

**Therapeutic Drug Monitoring and Population Analysis as Applied to
Leflunomide and Cyclosporine Therapy in Dogs with Immune-mediated Diseases**

by

Jameson Smoak Sofge

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Approved by

Dawn Boothe, Chair, Professor of Veterinary Medicine
Robert Judd, Boshell Associate Professor of Veterinary Medicine
James Wright, Professor of Veterinary Medicine

Abstract

Two immunomodulator drugs, leflunomide (teriflunomide is its active metabolite) and cyclosporine, are often monitored in veterinary patients in order to support the design of dosing regimens when used to treat immune-mediated diseases. Such information can be compiled to offer population-based recommendations. However, while cyclosporine has been well studied in normal animals, leflunomide has not been. This thesis describes the disposition of leflunomide in normal dogs, and an assessment of factors that impact drug concentrations for either drug in patients with clinical disease.

For leflunomide, apparently normal, healthy dogs (n=4) were orally dosed with 4 mg/kg and it and teriflunomide were quantified in plasma using HPLC. No leflunomide was detected in any of the samples. Mean pharmacokinetic values for teriflunomide were: $C_{max}=18.9\pm 8.5$ mcg/mL and half-life= 25 ± 10 hr. For clinical patients, samples were monitored between January 2012 and May 2014. In animals that responded to treatment, the recommended therapeutic range of teriflunomide is 25-45 mcg/mL. Approximately 77% of dogs on leflunomide were considered responders. The mean dose of leflunomide used to achieve control was 2.8 mg/kg once daily. The correlation (r) between leflunomide dose and teriflunomide plasma concentrations was 0.26.

For cyclosporine, clinical samples were monitored between October 2010 and May 2014. Marked variability was seen amongst cyclosporine plasma concentrations. The correlation (r) between cyclosporine dose and plasma concentration ranged from 0.0007-0.32 depending on

dosing interval and sample collection time. Half-life also showed marked variability.

Approximately 60% of dogs on cyclosporine were considered responders. Covariate analysis revealed the following potentially significant relationships with cyclosporine plasma drug concentrations: breed, disease, ketoconazole, and generic preparations. Covariates with a significant relationship with response were co-treatment of cyclosporine and prednisone or prednisolone, or disease (arthritis, anemia, and inflammatory bowel disease). This study supports the need for monitoring based on the lack of dose-response relationships for either drug. Several cofactors appear to influence concentrations.

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List of Abbreviations

A77-1726 Teriflunomide

Atopy Atopic dermatitis

AUC Area under the curve

BID Every 12 hours (twice daily)

CI Confidence interval

Cl Clearance

C_{max} Maximum plasma drug concentration

CPL Auburn University Clinical Pharmacology Laboratory

CsA Cyclosporine

ELISA Enzyme-linked immunosorbent assay

F Bioavailability

FL Fluorescence

GC Gas chromatography

GME Granulomatous meningoencephalitis/myelitis

HPLC High performance liquid chromatography

IBD Inflammatory bowel disease

IMHA Autoimmune hemolytic anemia

ITP Immune-mediated thrombocytopenia

LLOD Lower limit of detection

LLOQ	Lower limit of quantitation
LOD	Limit of detection
LOQ	Limit of quantitation
MS	Mass spectrometry
MRT	Mean residence time
PDC	Plasma drug concentration
PRCA	Pure red cell aplasia
QD	Every 24 hours (once daily)
RIA	Radioimmunoassay
TDM	Therapeutic Drug Monitoring
Tmax	Time to maximum plasma drug concentration
ULOD	Upper limit of detection
ULOQ	Upper limit of quantitation
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
Vd	Volume of distribution

Chapter 1: Background

1.1: Drug Movement

Drug movement may be described in three different phases: absorption (A) of the drug from the site of administration into systemic circulation, distribution (D) of the drug from circulation into tissues and back into peripheral circulation, metabolism (M) of the drug (most commonly in the liver), and excretion (E) of the drug from the body either unchanged by metabolism or as a metabolite. In humans and veterinary medicine, metabolism most commonly occurs in the liver, and excretion occurs through the kidney or bile.

Each drug movement (ADME) is influenced by physiologic as well as pharmacologic properties which may reflect the patient's ability to respond to treatment with a drug. For oral absorption, the major determinants of drug absorption into peripheral circulation are: gastrointestinal pH (weak acids are readily absorbed, however, weak bases are trapped and are unable to be absorbed), surface area (the small intestine), thickness of the mucosa (absorption inhibited by inflammatory infiltrates), epithelial permeability (vascular compromise and subsequent lack of epithelial barrier), and motility. Inherent properties of a drug may also alter the rate of absorption through manipulation of the drug's chemistry that alters the pH or concurrent administration with food or other drugs which inactivate the product.

Distribution is influenced by the degree of drug binding to plasma proteins, organ blood flow, capillary fenestration, and environmental pH. Metabolism, which occurs most frequently in the liver, is responsible for activating a drug (pro-drug to active drug), inactivating a drug, or forming active metabolites. This is accomplished by a two stage process involving a change of

the original lipid soluble drug to polar molecules (phase I) in preparation for conjugating the molecule (phase II) with a large water soluble molecule such as glucuronide or glutathione.

Excretion of a drug most commonly occurs through the kidneys, and can be influenced by blood flow, glomerular filtration, active tubular secretion, and passive reabsorption. A drug excreted solely by filtration should be cleared at a rate similar to the patient's glomerular filtration rate; where as a drug that is actively secreted in the urine may exceed the filtration rate. Excretion into the bile is most commonly seen with drugs that are very lipid soluble such as drugs that are greater than 600 MW in size. Accounting for these factors that affect a drug's absorption, distribution, metabolism, and excretion can be used to predict changes in drug concentration that will occur in the patient such that the dosing regimen can be appropriately modified to facilitate safe and effective therapy (Boothe, 2012).

1.2: Pharmacokinetics/Pharmacodynamics

Simplistically, pharmacokinetics is the study of what the body does to the drug. More specifically, it is the science of the time course of (generally plasma) drug concentrations and as such, is a means of describing mathematically the absorption (if extravascular), distribution, metabolism and excretion of a drug. Pharmacokinetic parameters are modeled based on measurable criteria such as dose, body weight, and plasma drug concentration. In general, drugs most commonly move by passive diffusion, with drug movement generally following first order kinetics, meaning that a constant fraction of drug moves per unit time. The line that is derived from drug movement over time is defined by a slope (rate constant = k) and Y intercept (C_0). C_p is the predicated concentration at any time (t) ($C_p = C_0 e^{-kt}$). For each drug movement, or change

in the rate of elimination of drug from the body, a Y intercept and slope will contribute to the equation (eg, two compartment model: $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where A and B and α and β are the respective Y intercepts and slopes for the first and second components respectively).

Pertinent pharmacokinetic parameters that describe drug behavior that can be determined from plasma drug concentration versus time data. These include (example units) but are not limited to:

1. C_{max} (mg/ml) is the maximum plasma drug concentration achieved at the given dose, after extravascular administration with C_0 being the extrapolated (from the Y axis) “maximum” concentration after IV administration.

2. Time to C_{max} (T_{max} ; min) is the time to maximum plasma drug concentration after extravascular administration.

3. Area under the curve (AUC; mg/L/min) which describes the total time course of the drug (usually calculated by C_0/k_{el} if one compartment, $A/\alpha + B/\beta$, if two, etc.).

4. Volume of distribution (V_d ; L/kg), that is, the (theoretical or apparent) the volume that the drug would have to be distributed to if present throughout the body at the same concentration as that measured in plasma. V_d inversely influences PDC and is a direct determinant of dose (larger V_d leads to lower PDC). Volume of distribution also influences half-life: a large V_d pulls the drug into tissues, away from the organs of clearance, thus prolonging its elimination from the body.

5. Elimination half-life. Defined as an inverse relationship with the slope such that half-life = $0.693/k$; for each component of the curve, a half-life can be determined. In the case of multicomponent curves, the half-life which describes the elimination of drug from the body is derived from the slope of the terminal component of the plasma drug concentration versus time

curve. Elimination half-life is important because it determines the amount of fluctuation between C_{\max} and C_{\min} and is the basis for determining the dosing interval. A short elimination half-life – such as occurs for cyclosporine – may be undesirable if response to therapy is dependent on the presence of the drug throughout a dosing interval. The elimination half-life is the pharmacokinetic variable used to determine duration of time necessary to remove the drug from the patient's body. At one half-life, 50% is eliminated; with the next half-life, 50% of the remaining drug is eliminated (25%) for a total of 75%; at 3 half-lives, another 12.5% is eliminated (half of 25%) for a total of 87.5%; by 4 half-lives, 93.75% is eliminated, and by 5 half-lives, 96.9% is eliminated. In general, 3 half-lives is enough time for drug movement to be clinically complete.

Half-life also determines the time to reach steady-state equilibrium (steady-state), the time point at which the amount of drug leaving the body is equal to the amount reaching systemic circulation, thus maintaining consistent PDC. Steady-state is reached after 3 to 5 drug half-lives have lapsed once a new dosing regimen has started, and only with drugs that accumulate with multiple dosing will reach steady-state. A drug with a long half-life, such as leflunomide, may present challenges in that the time to steady-state may be unacceptably long. However, in such instances, once steady-state is achieved, the patient might be “protected” from therapeutic failure that might otherwise occur with dramatic fluctuation in drug concentrations that is typical of drugs with short half-lives. Knowing whether a drug has a short or long half-life may be important for therapeutic success. Monitoring approaches also will vary for drugs with short half-lives, for which both a peak and trough concentration might be important, versus drugs with a long half-life, for which a sample collected any time during the dose interval may be sufficient.

