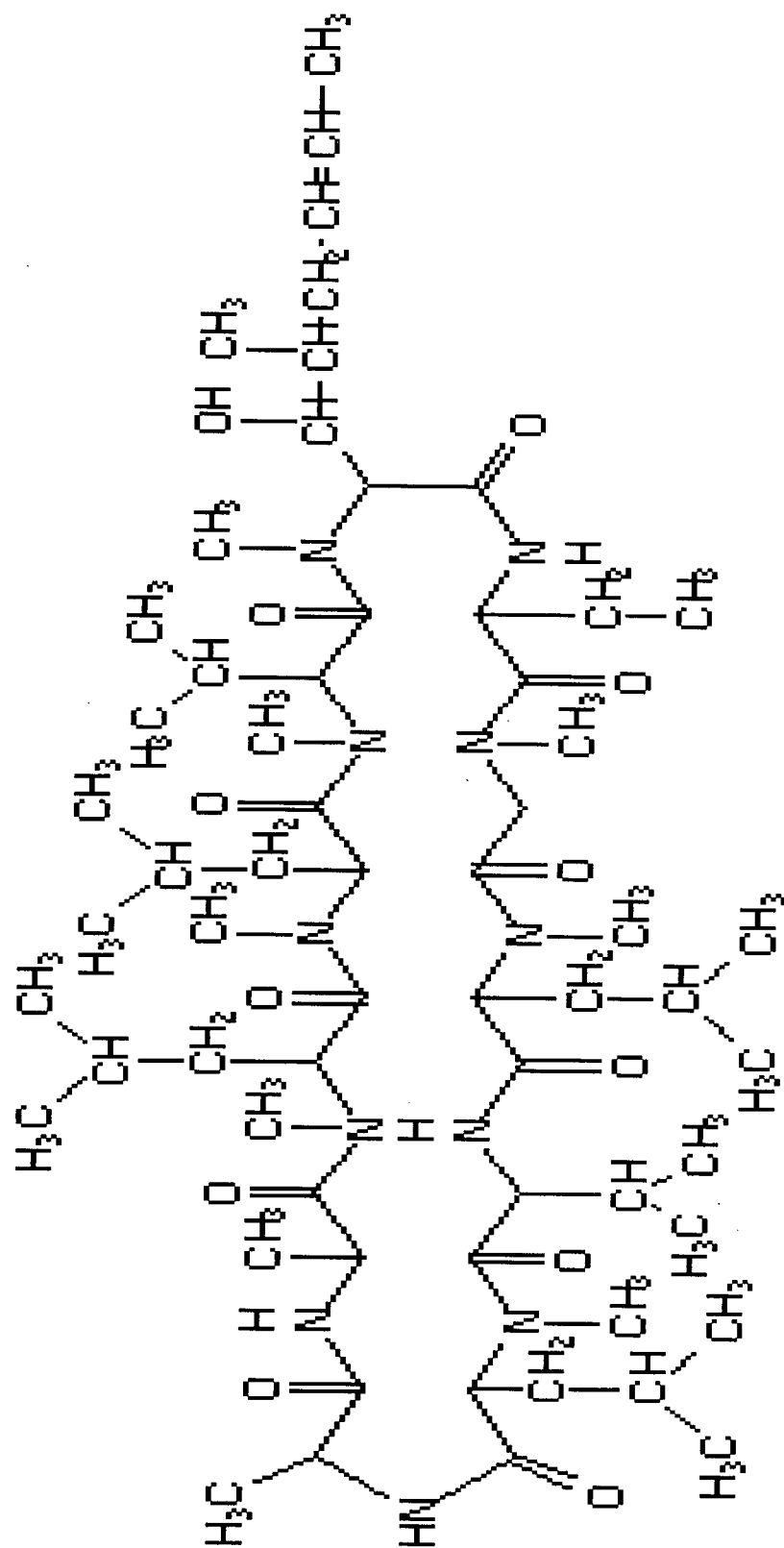
Figure 1: The chemical structure of cyclosporine A ($C_{62}H_{111}O_{12}$, molecular weight 1202.6). Cyclosporine A is a large, neutral, hydrophobic cyclic peptide composed of 11 amino acid residues. (Adapted from the Sigma Product Information Sheet for C3662: Cyclosporin A. http//www.sigma.sial.com/sigma/proddata/c3662.htm).

The immunosuppressive activity of CSA (Figure 2) appears to be mediated by intracellular, rather than cell membrane, receptors (Freeman 1991). CSA binds to several cytosolic proteins including calmodulin, cyclophilin, and peptidyl-prolyl *cis-trans* isomerase, an enzyme involved in the refolding of intracellular proteins, which is thought to be the same protein as cyclophilin (Freeman 1991, Halloran and Madrenas 1991, Zenke *et al* 1993). It is thought that the immunosuppressive activity of CSA is due to its binding to cyclophilin, which results in the formation of a complex and inhibition of *cis-trans* isomerase activity (Freeman 1991, Halloran and Madrenas 1991, Zenke *et al* 1993). This complex acts on calcineurin, a serine-threonine phosphatase that requires calcium binding for activity (Pai *et al* 1994). Under normal cellular conditions, calcineurin dephosphorylates the cytoplasmic subunit of the transcription factor NF-AT, allowing it to translocate to the nucleus where it is involved in transcriptional activation of lymphokine genes (Fruman *et al* 1992). Expression of these genes results in production of lymphokines, of which interleukin-2 is the most important in T cell activation. *In vitro* studies have shown that inhibition of calcineurin activity by CSA results in inhibition of the T cell signal transduction cascade and, therefore, activation of T cells (Clipstone and Crabtree 1993, Fruman *et al* 1992). At typical therapeutic dosages, CSA inhibits calcineurin activity by about 50% (Batiuk *et al* 1995a, 1995b, Cantarovich *et al* 1998, Quien *et al* 1997), which is likely one of the reasons why transplant recipients have sufficient immune system activity to fight infection and experience periods of rejection (Batiuk *et al* 1995b).

1.4 Considerations For Cyclosporine Use In Lung Transplantation

CSA immunosuppression has helped to make lung transplantation a viable therapeutic option for the management of patients with end-stage lung disease (Higenbottam *et al* 1990,

Trull *et al* 1999). Compared to other types of transplant recipients, higher target CSA concentrations are used in lung transplant patients due to the severe consequences of graft failure. In spite of this, most patients still experience at least 1 acute rejection episode in the first 3 months post-transplant (Trull *et al* 1999). Acute rejection (both the incidence and severity) appears to be the most important risk factor for chronic graft failure (Trull *et al* 1999, Best *et al* 1996). Also, continued exposure to high concentrations of cyclosporine could result in a higher incidence of adverse effects such as nephrotoxicity, hepatotoxicity, hypertension, and severe infection (Trull *et al* 1999). Lung transplant patients may eventually require dialysis and subsequent kidney transplantation due to CSA nephrotoxicity.

Differences in pharmacokinetics between Neoral® (NEO) and Sandimmune® (SIM), due to differences in formulation between the 2 products (which will be described in a later section), have implications in lung transplantation. Because CSA absorption with NEO is enhanced, it is possible that patients are exposed to greater amounts of CSA with NEO than with SIM when the same trough levels are maintained (Friman and Backman 1996). This could have implications in terms of incidence of adverse effects, although studies have shown that the two formulations are tolerated equally well (Kesten *et al* 1998a, Trull *et al* 1999, Zaldonis *et al* 1998). Increased absorption with NEO may also reduce CSA dosage requirements in lung transplant patients with cystic fibrosis, who typically have high SIM dosage requirements due to erratic absorption characteristics, poorer bioavailability, and delayed absorption (Trull *et al* 1999). However, one study (Trull *et al* 1999) has shown that there were no significant differences in doses of NEO and SIM required to achieve similar concentrations in lung transplant patients with cystic fibrosis. Reduced inter- and intra- patient variability with NEO may also help to reduce acute rejection

and possibly graft failure due to obliterative bronchiolitis since variability in trough levels with SIM has been identified as a significant risk factor for acute rejection (Best *et al* 1992).

1.5 Formulations Of Cyclosporine Used In Lung Transplantation

CSA is available in both parenteral and oral dosage forms. Cyclosporine concentrate for injection appears as a clear, faintly brownish-yellow solution. This formulation is a sterile solution of CSA in polyoxyl 35 castor oil (Cremophor® EL,polyethoxylated castor oil) with 32.9% alcohol (AHFS Drug Information 1999). At the time of manufacture, the air in the ampules is replaced with nitrogen (AHFS Drug Information 1999). The purpose of this is to help prevent oxidation of the formulation ingredients.

Oral dosage forms of the original SIM formulation of CSA consist of an oral solution and conventional liquid-filled, soft gelatin capsules. SIM oral solution has a clear, yellow, oily appearance. The oral solution contains the drug in an olive oil and peglicol 5 oleate (Labrafil® M 1944CS) vehicle with 12.5% alcohol (AHFS Drug Information 1999). SIM capsules were available in 25, 50, and 100 mg strengths. The SIM formulation of CSA is no longer available in Canada.

CSA is currently commercially available as a non-aqueous liquid formulation of the drug (NEO) that immediately forms an emulsion in aqueous fluids. This is done via mized micelles, thus forming an oil-in-water type of emulsion. The formulation is available as an oral solution for emulsion and as oral 25 and 100 mg liquid-filled soft gelatin capsules containing the oral solution for emulsion (AHFS Drug Information 1999). When exposed to an aqueous environment such as that found in the digestive tract, the oral solution for emulsion forms a homogenous transparent emulsion with a droplet size smaller than 100 nm in diameter (AHFS
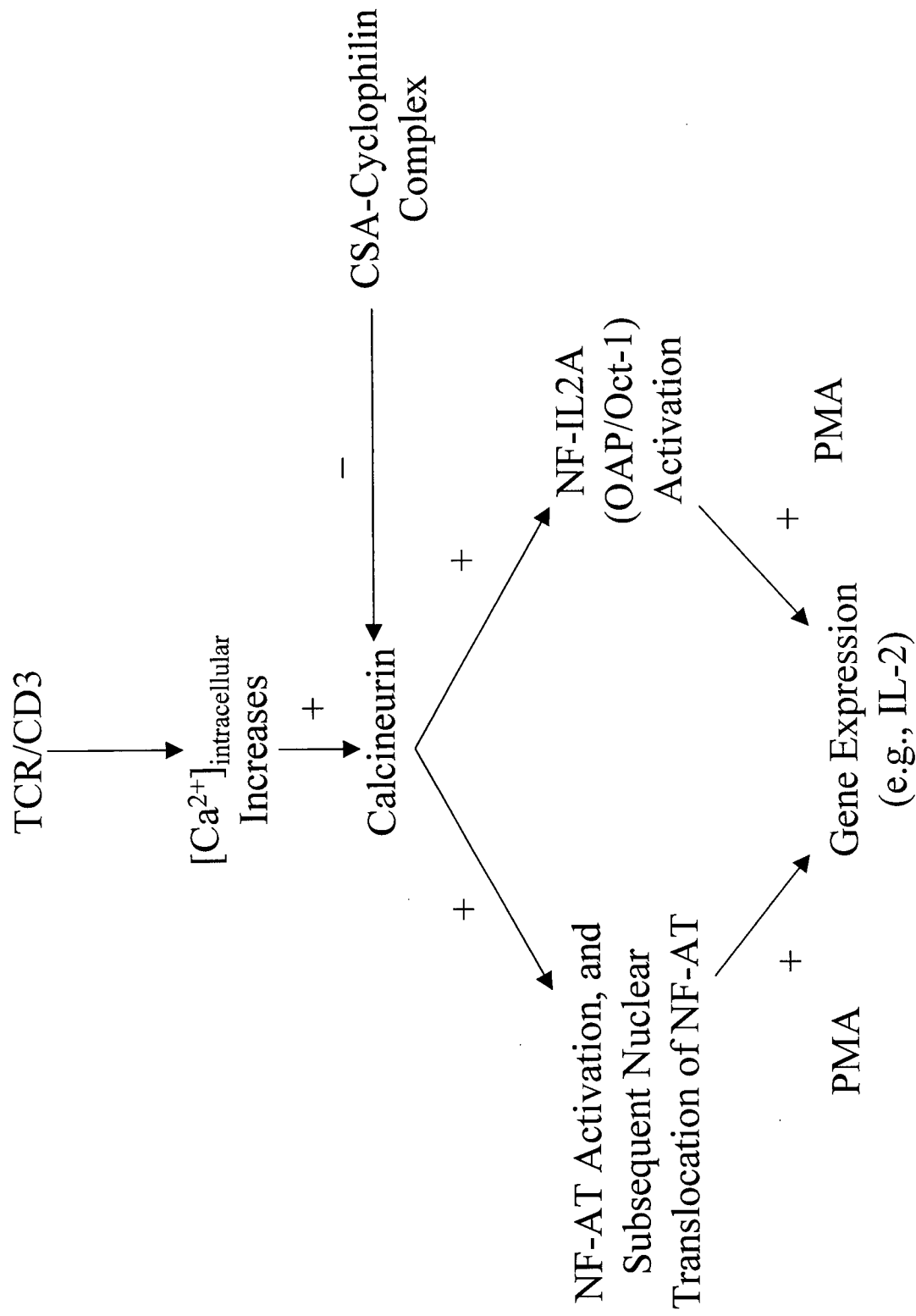
Figure 2: The mechanism of action of cyclosporine A. (Adapted from *Liu et al* 1991).

Drug Information 1999). Because of this, NEO is often referred to as a microemulsion formulation of CSA. In this formulation, the molecular structure of CSA is unmodified, and aqueous dilution results in formation of an emulsion without reprecipitation of the drug (AHFS Drug Information 1999). In NEO, CSA is dispersed in a mixture of propylene glycol, which functions as the hydrophilic solvent, and corn oil monoglycerides, diglycerides, and triglycerides, which function as lipophilic solvents (AHFS Drug Information 1999). When dispersed, polyoxyl 40 hydrogenated castor oil functions as a surfactant, and d,l-α-tocopherol functions as an antioxidant (AHFS Drug Information 1999). The oral solution for emulsion, both as the solution and in the capsules, also contains dehydrated alcohol in a maximum concentration of 9.5% (AHFS Drug Information 1999). NEO and SIM are not bioequivalent due to formulation and pharmacokinetic differences. Thus, they cannot be used interchangeably, although this is no longer an issue in Canada where, as previously mentioned, SIM is no longer available.

1.6 Pharmacokinetics Of Cyclosporine

(This section refers to the SIM formulation of CSA unless otherwise noted).

1.6.1 Absorption

Following oral administration, CSA (SIM) is incompletely and erratically absorbed (Freeman 1991, Friman and Backman 1996, Lemaire *et al* 1990, McMillan 1989, Yee and Saloman 1992). Mean bioavailability (F) is about 30% (Freeman 1991, Friman and Backman 1996, Lemaire *et al* 1990, McMillan 1989, Yee and Saloman 1992) with ranges of 8 to 60% (Lemaire *et al* 1990) and less than 5% to 90% being reported (Yee and Saloman 1992), depending on the reference consulted. In addition, bioavailability can increase with increased

time post-transplant in renal transplant patients (Lemaire *et al* 1990, Yee and Saloman 1992). The reasons for this are unknown. It is possible that the surgery disrupts homeostasis or some normal physiological parameter responsible for CSA absorption. If CSA is absorbed across the gastrointestinal tract membrane by transporters, it is possible that the number of transporters increases post-transplant. There may be an endogenous compound that increases absorption of CSA, similar to the situation involving vitamin D and calcium absorption. Of course, this is purely speculation. Factors which can affect absorption of CSA include availability of bile, gastrointestinal status, and coadministration with food.

The effect of bile on absorption of CSA was discovered from observations in liver transplant patients. In the immediate post-transplant period, absorption of CSA is poor due to gastrointestinal disturbances and disruption of the biliary system (van Mourik 1998). NEO, due to its microemulsion formulation, does not require solubilization by bile for absorption, and liver transplant patients have been successfully treated orally in the immediate post-transplant period (Tredger 1995).

The effect of food on CSA absorption is controversial, with food being reported to both increase and decrease CSA absorption (Friman and Backman 1996). Administration with food or food and bile acid tablets resulted in two peaks being observed in the concentration versus time curves obtained in a study published in 1990 (Lindholm *et al* 1990). CSA given under fasting conditions did not produce two peaks, suggesting that enterohepatic circulation occurs under non-fasting conditions (Lindholm *et al* 1990). In healthy volunteers, administration of SIM with a fat-rich meal nearly doubled time to the maximum concentration encountered during the dosing interval (T_{max}) and resulted in a 37% increase in area under the concentration versus time curve (AUC) (Mueller *et al* 1994). This was associated with significant elevations in CSA

concentrations when compared to CSA concentrations obtained under fasting conditions. The influence of a fat-rich meal on the pharmacokinetics of NEO was less pronounced. The maximum concentration observed during the dosing interval (C_{max}) decreased by 26%, there was no significant change in T_{max}, and AUC decreased by 15% (Mueller *et al* 1994).

Gastrointestinal status can also alter CSA absorption. Chemoradiation-induced enteritis, acute graft-versus-host-disease involving the intestine, diarrhea, and metoclopramide therapy have all been shown to decrease CSA absorption (Freeman 1991, McMillan 1989, Yee and Saloman 1992).

In renal transplant patients, conversion from SIM to NEO resulted in increases in C_{max} and AUC in one study (Kovarik *et al* 1994) and significant increases in C_{max}, AUC, and average steady-state concentrations in another (Sketris *et al* 1994). An additional study in renal transplant patients (Browne *et al* 1994) found significantly higher C_{max} values and significantly shorter T_{max} values in the same patients given SIM and then NEO.

One study published in 1998 involving lung transplant patients (Kesten *et al* 1998a) evaluated changes in pharmacokinetic parameters following conversion from SIM to NEO. A significant increase in C_{max} and AUC was found after conversion to NEO.

1.6.2 Distribution

CSA has a large apparent volume of distribution (Vd) of 4-6 L/kg in kidney and bone marrow transplant patients (Yee and Saloman 1992). A range of 1.8-13.8 L/kg has also been reported (Lemaire *et al* 1990), indicating that CSA is highly tissue bound. In addition, CSA can be measured in tissues for at least two weeks after CSA therapy is discontinued (Yee and

Saloman 1992). CSA is able to partition into many body tissues due to its lipophilic nature and the wide distribution of its binding protein, cyclophilin, throughout the body (Freeman 1991).

In vitro, 40 to 50% of CSA distributes into the erythrocyte fraction, 10 to 20% distributes into the leukocyte fraction, and 30 to 40% distributes into the plasma fraction (Christians and Sewing 1993, Lemaire *et al* 1990, Yee and Saloman 1992). CSA binding to erythrocytes is saturable (Yee and Saloman 1992). In addition, erythrocyte binding is both temperature and hematocrit dependent, such that increases in both result in an increase in CSA recovered in the cellular fraction (Yee and Saloman 1992). In plasma, CSA is highly bound to lipoproteins, which account for 10 to 15% of all plasma proteins (Yee and Saloman 1992). Forty-six percent of CSA is bound to high density lipoproteins, 31% to low density lipoproteins, 16% to very low density lipoproteins, and 8% to proteins, primarily albumin (Christians and Sewing 1993). It has been further confirmed in a more recent study that the majority of CSA distributes into HDL and LDL, with lesser amounts distributing into VLDL and proteins (Wasan *et al* 1997). Protein binding is concentration-independent between the concentration range of 25 to 500 ng/mL *in vitro* and temperature-dependent, such that CSA is 90 to 95% protein bound at 20 °C and 70% bound at 4 °C (Yee and Saloman 1992). Fraction unbound in plasma of 1-1.5% (Christians and Sewing 1993) and 1-17% (depending on the method of measurement) (Yee and Saloman 1992) have been reported.

1.6.3 Metabolism

CSA is extensively metabolized by the liver and in the gastrointestinal tract by cytochrome P450 3A4. Because this isoenzyme is responsible for the metabolism of many other drugs used therapeutically, there is a potential for clinically significant drug interactions

(Michalets 1998). It appears that following metabolism, the cyclic peptide structure remains intact, with most of the metabolites being hydroxylated, N-demethylated, or both (Lemaire *et al* 1990, Yee and Saloman 1992). A carboxylated metabolite and a conjugated (sulfate) metabolite have also been identified (Freeman 1991). Cyclization at amino acid 1 is another mechanism of metabolite production (Christians *et al* 1995, 1988). More than 30 metabolites have been identified from human bile, feces, blood, and urine (Christians *et al* 1995, Liu *et al* 1995). The primary metabolites are M1, M17, and M21 (Freeman 1991). More than 50% of the concentrations of M1, M9, M19, M69, M49, and M4N69 are associated with the cellular components of blood, while M4N9, M1c, M1c9, M14N, and M1A are found mainly in plasma (Christians and Sewing 1993). One of the reasons for using whole blood as the sampling fluid in routine clinical monitoring is because CSA metabolites detectable in blood preferentially distribute into blood cells. Thus, higher metabolite concentrations can be found in whole blood rather than plasma samples (Christians and Sewing 1993). CSA metabolites, like the parent compound, are widely distributed in tissue. Distribution into blood cells is also temperature- and hematocrit-dependent. It appears that partitioning into blood cells is facilitated by hydroxylation, while N-demethylation, carboxylation, and cyclization at amino acid 1 decrease cellular affinity (Christians and Sewing 1993). The clinical significance of CSA metabolites (i.e., their immunosuppressive and toxic activities) remains unclear (Christians *et al* 1995, Copeland *et al* 1990, Yee and Saloman 1992).

CSA is a low to intermediate extraction ratio drug (Freeman 1991, Yee and Saloman 1992). Thus, upon exposure to drug metabolizing enzymes, CSA is not extensively metabolized. Since there is not a large first pass effect, more CSA is systemically available. Different apparent clearance values have been reported for different transplant types. Apparent clearances

of 0.34 to 0.71 L/hr/kg have been reported for kidney transplant patients, 0.33 to 0.56 L/hr/kg for liver transplant patients, 0.34 to 0.79 L/hr/kg for bone marrow transplant patients, and 0.24 to 0.31 L/hr/kg for heart transplant patients (Yee and Saloman 1992). In addition, apparent clearance is lower in elderly patients and patients with decreased levels of serum triglyceride and cholesterol, as well as in patients with hepatic impairment (Kahan 1985). It is recommended that dosing intervals should be increased in the presence of elevated serum levels of bilirubin or alanine aminotransferase, but not aspartate aminotransferase, lactate dehydrogenase, or alkaline phosphatase (Yee et al 1984).

1.6.4 Excretion

Less than 1% of an administered CSA dose is excreted in the urine unchanged. In addition, very little CSA, in the form of metabolites, is excreted in the urine (Yee and Saloman 1992). The metabolites M1, M1c, and M9 are found in the urine, often in higher concentrations than the parent drug (Yee and Saloman 1992).

The major route of CSA excretion is via the biliary route (Freeman 1991, Yee and Saloman 1992). However, very little parent drug is found in the bile. Nearly all of the compounds detectable in the bile are metabolites, at concentrations much higher than those found in blood (Yee and Saloman 1992). It has been found that hepatic impairment reduces metabolite elimination (Kahan 1989).

The terminal elimination half-life ($t_{1/2}$) of CSA is considered long, although there is considerable variability in reported values. An elimination half-life of 6.2 to 23.9 hours has been reported (Freeman 1991).

1.7 Cyclosporine Toxicity

The majority of studies involving CSA and its associated toxicities were performed using the SIM formulation. Studies published 1995 and later, in the majority of cases, were performed with the NEO formulation.

Clinical use of CSA demonstrated the potent immunosuppressive activity of the compound. Unfortunately, it was also discovered that CSA has a narrow therapeutic index with several potential toxicities associated with its use. While nephrotoxicity is the primary adverse effect, other cardiovascular, nervous system, dermatologic, hepatic, gastrointestinal, infectious (due to bacterial, fungal, and viral pathogens), hematologic, lymphatic, and hypersensitivity effects can occur (Cyclosporine AHFS monograph 1998, Kahan 1989, Mihatsch *et al* 1998, Shihab 1996).

1.7.1 Nephrotoxicity

One of the most well known and commonly encountered toxicities of CSA is nephrotoxicity. It is also the most widely studied toxicity. Nephrotoxicity complicates the management of transplant patients. In renal transplant patients, it is difficult to differentiate between nephrotoxicity and acute rejection, and diagnosis of one or the other often involves considerable guesswork and expense. In heart, lung, and heart-lung transplant patients, who require higher CSA levels to prevent rejection, dialysis and subsequent renal transplantation are not uncommon.

In both experimental models and human clinical studies, it has been well established that CSA produces dose-dependent, acute and reversible vasoconstriction of renal arterioles (Olyaei *et al* 1999, Shihab 1996). CSA-induced acute renal failure may occur as early as a few weeks or

months following initiation of therapy (Flechner *et al* 1983). Prolonged cold ischemic time, advanced donor age, hypotension, perioperative surgical complications, and donor history of acute renal failure may increase the incidence and appearance of acute renal impairment (Olyaei *et al* 1999). The clinical signs of renal arteriolar vasoconstriction or acute renal dysfunction include reduction in glomerular filtration rate, hypertension, hyperkalemia, tubular acidosis, increased reabsorption of sodium, and oliguria (Remuzzi 1995). The adverse effects on renal hemodynamics caused by CSA are thought to be directly related to blood concentrations (Olyaei *et al* 1999).

Despite much research in the area, the exact mechanism and the mediators involved in the alteration of renal hemodynamics by CSA are not known. Increased production of endothelin and thromboxane A_2, decreased production of renal vasodilatory prostaglandins, inhibition of nitric oxide, as well as activation of the sympathetic nervous system have all been proposed as causes of renal arteriolar vasoconstriction (Auch-Schwelk *et al* 1994, Bobadilla *et al* 1994, Bunchman and Brookshire 1991, Lanese and Conger 1993, Morgan *et al* 1991, Scherrer *et al* 1990).

In solid organ transplant patients treated with CSA, chronic progressive nephrotoxicity is the major long-term toxic effect (Olyaei *et al* 1999). The effect of hemodynamic changes encountered in acute CSA nephrotoxicity may not be the only cause of progressive CSA nephrotoxicity. Studies of biopsies from experimental models, patients with autoimmune diseases and extrarenal solid organ transplants have shown distinct and specific pathological and morphological changes characteristic of CSA-associated chronic progressive nephropathy (Bertani *et al* 1987, Dische *et al* 1988, Nussenblatt and Palestine 1986, Palestine *et al* 1986). Histologically, there is destruction of arterial walls, myointimal necrosis, and progressive

narrowing of the arterial lumen (Olyaei *et al* 1999). There is also tubulointerstitial fibrosis in a striped pattern, beginning in the medulla and extending to the medullary rays of the cortex (Olyaei *et al* 1999). It is interesting to note that unlike CSA-induced acute renal impairment, chronic progressive nephropathy is not dose-dependent (Messana *et al* 1995). In most cases, chronic progressive nephropathy is associated with mild to moderate renal dysfunction.

Although it is difficult to distinguish and separate chronic rejection and chronic progressive nephropathy induced by CSA in renal transplant patients, the morphological changes associated with rejection involve mostly large blood vessels, while chronic progressive nephropathy affects mainly arterioles (Mihatsch *et* al 1995). It is also conceivable that a given patient could have both conditions present simultaneously. Renal function, as estimated by serum creatinine, is the most widely used surrogate marker of the presence or absence of chronic CSA nephropathy.

1.7.2 Cardiovascular Toxicity

An increase in arterial blood pressure is frequently observed in CSA-treated patients (Bennett and Porter 1988). This increase leads to hypertension, which in turn can result in initiation of antihypertensive therapy. The mechanism of this increase in blood pressure is not currently known. It has been shown that angiotensin-converting enzyme inhibitors have little effect in reducing blood pressure (Curtis *et al* 1988), suggesting that the renin-angiotensin system is not involved. Defects in endothelium-dependent relaxation and in response of vascular smooth muscle to vasodilators have been associated with CSA-induced hypertension in experimental models (Roullet *et al* 1994).

1.7.3 Neurotoxicity

Even though CSA is not believed to cross the intact blood-brain barrier, neurologic side effects occur in approximately 20 percent of kidney (Kahan *et al* 1987) and liver (de Groen *et al* 1987) transplant patients, resulting in syndromes of tremor, burning palmar and plantar paresthesias, headache, flushing, depression, confusion, and somnolence. CSA neurotoxicity is also an important cause of morbidity and mortality in bone marrow transplant patients (Shah 1999). Seizures of new onset may be triggered by hypocholesterolemia, hypertension, intravenous methylprednisolone therapy, hypomagnesemia, infection, hemorrhage, or cerebral infarction (Kahan 1989). Visual disorders, paresis, disorientation, and coma improve when CSA treatment is discontinued, but reoccur when CSA treatment is initiated again (de Groen *et al* 1987). As is the case with the other toxicities, the mechanism of neurotoxicity is not definitively known. On computerized axial tomography, the presence of neurotoxicity is associated with white matter hypodensity, which suggests increased water content in the brain (Kahan 1989). It has also been proposed that CSA has a direct cytotoxic effect on brain capillary endothelial cells, and the inhibition of P-glycoprotein by CSA may be partly involved in the occurrence of CSA neurotoxicity (Kochi *et* al 1999).

1.7.4 Dermatologic Toxicity

Hypertrichosis of the face, arms, shoulders, and back develops in at least 50 percent of renal transplant patients receiving CSA as part of their immunosuppressive protocol (Kahan *et al* 1987). Coarsening of facial features has also been reported (Reznik *et al* 1987). CSA also increases the number of gingival fibroblasts, as well as collagen production by the gingival fibroblasts, producing primarily anterior gingival hyperplasia (Kahan 1989).

1.7.5 Hepatotoxicity

CSA treatment nearly doubles the incidence of cholestasis with hyperbilirubinemia and elevation of serum levels of aminotransferases (especially alanine aminotransferase) in renal transplant patients (Pickrell *et al* 1988). Chronic hepatic dysfunction is associated with an increased incidence of cholelithiasis and choledocholithiasis (Kahan 1989). Experimental models of hepatotoxicity reveal centrilobular fatty changes and hepatocyte necrosis, dilated endoplasmic reticulum, and an increased number of autophagic vacuoles (Kahan 1989). In addition, it has been shown that CSA alters calcium fluxes across hepatocyte cell membranes (*in vitro*), elevates serum bile acids, and decreases bile flow (Kahan 1989).

1.7.6 Gastrointestinal Toxicity

When the Sandimmune® formulation of CSA was still in widespread clinical use, anorexia, bloating, nausea, and/or vomiting were frequently reported following ingestion of Sandimmune® oral suspension (Kahan 1989). CSA has no direct toxic effects on the structure or function of gastrointestinal mucosa. Even in the case of oral CSA overdose, only a mild syndrome of hypertension, dysesthesias, flushing, and stomach upset is encountered (Kahan 1989).

1.7.7 Hematologic Toxicity

There are reports of increased incidences of thrombosis of arterial and venous limbs of renal allografts (Kahan *et al* 1985, Najarian *et al* 1985), as well as thrombosis of systemic veins (Varenterghem *et al* 1985). This increased incidence of thrombosis may be due to increases in

platelet aggregation, thromboxane A_2 release, thromboplastin generation, and factor VII activity (Carlesen and Prydz 1987, Grace *et al* 1987). Another common hematologic-related problem is atherogenic changes in serum lipids, with increased cholesterol, apolipoprotein B, and triglyceride concentrations. This increase in serum lipid levels often requires lipid-lowering therapy, and may also exacerbate the adverse effects of CSA on vascular endothelium (Ellis *et al* 1986).

1.7.8 Lymphatic And Related Toxicity

The risk of non-Hodgkin lymphoma in organ transplant patients receiving CSA immunosuppression is high (Opelz and Henderson 1993). It appears that the greatest risk is during the first year post-transplant, when target CSA concentrations are higher. In the first year post-transplant, kidney and heart transplant patients had occurrence rates of 20 and 120 times higher than the general population, respectively (Opelz and Henderson 1993). The higher incidence in heart transplant patients is thought to be due to the higher amounts of CSA that heart transplant patients are exposed to when compared to kidney transplant patients (Land 1987). Proliferation of B cells that escape T-cell control, driven by Epstein-Barr virus, is believed to be the primary mechanism of lymphoma development (York and Qualtiere 1990).

Immunosuppression is also associated with an increased incidence of certain other cancers (Penn 1986). It is important to point out that these cancers occur not only in transplant patients, but also in patients receiving immunosuppressants for autoimmune and chronic inflammatory disorders, patients who are immunosuppressed as a side effect of chemotherapy, patients with primary or genetically determined immunodeficiency disorders, patients with AIDS, and patients with chronic renal failure who are on dialysis (Penn 1986).

Patients receiving transplants have a 3-fold increase in various cancers in when compared with age-matched controls (Kinlen 1985). Cancers commonly encountered in the general population, including cancer of the lung, prostate, colon, rectum, female breast, and invasive cancer of the uterine cervix were not increased in incidence in transplant patients (Penn 1986). However, other cancers, such as skin, lip, Kaposi's sarcoma, other uterine, vulva, perineum, scrotum, penis, perianal skin, anus, and hepatobiliary, showed an increased incidence in transplant patients when compared to the general population (Penn 1986). While earlier data showed that the incidence of lung cancer was not increased in transplant patients, more recent data have shown that there is a high incidence of lung cancer following heart transplantation, particularly in patients who were smokers prior to transplantation (Goldstein *et al* 1995, 1996, Curtil *et al* 1997).