6. Clearance. As important as half-life is, it is also the parameter that is most subject to change in response to physiologic, pharmacologic and pathologic factors. The rate of elimination (min^{-1}) or $t_{1/2}$ (min) of a drug reflects the effect of two pharmacokinetic parameters. Elimination is directly proportional to the volume of blood cleared per unit time, referred to as clearance (CL: ml/kg/min) and indirectly (inversely) proportional to volume to which the drug is distributed, referred to as volume of distribution (a drug that is distributed to tissues is not available for clearance by the liver or kidney). For example, if volume of distribution of a drug decreases (eg, in a dehydrated patient), more drug is in each volume cleared, and elimination increases (half-life is shorter) even if clearance does not change. However, if clearance also were to decrease (e.g., decreased GFR), less volume would be going through the kidney and thus elimination would not change, despite profound differences in drug disposition. Like V_d , however, CL only can be determined after IV administration of a drug since it reflects the proportion of the V_d that is irreversibly cleared per unit time ($CL = V_d \cdot K$) (Boothe, 2012).

1.3: Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is a tool that is used to guide dosing regimens for drugs that are likely to be characterized by marked variability in disposition and thus are not characterized by a strong relationship between dose and plasma drug concentration or response. It can replace the trial and error approach to design of dosing regimens. As such, it is most important for drugs that are potentially toxic, characterized by a poor dose-plasma drug concentration–response relationship or for disease for which success or failure indicators are insensitive or put the patient at risk (i.e., epilepsy, immune-mediated disease). TDM is based on

validated methods that quantitate the amount of drug (parent or active metabolite) in plasma, serum, or whole blood. There are multiple methods by which drugs can be quantitated in body tissues, including plasma. Among the more common methods are immunoassays, which use compound-specific antibodies in conjuncture with a reporter molecule (such as enzyme-linked immunosorbent assays (ELISA) or radioimmunoassay (RIA)). However, the disadvantage of this method is that the antibodies directed toward the drug may not sufficiently discriminate between parent and metabolite. The use of monoclonal – as opposed to polyclonal- antibodies increases the accuracy of immunoassays in the presence of metabolites. Further, if the metabolite is active, then the assay ideally will detect all active components. Immunoassays have been developed for a number of drugs using automated instrumentation, thus facilitating the accurate, yet rapid measurement of drug in the body, at a reasonable cost. Cyclosporine is among the drugs for which an immunoassay for use in humans has been approved by the Food and Drug Administration. However, although based on a monoclonal antibody, it nonetheless does detect several metabolites.

Alternative methods for quantitating drug in the body include, but are not limited to, high performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), or gas chromatography (GC) separation with ultraviolet (UV), fluorescence (FL), or mass spectrometry (MS). The method must be rapid, cost effective, precise (repeatable), accurate, and specific (able to distinguish the target drug from other drugs) (Boothe, 2012). An advantage of these methods is that parent compounds can be discriminated from metabolites. However, these methods often are tedious, requiring calibration with each “run”, and require costly, high maintenance instrumentation. Further, method based on these methods can generally be developed for most drugs without the need of producing antibodies.

TDM is a tool that can be helpful in designing and maintaining a dosing regimen. Therapeutic ranges offer guidelines for target PDC based on the PDC at which most patients might respond. However, a “therapeutic range” is a population statistic, indicating the range of concentrations between which a sample population is expected to respond to the drug. As such, a therapeutic range offers a population target. However, for the individual patient, each patient is different and a patient-specific concentration should be the basis for TDM. When to draw the sample(s), both amount of time after starting the treatment and time post dose is dictated by the pharmacokinetics of the drug, which should be established in the target species. TDM can provide useful information at several different time-periods in the cycle of treatment (Boothe, 2012).

Once a loading dose is complete (for drugs with a long elimination half-life), or once a maintenance dose has reached steady state (typically 3 – 5 half-lives), a baseline value can provide valuable information regarding the therapeutic target for the patient. Periodic rechecks can ensure that effective concentrations are maintained and that toxic concentrations are avoided, potentially preventing adverse events such as therapeutic failure or drug toxicity. If an adverse event does occur, monitoring can help identify the cause of therapeutic failure or confirm toxicity. Changes in patient factors (such as dose, dose interval, disease, diet, or other treatments) may also provide grounds for checking levels to identify impact on treatment exposure (Boothe, 2012).

1.4: Clinical Pharmacology Laboratory

The Auburn University Clinical Pharmacology Laboratory (CPL) performs veterinary TDM on canine, feline, equine, and exotic species. Whole blood, serum or plasma samples to be tested for specific drugs being used to control disease are submitted from laboratories and veterinary clinics throughout the United States and internationally. These results in conjunction with recommendations by the clinical pharmacologist or residents are used to make medication decisions.

The CPL results are kept in a Microsoft® Access (2007-2013) database. The current database contains results from January, 2003 through the present. In this database are greater than 60,000 submissions representing more than 70,000 results. Along with measured drug concentrations, the database also contains other pieces of patient medical records. This includes, but is not limited to: species, breed, gender, age, history, drugs, dosages, clinical response, and drug side effects. The data collected in this database has served to provide clinically relevant retrospective analysis for the areas of small animal seizure management, anti-microbial recommendations for both small and large animals, as well as immunomodulator therapy in small animal practice.

1.5: Study Design

Among the drugs for which TDM can provide effective guidance are those that target the immune system. Both therapeutic failure and overzealous therapy can result in life-threatening consequences to the veterinary patient. Among the most common immunomodulating drugs used

in veterinary medicine is cyclosporine. Cyclosporine is approved for use in dogs and as such, has a long history of successful use. Surprisingly, however, little information is available regarding relationships among dose, plasma drug concentrations and response in dogs. Recently, the use of leflunomide, an immunomodulating drug with a differing mechanism of action, has emerged in dogs that are not sufficiently responding to cyclosporine.

In this study we focused on improving the use of both of these drugs in animals through TDM and two approaches were used. First, because essentially no information is available regarding monitoring of leflunomide in animals, we set out to determine the conditions under which TDM of leflunomide might take place, including determining a therapeutic range. This necessitated development of an HPLC method for quantitation of leflunomide and its active metabolite teriflunomide in canine serum and plasma. Because little information was available regarding the pharmacokinetics of leflunomide in dogs, its disposition after oral administration was evaluated by giving leflunomide to dogs and measuring the leflunomide and teriflunomide concentrations in plasma. The conditions for monitoring leflunomide were also determined. Signalment, history, presenting complaint and response to therapy of clinical samples submitted for the leflunomide assay, were examined to identify trends and relationships among PDC, controlled clinical response, and potential covariates.

Secondly, we attempted to answer questions regarding the proper methods for monitoring cyclosporine in dogs. This approach was based on retrospective information collected from samples submitted for TDM by small animal practices in patients receiving cyclosporine for immunosuppressive therapy. This information was used to describe the population demographics and statistics as well as to identify covariates for variability in PDC associated with cyclosporine

administration. In both arms of this study we explored the relationship between PDC and the control of clinical signs.

Chapter 2: Leflunomide

2.1: Introduction

Leflunomide is a human approved immunomodulatory drug commonly used in dogs for a variety of immune-mediated diseases. Leflunomide is a prodrug with its activity solely dependent on rapid and complete conversion to the active metabolite, teriflunomide (A77-1726) (Figure 1). Studies have demonstrated leflunomide to be useful for treatment of a variety of immune mediated diseases in dogs and cats (Mehl, 2012; Gregory, 1998; Kyles, 2001). However, studies describing the pharmacokinetics of leflunomide or its metabolite in dogs are limited (Singer, 2011). Further, the therapeutic range has not been described for dogs. The purpose of this study was to describe the disposition of teriflunomide in dogs after single oral dosing of leflunomide (pharmacokinetics) and then to determine a therapeutic range for teriflunomide in dogs with spontaneous immune-mediated disease (pharmacodynamics).

2.2: Materials and Methods

This study was implemented using two approaches: a pharmacokinetic study which established the disposition of leflunomide in normal dogs. From this, the most appropriate time for collection of samples and the impact of sample handling on drug concentrations was determined; and a prospective TDM trial that established dose, concentration, and response

relationship in dogs with spontaneously occurring immune-mediated disease. All studies were approved by the Institutional Animal Care and Use Committee (IACUC), and the Auburn University College of Veterinary Medicine Clinical Research Review Committee (CRRC). Samples were collected and analyzed by the Auburn University Clinical Pharmacology TDM service.

In vitro study

Dog plasma was analyzed for Teriflunomide (A77-1726) concentrations by HPLC with ultraviolet (UV) detection. The HPLC system consisted of a Waters 717 plus autosampler, Waters 1525 Binary pump and a 2487 UV-Visible detector (Waters Corporation™, Milford, MA, USA). Separation was achieved with a Luna C18, 5 μm, 150 x 4.6 mm column (Phenomenex Inc.™, Torrance, CA, USA). The mobile phase consisted of 40:60, 0.05 M Potassium dihydrogen phosphate, KDP, (pH adjusted to 3.0 w/ phosphoric acid):Acetonitrile (VWR®, Radnor, PA, USA) with the flow rate set to 1 mL/min. The standard curve was generated ranging from 2.5 to 200 mcg/mL by fortifying canine plasma with known amounts of Teriflunomide (VWR®, Radnor, PA, USA) reference standard and accepted if the coefficient of determination (r^2) was at least 0.999 and the predicted concentrations were within 10% of the actual concentrations. Prior to analysis 500 μL of acetonitrile were added to tubes containing 250 μL of reference standard. The contents of each tube were mixed vigorously for 20 seconds through vortexing, and then subjected to centrifugation for 10 minutes at 3000 x g. The volume of clear supernatant injected into the column was 20 μL. The retention time for Teriflunomide was 3.8-4.3 min and UV absorbance was monitored at 275 nm.