1.7.9 Hypersensitivity And Other Effects

It has long been known that hypersensitivity to the older olive oil based and castor oil based drug vehicles manifests as a spectrum of clinical symptoms, ranging from mild flushing and hypertension to hypotension, crushing chest pain, dyspnea, and respiratory distress after oral, and especially after intravenous bolus administration of CSA (Kahan 1985).

CSA occasionally produces hyperglycemia not accompanied by ketosis, ketonuria, or altered responses of endogenous insulin and C peptide to intravenous glucose administration (Gunnarsson *et al* 1984). Since CSA has no effect on the number or binding affinity of cellular insulin receptors, the hyperglycemia may be due to impaired hepatic synthesis of glycogen rather than hormone resistance (Betschart *et al* 1988). CSA increases serum prolactin but decreases testosterone levels, which leads to gynecomastia in men (Kahan 1989). This change has also

caused impaired spermatogenesis or sperm maturation in experimental models (Rajfer *et al* 1987).

CSA, when used as monotherapy or as polytherapy, can potentially affect skeletal muscle (Briel and Chariot 1999, Rush 1990). Myopathy manifesting by myalgia, muscle weakness, and plasma creatine kinase elevation have all been attributed to CSA use (Briel and Chariot 1999). Because of a potential drug interaction between CSA and hydroxymethylglutaryl coenzyme A reductase inhibitors, colchicine, and pyrazinamide, the frequency of muscular complications may be increased (Briel and Chariot 1999). This is especially a concern with hydroxymethylglutaryl coenzyme A reductase inhibitors, as these agents are commonly used to treat hyperlipidemia in transplant patients. The mechanism of myotoxicity is not known, but some clinical and experimental findings have provided some clues: 1) some patients with myopathy attributed to CSA have mitochondrial abnormalities and lipid droplet accumulation in skeletal muscle (Fernandez-Sola *et al* 1990, Larner *et al* 1994); and 2) CSA decreases mitochondrial respiration in rat skeletal muscle (Hokanson *et al* 1995, Mercier *et al* 1995).

Therapy is often required to prevent bone loss following institution of CSA therapy. In cardiac transplant patients, bone loss and fractures are frequent complications, particularly during the first year post-transplantation when higher CSA levels are targeted (Boncimino *et al* 1999). Magnesium depletion also frequently occurs with CSA therapy, and this can adversely affect many phases of skeletal metabolism. Magnesium depletion has also been implicated as a risk factor in several forms of osteoporosis (Boncimino *et al* 1999). However, recent evidence has shown that cardiac transplant patients with low serum magnesium levels had significantly lower rates of bone loss, lower serum parathyroid hormone concentrations, and lower bone

turnover (Boncimino *et al* 1999). The exact mechanism of this protective effect is not currently known.

Developing A Limited Sampling Strategy For Cyclosporine Area Under The Curve Monitoring In Lung Transplant Patients

1.1 Introduction

The development of immunosuppressive agents has revolutionized the practice of organ transplantation and brought about significant improvements in survival rates (Trulock 1997, Tsunoda and Aweeka 1996). CSA is the mainstay of immunosuppressive therapy for solid organ transplants (Trulock 1997, Tsunoda and Aweeka 1996). Early clinical studies have demonstrated the immunosuppressive efficacy of CSA (Calne *et al* 1978, Powles *et al* 1978, Starzl *et al* 1981). Since its introduction into clinical practice in 1983, CSA has been shown to increase survival in liver (Starzl *et al* 1983), heart (Macoviak *et* al 1985, Oyer *et al* 1983), heart-lung (Macoviak *et al* 1985), and renal (The Canadian Multicentre Transplant Group 1986) transplant patients. Despite its widespread use, however, the optimum strategy for the therapeutic drug monitoring of this drug remains undetermined. Measurement of CSA blood concentrations is essential in guiding optimal dosages to minimize its toxicity and promote its efficacy (Yee and Saloman 1992). It has been demonstrated that $AUC_{0-\tau}$ pharmacokinetic monitoring of CSA is superior to trough level monitoring (Grevel *et al* 1989). However, the AUC method requires numerous serial blood samples (i.e., typically 7 or more) and, thus, is expensive, cumbersome, and impractical for routine clinical use. Consequently, the usual practice consists of sampling trough levels only. Patients with identical trough levels, however, may have significantly different systemic exposure to CSA as measured by AUC (Yee and Saloman 1992). For example, two patients

could have identical trough levels of 200 ng/mL. However, if concentration-time data were gathered over a dosing interval for these two patients, the resulting concentration-time curves would not be superimposable. If AUC values were calculated for the dosing interval, the values obtained would not be identical. Thus, despite similar trough levels, the patients will not have identical AUC, and therefore, exposure to CSA.

Recent studies conducted primarily in kidney transplant patients (Johnston *et al* 1990, Grevel and Kahan 1991a, Meyer *et al* 1991, Meyer *et al* 1993, Serino *et al* 1994, Foradori *et* al 1995, Johnston *et al* 1996, Serafinowicz *et al* 1996, Amante and Kahan 1996, Keown *et al* 1996, Cooney *et al* 1996, Gaspari *et al* 1997, Primmett *et al* 1998, Meier-Kriesche *et al* 1998, Lemire *et al* 1998, Marsh 1999) and a small number of heart (Johnston *et al* 1990) and liver (Cooney *et al* 1996) transplant patients indicate that a limited number of blood samples (i.e., a limited sampling strategy) provides a reliable alternative to AUC monitoring. Some studies involved patients taking the older CSA formulation, SIM (Johnston *et al* 1990, Grevel and Kahan 1991a, Meyer *et al* 1991, Meyer *et al* 1993, Serino *et al* 1994), while others involved patients taking the new formulation, NEO (Foradori *et* al 1995, Johnston *et al* 1996, Serafinowicz *et al* 1996, Amante and Kahan 1996, Keown *et al* 1996, Cooney *et al* 1996, Gaspari *et al* 1997, Primmett *et al* 1998, Meier-Kriesche *et al* 1998, Lemire *et al* 1998, Marsh 1999). CSA absorption appears to be enhanced and inter- and intra-patient variability reduced with NEO, compared with SIM (Kovarik *et al* 1994a, 1994b, 1994c). As such, a limited sampling strategy may be an even better predictor for NEO than for SIM. However, studies involving these abbreviated monitoring strategies for NEO are nonexistent in the lung transplant population.

Several studies have described CSA pharmacokinetic parameters in renal transplant patients (Ptachcinski *et al* 1985, Awni *et al* 1989, Awni *et al* 1990, Honcharik *et al* 1991, Kahan

et al 1992, Cooney *et al* 1994, Kovarik *et al* 1994, Sketris *et al* 1994, Kahan *et al* 1995, Kahan *et al* 1996, Amante *et al* 1997). Studies involving healthy subjects (Lindholm *et al* 1990, Gupta *et al* 1990, Drewe *et al* 1992, Gardier *et al* 1993, Kovarik *et al* 1994b, Mueller *et al* 1994), heart-lung transplant patients (Tan *et al* 1993, Tsang *et al* 1994, Eadon *et al* 1995), and liver transplant patients (Trull *et al* 1995, Tredger 1995, van Mourik *et al* 1999, Reynaud-Gaubert *et al* 1997) have also been published. However, relatively few studies describing CSA pharmacokinetics in lung transplant patients are available (Eadon *et al* 1995, Reynaud-Gaubert *et al* 1997, Kesten *et al* 1998a, 1998b).

1.2 Background Studies

Trough level monitoring (TLM) is the traditional method of monitoring CSA therapy. Some advantages of TLM are that it is simple, practical for routine clinical use in both inpatients and outpatients, provides qualitative insight into a patient's absorption, and is the most widely studied method of drug monitoring. However, its disadvantages are that it is not a good indicator of total drug exposure, is not a good predictor of outcome since single concentration-effect relationships are weak, and provides only a rough estimation of absorption and/or elimination and no information on other pharmacokinetic parameters (Keown *et al* 1998, Lindholm and Sawe 1995).

The therapeutic ranges of many drugs have been questioned recently, mainly because these ranges were derived from studies that used empirical observations and approximations (usually in studies with small sample sizes) rather than population studies with appropriate statistical analysis (Morris 1997). There is similar controversy with the therapeutic range of CSA, as not all studies show a strong correlation between CSA levels and efficacy or toxicity

(Morris 1997). Due to wide inter-patient variability in CSA pharmacokinetics and the fact that the upper and lower concentration values are, in the majority of cases, arbitrary values within which "efficacy" was achieved with a comparatively low incidence of "toxicity", there are patients who have periods of rejection despite a therapeutic trough level. Similarly, there are patients who exhibit toxicity at therapeutic levels. The therapeutic range can be considered analogous to a confidence interval in that it specifies a range of possible concentrations that are associated with efficacy (e.g., no overt rejection in the case of CSA). Using a simplified and fictional example, we could say that a given therapeutic range for CSA is an 80% confidence interval. Thus, in 80% of cases, efficacy will be achieved, but in 20% of cases, rejection or toxicity may occur (Dumont and Ensom 2000).

The therapeutic range method of TDM has been criticized (Morris 1997, Ensom *et al* 1998, Holford 1999). Because there is a range of possible target values, there is uncertainty regarding what initial dose to prescribe (Holford 1999) and initial dosing is often a "hit and miss" process. The therapeutic range concept also implies that all concentrations within the range are equally desirable. This further implies that there is a 3-step concentration-effect relationship (Holford 1999). Thus, rejection will be encountered with sub-therapeutic levels and toxicity will occur with supra-therapeutic levels. If, for example, a level comes back a few concentration values above "therapeutic", the dose may be immediately lowered to prevent toxicity when in fact, that level is probably efficacious and the patient is experiencing no toxicity.

Because of the problems with TLM of CSA therapy, researchers looked for other methods of monitoring. It is widely known that AUC gives an indication of extent of exposure to a drug. Proper calculation of AUC requires administration of a dose, followed by blood

collection according to an intensive sampling strategy. Concentration values obtained are used to calculate AUC, usually by the trapezoidal method. A study published in 1989 by Grevel and colleagues (Grevel *et al* 1989) is one of the most commonly cited studies demonstrating that AUC monitoring is better than TLM. Patients involved in the study had previously taken part in a pre-transplant pharmacokinetic study to determine individualized oral dosing. Serum concentrations of CSA were measured by polyclonal radioimmunoassay. Correlation analysis was based on 71 observations in 36 renal transplant patients 0-36 months following transplantation. Performance analysis was based on 26 observations in 14 different patients who were 0 - 4 months post-transplant. The conclusion of the study was based on two observations. First, AUC ($r = 0.381$, $p = 0.001$ for dose in mg; $r = 0.538$, $p = 0.0001$ for dose in mg/kg) but not trough level ($r = 0.154$, $p = 0.20$ for dose in mg; $r = 0.136$, $p = 0.26$ for dose in mg/kg) was significantly, albeit poorly, correlated with dose. Second, after adjusting the oral dosage to achieve the target CSA concentration, the absolute deviation of the AUC prediction was significantly smaller than the absolute deviation of the trough level prediction ($14.6 \pm 13.6\%$ for AUC versus $36.0 \pm 27.5\%$ for TL, $p = 0.0005$; mean $\pm$ standard deviation) (Grevel *et al* 1989). A subsequent study (Grevel and Kahan 1991b) in renal transplant patients showed that CSA inter-patient variability could be counterbalanced by dosage individualization through the use of AUC monitoring.

Some advantages of AUC monitoring are that it is the most precise indicator of drug exposure, can characterize abnormal absorption patterns, appears to be a predictor of clinical outcomes in the majority of studies (Table 3) (Kahan *et al* 1986, Savoldi and Kahan 1986, Kasiske *et al* 1988, Grevel *et al* 1991, Lindholm *et al* 1993, Schroeder *et al* 1994, Barone *et al* 1996, Bowles *et al* 1996), generates a concentration-time profile, allows calculation of oral

Table 3. Clinical studies evaluating area under the curve monitoring of cyclosporine A and outcome.

Reference	Patient Group	Sample Matrix/ Analysis Method	Indicator(s) of Outcome	Findings
Kahan et al 1986	Renal	Serum/P RIA	NT	CSA AUC : dose was significantly higher in patients with NT.
Savoldi and Kahan 1986	Renal	Serum/RIA	RJ, NT, Hepatotoxicity, Infection	For IV dosing, RJ was associated with a significantly lower AUC with twice-daily but not once-daily dosing; NT was associated with a significantly higher AUC with once-daily and twice-daily dosing; Hepatotoxicity was associated with a significantly lower AUC with twice-daily dosing only; With infection, there was no significant difference in AUC. For PO dosing, infection was associated with a significantly higher AUC with once-daily dosing. There was no significant difference in AUC with the other indicators of outcome.
Kasiske et al 1988	Renal	WB/HPLC	RJ, NT, Hypertension	No correlation was found between AUC and NT.
Grevel et al 1991	Renal	Serum/P RIA, WB/P FPIA	RJ	A significant difference in AUC was found only with measurement by P RIA.
Lindholm et al 1993	Renal	WB/M RIA	RJ, Graft Loss, Renal Function	AUC values were used to calculate pharmacokinetic parameters. A significantly higher clearance was found with RJ (IV dosing). With PO dosing, those with RJ had a significantly lower average steady-state concentration and a significantly higher oral clearance. Significantly lower average steady-state concentration and bioavailability and significantly higher oral clearance were found with graft loss. AUC-monitored patients had significantly better renal function (as assessed by creatinine clearance) at 6 months post-transplant, with trends for better renal function at 1 and 2 years post-transplant

				(historical control).
Schroeder *et al* 1994	Renal	WB/HPLC, WB/P FPIA	Length of Stay, Number of Readmissions, RJ	Retrospective. Acute RJ resulted in a significantly greater number of readmissions and length of stay for re-hospitalization the first year post-transplant. AUC was significantly lower in both acute and chronic rejection.
Barone *et al* 1996	Renal	WB/HPLC, WB/FPIA	Time to Death, Time to Graft Loss, Time to First RJ Episode	No correlation was found between AUC and any indicators of outcome.
Bowles *et al* 1996	Renal	WB/FPIA	RJ, NT	No significant difference in AUC was found between nephrotoxic and non-nephrotoxic groups.
Mahalati *et al* 1999	Renal	WB/M RIA	RJ	There was a significant difference in AUC between patients with and without acute rejection. However, there was no significant difference in trough levels between patients with and without acute rejection.
Grant *et al* 1999	Liver	WB/FPIA or M RIA, or EMIT	RJ	There was a significant correlation between AUC and rejection, but not between trough level and acute rejection.

Abbreviations: AUC = area under the concentration versus time curve; P RIA = polyclonal radioimmunoassay; NT = nephrotoxicity; CSA = cyclosporine A; RIA = radioimmunoassay; WB = whole blood; HPLC = high performance liquid chromatography; RJ = rejection; M RIA = monoclonal radioimmunoassay; PO = oral; FPIA = fluorescence polarization immunoassay; EMIT = enzyme multiplied immunotechnique

pharmacokinetic parameters, and reduces the problems associated with lab errors and single concentrations. Despite its appealing potential advantages, the major disadvantage of AUC monitoring is its inherent need for multiple blood samples. The increased number of samples required, when compared to TLM, makes AUC monitoring impractical for routine clinical use, more expensive in the short-term due to increased sample collection, analysis and interpretation

of results, and inconvenient for patients, especially those in an outpatient setting (Keown *et al* 1998, Lindholm and Sawe 1995).

AUC monitoring showed promise, but was not possible to use in routine clinical practice. A search for methods to accurately approximate AUC without the intensive sampling led to the development of limited sampling strategies (LSS). Briefly, an LSS is developed by full AUC calculations in a sample population. Stepwise multiple regression analysis is then performed on the concentration-time points sampled. The points that do not correlate well with AUC are removed until a regression equation consisting of two or three concentration-time points is left. A number of limited sampling strategies have been developed in a variety of transplant patients (Johnston *et al* 1990, Grevel and Kahan 1991a, Meyer *et al* 1991, Meyer *et al* 1993, Serino *et al* 1994, Foradori *et* al 1995, Johnston *et al* 1996, Serafinowicz *et al* 1996, Amante and Kahan 1996, Keown *et al* 1996, Cooney *et al* 1996, Gaspari *et al* 1997, Primmett *et al* 1998, Meier-Kriesche *et al* 1998, Lemire *et al* 1998, Marsh 1999). (Table 4).

Table 4. Summary of clinical studies that have developed a limited sampling strategy for a transplant population.