Pharmacokinetic study

Four, healthy, client owned dogs (two female, two male, all neutered; 2 Rottweilers, 1 Shetland sheepdog and 1 mixed breed) ranging in 3 to 6 years of age were studied. Healthy participants were solicited from students, faculty, or staff. Each person was provided an informed consent approved by Auburn University's Clinical Research Review Committee.

Each dog was determined as healthy based on a complete blood cell count and serum chemistries. The morning of each pharmacokinetic study, an indwelling catheter (14 G over the wire Arrow catheters) was placed in the external jugular vein of each dog, using manual restraint and topical anesthetic gel.

Dogs were fasted at least 4 hours prior to the study. Each dog received 4.0 mg/kg leflunomide orally. Selection of this dose was based on previously published literature in both dogs and cats indicating both a safe as well as clinically comparable dose (Mehl 2011, Singer 2011). Whole blood was collected from dogs at: 0, 5, 10, 15, 20, 30, 45, 60, 90 minutes, and 2, 6, 12, 24, and 48 hours after administration. For samples 0-60 minutes, the whole blood sample (5 mLs) was treated with acetonitrile (0.5 mL) to inhibit conversion of parent compound to active metabolite. For the remaining samples, whole blood was centrifuged at 3000 rpm for 10 minutes at 18 °C. The plasma was then harvested from the packed red blood cells and treated with acetonitrile (0.5 ml). Once processed, samples were immediately analyzed via HPLC. Parameters of interest included AUC (ng*hr/ml), C_{max} (oral, mcg/mL) at time (T_{max} ; h); $t_{1/2}$ (h) and mean residence time (MRT; h). For *in vitro* samples, only AUC were determined. From these time collection points (both *in vivo* and *in vitro*), the optimum time of sample collection,

storage conditions for shipment, and processing of samples were established for the purpose of the prospective therapeutic monitoring portion of the study.

Therapeutic Drug Monitoring

Clinical samples were received from practitioners treating immune-mediated disorders in dogs as part of the CPL TDM Service between January 2012 and May 2014. Inclusion criteria included canine patients receiving once daily therapy of leflunomide for at least 5 days (steady state). Submission forms must have provided leflunomide dose in mg/kg or ability to calculate mg/kg based on dose and body weight. Also, the teriflunomide plasma drug concentration must have been above the LLOQ. To maintain the independence of each observation, patients (identified by clinic, animal name, and owner last name) with multiple accessions meeting inclusion criteria were only included once. To account for potential dose changes to achieve control, the accession with the highest dose (mg/kg) was selected from those patients that had multiple accessions.

Data Management and Analysis

All statistics were performed using SAS® statistical software (version 9.3). All pairwise comparisons were adjusted for using a Tukey-Kramer adjustment. Population demographics were counted using Proc Freq. Group comparisons were performed by analysis of variance (ANOVA) using Proc GLM. Descriptive statistics were generated by Proc Means. Correlations were performed using Proc Corr. Regression plots were generated using Proc Sgplot. The

relationship between leflunomide dose (mg/kg) and teriflunomide plasma concentration ($\mu\text{g/ml}$) was estimated by correlation. Samples for which clinical control was considered positive (meaning disease had responded to leflunomide treatment) were used to calculate a therapeutic range.

2.3: Results

Assay

The linear correlation coefficient for Teriflunomide was 0.9995. The limit of detection (LOD) was 2.5 mcg/mL and the lower and upper limits of quantification (LOQ) for Teriflunomide were 5.0 mcg/mL and 200 mcg/mL respectively. The RSD% for Teriflunomide, 5.0, 10, 50, 100, 150, 200 mcg/mL were 4.5%, 4.6%, 3.4%, 2.5%, 4.2%, and 4.5% respectively.

During the development of our assay, we found that the parent compound is spontaneously converted to metabolite in plasma in-situ (Figure 2.1, 2.2, and 2.3). However, lower temperatures (-20°C , -80°C) slowed the rate of conversion (Figure 2.4 and 2.5). As such, standard curves only contained the active metabolite.

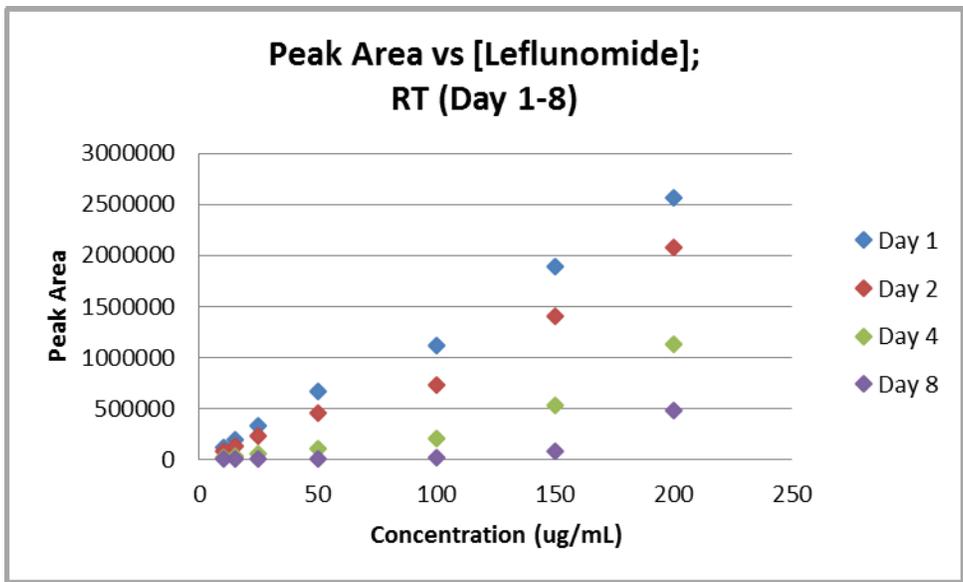


Figure 2.1: Peak area vs concentration plot for leflunomide at room temperature calibration standards run on days 1-8.

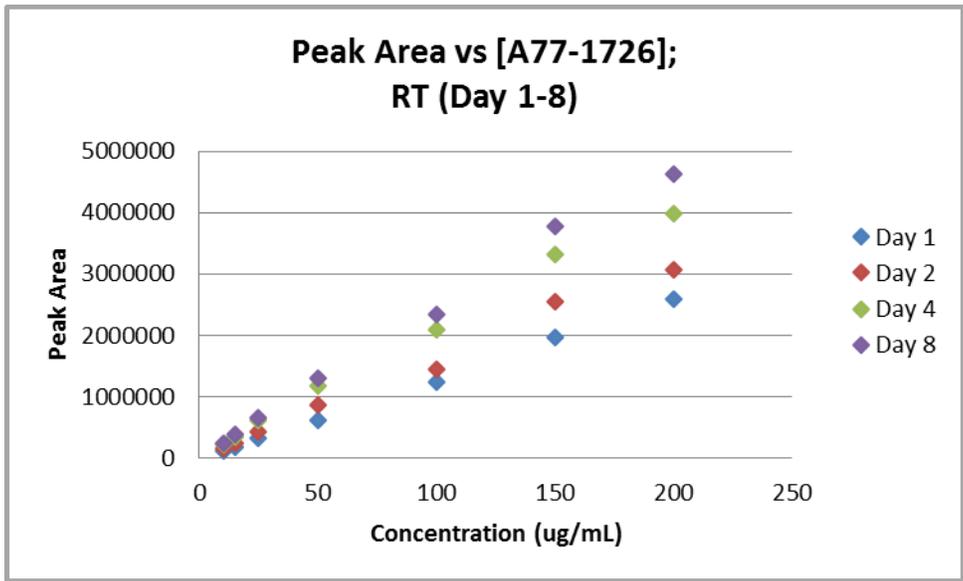


Figure 2.2: Peak area vs concentration plot for A77-1726 at room temperature calibration standards run on days 1-8.

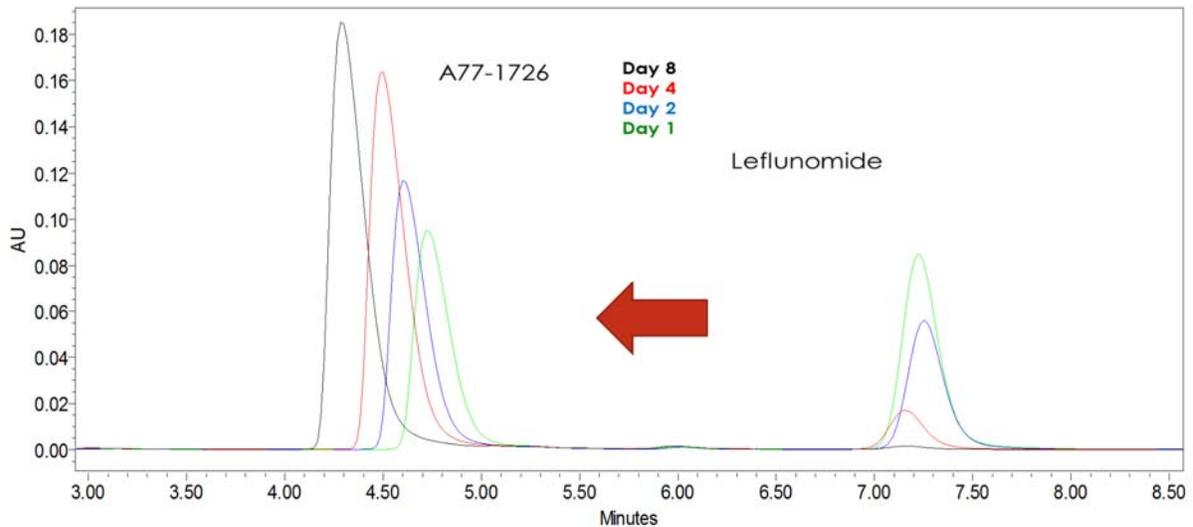


Figure 2.3: Leflunomide and Teriflunomide (A77-1726) peak areas in the same standard (SK9-5, 50 $\mu\text{g}/\text{mL}$) over multiple days at room temperature, demonstrating gradual spontaneous metabolism of the parent compound to the active metabolite. The red arrow represents the direction of conversion