Reference	Patient Group	Formulation	AUC Interval	Equation (C = concentration)
Johnston *et al* 1990	Renal and Heart	SIM	12 hour	$AUC = 4.3 \times C_{3.5} + 5.5 \times C_8 + 3.1 \times C_{10} - 333$
Grevel and Kahan 1991	Renal	SIM	24 hour	$AUC = 2.91 \times C_2 + 5.95 \times C_6 + 11.68 \times C_{14} + 153$
Meyer *et al* 1991	Renal	SIM	24 hour	$AUC = 2.0 \times C_2 + 10.2 \times C_6 + 0.2$
Meyer *et al* 1993	Renal	SIM	24 hour	$AUC = 8.6 \times C_{24} + 1.4 \times C_2 + 6.2 \times C_6 + 1.57$
Serino *et al* 1994	Renal	SIM	12 hour	$AUC = 2.11 \times C_2 + 3.23 \times C_4 + 5.63 \times C_9 + 250$

Foradori *et al* 1995	Renal	NEO	12 hour	$AUC = 0.681 \times C_1 + 1.859 \times C_{2.5} + 3.411 \times C_5 + 791.74$
Johnston *et al* 1996	Renal	NEO	12 hour	$AUC = 1.96 \times C_2 + 11.5 \times C_8 + 355.2$
Serafinowicz *et al* 1996	Renal	NEO	12 hour	$AUC = 9.131 \times C_0 + 0.784 \times C_1 + 2.617 \times C_2 + 193.561$
Amante *et al* 1996	Renal	NEO	12 hour	$AUC = 2.4 \times C_2 + 7.7 \times C_6 + 195.8$ $AUC = 1.5 \times C_{1.5} + 4.1 \times C_4 + 5.6 \times C_9 + 105.5$
Keown *et al* 1996	Renal	NEO	12 hour	$AUC = 1.84 \times C_0 + 4.39 \times C_2 + 312.66$
Cooney *et al* 1996	Liver (pediatric)	NEO	8 hour	$AUC = 10.19 \times C_0 + 4.47 \times C_3 + 749.7$ $AUC = 15.13 \times C_0 + 6.78 \times C_3 - 2.94 \times C_{3.5} + 301.3$ $AUC = 3.69 \times C_3 + 13.76 \times C_{12} + 402.8$ $AUC = 5.32 \times C_0 + 3.84 \times C_3 + 10.83 \times C_{12} - 33.6$
Gaspari *et al* 1997	Renal	NEO	12 hour	$AUC = 5.189 \times C_0\ 1.267 C_1 + 4.150 \times C_3 + 135.079$
Primmett *et al* 1998	Renal	NEO	4 hour	$AUC = 12.34 \times C_0 + 2.48 \times C_2 + 441.42$ $AUC = 9.55 \times C_0 + 0.96 \times C_1 + 2.05 \times C_2 + 112.07$
Meier-Kriesche *et al* 1998	Renal (pediatric and adult)	NEO	8 hour	$AUC = 1.84 \times C_2 + 4.39 \times C_4 + 129$
Lemire *et al* 1998	Renal	NEO	12 hour	$AUC = 1.023 \times C_1 + 13.10 \times C_6 + 242$
Marsh 1999	Renal	NEO	12 hour	$AUC = 6 \times (C_0 + C_4)$

Earlier studies involved the Sandimmune® formulation, and 3 of these 5 studies used once daily dosing. Because the majority of centers dose cyclosporine twice daily, and because once daily dosing is no longer used, these LSS may no longer be applicable.

LSS are generally considered superior to TLM because trough concentrations do not provide an adequate estimate of exposure to CSA. Also, equations for some LSS were chosen solely on the basis of a high coefficient of determination value, rather than looking at other

criteria such as prediction error (Gaspari *et al* 1998). LSS developed using the original Sandimmune® formulation are likely no longer applicable due to the differences between Neoral® and Sandimmune®. In addition, LSS are primarily available for renal allografts. Because of pharmacokinetic differences between transplant types, these LSS are not applicable to, and warrant further study in, other transplant types. LSS may be center-specific, as studies that have evaluated LSS developed in other centers have found that they do not perform as well in terms of percent prediction error for AUC (Gaspari *et al* 1997, 1998, 1993). As well, not all LSS have worked (Gaspari *et al* 1993).

1.3 Significance Of The Research Project

To our knowledge, this is the first study to develop a limited sampling strategy for use in the therapeutic drug monitoring of CSA in lung transplant patients. A previously published report by Trull and colleagues (Trull *et al* 1999) stated in the abstract that they had utilized a limited sampling strategy for their population of lung transplant patients, although this limited sampling strategy was not mentioned in the body of the paper. Also, this "limited sampling strategy" was not an LSS like the ones reported in this thesis and previously published papers. They did not use an equation obtained from multiple regression analysis of concentration-time data to predict total $AUC_{0-\tau}$. Instead, they calculated an AUC_{0-6} and performed repeated measures analysis of variance on the AUC_{0-6} obtained from pharmacokinetic studies performed at the end of weeks 1 to 4 and at the end of weeks 13, 26, 39, and 52 (Trull *et al* 1999). Thus, because they did not develop or even utilize an LSS developed according to previously published methods, our LSS developed specifically for lung transplant patients is, indeed, novel. The information generated from the proposed study could prove to be crucial for effective

management of lung transplant patients. Using an individualized limited sampling strategy for CSA developed uniquely for lung transplant patients is expected to be a convenient and accurate method to minimize toxicity and maximize efficacy of long-term immunosuppressant therapy in this patient subpopulation. Calculation of pharmacokinetic parameters in lung transplant patients taking the Neoral® formulation of CSA will add to the existing, limited data.

Research Hypothesis

A limited sampling strategy for CSA (Neoral®) is a precise and unbiased predictor of AUC in lung transplant patients.

Objectives

The objectives of this study are:

1) To define the optimal limited sampling strategy for CSA monitoring in lung transplant patients.

2) To compare the predictive performance of this optimal limited sampling strategy (with other models derived from previous studies) in lung transplant patients.

3) To determine the mean steady-state pharmacokinetic parameters of CSA (Neoral®) in lung transplant patients.

Rationale

Of all organs, the lung is most susceptible to rejection (Trulock 1997). Although CSA has been largely responsible for the success of lung and other organ transplantation, it is not

without problems. CSA has a narrow therapeutic window, thus requiring measurement of blood concentrations to maintain efficacy and minimize toxicity (Yee and Saloman 1992).

The uniqueness of CSA dosing and monitoring in lung transplant patients is further illustrated by the large proportion (approximately 1/3 in the VHHSC lung transplant program) of them who have cystic fibrosis. Compared to patients without cystic fibrosis, those with cystic fibrosis are known to have greater CSA clearance, more erratic absorption, and more variable pharmacokinetics (Tan *et al* 1993).

In the lung transplant population, the newer microemulsion formulation of CSA, NEO, demonstrates greater bioavailability and reduced variability in CSA blood concentrations compared with older CSA formulations (Kesten *et al* 1998b, Zaldonis *et al* 1998, Mikhail *et al* 1997, 1998, Wilczek *et al* 1997, Svendsen *et al* 1995, 1996, Girault *et al* 1995). In these studies, the improved pharmacokinetic characteristics of NEO were demonstrated via calculations of AUC obtained by serial blood sampling (i.e., typically 7 or more concentrations). In clinical practice, collection of multiple serial samples to monitor CSA (NEO) therapy is not possible and collection of only trough samples is the routine.

It has been demonstrated that AUC monitoring of CSA is superior to trough level monitoring (Grevel *et al* 1989). Patients with identical trough levels, however, may have significantly different systemic exposure to CSA as measured by AUC (Yee and Saloman 1992). Suboptimal exposure to CSA can lead to acute rejection. Furthermore, multiple acute rejection episodes lead to a greater risk of chronic rejection, ultimately ending in graft failure and death (Trulock 1997).

A limited sampling strategy has the advantage of minimizing the number of blood samples required by AUC monitoring and at the same time, providing relevant pharmacokinetic

data to guide optimal CSA dosing. Information on the utility of limited sampling strategies for CSA, either Neoral® or Sandimmune®, in lung transplant patients, however, is glaringly lacking. To our knowledge, no published reports are available in this patient subpopulation.

A limited sampling strategy has served as a good predictor for CSA (older formulations and NEO) AUC in non-lung transplant patients (Johnston *et al* 1990, Grevel and Kahan 1991a, Meyer *et al* 1991, Meyer *et al* 1993, Serino *et al* 1994, Foradori *et* al 1995, Johnston *et al* 1996, Serafinowicz *et al* 1996, Amante and Kahan 1996, Keown *et al* 1996, Cooney *et al* 1996, Gaspari *et al* 1997, Primmett *et al* 1998, Meier-Kriesche *et al* 1998, Lemire *et al* 1998, Marsh 1999). Because of reduced inter- and intra-patient variability in CSA blood concentrations associated with NEO administration to lung transplant patients, we expect such a limited sampling strategy to be an even better predictor for NEO than for older CSA formulations. Furthermore, the inability to apply some limited sampling strategies for CSA (derived from kidney transplant patients from a particular institution to another institution) (Gaspari *et al* 1997, 1998, 1993) underscores the need to develop limited sampling strategies unique to the lung transplant patient subpopulation.

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental Design

This was an open-label, single center, pilot clinical study.

2.2 Clinical Research Subjects

Stable, adult lung transplant patients (both single and double lung graft recipients) were recruited from the outpatient solid organ transplant clinic, Vancouver Hospital and Health Sciences Centre, Vancouver, British Columbia, Canada.

2.3 Clinical Research Subject Inclusion Criteria

1) Lung transplant patients who are on a steady-state dosage of CSA (Neoral®) (Attainment of steady state was assumed when patients have taken CSA for at least 2 weeks without a dosage adjustment or change in a concurrent medication that can affect CSA metabolism).

2) Patients over 16 years of age, in accordance with a predetermined arbitrary limit between adult and pediatric age.

3) Patients able to provide informed consent

2.4 Clinical Research Subject Exclusion Criteria

1) Patients refusing or unable to provide informed consent

2) Patients younger than 16 years of age

3) Patients whose CSA (Neoral®) therapy was not at steady state, as defined previously in section 2.3, Clinical Research Subject Inclusion Criteria.

2.5 Clinical Research Study Protocol

Subjects reported to the research clinic at the BC Transplant Society office (Vancouver, BC, Canada) prior to their morning dose of CSA (NEO). In order to have consistent start times for all patients, the start time was 0700 for all patients. Prior to arrival for the study visit, all patients were interviewed by phone regarding medication history and administration time. If a given patient did not normally take CSA at 0700, they were asked to switch to this time 2 weeks prior to the study visit. In addition, all relevant medical chart data, including date and type of transplant, serum creatinine level, demographic information, and transplant and non-transplant related medical conditions were gathered prior to the study visit. Prior to administration of their morning CSA dose, an indwelling intravenous catheter (i.e., a "butterfly") was placed in a forearm vein if they did not have a central line or if they preferred a "butterfly" over multiple venipunctures. Ethyl chloride spray U.S.P. (Xenex Laboratories Inc., Coquitlam, BC, Canada) was used as a local anesthetic prior to venipuncture. All patients, with the exception of 1, chose the intravenous catheter over multiple venipunctures. Depending on the patient, 1 of 2 types of catheter devices were used. One was an Insyte™ 22 gauge × 1 inch intravenous catheter (Becton Dickinson Infusion Therapy Systems Inc., Sandy, Utah, USA). The other was a shorter unit, which was an Argyle® intermittent infusion plug (Sherwood Medical, St. Louis, Missouri, USA). Routine clinical measurements (blood pressure, pulse, height, and weight) were also taken. All patients took the morning CSA dose in a fasted state. This likely does not represent the normal clinical situation, but was done for the sake of consistency, although approximately 25% of the patients who participated did not eat breakfast at all. Patients were allowed to eat normally and move about following collection of the 0-hour blood sample, routine clinical measurements, and ingestion of the dose. Because patients were allowed to eat normally immediately following

dosage ingestion if desired, any benefits of fasting on absorption were negated and likely made our protocol more closely resemble the normal clinical situation. Blood samples (approximately 3 mL each) were collected at 0, 1, 2, 3, 4, 5, 6, 8, 9, 10, and 12 hours following CSA administration. A Vacutainer™ 22 gauge × 1 inch needle (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) and a Vacutainer® holder (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) were used for blood withdrawal. All samples were collected into Vacutainer® (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) collection tubes. These collection tubes contained 0.057 mL of 0.34 molar potassium ethylene diamine tetraacetic acid, had a draw volume of 5 mL, and were equipped with a Hemogard™ closure. In cases of a difficult draw, a Monoject® 25 guage × 5/8 inch 3 cc luer lock syringe with needle was used to withdraw the sample from either the forearm vein (in 1 case) or the collection device. The blood was then transferred from the syringe to a collection tube. Immediately after sample collection, the collection tubes were gently inverted several times, and kept refrigerated until transported to the laboratory. The collection device was then flushed with 1 mL of sodium chloride injection U.S.P. 0.9%, followed by 0.5 mL of heparin lock flush solution U.S.P. 100 U.S.P. units per mL. The heparin used in the lock flush solution was of porcine intestine mucosal origin. Prior to collection of the next sample, approximately 1 mL of blood was collected into a collection tube as described previously in order to ensure that there was no flush solution collected into the sample collection tubes. Patients ingested other medications in their medication regimen as normal, in order to simulate the normal clinical situation. In all cases, other medications were taken along with the morning CSA dose.

2.6 Sample Analysis

Whole blood CSA concentrations were analyzed by fluorescence polarization immunoassay using a specific monoclonal antibody kit (TDx®, Abbott Inc., Abbott Park, Illinois, USA) in the central hospital laboratory located in the Department of Pathology and Laboratory Medicine at VHHSC. A typical sample run on the FPIA machine consists of a treated sample containing fluorescein tagged CSA, CSA from the patient sample, and a monoclonal antibody specific for the parent CSA molecule. When low levels of CSA are present in the patient sample, more of the monoclonal antibody is bound to the tagged CSA molecules. This binding fixes the tagged CSA molecule, preventing it from rotating freely when exposed to polarized light. Thus, with low levels of CSA in the patient sample, there are high levels of polarization, which the FPIA machine detects and uses to quantitate CSA present in the patient sample through the use of a standard curve. The opposite is true when the patient sample contains high levels of CSA.

2.6.1 Reagents And Standards

2.6.1.1 Reagent Solutions

The CSA monoclonal kit consists of a 3-pot reagent pack labeled as *S, *T, or *P. *S is less than 25% CSA antiserum (mouse monoclonal) in buffer with protein stabilizer and sodium azide as preservative. *T is less than 0.01% CSA fluorescein tracer in buffer containing surfactant, protein stabilizer, and sodium azide as preservative. *P is pretreatment solution containing surfactant in buffer, with sodium azide as preservative. The reagent packs were stored at 2 to 8 °C and are stable until expiry. Prior to use, the reagent was mixed gently, the cap was removed, and all bubbles were removed with an applicator stick. The reagents did not need

to be warmed to room temperature before use. Accessories to the reagents include whole blood precipitation reagent/probe wash, which is zinc sulfate solution in methanol and ethylene glycol and solubilization reagent, which contains surfactants in water with sodium azide as preservative. The accessories were stored at room temperature, and no additional preparation was required prior to use.

2.6.1.2 Calibrator Solutions

There are 6 CSA monoclonal whole blood calibrators, and they were stored in 6 vials labeled A through F. The concentrations of the 6 standards, with the lowest concentration stored in vial A and the highest concentration stored in vial F, were as follows: 0, 100, 250, 500, 1000, and 1500 ng per mL. These 6 concentrations comprised the 6-point calibration curve used for quantitation of patient samples. Opened and unopened calibrator packs were stored at 2 to 8 °C, and were stable until expiry. All calibrators were mixed gently before use.

2.6.2 Sample Preparation

A pretreatment step was performed on each matrix to be measured (calibrators, controls, and patient samples) prior to testing. The purpose of the pretreatment step was to minimize interference from endogenous protein-bound fluorescent compounds. The pretreatment consisted of addition of solubilization reagent (in order to solubilize the cells) and whole blood precipitation reagent/probe wash to the sample (in order to precipitate protein), followed by centrifugation to obtain a clear supernatant. The supernatant was then used for further analysis.

2.6.3 Assay Controls

Three different controls, stored in vials labeled L, M, and H were utilized throughout sample analysis. Control solutions were reconstituted by volumetrically pipetting 2.0 mL of Barnstead water into 1 vial. The solution was then left to stand at room temperature for 20 minutes, with occasional swirling. Prior to use, the vial was inverted several times to ensure homogeneity. Reconstituted vials were stored at 2 to 8 °C and were stable for 30 days following reconstitution. If the controls were not freshly reconstituted, they were warmed to room temperature and inverted several times prior to use. Two levels of controls were used in each run, and the levels used were alternated between runs. The control vials should read in the following ranges: vial L, 120 to 180 ng per mL; vial M, 340 to 460 ng per mL; vial H, 680 to 920 ng per mL.