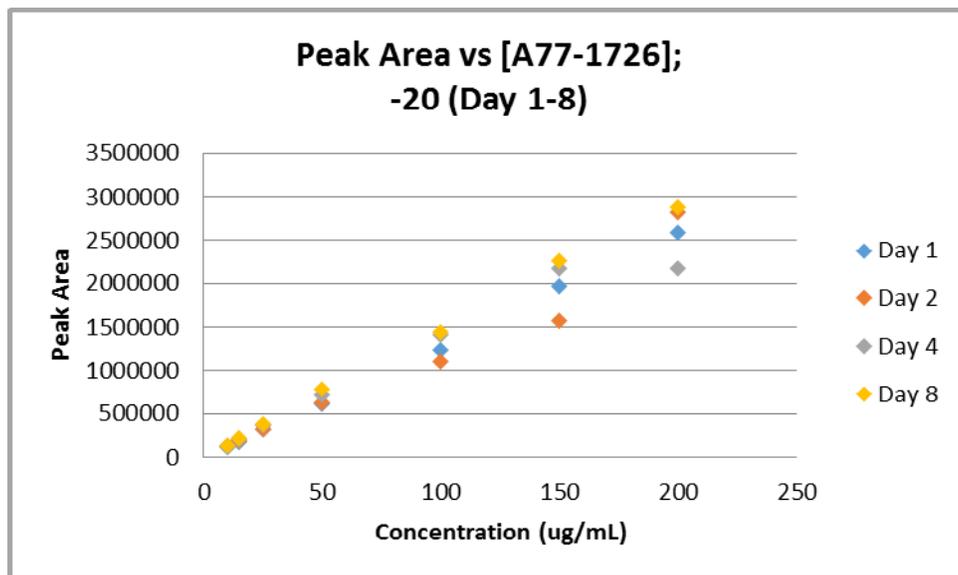


Figure 2.4: Peak area vs Concentration plot for A77-1726 at -20°C calibration standards run on days 1-8.

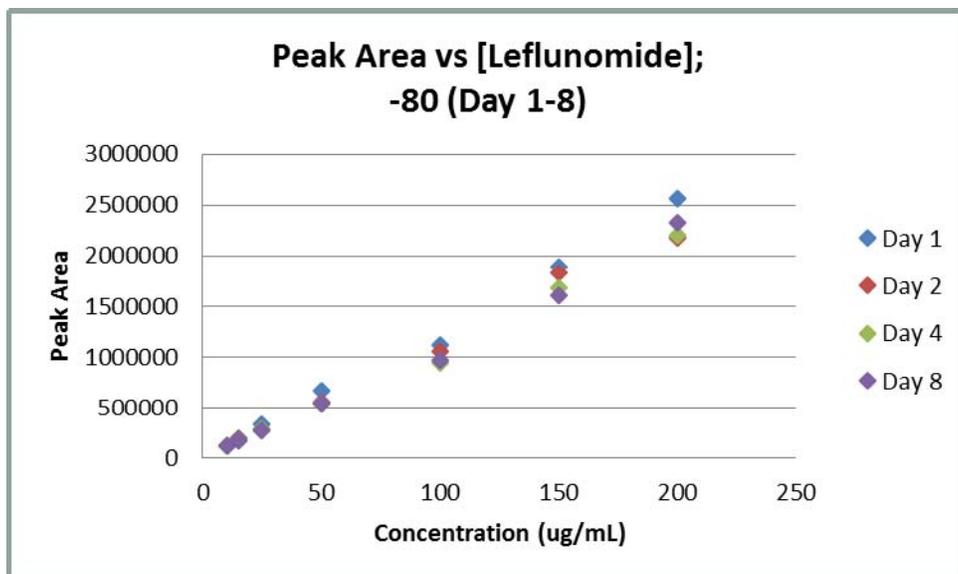


Figure 2.5: Peak area vs Concentration plot for Leflunomide at -80°C calibration standards run on days 1-8.

Pharmacokinetics (Experimental)

No leflunomide was detected in any of the pharmacokinetic study samples. For the active metabolite, teriflunomide, Table 2.1 delineates selected pharmacokinetic parameters for the time versus concentration curves demonstrated in Figure 2.6. Plasma drug concentrations remain in the therapeutic range for at least 12 hours after administration of a single, oral 4 mg/kg dose.

Parameter	Mean \pm SD
C_{max} ($\mu\text{g/ml}$)	18.9 ± 8.5
T_{max} (hr)	1.38 ± 0.25
AUC ($\text{hr} \cdot \mu\text{g/mL}$)	804 ± 285
Half-life (hrs)	25 ± 10
V_d/F (L/kg)	23 ± 32
Cl/F (L/hr/kg)	0.49 ± 0.6
MRT (hr)	175 ± 234

Table 2.1: Select pharmacokinetic parameters for teriflunomide after single dose oral administration of 4 mg/kg leflunomide in 4 normal dogs.

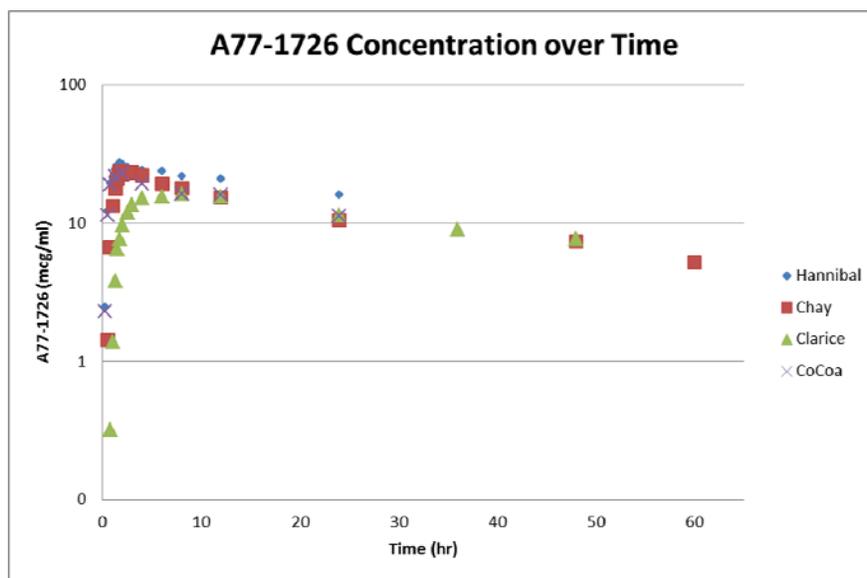


Figure 2.6: Teriflunomide (A77-1726) concentration ($\mu\text{g/ml}$) over time (hr) after single oral administration of 4mg/kg Leflunomide in 4 normal dogs.

Therapeutic Drug Monitoring (Clinical)

Out of 182 clinical samples, based on clinician assessment, 141 (77%) animals were controlled, 23 (13%) animals were not controlled, and 18 (10%) animals were unspecified. The most common indication was granulomatous meningoencephalitis (GME) at approximately 30% (n = 55). No leflunomide was detected in any clinical sample. There was a significant difference (F-value = 24.38, p < 0.0001) between mean concentrations for samples indicating no control of clinical signs versus those samples indicating control of clinical signs (See Figure 2.7). The mean PDC of uncontrolled patients was 52.2 ± 38.6 $\mu\text{g/ml}$ (CI95% of 43.9-60.4 $\mu\text{g/mL}$). The mean PDC of controlled patients was 29.8 ± 15.3 $\mu\text{g/ml}$ (CI95% of 26.5-33.2 $\mu\text{g/ml}$). The double sided 95% distribution teriflunomide plasma concentrations in animals whose disease was considered controlled was 5.0-59.3 $\mu\text{g/ml}$. Sample collection occurred any time during the dosing interval. The mean dose of leflunomide used to achieve control was 2.8 mg/kg once daily (CI95% of 2.6-3.0 mg/kg). The correlation (r) between leflunomide dose and teriflunomide plasma concentrations was 0.26 (p = 0.0003) (Figure 2.8).

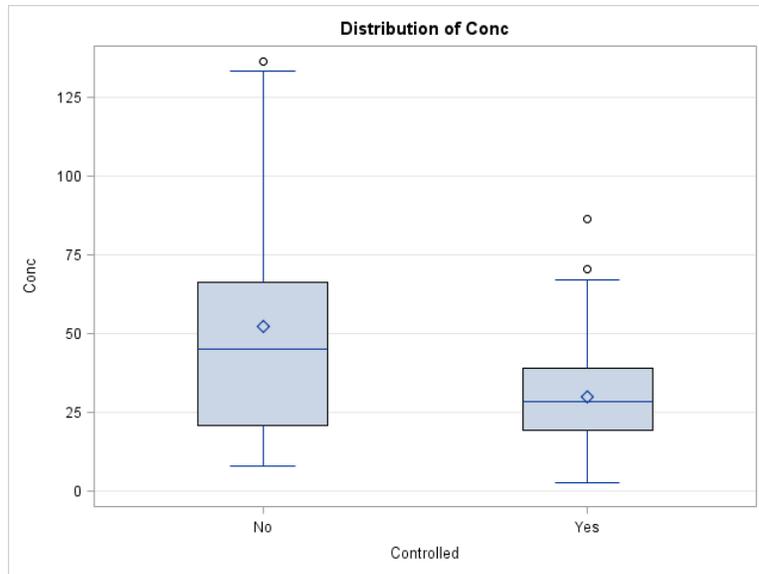


Figure 2.7: Box plot of trough teriflunomide (A77-1726) PDC ($\mu\text{g/ml}$) based on control of clinical signs (n= 23 No, 141 Yes). F-value = 24.38, p-value <0.0001. Outliers = $^{\circ}$.

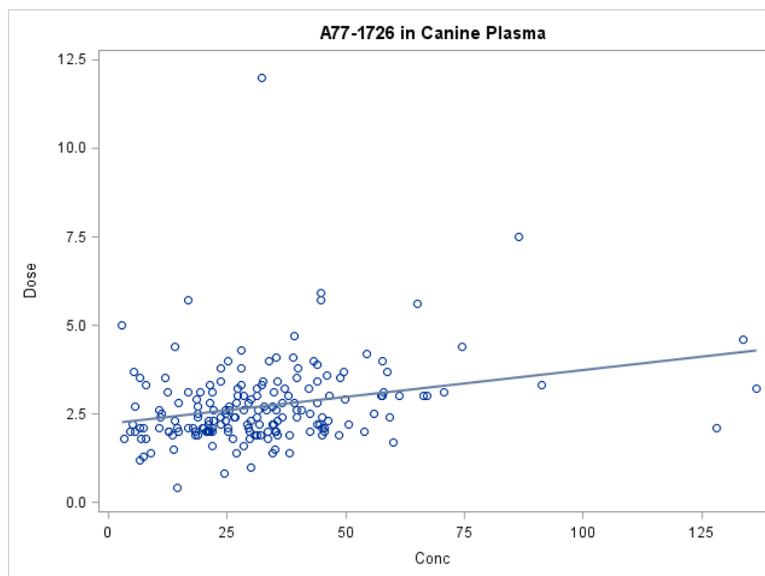


Figure 2.8: Leflunomide dose (mg/kg) vs. trough teriflunomide (A77-1726) concentration ($\mu\text{g/ml}$) based on clinical samples (n=182). Correlation (r) = 0.26 (p = 0.0003).

2.4: Discussion and Conclusions

Studies of leflunomide in canine clinical patients are limited. However, as early as 1998, Gregory and coworkers (Gregory 1998) reported the use of the drug (1.5 mg/kg twice daily to 2 to 4 mg/kg once daily) in dogs (n = 29) afflicted with various immune-mediated disorders. These include idiopathic thrombocytopenic purpura (n = 3; good to excellent response), IMHA with idiopathic thrombocytopenic purpura (Evans syndrome), systemic histiocytosis (n = 3; one failure and two total remissions), multifocal nonsuppurative encephalomyelitis (n = 5; good to excellent response), and a variety of other immune-mediated disorders that had not responded to glucocorticoids or azathioprine. Side effects included decreased appetite, mild anemia, and bloody vomitus or stools (n = 3). This study led to experimental studies. Using a model of canine renal transplantation, Kyles (2001, 2003) demonstrated a synergistic effect with cyclosporine when combined with a leflunomide analogue. In similar studies, leflunomide has been studied at doses ranging from 2 to 16 mg/kg/day as sole therapy or in combination with cyclosporine in dogs undergoing experimental renal transplantation (Kyles 2001, 2003). Mean trough leflunomide concentrations ranged from 10 µg/mL (2 mg/kg) to 55 µg/mL (16 mg/kg). By using combination therapy, of cyclosporine and leflunomide at 4 mg/kg, survival time increased from 28 to 68 days.

Since leflunomide was not detected in any of the pharmacokinetic study samples, it is reasonable to conclude that leflunomide is rapidly and completely converted to teriflunomide in dogs. Therefore, TDM of leflunomide can be solely based on teriflunomide plasma concentrations. A correlation (r) between leflunomide dose and teriflunomide of 0.26 (p = 0.0003) indicates that prediction of plasma teriflunomide levels based on leflunomide dose will

be difficult. Therefore, TDM is recommended to ensure patients are reaching target levels of teriflunomide. Based on a 25 hr half-life indicated by the pharmacokinetic study, a dose of 3.5 (3-4 mg/kg) once daily initially should achieve concentrations within the therapeutic range at steady-state (3-5 days) and the target concentration at 24 hours post dose is 5.0-59.3 mcg/mL. This concentration is inconsistent with ranges recommended in humans ($>16 \mu\text{g/ml}$) (van Roon, 2005). Therefore, when monitoring, a single trough sample (approximately 24 hours post dose) 1 week post dose change is recommended. Due to the lack of correlation between dose and A77-1726 plasma concentrations recommends TDM to adjust doses for chronic therapy. Timing of sample collection can be throughout a 24 hour dosing interval.

Chapter 3: Cyclosporine

3.1: Introduction

Cyclosporine (cyclosporine A, CsA), a very effective immune modulator, commonly is used in dogs for a variety of immune-mediated diseases. Its use in the treatment of canine and feline diseases has recently been reviewed (Archer, 2014). The CsA works by forming a complex with cyclophilin that inhibits calcineurin. Inhibition of calcineurin prevents promotion of cytokine genes, and subsequently T cell activation (Matsuda, 2000). As a drug, CsA is a large molecule drug with a molecular weight of 1202.61 daltons (Da), and is extremely lipophilic, which contributes to its poor oral absorption in the patient.

Metabolism of CsA occurs primarily in the liver, with a lesser extent in the intestinal epithelial cells, through the cytochrome p450 enzyme CYP3A4. Metabolism leads to formation of more than 20 metabolites, with some being active and others inactive. CsA is a known substrate of P-glycoprotein, a prominent transport system in portals of entry (such as the intestine), sanctuaries (such as the blood brain barrier), and drug excretion organs (such as the kidney) (Boothe, 2012). In 2004, Steffan reported a mean half-life of 7.6 ± 1.1 hours in healthy dogs. In 2006, Allenspach reported a mean half-life of 5.6 ± 1.2 in dogs with IBD.

In veterinary medicine, some of the most common uses for CsA include autoimmune hemolytic anemia (IMHA), granulomatous meningoencephalitis/myelitis (GME), anemia, inflammatory bowel disease (IBD), immune-mediated thrombocytopenia (ITP), atopic dermatitis (atopy), arthritis, peri-anal fistulas, and pure red cell aplasia (PRCA). Because these diseases are often life-threatening, it is not at all unusual for patients to be receiving multiple drugs.

The disposition of cyclosporine is markedly variable among animals. This reflects a number of factors. Breed differences, although not documented, likely are to be significant, in part because of the recognized deficiency in p-glycoprotein activity resulting from mutations in the ABCB (MDR-1) (Mealey, 2010). Genetic differences in CYP3A4 metabolism are also likely (Martinez, 2013). Because of the common role of p-glycoprotein and CYP3A4 in the disposition of other drugs, drug interactions involving cyclosporine, which are well documented in humans, are also likely in animals (Campana 1996). A likely major contributor to variability in disposition is its oral formulation and variability in oral absorption. Cyclosporine is prepared as a microemulsion in order to facilitate its oral absorption. Although a veterinary product is approved for use in both dogs and cats (Atopica®; <http://www.us.atopica.com/#>), it is common for veterinarians to prescribe alternatives in order to decrease cost. Among the alternatives are human generics, of which currently more than 11 preparations are available (FDA Orange Book (<http://www.accessdata.fda.gov/scripts/cder/ob/docs/queryai.cfm>)). Although bioequivalence of these products has been demonstrated in humans, this information cannot be extrapolated to animals.

Further, and more disconcertingly, it is not at all unusual for veterinarians to prescribe compounded cyclosporine. Variability among cyclosporine products compounded for animals recently has been demonstrated (Umstead, 2012). Because of these and other factors, plasma drug concentrations of cyclosporine are likely to be markedly variable among animals. Yet, because it is used to treat life-threatening disorders, clinicians can monitor drug concentrations in order to maintain cyclosporine concentrations within an assumed therapeutic range (Archer, 2014).

The CPL TDM monitors CsA for patients from animal hospitals across the United States. The purpose of this study is to use the data in the TDM database to perform a retrospective study with several goals: describe population demographics and pharmacokinetics, identify therapeutic ranges, and identify potential covariates for changes in PDC or half-life.

3.2: Materials & Methods

Clinical submissions were selected from the TDM database between October 2010 and May 2014. CsA was detected in canine EDTA whole blood using the Siemens (New York, NY) Cyclosporine Immunoassay® (CSA) and the Siemens Cyclosporine Extended Range Immunoassay® (CSAE) on a Siemens (New York, NY) Dimension Xpand Plus® general chemistry analyzer. For CSA, the upper limit of quantitation was 500 ng/mL, and the lower limit of quantitation was 25 ng/mL. CSA was calibrated using the Siemens CSA Calibrator® and quality control was performed using More Diagnostics (Los Osos, CA) RAP/TAC/CSA Controls®. For CSAE, the upper limit of quantitation was 2000 ng/mL, and the lower limit of quantitation was 350 ng/mL, with ability to dilute up to 6000 ng/mL. CSAE was calibrated with the Siemens CSAE Calibrator® and quality control was performed using More Diagnostics Cyclosporine C2 Controls®.

To be included in this study submissions required the following information: canine patients, dose in mg/kg, dose interval of twice daily (BID) or once daily (QD), dose of less than or equal to 10 mg/kg on BID dosing, dose of less than or equal to 15 mg/kg on QD dosing. If a patient had multiple accessions meeting the previous criteria, the earliest sample received that specified clinical response regarding control was selected. Data collected from each patient

included: patient demographic information including species, breed, gender, and age. Additional patient information included the disease being treated and drugs being co-administered. Drug information included drug preparations (Atopica®, generic (human), or compounded; dose (mg/kg), and interval; and monitoring-specific information, including peak versus trough sample submission times. Response to therapy was recorded, when available, as “yes” or “no”, and was based on assessment by the submitting veterinarian.