2.6.4 Calibration

Calibration was always required following the machine activation procedure, and if the memory circuit is replaced. Calibration may be required when quality control is unacceptable, reagents with new lot numbers are used, buffer with a new lot number is used, when any dispenser component is replaced, and when any instrument calibration is performed. During the calibration procedure, all calibrators are run in duplicate, and all levels of control solutions are run. If possible, a fresh reagent pack and a fresh calibrator pack are used.

2.6.5 Sample Analysis Procedure

A maximum of 20 samples can be run in 1 batch. All samples (calibrators, controls, and patient samples) were prepared as described in section 2.6.2 Sample Preparation. Following

preparation, the samples were mixed by gentle inversion in order to prevent foaming, which could affect the results. If excessive foaming did develop, the samples were left to stand for a few minutes until the foam dissipated. One hundred fifty μL of the sample to be analyzed was pipetted into an appropriately labeled centrifuge tube. The same pipette tip was used throughout, with the pipette tip being rinsed with normal saline several times between each transfer of sample. Fifty μL of solubilization reagent was then pipetted into the centrifuge tube, followed by 300 μL of whole blood precipitation/probe wash solution. The centrifuge tube was then capped and vortexed for 10 seconds to ensure thorough mixing. The samples were then centrifuged for 5 minutes at 9500 g, or until a clear supernatant and a hard, compact pellet of denatured protein were obtained. After centrifugation was complete, the centrifuge tubes were uncapped and the supernatant was immediately decanted into the corresponding sample well of a sample cartridge. (A minimum of 150 μL of sample supernatant is required to perform the assay). Samples were run immediately following transfer to the sample cartridges.

2.6.6 Linearity

The standard curve is linear and useable in the range of 25 to 1500 ng/mL. Samples with a concentration of greater than 1500.00 ng/mL are diluted with an equal amount of whole blood calibrator A (0.00 ng/mL) prior to performing the solubilization step. The final sample concentration is then determined by doubling the result obtained.

2.6.7 Precision

Reproducibility was determined by assaying the three CSA monoclonal whole blood controls, L (low, 150 ng/mL), M (medium, 400 ng/mL), and H (high, 800 ng/mL) in replicates of 5 in each of 10 independent runs on 11 instruments at 10 laboratories. Within run coefficient of variation (CV), between run CV, and between laboratory CV were calculated (Table 5).

Table 5. Expected precision of the monoclonal fluorescence polarization immunoassay used for sample analysis. (Adapted from the VHHSC laboratory standard procedure manual 1999).

Target Cyclosporine Concentration (ng/mL)	150.0	400.0	800.0
Mean Cyclosporine Concentration Obtained (ng/mL)	146.7	390.8	791.8
CV Within Run (%)	3.2	2.3	2.3
CV Between Run (%)	3.9	2.9	3.0
CV Between Laboratory (%)	4.7	3.5	3.5

2.6.8 Standard Reference Intervals For Cyclosporine Trough Levels Utilized At Vancouver Hospital And Health Sciences Centre

The standard trough concentrations targeted at VHHSC vary with the type of transplant and the time post-transplant (Table 6). Results are reported as whole numbers, and if the result is less than the lower limit of quantitation of the assay, the result is reported as "<25 ng/mL".

Table 6. Standard reference intervals for target trough cyclosporine concentrations at Vancouver Hospital and Health Sciences Centre.

Transplant Type	Time Post-Transplant	Target Trough Concentration (ng/mL)
Kidney, Liver, and Heart	Less than 1 month	400 to 450
	Less than 2 months	300 to 400
	2 to 6 months	200 to 300
	6 to 12 months	150 to 250
	Greater than 12 months	125 to 200
Lung	0 to 1 month	350 to 400
	1 to 3 months	300 to 350
	3 to 6 months	250 to 300
	6 to 12 months	200 to 250
	Greater than 12 months	150 to 200

2.6.9 Specificity Of The Fluorescence Polarization Immunoassay

The specificity of the high volume assay methods utilized in the routine clinical management of transplant patients receiving CSA as the primary form of immunosuppression was improved greatly when polyclonal antibodies were replaced with monoclonal antibodies that were more specific for the parent drug. However, because of the large structure of CSA and because the large cyclic structure remains intact following metabolism, there is still cross-reactivity with metabolites even with the monoclonal antibodies.

The cross-reactivity of the monoclonal antibodies used in the fluorescence polarization immunoassay used at VHHSC was determined by spiking whole blood specimens containing 200 ng/mL of CSA with known amounts of the CSA metabolites M1, M8, M17, M18, and M21. The percent cross-reactivity was determined according to the following equation:

$$\text{Percent Cross-Reactivity} = \frac{(\text{Metabolite concentration obtained} - \text{Control concentration obtained})}{\text{Metabolite concentration added}} \times 100$$

The results are summarized in Table 7. Occasionally, patient samples are obtained which contain unusually high levels of metabolites. This primarily occurs in patients with hepatic dysfunction or in patients who have inadequate biliary flow. Although the cross-reactivity of the assay with the metabolites tested is low (with 1 exception), the presence of these metabolites in high concentrations can result in overestimation of the trough CSA concentration. In this case, it is recommended by the manufacturer that an alternate methodology that quantitates the parent drug only, such as HPLC, be used instead.

Table 7. Cross-reactivity of the monoclonal fluorescence polarization immunoassay used at Vancouver Hospital and Health Sciences Centre.

Metabolite	Concentration Added (ng/mL)	Average Cross-Reactivity In The Presence Of Cyclosporine (%)	Standard Deviation
M1	250	19.4	2.6
M8	250	Less than 5	
M17	500	6.7	1.7
M18	250	Less than 5	
M21	250	Less than 5	

2.7 Pharmacokinetic Analysis

Drug concentration-time data obtained from the clinical study was modeled with WinNonlin® pharmacokinetic modeling software (Version 1.1, SCI Software, North Carolina, USA). For the WinNonlin® pharmacokinetic analysis, model 200, a noncompartmental model with extravascular input and plasma/blood data, was utilized. Initial estimates of the pharmacokinetic parameters were calculated manually using Microsoft® Excel 2000.

The area under the concentration versus time curve over one dosing interval ($AUC_{0-\tau}$) for each subject was calculated using the linear trapezoidal method. Briefly, the trapezoidal method involves the description of the concentration versus time curve by a function that depicts the curve as a series of straight lines. Hence, the area under this curve can be divided into multiple

trapezoids. Then, the area of each trapezoid is calculated and the sum of the areas of each trapezoid yields an estimate of the true AUC. Dose corrected $AUC_{0-\tau}$ was calculated by dividing the value obtained for $AUC_{0-\tau}$ by the cyclosporine dosage in mg/kg.

The area under the first moment curve for one dosing interval ($AUMC_{0-\tau}$) for each subject was calculated in a manner similar to the trapezoidal method, except a concentration × time versus time curve was described by a function that depicts the curve as a series of straight lines.

Mean residence time (MRT) for each subject was calculated according to a previously published method (Pfeffer 1984):

$$MRT = [AUMC_{0-\tau} + (\tau \times AUC_{0-\infty})]/AUC_{0-\tau}$$

The maximum concentration observed during the dosing interval (C_{max}) and the time of the maximum concentration observed during the dosing interval (T_{max}) for each subject were elucidated by visual inspection of the appropriate concentration versus time curves.

The terminal elimination rate constant (λz) for each subject was determined from the slope of the terminal elimination phase. This slope was determined by least-squares linear regression of the terminal elimination phase. For the WinNonlin$^{®}$ pharmacokinetic analysis, the points used in the calculation of λz were determined by the program. For the determination of the initial estimate of λz, the 8-hour to 12-hour concentration-time points were utilized.

The terminal elimination half-life ($t_{1/2}$) for each subject was calculated initially according to the following equation:

$$t_{1/2} = 0.693/\lambda z$$

The apparent oral clearance (CL/F) for each subject was calculated as follows:

$$CL/F = Dose/AUC_{0-\tau}$$

The apparent volume of distribution (Vd/F) for each subject was calculated as follows:

$$Vd/F = Dose/(\lambda z \times AUC_{0-\tau})$$

2.8 Sample Size

The total sample size used for the clinical study was a convenience sample size. A minimum of 14 patients (providing a sample size of 8 for Phase I and 6 for Phase II) was deemed reasonable given that only 23 lung transplant patients are followed at the solid organ transplant clinic based at VHHSC. One of these patients no longer lives in British Columbia, and is followed at Foothills Hospital in Calgary, Alberta, Canada. The remainder of the lung transplant patients above the 14 selected either refused to participate or were unable to participate due to medical reasons. Members of the investigative group with experience in determining pharmacokinetic parameters (in Phase I) and testing their predictive performance (in Phase II) (Radomski *et al* 1997, Birt and Chandler 1990, Rhoney *et al* 1993) suggested that 14 patients was a reasonable number to use for the clinical study.

2.9 Phase I Of The Clinical Study

Data from the first 8 subjects were used to develop the limited sampling strategy (LSS) for lung transplant patients. Multiple regression analysis, with AUC as the dependent variable and the blood concentrations grouped by time as the independent variables, was performed using Statistica® statistical software (StatSoft Inc., Tulsa, Oklahoma, USA). The backward elimination method was used to calculate the initial regression equation. Briefly, this procedure involves an initial regression using all concentration-time data. Then concentration-time data are removed one collection time (e.g., all of the 6 hour post-dose samples) at a time. If this deletion made

little difference to the coefficient of determination, it was not included in the final equation. If the deletion was important to the regression, then it was included. Following this initial regression analysis, the remainder of the analysis was restricted to the first 3 hours post-dose. In order to generate as many potential equations as possible, the concentration-time data to be analyzed were chosen manually, and the standard regression analysis technique was performed.

2.10 Phase II Of The Clinical Study

Data from the remaining 6 subjects was used to test the predictive performance of the limited sampling strategy developed in Phase I. Predictive performance of our limited sampling strategy was compared with the predictive performance of other published limited sampling strategies derived from non-lung transplant patients.

Predictive performance was calculated according to the methods proposed by Sheiner and Beal (Sheiner and Beal 1981). First, concentration-time data was used to calculate AUC from the LSS for lung transplant patients, which was determined in Phase I. For each LSS evaluated (the optimal LSS developed specifically for lung transplant patients and other previously published LSS developed for other transplant populations), prediction error (pe) was determined using the following equation:

$$pe = \text{predicted AUC} - \text{actual AUC}$$

Percent prediction error (%pe) was calculated according to the following equation:

$$\%pe = 100 \times \frac{(\text{predicted AUC} - \text{actual AUC})}{(\text{actual AUC})}$$

We arbitrarily chose an acceptable error of 10% for %pe. Thus, AUC predictions that were within ± 10% of the actual value were deemed acceptable. These error limits are widely

regarded as standard in basic science and the pharmaceutical industry, and are likely considered strict for therapeutic drug monitoring of CSA.

Following calculation of pe and %pe for each LSS evaluated, the mean prediction error (ME, bias) and the mean absolute error (MAE, precision) were determined according to the following equations:

$$ME = (\Sigma pe)/n$$

$$MAE = (\Sigma |pe|)/n$$

Comparisons of predictive performance were done in a pairwise fashion. All comparisons of predictive performance involved the optimal LSS for lung transplant patients and 1 previously published LSS derived for other transplant patients. Using pe values for each LSS, the mean difference in prediction error (MDPE) was calculated by taking the mean of the differences between all of the respective pe. Similarly, the mean difference in absolute error (MDAE) was calculated by taking the mean of the differences between all of the respective absolute errors. Finally, the 95% confidence intervals (CI) are calculated for MDPE and MDAE. If the 95% confidence interval (CI) for the mean difference between two equations' prediction errors does not contain zero, then the respective equations differ in bias at a significance level of < 0.05. If the 95% CI for the mean difference between two equations' absolute errors does not contain zero, then those equations differ in precision at a significance level of < 0.05 (Sheiner and Beal 1981, Radomski *et al* 1997, Rhoney *et al* 1993, Welch *et al* 1993, Leader *et al* 1994, Cropp *et al* 1998, Davis and Chandler 1996).

CHAPTER 3

RESULTS

3.1 Lung Transplant Patient Characteristics

Fourteen stable, adult lung transplant patients participated in the clinical study. There was a 50:50 distribution in sex. All of the various types of lung transplant (double lung, right single lung, and left single lung) were represented. In addition, most of the common indications for lung transplantation were represented (Table 8). One patient, who participated in Phase II of the clinical study, was also the recipient of a kidney transplant necessitated by CSA nephrotoxicity. This patient had received a double lung transplant for cystic fibrosis, and had the longest time post-transplant. Another patient, who also participated in Phase II, was on dialysis due to CSA nephrotoxicity. The patient had also received a double lung transplant for cystic fibrosis, and received CSA every 8 hours. Because dialysis does not affect CSA levels due to the large size and lipophilicity of the CSA molecule, and because dialysis was not an exclusion criterium, the patient's data were included in Phase II data analysis.

Table 8. Patient characteristics for 14 stable, adult lung transplant patients.

Age	48 ± 12 years
Sex	7 male, 7 female
Weight	69 ± 17 kg
Transplant Type	6 double lung, 6 right single lung, 2 left single lung
Time Post-Transplant	5.1 ± 3.4 years
Reason For Transplant	Chronic obstructive lung disease (6 cases), alpha-1 antitrypsin deficiency emphysema (3 cases), cystic fibrosis (4 cases), idiopathic pulmonary fibrosis (1 case)
Immunosuppressive Regimen	Triple therapy: cyclosporine (Neoral®) + mycophenolate mofetil or azathioprine + prednisone
CSA Dose	4.3 ± 1.7 mg/kg/day
Note: Data are presented as mean ± standard deviation	

3.2 Cyclosporine Concentration-Time Data

A total of 160 blood samples were gathered for analysis by FPIA at the hospital laboratory, VHHSC. Of the 160 blood samples dropped off at the laboratory, 158 were analyzed

(Table 9 and 10). Two samples from 1 patient in Phase I were lost prior to analysis. Fortunately, the samples were in the middle of the terminal elimination phase and thus, were not crucial for the pharmacokinetic and multiple regression analysis. In the case of 12 patients, 11 blood samples were gathered over 1 dosing interval of 12 hours. In the remaining 2 cases, 8 blood samples were gathered over 1 dosing interval of 8 hours. Both of these patients were young (with ages greater than 1 SD less than the mean) cystic fibrosis patients with double lung transplants. Both were documented as poor absorbers of CSA in their respective medical chart. One of these patient's data were included in Phase I analysis, and the other patient's data were included in Phase II analysis.

Table 9. Concentration-time data for 14 stable, adult lung transplant patients.

Time	Pt. 001	Pt. 002	Pt. 003	Pt. 004	Pt. 005	Pt. 006	Pt. 007	Pt. 008	Pt. 009	Pt. 010	Pt. 011	Pt. 012	Pt. 013	Pt. 014
0	154	193	274	169	154	213	319	177	184	169	120	302	223	123
1	935	992	691	1137	1203	1219	1323	320	1096	1588	827	3076	1818	301
2	722	749	1093	724	737	746	1482	1184	1276	1120	627	1752	972	789
3	423	457	775	472	462	518	957	751	895	651	405	949	613	314
4	310	371	637	348	397	415	633	497	705	454	266	666	485	257
5	243	308	546	286	326	338	547	395	594	319	215	528	430	194
6	198	272	420	242	295	Lost	476	341	500	268	189	454	339	160
8	148	222	238	198	218	Lost	349	245	350	200	156	376	267	109
9	142	215	–	192	200	250	300	207	327	177	138	362	240	–
10	133	188	–	180	193	223	298	194	300	162	131	359	211	–
12	123	163	–	157	163	203	257	178	251	130	124	296	180	–
Note: Time is in units of hours, and concentration is in units of ng/mL														

Table 10. Mean concentration-time values for 14 stable, adult lung transplant patients.

Time (hours)	Mean Concentration (ng/mL)	Standard Deviation (ng/mL)
0	198	62
1	1180	689
2	998	336
3	617	215
4	460	150
5	376	133
6	320	114
8	237	82
9	229	70
10	214	70
12	185	56

3.3 Steady-State Pharmacokinetic Parameters For Cyclosporine (Neoral®)

Individual (Table 11) and mean (Table 12) steady-state pharmacokinetic parameters for CSA (Neoral® [NEO]) were calculated using concentration-time data gathered from 14 stable, adult lung transplant patients. The pharmacokinetic characteristics of the NEO formulation are more predictable than those of the SIM formulation, thus reducing inter- and intra-patient variability in pharmacokinetic parameters. Because there was only 1 study visit per patient, it was not possible to evaluate intra-patient variability in pharmacokinetic parameters. As can be seen from the ranges observed in the individual pharmacokinetic parameters (Table 13), there is noticeable inter-patient variability. The results confirm the generalizations in pharmacokinetic parameters reported in the literature for the various transplant populations. That is, CSA NEO, in stable, adult lung transplant patients, has a long $t_{1/2}$, a large apparent Vd, and a T_{max} that occurs predictably within 2 hours following dosage administration. Figure 3 depicts the mean concentration versus time curve developed from data from 14 stable lung transplant patients. Figure 4 depicts the mean log concentration versus time curve developed from the same data.

Figures 5 and 6 depict all 14 concentration versus time curves on one graph, and all 14 log

concentration versus time curves on one graph, respectively

Table 11. Individual steady-state pharmacokinetic parameters for cyclosporine (Neoral®) in 14
stable, adult lung transplant patients.

Parameter	Pt. 001	Pt. 002	Pt. 003	Pt. 004	Pt. 005	Pt. 006	Pt. 007
$AUC_{0-\tau}$ (ng×hr/mL)	3669	4378	4688	4381	4615	5044	7301
Dose-Corrected $AUC_{0-\tau}$ (ng×hr/mL)	2422	2977	2297	3637	3655	4237	2434
$AUMC_{0-\tau}$ (ng×hr²/mL)	14366	18539	15955	17677	19098	21605	31001
MRT (hr)	24.44	21.23	22.41	22.43	22.05	22.78	19.86
C_{max} (ng/mL)	935	992	1093	1137	1203	1219	1482
T_{max} (hr)	1.08	0.93	2.00	0.97	1.00	1.00	2.00
CL/F (L/hr)	15.94	20.24	27.08	14.89	12.87	12.87	15.79
CL/F (L/hr/kg)	0.24	0.24	0.37	0.18	0.15	0.15	0.32
Vd/F (L)	337	226	97	222	253	173	135
Vd/F (L/kg)	5.11	2.66	1.32	2.67	2.06	2.06	2.70
λz (hr⁻¹)	0.047	0.089	0.037	0.067	0.072	0.074	0.117
$t_{1/2}$ (hr)	14.67	7.76	2.48	10.32	9.32	5.93	5.93
Parameter	Pt. 008	Pt. 009	Pt. 010	Pt. 011	Pt. 012	Pt. 013	Pt. 014
$AUC_{0-\tau}$ (ng×hr/mL)	4787	6984	5450	3368	9553	6077	2272
Dose-Corrected $AUC_{0-\tau}$ (ng×hr/mL)	3532	3179	1526	1437	2809	2139	1263
$AUMC_{0-\tau}$ (ng×hr²/mL)	21806	31192	19551	13324	35395	23442	7087
MRT (hr)	25.61	21.29	18.31	32.20	20.55	19.18	13.15
C_{max} (ng/mL)	1184	1276	1588	827	3076	1818	789
T_{max} (hr)	2.00	1.98	1.00	1.00	1.00	1.00	1.97
CL/F (L/hr)	11.91	12.77	22.43	19.01	18.65	28.99	26.31
CL/F (L/hr/kg)	0.16	0.22	0.53	0.30	0.25	0.37	0.63
Vd/F (L)	242	143	213	699	243	271	140
Vd/F (L/kg)	3.27	2.51	5.08	10.93	3.30	3.42	3.35
λz (hr⁻¹)	0.049	0.089	0.105	0.027	0.077	0.107	0.189
$t_{1/2}$ (hr)	14.07	7.76	6.59	25.49	9.02	6.48	3.68

Table 12. Mean steady-state pharmacokinetic parameters for cyclosporine (Neoral[®]) in 14 stable, adult lung transplant patients.

Parameter	Mean Value	Standard Deviation	Range
$AUC_{0-\tau}$ (ng×hr/mL)	5183	1835	2272 to 9553
Dose-Corrected $AUC_{0-\tau}$ (ng×hr/mL)	2682	912	1263 to 4237
$AUMC_{0-\tau}$ (ng×hr^2/mL)	20712	7655	7087 to 35395
MRT (hr)	21.82	4.22	13.15 to 32.20
C_{max} (ng/mL)	1330	578	789 to 3076
T_{max} (hr)	1.35	0.49	0.93 to 2.00
CL/F (L/hr)	18.93	5.52	11.91 to 28.99
CL/F (L/hr/kg)	0.30	0.14	0.15 to 0.63
Vd/F (L)	242	146	97 to 699
Vd/F (L/kg)	3.64	2.33	1.32 to 10.93
λz (hr^{-1})	0.082	0.041	0.027 to 0.189
$t_{1/2}$ (hr)	9.52	5.71	2.48 to 25.49 (5.93 to 25.49 if the patients receiving CSA q8h are excluded)

3.4 Phase I: Multiple Regression Analysis

Multiple regression analysis using all concentration-time data, as well as concentration-time data restricted to the first 3 hours post-dose (for clinical feasibility purposes) yielded a total of 17 LSS to choose from. Of these 17 LSS, 2 required 4 blood samples. One of these LSS had the highest coefficient of determination (0.999), but required a wait time of 12 hours. This equation was derived from all of the concentration-time data. Fifteen of the other equations were derived from concentration-time data gathered during the first 3 hours post-dose. The other equation requiring 4 blood samples had a patient wait time of 3 hours. Four LSS required 3 blood samples, 6 LSS required 2 blood samples, and the remaining 4 LSS required 1 blood sample. Because these equations were derived from concentration-time data in the first 3 hours post-dose, the maximum patient wait time was 3 hours, and in 7 cases, it was less than this. Coefficients of determination were obtained for all LSS generated. In addition, in order to aid in

the choosing of the optimal LSS developed specifically for lung transplant patients, the bias, precision, and percent prediction error (%pe) range of each LSS was calculated using the concentration-time data gathered from the 6 patients in Phase II (Table 14). Taking into account the number of blood samples required, patient wait time, coefficient of determination, bias, precision, and %pe range, we defined the following equation as the optimal LSS for the population of lung transplant patients studied:

$$AUC = 1.46 \times C_1 + 5.36 \times C_3 + 274.49$$

In order to confirm this LSS was the optimal LSS, the predictive performance of all of the LSS developed was compared (Table 15). This comparison was performed primarily to determine if the LSS compared favorably to equations that required more blood samples in terms of bias and precision.

When compared to the 1-point LSS, there were no significant differences in bias. However, the recommended LSS was significantly more precise than 3 of the 4 1-point LSS.

When the 2-point LSS were compared, it was found that there were also no significant differences in bias, while the recommended LSS was significantly more precise than 4 of 5 2-point LSS compared.

There were no significant differences in bias or precision when the recommended LSS was compared to the various 3-concentration LSS, with 3 exceptions. The recommended LSS was significantly less biased than 1 equation, was significantly more precise than another, but was significantly more biased than a 3rd 3-concentration equation. (The only unfavorable comparison, with the 3-concentration requiring blood samples at 0, 1, and 3 hours post-dose, is italicized, boldfaced, and underlined in Table 15).

Figure 3: The mean log concentration versus time curve for 14 stable, adult lung transplant patients receiving a steady-state dosage of cyclosporine (Neoral®).

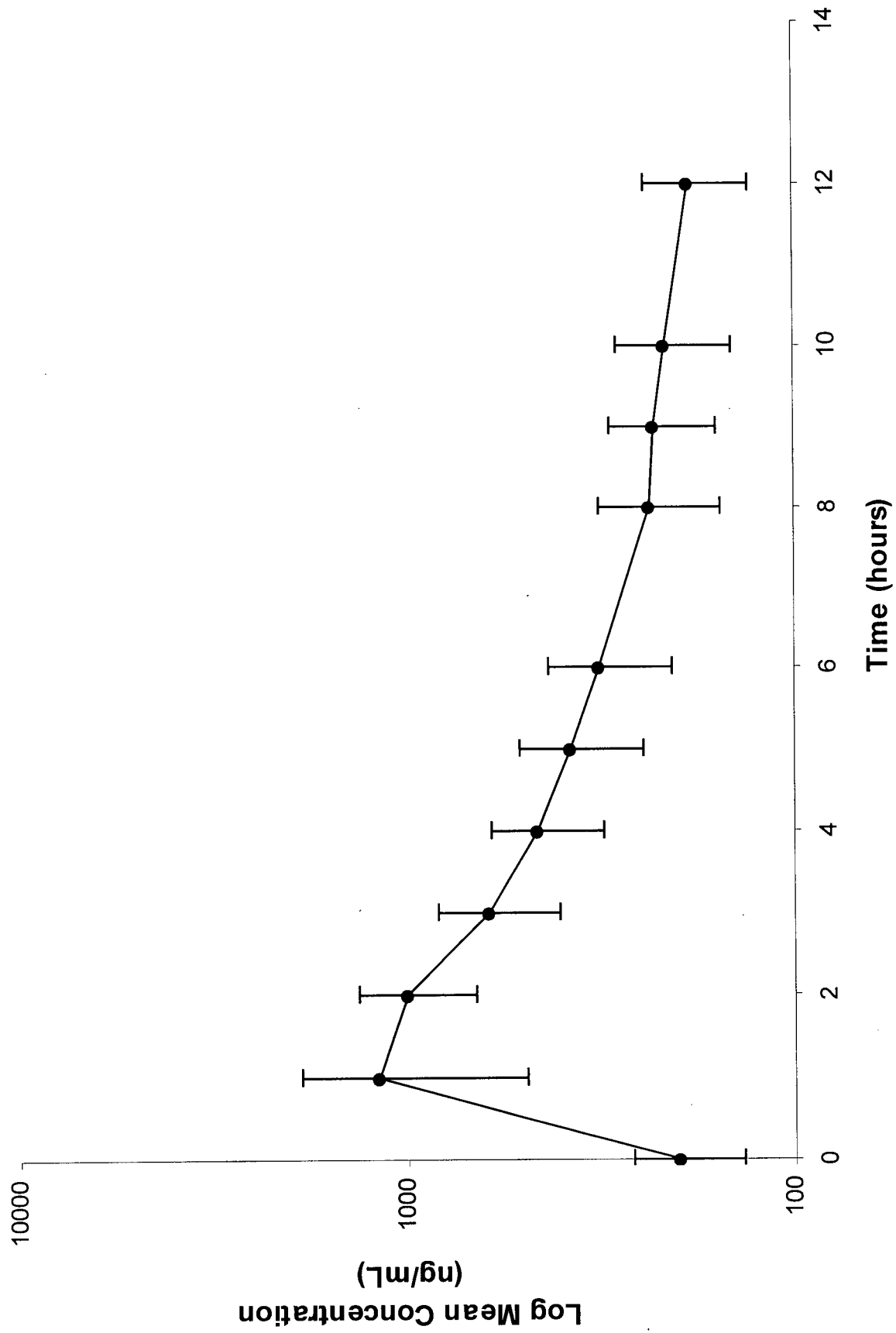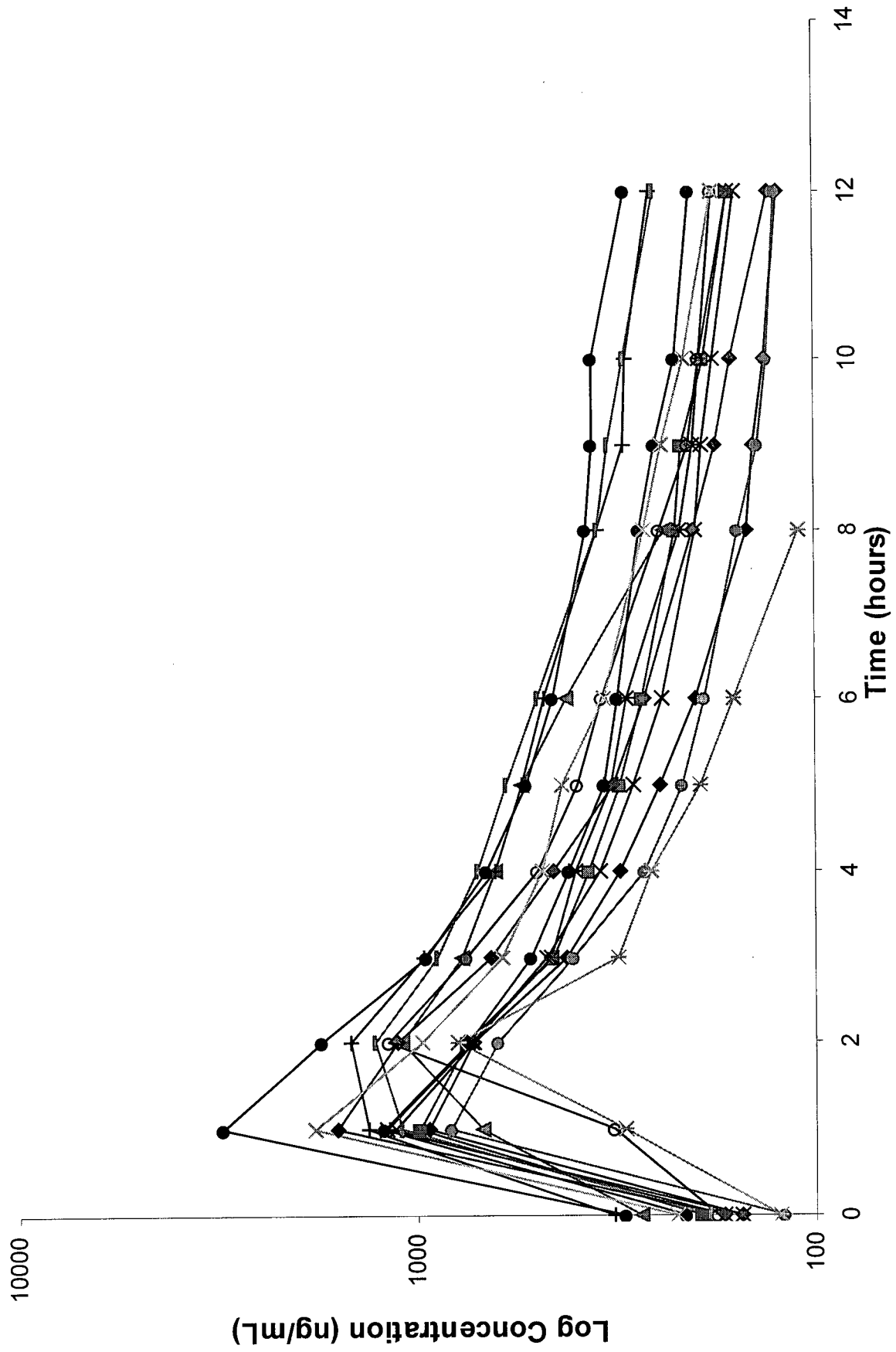**Figure 4:** The log concentration versus time curves for 14 stable, adult lung transplant patients receiving a steady-state dosage of cyclosporine (Neoral®).

Finally, there were no significant differences in bias or precision between the recommended LSS and the 4-point equation derived from all of the concentration-time data gathered in the first 3 hours post-dose.

Table 13. The limited sampling strategies, and their respective predictive performance, derived from concentration-time data from 8 stable, adult lung transplant patients.

AUC Equation	r^2	%pe (range)	ME (ng×hr/mL)	MAE (ng×hr/mL)
a) Equation from multiple regression analysis of all concentration-time data				
AUC = $17.24 \times C_6 - 58.96 \times C_8 + 23.39 \times C_9 + 52.29 \times C_{12} - 796.07$	0.999	-45.50 to 13.99	-876.83	1267.77
b) Equation using all concentration-time points in the first 3 hours post-dose				
AUC = $2.99 \times C_0 + 1.17 \times C_1 - 2.69 \times C_2 + 8.60 \times C_3 - 592.31$	0.936	-11.05 to 10.08	21.29	277.29
c) Three-concentration equations using concentration-time points in the first 3 hours post-dose				
AUC = $0.96 \times C_0 + 1.46 \times C_1 + 3.42 \times C_2 + 34.52$	0.945	-11.58 to 44.80	440.73	710.28
AUC = $1.56 \times C_0 + 1.34 \times C_1 + 4.96 \times C_3 + 320.54$	0.964	-6.71 to 9.01	102.86	259.02
AUC = $13.05 \times C_0 - 8.24 \times C_2 + 14.27 \times C_3 + 1634.10$	0.908	-51.00 to 13.23	-1419.09	1567.58
AUC = $1.42 \times C_1 - 1.52 \times C_2 + 7.61 \times C_3 + 403.53$	0.964	-2.33 to 10.08	44.94	253.63
d) Two-concentration equations using concentration-time points in the first 3 hours post-dose				
AUC = $19.13 \times C_0 - 0.03 \times C_1 + 1167.63$	0.846	-33.35 to 54.53	-918.91	1355.62
AUC = $14.13 \times C_0 + 1.13 \times C_2 + 1053.43$	0.886	-27.04 to 62.08	-692.80	1193.02
AUC = $13.32 \times C_0 + 1.95 \times C_3 + 1119.87$	0.899	-26.79 to 48.33	-764.87	1177.76
AUC = $1.54 \times C_1 + 3.59 \times C_2 - 8.61$	0.963	-10.36 to 44.67	519.50	760.78
AUC = $1.46 \times C_1 + 5.36 \times C_3 + 274.49$	0.975	-4.47 to 8.47	194.60	298.62
AUC = $-9.10 \times C_2 + 18.74 \times C_3 + 2342.01$	0.741	-53.95 to 25.49	-1235.06	1694.17
e) One-concentration equations using concentration-time points in the first 3 hours post-dose				
AUC = $19.06 \times C_0 + 1150.60$	0.884	-33.31 to 53.80	-905.49	1336.46
AUC = $1.28 \times C_1 + 3597.49$	0.130	-28.40 to 75.27	-162.37	1222.13
AUC = $3.45 \times C_2 + 1638.14$	0.737	-19.58 to 91.88	-220.80	1078.78
AUC = $5.22 \times C_3 + 1789.53$	0.770	-29.41 to 50.88	-498.12	1062.24

Table 14. Comparisons of predictive performance between the recommended limited sampling strategy for lung transplant patients and the remaining limited sampling strategies for lung transplant patients resulting from Phase I multiple regression analysis.