The results are stored in a Microsoft Access database. In addition to CsA plasma drug concentration (PDC), patient demographic information, dosing information, and control of clinical signs are kept when provided on the submission form. All statistics were run using SAS® Statistical Software. All pairwise comparisons were adjusted for using a Tukey-Kramer adjustment. Population demographics were counted using Proc Freq. Group comparisons were performed by analysis of variance (ANOVA) using Proc GLM. Descriptive statistics were generated by Proc Means. Correlations were performed using Proc Corr. Regression plots were generated using Proc Sgplot. Testing for covariates was performed using Proc Reg or Proc Logistic with backward selection. For many comparisons, due to the short half-life of CsA, the samples were separated into dosing (BID vs QD) groups. These groups were further separated into peak (less than 4 hours post dose) and trough (between 10 and 14 hours) for BID dosing; and peak (less than 4 hours post dose), mid (between 10 and 14 hours), and trough (between 20 and 28 hours post dose) for QD dosing.

3.3: Results

Population Demographics

Samples from 916 dogs were analyzed. 320 (35%) cases did not specify clinical response, while 185 (20%) indicated no control, and 411 (45%) indicated positive control of clinical signs. There were 859 CsA PDC and 104 (83%) half-life values on BID dosing. There were 189 CsA PDC and 22 (17%) half-life values on QD dosing. Age range was 0.5 to 17 years with a mean value of 6.9 ± 3.2 years. Genders represented were unspecified female (n = 38), intact female (n = 5), spayed female (n = 460), unspecified male (n = 55), intact male (n = 8), and neutered male (n = 325). The top 5 most common breeds were mixed breed (n = 171), German shepherd (n = 45), Dachshund (n = 43), Maltese (n = 42), and Chihuahua (n = 40).

As indicated by the patient history in the database, some of the concurrent medications were pred (unspecified) (n = 94), prednisone (n = 342), prednisolone (n = 32), azathioprine (n = 63), omeprazole (n = 40), ketoconazole (n = 29), leflunomide (n = 15), and azithromycin (n = 5). These drugs were checked for covariance throughout the analysis. The most commonly cited diseases being treated were (IMHA) (n = 116), GME (n = 84), anemia (n = 68), IBD (n = 51), ITP (n = 39), atopy (n = 12), arthritis (n = 12), and PRCA (n = 6). These diseases were checked for covariance throughout the analysis. See Table 3.1 for CsA dosing interval (how often dose is given; BID = every 12 hours; QD = every 24 hours) breakdown by disease.

Disease	BID Dosing	QD Dosing	Total
IMHA	100	16	116
GME	77	7	84
Anemia	64	4	68
IBD	37	14	51
ITP	36	3	39
Atopy	3	9	12
Arthritis	9	3	12
PRCA	6	0	6

Table 3.1: Dosing interval (how often dose is given; BID = every 12 hours; QD = every 24 hours) for cyclosporine in dogs for each disease being treated (see text for key to abbreviations).

Population Kinetics

The correlation (r) between log of PDC adjusted for dose over time was 0.24 ($p < 0.0001$) (See Figure 3.1). The correlation (r) between log of PDC adjusted for dose over time for BID dosing was 0.24 ($p < 0.0001$) (See Figure 3.2). The correlation (r) between log of PDC adjusted for dose over time for BID dosing was 0.33 ($p < 0.0001$) (See Figure 3.3).

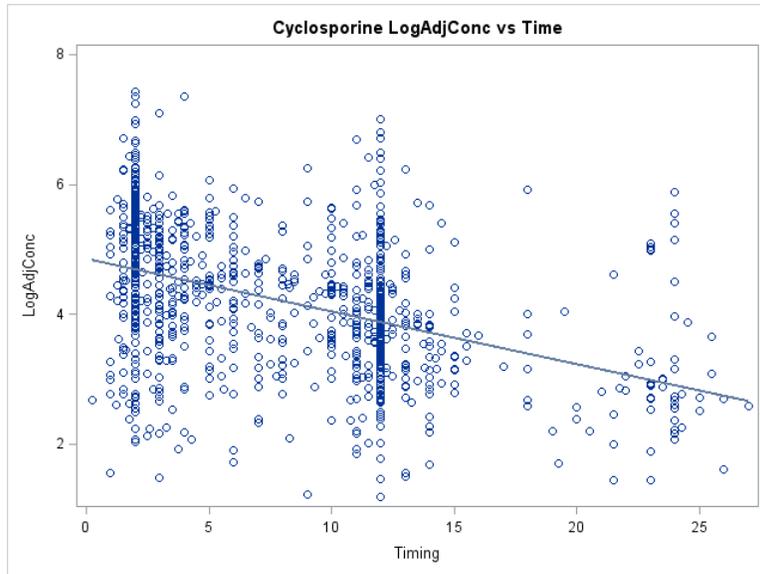


Figure 3.1: Log PDC adjusted for dose (ng/mL / mg/kg) over time (hours) (BID and QD dosing).

Correlation (r) = 0.24 ($p < 0.0001$).

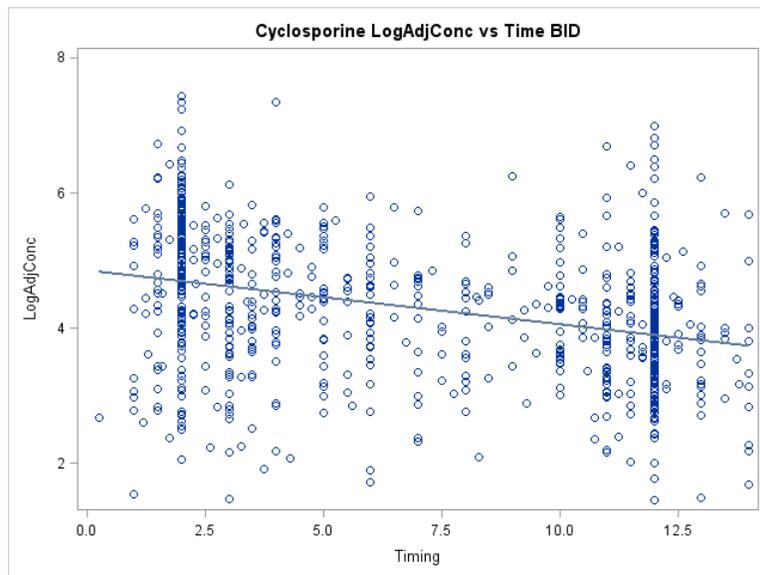


Figure 3.2: Log PDC adjusted for dose (ng/mL / mg/kg) over time (hours) (BID dosing).

Correlation (r) = 0.24 ($p < 0.0001$).

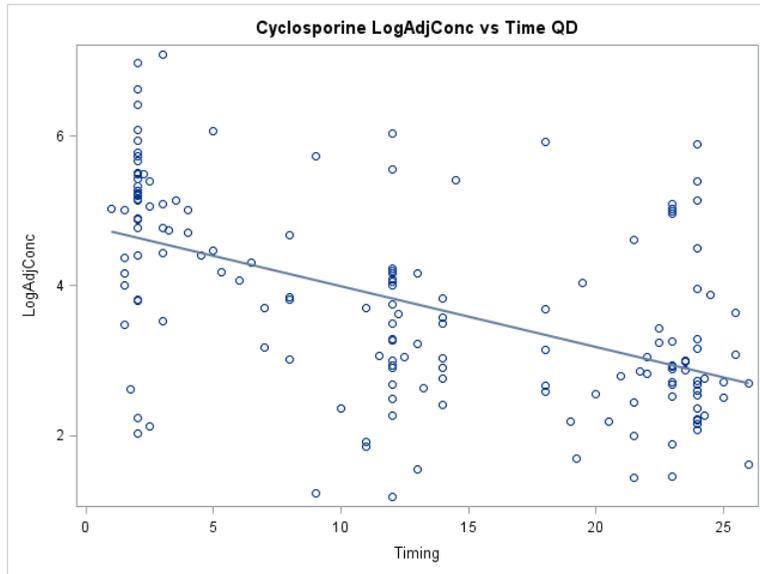


Figure 3.3: Log PDC adjusted for dose (ng/mL / mg/kg) over time (hours) (QD dosing).
 Correlation (r) = 0.33 ($p < 0.0001$).

The correlation (r) between dose (mg/kg) and PDC (ng/mL) was 0.04 ($p = 0.42$) for peak samples (less than 4 hours post dose) on BID dosing (See Figure 3.4). The correlation (r) between dose (mg/kg) and PDC (ng/mL) was 0.0007 ($p = 0.99$) for trough samples (between 10 and 14 hours post dose) on BID dosing (See Figure 3.5); 0.32 ($p = 0.02$) for peak samples (less than 4 hours post dose) on QD dosing (See Figure 3.6); 0.20 ($p = 0.23$) for mid-interval samples (between 10 and 14 hours post dose) on QD dosing (See Figure 3.7) and 0.15 ($p = 0.29$) for trough samples (between 20 and 28 hours post dose) on QD dosing (See Figure 3.8).

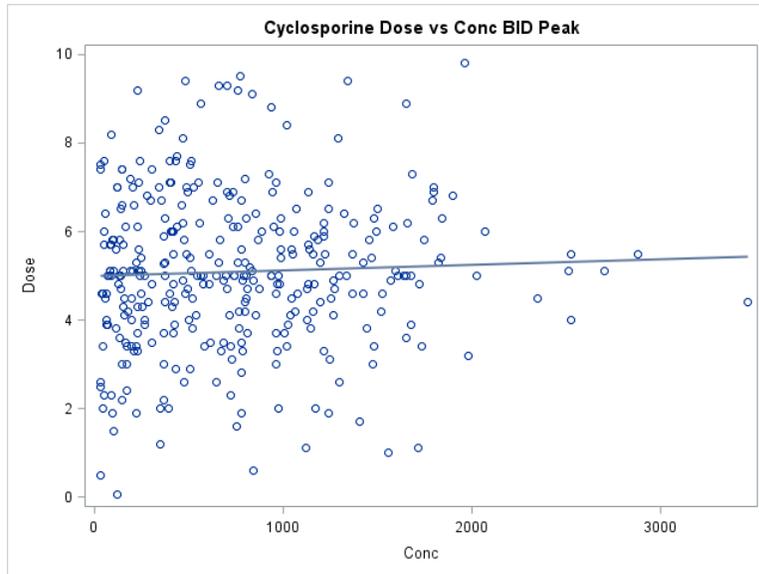


Figure 3.4: CsA dose (mg/kg) vs. peak (less than 4 hours) CsA PDC (ng/mL) based on clinical samples in dogs receiving CsA with BID dosing (n = 240). Correlation (r) = 0.04 (p = 0.42).

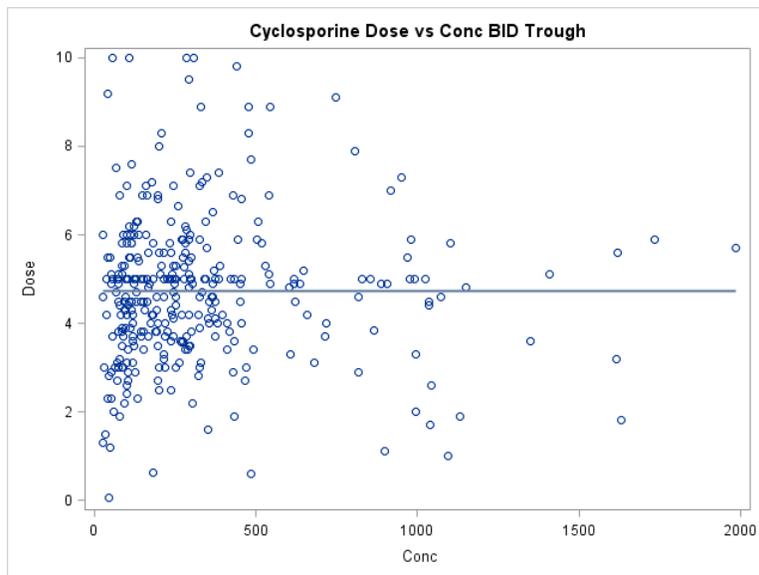


Figure 3.5: CsA dose (mg/kg) vs. trough (between 10 and 14 hours post dose) CsA PDC (ng/mL) based on clinical samples on BID dosing (n = 219). Correlation (r) = 0.0007 (p = 0.99).

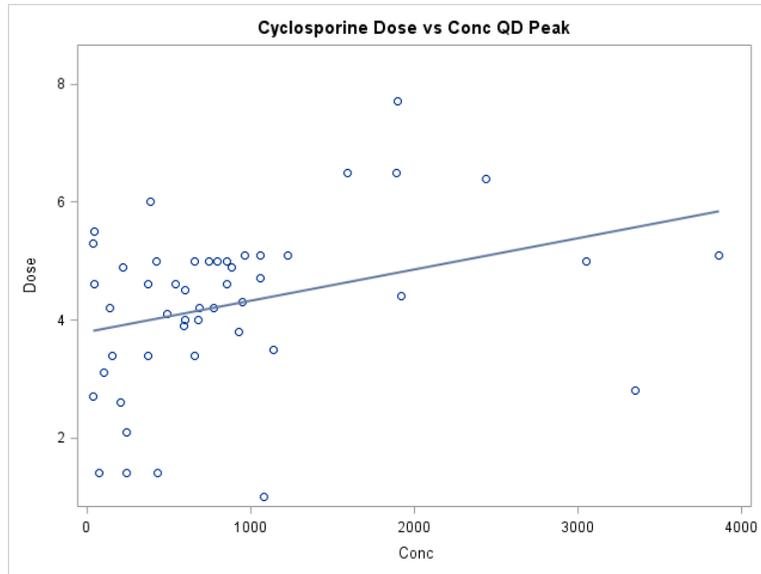


Figure 3.6: CsA dose (mg/kg) vs. peak (less than 4 hours post dose) CsA PDC (ng/mL) based on clinical samples in dogs receiving CsA with QD dosing (n = 31). Correlation (r) = 0.32 (p = 0.02).

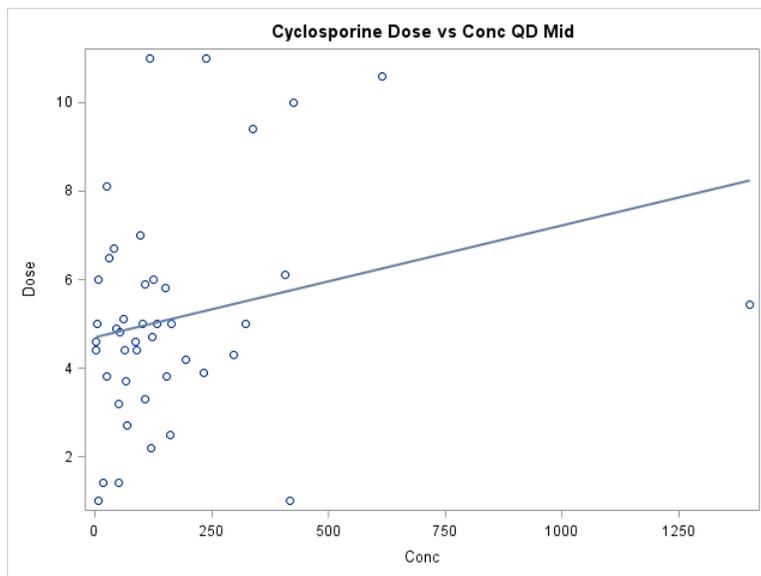


Figure 3.7: CsA dose (mg/kg) vs. mid-interval (between 10 and 14 hours post dose) CsA PDC (ng/mL) based on clinical samples from dogs receiving CsA with QD dosing (n = 23). Correlation (r) = 0.20 (p = 0.23).

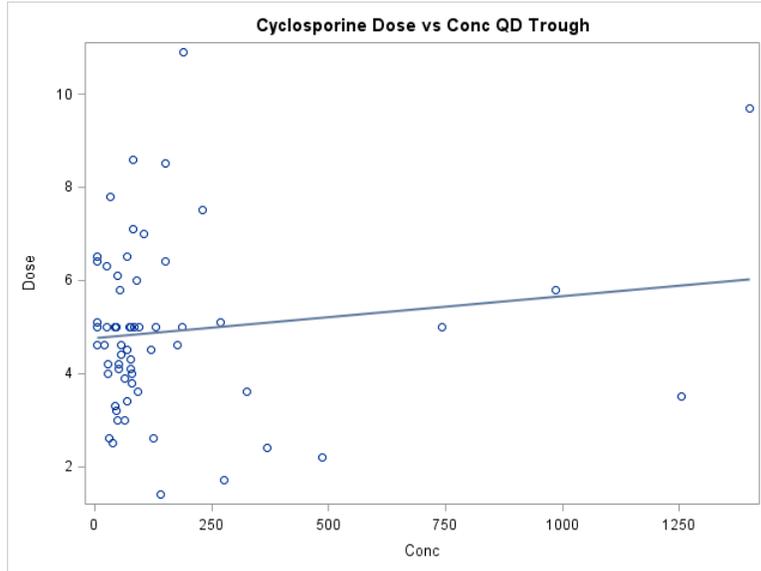


Figure 3.8: CsA dose (mg/kg) vs. trough (between 20 and 28 hours post dose) CsA PDC (ng/mL) based on clinical samples on QD dosing (n = 30). Correlation (r) = 0.15 (p = 0.29).

Due to the lack of correlation between dose and PDC, the marked variability was examined for covariates in the next section. The mean half-life for all animals was 11.1 ± 12.9 hours. The median and mode were 4.8 hours and 3.7 hours respectively. The minimum half-life was 2.2 hours, while the maximum half-life was 69.7 hours. The 95% confidence interval of the mean was 8.7 – 13.6 hours. No concurrent medications (or combination of medications) or diseases (see above) were significant covariates for half-life ($p > 0.05$). Age and gender were also not significant covariates for half-life ($p > 0.05$).

“Responders” vs “Non-responders”

Analysis attempted to answer the question whether or not CsA concentrations varied between patients in which clinical signs were controlled (“responders”) versus those in which clinical signs were not controlled (“non-responders”). This question was asked for peak versus trough concentrations in patients receiving CsA every 12 hrs (BID) or every 24 hrs (once daily (QD)). For the latter group, because samples also were collected mid interval, a 3rd category was considered between responders and non-responders.

For BID dosed peak samples (less than 4 hours post dose) (n = 240), a significant difference could not be detected between mean CsA PDC (F-value = 1.66, p = 0.20) for responders versus non-responders. Sixty-one non-responders had a mean PDC of 847.4 ng/mL (95% CI = 696.3 – 998.4 ng/mL). One hundred seventy-nine responders had a mean PDC of 733.1 ng/mL (95% CI = 644.9 – 821.2 ng/mL) (See Figure 3.9).