LSS Equation	MDPE	95% CI	MDAE	95% CI
$AUC = 19.06 \times C_0 + 1150.60$	1100	-49 to 2249	-1038	**-1825 to -250**
$AUC = 1.28 \times C_1 + 3597.49$	357	-841 to 1555	-924	**-1670 to -178**
$AUC = 3.45 \times C_2 + 1638.14$	415	-703 to 1534	-780	**-1524 to -36**
$AUC = 5.22 \times C_3 + 1789.53$	693	-453 to 1839	-764	-1565 to 37
$AUC = 19.13 \times C_0 - 0.03 \times C_1 + 1167.63$	1114	-52 to 2279	-1057	**-1853 to -261**
$AUC = 14.13 \times C_0 + 1.13 \times C_2 + 1053.43$	887	-167 to 1942	-894	**-1574 to -215**
$AUC = 13.32 \times C_0 + 1.95 \times C_3 + 1119.87$	959	-78 to 1997	-879	**-1571 to -188**
$AUC = 1.54 \times C_1 + 3.59 \times C_2 - 8.61$	-325	-812 to 162	-462	**-855 to -69**
$AUC = -9.10 \times C_2 + 18.74 \times C_3 + 2342.01$	1430	-438 to 3297	-1396	-2859 to 68
$AUC = 0.96 \times C_0 + 1.46 \times C_1 + 3.42 \times C_2 + 34.52$	-246	-711 to 219	-412	**-775 to -48**
$AUC = 1.56 \times C_0 + 1.34 \times C_1 + 4.96 \times C_3 + 320.54$	92	***5 to 179***	40	-74 to 153
$AUC = 13.05 \times C_0 - 8.24 \times C_2 + 14.27 \times C_3 + 1634.10$	1614	**83 to 3145**	-1269	-2586 to 48
$AUC = 1.42 \times C_1 - 1.52 \times C_2 + 7.61 \times C_3 + 403.53$	150	-59 to 358	45	-49 to 139
$AUC = 2.99 \times C_0 + 1.17 \times C_1 - 2.69 \times C_2 + 8.60 \times C_3 - 592.31$	173	-12 to 359	21	-62 to 104

3.5 Phase II: Comparisons Of Predictive Performance

Of the 21 LSS reported from the 16 clinical studies identified, 11 were deemed eligible for comparison in Phase II of the clinical study (Table 16). Five of the LSS deemed not eligible for comparison were developed using the SIM formulation, and 3 of these studies with SIM used once daily dosing. These LSS were excluded from analysis because of the pharmacokinetic differences between the SIM and NEO formulations of CSA. Also, once daily dosing is no longer used in clinical practice.

The LSS proposed by Foradori and colleagues (Foradori *et al* 1995) was appropriate for comparison. However, the 3-concentration equation required a blood sample at 2.5 hours post-

dose, for which we did not have concentration-time data. For this reason, the LSS was excluded from analysis.

The 3-concentration LSS proposed by Amante and colleagues (Amante *et al* 1996) required a blood sample for which we did not have concentration-time data (at 1.5 hours post-dose).

For the 4 LSS reported by Cooney and colleagues (Cooney *et al* 1996), only 1 was eligible for further evaluation. One LSS required a blood sample at 3.5 hours post-dose, for which we did not have concentration-time data. The other 2 LSS, one a 2-concentration LSS, and the other, a 3-concentration LSS, were deemed not eligible because they both required a 12 hour post-dose trough concentration. This is not clinically feasible, except perhaps in an inpatient setting. However, because the stable, adult lung transplant patients studied were followed exclusively on an outpatient basis, this was not appropriate.

All of the LSS compared with our derived, optimal LSS for lung transplant patients involved either 2-concentration or 3-concentration LSS. There were no significant differences in bias or precision with 5 exceptions. Of these 5 exceptions, only 1 was unfavorable. Our optimal LSS was significantly less biased than the LSS proposed by Meier-Kriesche and colleagues (Meier-Kriesche *et al* 1998) and Marsh (Marsh 1999), and significantly more precise than the LSS proposed by the same investigators. The derived LSS was significantly more biased than the 3-concentration LSS proposed by Gaspari and colleagues (Gaspari *et al* 1997).

Table 15. Comparisons of predictive performance between the recommended limited sampling strategy for lung transplant patients and other previously published limited sampling strategies developed for non-lung transplant patient population.

Study	Population	Equation
Johnston et al 1996	Renal	$AUC = 1.96 \times C_{2h} + 11.5 \times C_{8h} + 355.2$
Serafinowicz et al 1996	Renal	$AUC = 9.131 \times C_{0h} + 0.784 \times C_{1h} + 2.617 \times C_{2h} + 193.561$
Amante et al 1996	Renal	$AUC = 2.4 \times C_{2h} + 7.7 \times C_{6h} + 195.8$
Keown et al 1996	Renal	$AUC = 1.84 \times C_{0h} + 4.39 \times C_{2h} + 312.66$
Cooney et al 1996	Pediatric Liver	$AUC = 10.19 \times C_{0h} + 4.47 \times C_{3h} + 749.7$
Gaspari et al 1997	Renal	$AUC = 5.189 \times C_{0h} \ 1.267 C_{1h} + 4.150 \times C_{3h} + 135.079$
Primmett et al 1998	Renal	$AUC = 12.34 \times C_{0h} + 2.48 \times C_{2h} + 441.42$
Primmett et al 1998	Renal	$AUC = 9.55 \times C_{0h} + 0.96 \times C_{1h} + 2.05 \times C_{2h} + 112.07$
Meier-Kriesche et al 1998	Renal	$AUC = 1.84 \times C_{2h} + 4.39 \times C_{4h} + 129$
Lemire et al 1998	Renal	$AUC = 1.023 \times C_{1h} + 13.10 \times C_{6h} + 242$
Marsh 1999	Renal	$AUC = 6 \times (C_{0h} + C_{4h})$

Table 15. (Continued)

Study	Population	%pe (range)	ME (ng×hr/mL)	MAE (ng×hr/mL)	95%CI (ng×hr/mL; bias/precision)
Johnston et al 1996	Renal	-15.07 to 38.85	-332.34	630.14	-219.43 to 1273.31/ -798.75 to 135.72
Serafinowicz et al 1996	Renal	-13.05 to 59.19	270.77	574.67	-563.93 to 411.59/ -704.79 to 152.70
Amante et al 1996	Renal	-17.34 to 46.16	-355.77	746.87	-307.24 to 1407.98/ -977.45 to 80.95
Keown et al 1996	Renal	-17.88 to 76.15	-178.53	785.51	-467.31 to 1213.57/ -1111.59 to 137.82
Cooney et al 1996	Pediatric Liver	-17.88 to 49.92	-112.49	629.03	-438.99 to 1053.17/ -853.29 to 192.47
Gaspari et al 1997	Renal	-11.33 to 8.16	-27.15	241.50	**62.74 to 380.77/** -184.32 to 298.56
Primmett et al 1998	Renal	-20.71 to 40.52	-94.71	433.87	-211.36 to 789.99/ -671.77 to 401.28
Primmett et al 1998	Renal	-15.88 to 72.33	-168.65	753.07	-432.89 to 1159.39/ -1034.64 to 125.75
Meier-Kriesche et al 1998	Renal	-34.30 to 19.21	-1410.95	1556.49	**569.73 to 2641.37/** **-2062.55 to –453.18**
Lemire et al 1998	Renal	-2.27 to 16.44	279.40	375.70	-643.61 to 474.00/ -405.73 to 251.57
Marsh 1999	Renal	-39.20 to 0.34	-1663.14	1665.68	**808.31 to 2907.17/** **-2318.53 to –415.58**

CHAPTER 4

DISCUSSION

The objectives of our study were threefold. First, we sought to define the optimal limited sampling strategy for CSA monitoring in lung transplant patients. Second, we wished to compare the predictive performance of this optimal limited sampling strategy with the predictive performance of previously published limited sampling strategies in other (i.e., non-lung) transplant populations. Finally, we wished to determine the mean steady-state pharmacokinetic parameters of CSA (Neoral®) in our population of lung transplant patients.

To meet the first objective, we defined the optimal limited sampling strategy as a 2-concentration equation for AUC with blood samples required at 1 and 3 hours post-dose. This recommendation was based on the number of blood samples required, clinical feasibility, coefficient of determination, %pe range, bias, precision, as well as comparisons of predictive performance with the other 1-, 2-, 3-, and 4-concentration LSS developed specifically for our population of lung transplant patients. The equation is as follows:

$$AUC = 1.46 \times C_1 + 5.36 \times C_3 + 274.49$$

For the second objective, it was found that the predictive performance of the optimal LSS for lung transplant patients compared very favorably with the predictive performance of previously published LSS developed for non-lung transplant populations. In all comparisons except for 5, there were no significant differences in bias or precision. Of these 5 comparisons in which there were significant differences found in either bias or precision, only 1 was unfavorable. The optimal LSS was significantly less biased than the LSS proposed by Meier-Kriesche and colleagues (Meier-Kriesche *et al* 1998) and Marsh (Marsh 1999), and significantly more precise than the LSS proposed by the same investigators. The optimal LSS was significantly more biased than the 3-concentration LSS proposed by Gaspari and colleagues (Gaspari *et al* 1997).

Of the 14 lung transplant patients who participated in the study, 12 received CSA every 12 hours, and 2 received CSA every 8 hours. This brings to light possible concerns regarding sample homogeneity. We chose to include the 2 lung transplant patients (1 patient in Phase I, and 1 patient in Phase II) who received CSA every 8 hours in all analyses performed for several reasons. The most important reason was that we wanted to develop a monitoring method that was useable for all patients encountered in routine clinical practice. Also, our aim was to develop an LSS that gave a precise and unbiased estimate of AUC during the dosing interval. Because we were interested specifically in the $AUC_{0-\tau}$, the 2 lung transplant patients could be included, because we sampled sufficiently to calculate their $AUC_{0-\tau}$. Thus, our experiment was designed such that the length of the dosing interval did not matter, as long as the drug regimen was at steady-state.

4.1 Steady-State Pharmacokinetic Parameters For Cyclosporine (Neoral®)

The mean steady-state pharmacokinetic parameters for CSA (NEO) in lung transplant patients were determined using concentration-time data gathered from 14 stable, adult lung transplant patients. As mentioned previously, 12 of these patients received CSA every 12 hours, and the other 2 patients, both of whom were documented poor absorbers of CSA, received CSA every 8 hours. The pharmacokinetic parameters of interest clinically (i.e. CL/F, Vd/F, and $t_{1/2}$) were determined. Mean CL/F was 0.30 L/hr/kg, mean Vd/F was 3.64 L/kg, and mean $t_{1/2}$ was 9.52 hours. Mean bioavailability of CSA is generally regarded in the literature as 30%. However, this value is for the SIM formulation of CSA, which is erratically and incompletely absorbed when compared to NEO. To my knowledge, no mean apparent bioavailability figure is available for the NEO formulation. One study has shown that the relative bioavailability of CSA

in the NEO formulation was 1.84 and 2.09 times greater than SIM at dosages of 200 and 800 mg, respectively in heart-transplant candidates (Tan *et al* 1995). However, relative bioavailability cannot be used to convert CL/F to CL and Vd/F to Vd. Thus, I did not believe it was appropriate to calculate apparent clearance (CL) and apparent volume of distribution (Vd) using an F of 30%. The mean apparent bioavailability of CSA in the NEO formulation is certainly higher than 30%, but it is not possible to speculate by how much at this time.

CL/F is an important piece of information, as that parameter has been associated with outcome in renal transplant patients in 2 large clinical studies (Lindholm *et al* 1993, Lindholm and Kahan 1993). Both of these studies compared patients with and without rejection, and patients who had suffered graft loss, and patients who had not. Both studies found that patients with rejection and patients who suffered graft loss had a significantly higher oral clearance (Lindholm *et al* 1993, Lindholm and Kahan 1993). Lindholm and colleagues found that an oral clearance of ≥ 85 L/hr was associated with an increased incidence of rejection, while an oral clearance of ≥ 101 L/hr was associated with an increased incidence of graft loss. An oral clearance of ≤ 59 L/hr was associated with a decreased incidence of acute rejection, while an oral clearance of ≤ 63 L/hr was associated with a decreased incidence of graft loss. Lindholm and Kahan found that an oral clearance of ≥ 90 L/hr was associated with decreased graft survival and an increased incidence of acute rejection.

The mean oral clearance obtained in our current clinical study was approximately 19 L/hr. One possible explanation for the large difference in oral clearances between our study and the one by Lindholm and colleagues is that their study was conducted in the early post-transplant period.

The apparent volume of distribution observed in our clinical study confirms that CSA, likely due to its lipophilic nature, has a large apparent volume of distribution. However, because the parameter calculated is actually Vd/F and not Vd, this parameter also depends on bioavailability. To my knowledge, Vd/F has not been associated with outcome in a clinical trial. Thus, this parameter has limited use both clinically and academically until a bioavailability value for NEO becomes available.

CSA is regarded as a drug with a long terminal elimination half-life, and our value of 9.52 hours is consistent with that generality. A mean terminal elimination value of 5.5 hours has been reported in lung transplant patients (Kesten *et al* 1998a), and values of 6.3 and 8.1 hours have been reported in heart-lung transplant patients (Tsang *et al* 1994). Our value was higher than these. Because of the low numbers of patients involved in these studies (14 lung transplant patients for ours, and an additional 20 lung and 10 heart-lung transplant patients in the other 2), it is just possible that all of the values were within 1 standard deviation of the true mean of the total worldwide population of lung transplant patients. Another possibility may be that the differences are due simply to inter-patient variability. As mentioned previously, 2 patients, both of whom have cystic fibrosis and were documented poor-absorbers of CSA, received CSA every 8 hours. It is possible that the sampling time was not over at least one half-life as is required, resulting in half-life values that may be falsely low. This may be the case, as half-life values of 2.5 and 3.7 hours were obtained for these patients. A mean half-life of 10.6 hours, with a range of 5.9 to 25.5 hours was obtained if the data from these two patients were excluded from analysis. Terminal elimination half-lives of 10.7, 7.4, 15.1, 12.8, 7.4, and 8.7 hours have been reported in renal transplant patients, and a terminal elimination half-life of 6.4 hours has been reported for cardiac transplant patients (Yee and Saloman 1992). The half-life of 9.52 hours obtained in the

current clinical study is consistent with these values. Possible differences could be due to inter-individual variablility, assay methodology, sampling fluid, and differences in pharmacokinetics among different transplant types.

In general, the lung transplant patients who participated in this study were stable, long-term allograft recipients who were free of major adverse effects from CSA. One patient had received a kidney transplant necessitated by CSA nephrotoxicity, but is now stable and free of adverse effects from CSA. Another patient was on dialysis awaiting kidney transplantation, but otherwise was stable and free of major CSA adverse effects. Otherwise, there were no notable instances of major CSA adverse effects that negatively impacted quality of life. One patient had a case of CSA-associated tremor, and several others had well controlled hypertension and hyperlipidemia. Others had noticeable cases of hypertrichosis on the arms and torso.

The mean $AUC_{0-\tau}$ obtained was 5183 ng×hr/mL (range 2272 to 9553 ng×hr/mL), and the mean dose-corrected $AUC_{0-\tau}$ was 2682 ng×hr/mL/mg/kg (range 1263 to 4237 ng×hr/mL/mg/kg). Values of 5944 (Kesten *et al* 1998a), 5318 (Kesten *et al* 1998b), and 7013 (Reynaid-Gaubert *et al* 1997) ng×hr/mL have been reported in lung transplant recipients. Our value was close to 2 of these reported values, but was approximately 2000 units less than the third. The difference may be due to differences in dose, or inter-patient variability in absorption or gastrointestinal cytochrome P-450 3A4 activity. AUC values of 4543 ng×hr/mL (van Mourik *et al* 1999) have been reported in stable liver transplant patients. AUC values of 5951 (Awni *et al* 1989), 2085 (Honcharik *et al* 1991), 3485 (Kovarik *et al* 1994a), and 5020 (Sketris *et al* 1994) ng×hr/mL have been reported in stable renal transplant patients. Inter-patient variability in absorption or metabolism cannot be discounted as reasons for the differences in AUC between the various transplant populations and the population of lung transplant patients studied in the current

clinical study. Also, there are well-documented differences in pharmacokinetic parameters among the various transplant types. The most likely reasons, however, are that larger doses are given to lung transplant patients in order to achieve the higher target CSA concentrations for this type of transplant, and also due to pharmacokinetic differences between SIM and NEO in the older studies. AUC values of 10405 (Gardier *et al* 1993), 2234 (Drewe *et al* 1992), 4471 (Gupta *et al* 1990), 7453 (Lindholm *et al* 1990), 3441 (Kovarik *et al* 1994), and 2981 (Mueller *et al* 1994) ng×hr/mL have been reported in healthy subjects.

Despite several studies published involving AUC monitoring or LSS, the target AUC values for stable patients are uncertain even in the renal transplant population. A tentative "therapeutic AUC range" has been recently proposed for renal transplant patients (Belitsky *et al* 1999). They defined an AUC of 9500 to 11500 ng×hr/mL as the range in which the incidence of acute rejection was the lowest without an increased incidence of nephrotoxicity (Belitsky *et al* 1999). This range is not potentially applicable to our population of lung transplant patients, or even other types of stable transplant patients because the study was done in the immediate post-transplant period. In the study by Belitsky and colleagues (Belitsky *et al* 1999), concentration-time data were gathered after only 3 days on NEO therapy following 1 to 3 days of intravenous CSA. Only 5 blood samples were collected at 0, 1, 2, 3, and 4 hours post-dose, and AUC was extrapolated out to 12 hours. Another study, by the same investigative group (Mahalati *et al* 1999), proposed an AUC_{0-4} range of 4400 to 5500 ng×hr/mL, which corresponded to the same AUC_{0-12} range of 9500 to 11500 ng×hr/mL reported previously. As the study was conducted in the early post-transplant period, these ranges are of little interest to the stable, long-term transplant population. Because the lung transplant patients who participated in our clinical study were stable and free of major adverse effects, it would be reasonable to suggest that the target

AUC value may be in the 5000 to 6000 ng×hr/mL range for lung transplant patients. This suggestion is based on our results, as well as previously published values. However, much more study in this area is needed.

It is important to note that maximum absorption of CSA occurred predictably within 2 hours post-dose. Even in the cases of the 2 documented poor absorbers of CSA, T_{max} occurred within 2 hours post-dose. The parameter, $AUMC_{0-\tau}$, simply defined the mean of a probability distribution, is of little use clinically, and was included mainly to calculate MRT. It is interesting to note that the mean MRT value obtained (21.82 hours) was much greater than the terminal elimination half-life value obtained. It is not unusual for the values to be different, as they have different meanings. By definition, terminal elimination half-life is the time it takes for half of the amount/concentration of drug to be eliminated from the body. Mean residence time, on the other hand, is the arithmetic average of the time that each molecule remains in the body. It can also be more simply defined as the time it takes for 63% of the drug to be eliminated from the body. Because it takes longer to remove 63% than 50% of the body, the MRT value should theoretically be longer than $t_{1/2}$. However, the mean MRT value was approximately double the value for the mean terminal elimination half-life. The reasons for the result are uncertain. It is possible that the method used to calculate MRT somehow takes into account the time it takes for a given CSA molecule to exit the peripheral compartment.

4.2 Multiple Regression Analysis

Multiple regression analysis is an excellent way of generating an equation that allows the calculation of values of a dependent variable using values from one or more independent variables. Following development of a regression equation, a coefficient of determination is

generated, which gives an indication of the degree of association of the independent and dependent variables. It can also give an indication of variance. For example, an r^2 value of 0.999 explains 99.9% of the variance. Because it is not appropriate to use an r^2 value to recommend an LSS, no further related statistical calculations were required. Determination of a Pearson's correlation coefficient, for example, was inappropriate for the current analysis for 2 reasons. First, we wanted to be consistent with the methods of previous investigators, who did not perform this test. Second, correlation analysis does not assume a relationship between the concentration and AUC data, and because our goal was to determine a relationship between these data (via our recommended LSS). Multiple regression analysis, with AUC as the dependent variable, and whole blood CSA concentrations grouped by time as the independent variable, yielded 17 potential LSS capable of providing unbiased and precise estimates of AUC.

To our knowledge, this is the first study that has developed an LSS from concentration-time data obtained specifically from lung transplant recipients. Even with restricting the multiple regression analysis to concentration-time data in the first 3 hours post-dose, 15 potential LSS for lung transplant recipients were delineated. Despite having a very high coefficient of determination, the 4-concentration LSS developed from all of the concentration-time data performed quite poorly in terms of the measures of predictive performance. This is an excellent example of why predictive performance should be evaluated before making a recommendation. Some of the earlier published LSS may not have been optimal, and it has been proposed that equations for some LSS were chosen solely on the basis of a high coefficient of determination value, rather than looking at other criteria such as prediction error (Gaspari *et al* 1998).

The 4-concentration LSS developed from concentration-time data developed in the first 3 hours post-dose performed well in all calculated measures. However, the requirement for 4

blood samples was deemed not clinically feasible, and later comparison with the recommended LSS confirmed that there was no additional advantage in using that LSS over the recommended LSS.

Two of the 3-concentration equations also performed well. There were no unfavorable significant differences in predictive performance with one exception. Specifically, our recommended LSS was significantly more biased than the LSS requiring C_0, C_1, and C_3. We believed that the advantage of one less blood sample outweighed the disadvantage of a small, albeit significant, over-prediction of AUC.

The recommended LSS outperformed the various 1- and 2-concentration LSS in all assessments of predictive performance. An additional, practical benefit of using C_1 instead of C_0 is that it eliminates the problem of patients inadvertently taking their CSA dose before the trough sample is drawn, thus making it essentially useless in helping to guide therapy.

Although the reason for inclusion of the 2 lung transplant patients who received CSA every 8 hours was adequately justified in a previous section, we wanted to be thorough. Multiple regression analysis was performed on concentration-time data obtained in the first 3 hours post-dose from the 7 lung transplant patients in Phase I who received CSA every 12 hours. The following equation was the result:

$$AUC = 1.52 \times C_1 + 5.31 \times C_3 + 267.89$$

An r^2 value of 0.971 was obtained for this equation. The concentration-time data from the 6 patients in Phase II was used to assess the predictive performance of this LSS. Percent pe ranged from –4.26 to 12.64%, which exceeded our previously defined limit of acceptable error in AUC prediction. Bias was 243 ng×hr/mL, and precision was 342 ng×hr/mL. When the predictive performance of this LSS was compared to the predictive performance of our recommended LSS,

there was no difference in bias. However, the recommended LSS for lung transplant patients was significantly more precise.

These results further support our reasons for inclusion of the lung transplant patients receiving CSA every 8 hours. Exclusion of 1 of these patients in the development of the LSS made it less than optimal for predicting the AUC of all of the different patients encountered in routine clinical practice. This is not surprising as AUC values were similar to patients receiving CSA every 12 hours. Also, because the data analysis was restricted to the first 3 hours post-dose, when all patients were sampled the same regardless of dosing interval, the distinction between patients receiving CSA every 12 hours and patients receiving CSA every 8 hours is not discernable.

4.3 Comparisons Of Predictive Performance

We chose to compare the predictive performance of our LSS with the predictive performance of other published LSS developed for other transplant populations, in part, to see how the AUC estimation in lung transplant patients was compared to AUC estimation by the other LSS. There is some evidence that LSS may be patient-specific, as studies that have evaluated LSS developed in other centers have found that they do not perform as well in terms of percent prediction error for AUC estimation (Gaspari *et al* 1993, 1997, and 1999). Because these studies did not assess predictive performance as suggested by Sheiner and Beal (Sheiner and Beal 1981), and because of the pharmacokinetic differences between transplant types, we believed that comparisons of predictive performance between the recommended LSS for lung transplant recipients and LSS derived from primarily renal transplant recipients would provide some additional insight.

No published LSS developed for other transplant populations had %pe within 10% of the actual AUC value. Because one study was close, and another study was within 20%, it is possible that these LSS would be acceptable for use in lung transplant recipients. However, as might be expected, the recommended LSS, developed specifically using concentration-time data from the population of interest, performed better in terms of %pe. In the majority of comparisons with other LSS, however, there were no differences in predictive performance. In two comparisons, the recommended LSS had more favorable predictive performance. In the only unfavorable comparison, the recommended LSS was significantly more biased than the LSS of Gaspari and colleagues (Gaspari *et al* 1997). However, this LSS required 3 blood samples, and we believe that the advantage of 1 less blood sample outweighed the disadvantage of a small (relative to total AUC) but significant over-prediction of AUC.

Thus, our recommended LSS is the most suitable for future use in our population of lung transplant recipients. However, before this LSS can replace routine trough level monitoring, prospective studies directly comparing the two monitoring strategies, looking at well-defined outcomes such as nephrotoxicity, episodes of rejection, and/or incidence of graft failure, are warranted. Currently, there are some prospective data evaluating outcomes in liver (Grant *et al* 1999), heart (Cantarovich *et al* 1999), and renal (Kelles *et al* 1999) transplant recipients. However, there are no published studies that compare the 2 monitoring methods in lung transplant patients. In addition, these 3 studies (Grant *et al* 1999, Cantarovich *et al* 1999, Kelles *et al* 1999) do not compare the two monitoring methods head-to-head. That is, 1 method (e.g., trough level monitoring) is utilized as a result of data gathering from another method (e.g., AUC_{0-4}). There are not 2 separate groups (i.e., 1 group of transplant patients followed with trough level monitoring, and 1 group of patients followed using an LSS).

Our full evaluation of predictive performance fully tested the appropriateness of our recommended LSS for further prospective use. Earlier studies of this nature in other transplant populations recommended LSS based solely on high r^2 values, which is completely inappropriate. Other studies went further and calculated %pe, which provided some additional, useful information. However, very few studies have determined these values and also carried out the full analysis of predictive performance according to the methods of Sheiner and Beal.

4.4 Conclusion

A limited sampling strategy developed specifically for lung transplant patients is a precise and unbiased method of estimating actual AUC to allow the AUC monitoring of lung transplant patients. The LSS developed in this clinical study clearly outperformed other previously published LSS developed for non-lung transplant populations in terms of percent prediction error range. When predictive performance was compared, in general, there were no significant differences with 3 exceptions. Two of these were favorable, and the unfavorable one indicated an over-prediction in AUC that was small relative to total AUC. In addition, the LSS that was significantly less biased than ours required 3 blood samples, and thus would be more expensive and less clinically feasible than the LSS for lung transplant patients.

The NEO formulation of CSA has a long terminal elimination half-life in lung transplant patients. The value of 9.52 hours was longer than previously published values for lung transplant patients. CSA has a large apparent volume of distribution (Vd/F = 3.64 L/kg), and an apparent oral clearance of 0.30 L/hr/kg. In addition, T_{max} occurred predictably within 2 hours of dosage administration, with a mean T_{max} of 1.35 hours.

In conclusion, based on the number of blood samples required, the patient wait time, coefficient of determination, %pe, and comparisons of predictive performance, the best clinically feasible LSS for cyclosporine AUC estimation for lung transplant patients requires 2 concentrations drawn at 1 and 3 hours post-dose:

$$AUC = 1.46 \times C1 + 5.36 \times C3 + 274.49$$

Few studies that have developed LSS have included appropriate comparisons of predictive performance, thus allowing statistical comparisons of the various LSS. Because we have done so with our LSS, our recommendation of the appropriateness of our LSS for lung transplant patients is more definitive than the recommendations of previous studies.

The most obvious limitation to the clinical study is the relatively small sample size. A convenience sample size was the only option available to the investigative group. Given the limited number of potential subjects available, and based on previously published studies that I have read, 14 is actually a fairly large sample size. Several useful insights were discovered despite this limitation. However, it would have been interesting to compare the pharmacokinetic parameters between lung transplant patients with and without cystic fibrosis. Such statistical comparisons could have been made as is, but given that only 6 patients had cystic fibrosis, there would not have been sufficient power to detect a difference.

Another limitation is the lack of blood samples, and therefore concentration-time data in the absorption phase. There was only 1 blood sample to characterize the upswing of the curve, and thus, it was not possible to calculate the absorption rate constant. This resulted in the loss of reporting of an oral dosing pharmacokinetic parameter. Knowledge of the absorption rate constant also would have allowed the calculation of the volume of distribution at steady state, thus giving an estimate of volume of distribution that did not depend on mean absolute

bioavailability, unlike the Vd/F parameter calculated in this study. Additional blood samples (e.g., at 15 minutes, 30 minutes, and 45 minutes post-dose) would have been useful. However, the primary concern was in obtaining enough samples to give an accurate calculation of AUC for multiple regression analysis, and not the calculation of pharmacokinetic parameters.

Because this study enrolled stable, long-term lung transplant patients, the results provide no insight into another common area of transplant medicine: the early post-transplant period. This period is critical for lung transplant patients given the common occurrence of acute rejection, and the prognostic significance of those episodes in terms of chronic rejection.

Pharmacokinetic data for lung transplant patients are relatively sparse in the literature, especially when compared with the pharmacokinetic data available for renal transplant patients. Some of the estimates of the mean pharmacokinetic parameters generated by this clinical study, such as apparent oral clearance, and especially terminal elimination half-life, can potentially aid in dosage adjustments, for example. Specifically, by using a combination of available concentration-time data, and literature values of pharmacokinetic parameters such as oral clearance, patient-specific parameters such as terminal elimination half-life and apparent volume of distribution could be calculated. These parameters could be used to calculate patient-specific dosages and dosing intervals for patients found to be not in the "therapeutic range".

We have developed a feasible LSS available for use in lung transplant patients followed at VHHSC solid organ transplant clinic. Following publication in a transplantation journal, the LSS will be available to other centers. Further randomized, prospective studies comparing the LSS with trough level monitoring are required before the LSS can replace routine trough level monitoring as the standard therapeutic drug monitoring method. We have provided a potentially

useful tool for future changes in the practice of therapeutic drug monitoring of CSA in lung transplant patients.

As stated in section 4.5, our LSS cannot be applied to lung transplant patients in the early post-transplant period, when prevention of acute rejection is of the utmost importance. In addition, the LSS was developed using lung transplant patients who were on a steady-state dosage of CSA. In the early post-transplant period, there are usually several dosage adjustments, and steady-states can often take 1 or more months to achieve.

Currently, it is not known what AUC values to target. There has been an AUC_{0-12} and an AUC_{0-4} range proposed for renal transplant patients in the very early post-transplant period. However, this is of little use for our target transplant population. A possible future clinical study is one that attempts to define a "therapeutic AUC range" for lung transplant patients. Because the mean AUC obtained from the current clinical study was approximately 5200 ng×hr/mL, it would be reasonable to include a patient group of randomly assigned lung transplant patients who are followed by targeting an AUC range of 5000 to 6000 ng×hr/mL. There could be an additional group followed by targeting a higher AUC range, and one followed by targeting a lower AUC range. The most appropriate AUC range could then be determined by looking at surrogate markers of outcome, such as incidence of acute rejection, or incidence of nephrotoxicity.

Once an appropriate "therapeutic AUC range" has been established, another clinical study that would allow the prospective comparison of the LSS for lung transplant patients with the current standard method of therapeutic drug monitoring (i.e., trough level monitoring) could be undertaken. Again, well defined surrogate markers of outcome could be assessed. Because the study needs to be of sufficient duration to assess the superiority of one method over another,

markers such as incidence of graft loss, number of hospital readmissions, length of stay, etc. could be assessed in addition to the usual markers. These 2 studies would then define the role of LSS in the routine monitoring of lung transplant patients.

Additional clinical studies using the pharmacokinetic data obtained from our clinical study could also be performed. A prospective clinical study, with a blood sampling schedule similar to the current clinical study, could be performed to see if any pharmacokinetic parameters could be associated with outcome, as has been done in renal transplant patients.

We have recommended a LSS developed specifically for lung transplant patients, and to our knowledge, we are the first to do so. Our recommendation was based on evaluation of all possible criteria. Our investigative group now has a monitoring strategy that is useable in further clinical studies involving lung transplant patients to see if AUC monitoring through the use of LSS has a place in the therapeutic drug monitoring of CSA in lung transplant patients. Because the results of our clinical study will be published in a transplantation journal, the monitoring strategy will be available for further clinical studies in lung transplant patients at other centers. Thus, the results of our study have contributed to, and filled a gap in, the existing transplantation and pharmacokinetic literature.

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