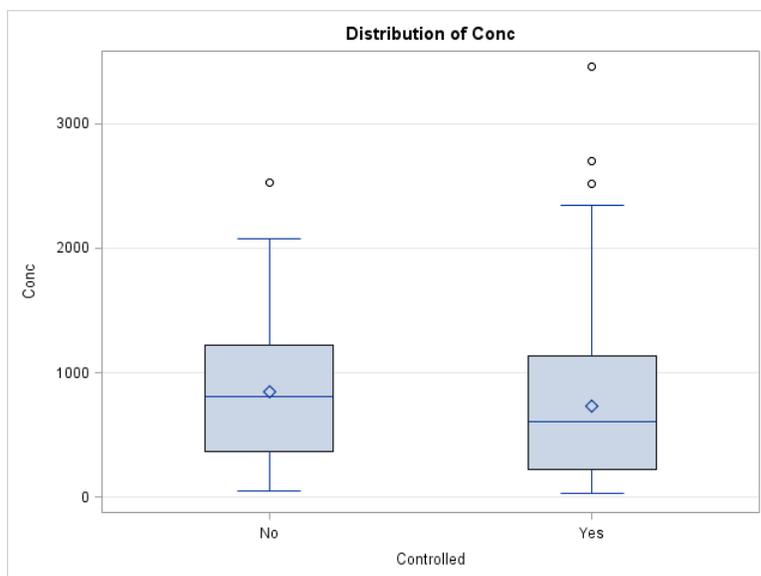


Figure 3.9: Box plot of peak (less than 4 hours post dose) CsA PDC (ng/mL) based on control of clinical signs (n= 61 No, 179 Yes) in dogs receiving CsA with BID dosing (F-value = 1.66, p =0.20). Outliers = °.

For BID dosed trough samples (between 10 hours and 14 hours post dose) (n = 219), a significant difference was detected between mean CsA PDC (F-value = 5.81, p = 0.017) for responders versus non-responders. Ninety-two samples collected from non-responders had a mean PDC of 268.7 ng/mL (95% CI = 202.4 – 335.0 ng/mL). One hundred twenty-seven samples collected from positive responders had a mean PDC of 375.1 ng/mL (95% CI = 318.7 – 431.51 ng/mL) (See Figure 3.10).

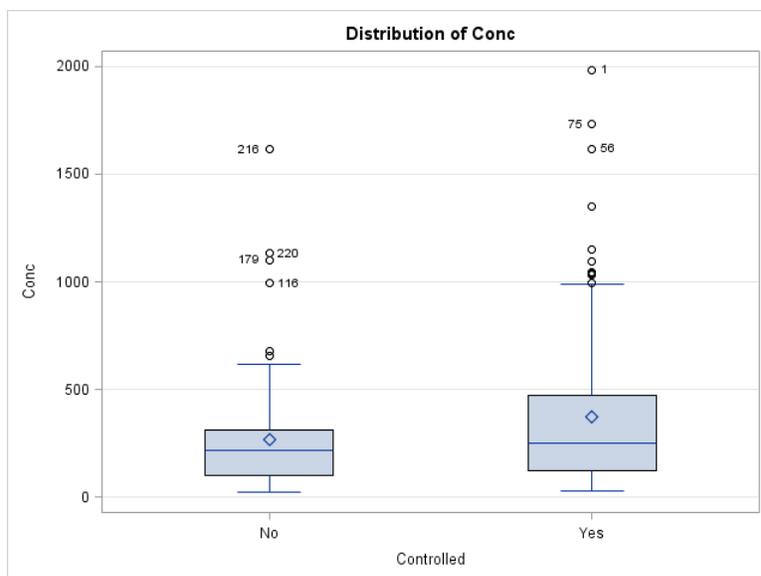


Figure 3.10: Box plot of trough (10 hours to 14 hours post dose) CsA PDC (ng/mL) based on control of clinical signs (n= 92 No, 127 Yes) in dogs receiving CsA with BID dosing (F-value = 5.81, p = 0.017). Outliers = °.

For QD dosed peak samples (less than 4 hours post dose) (n = 31), differences were not significant at $p < 0.05$, but were significant at $p < 0.10$) between mean CsA PDC (F-value = 3.22, $p = 0.083$) for responders versus non-responders. Fourteen samples collected from non-responders, had a mean PDC of 558.3 ng/mL (95% CI = 86.9 – 1029.7 ng/mL). Seventeen samples had from responders had a mean PDC of 1117.1 ng/mL (95% CI = 689.4 – 1544.9 ng/mL) (See Figure 3.11).

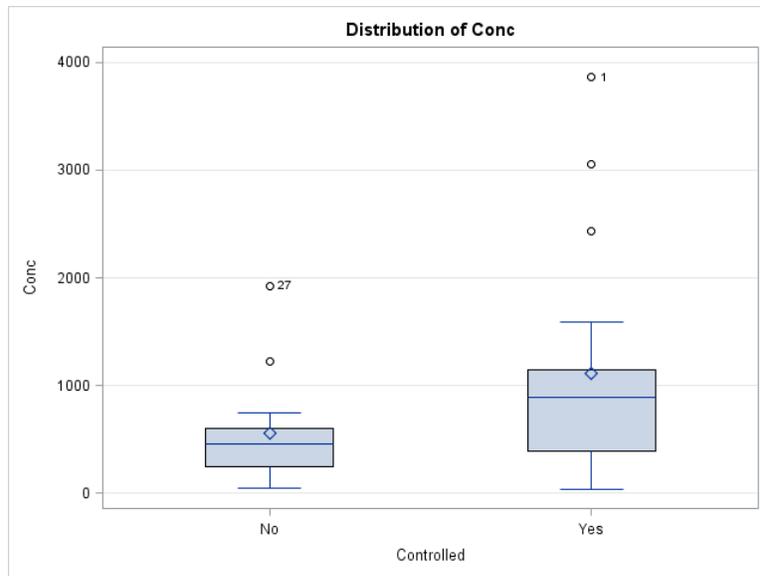


Figure 3.11: Box plot of peak (less than 4 hours post dose) CsA PDC (ng/mL) based on control of clinical signs (n= 14 No, 17 Yes) from dogs receiving CsA with QD dosing (F-value = 3.22, p = 0.083). Outliers = °.

For QD dosed mid-interval samples (between 10 hours and 14 hours post dose) (n = 23), a significant difference could not be detected between mean CsA PDC (F-value = 0.97, p = 0.34) for responders versus non-responders. Six samples were collected from non-responders, with a mean PDC of 209.8 ng/mL (95% CI = 89.6 – 330.0 ng/mL). Seventeen samples were collected from responders, with a mean PDC of 143.5 ng/mL (95% CI = 72.1 – 215.0 ng/mL) (See Figure 3.12).

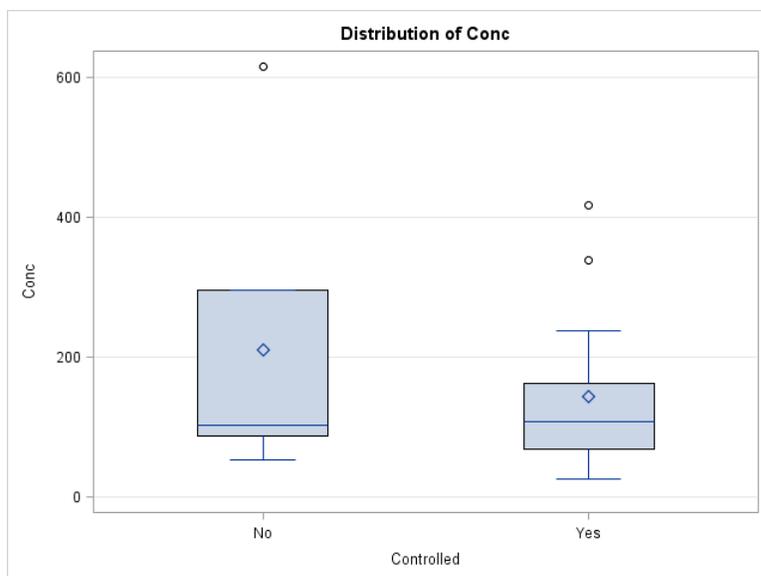


Figure 3.12: Box plot of mid-interval (between 10 and 14 hours post dose) CsA PDC (ng/mL) based on control of clinical signs (n= 6 No, 17 Yes) from dogs receiving CsA with QD dosing (F-value = 0.97, p = 0.34). Outliers = °.

For QD dosed trough samples (between 20 hours and 28 hours post dose) (n = 30), significant differences also could not be detected between mean CsA PDC (F-value = 1.5, p = 0.23) for responders versus non-responders. Twelve samples were collected from non-responders, with a mean PDC of 82.3 ng/mL (95% CI = 2.2 – 162.5 ng/mL). Eighteen samples were collected from responders, with a mean PDC of 144.1 ng/mL (95% CI = 78.7 – 209.6 ng/mL) (See Figure 3.13).

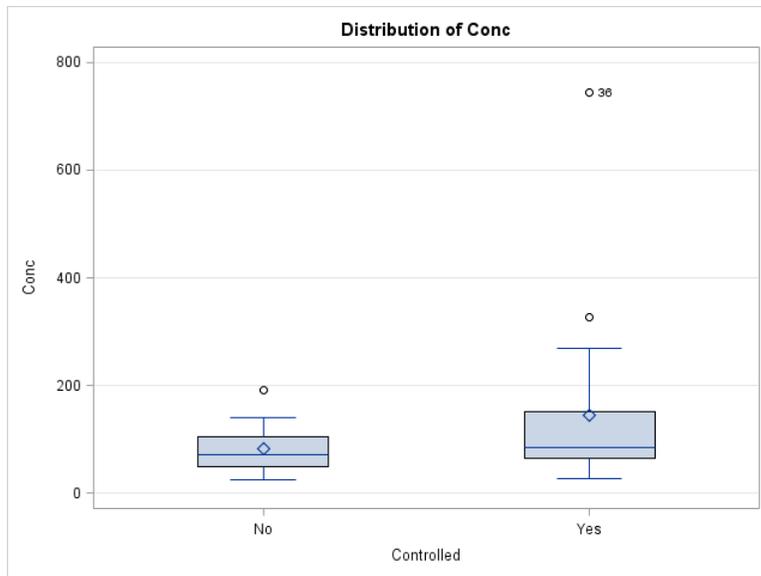


Figure 3.13: Box plot of trough (between 20 and 28 hours post dose) CsA PDC (ng/mL) based on control of clinical signs (n= 12 No, 18 Yes) for QD dosing (F-value = 1.5, p = 0.23). Outliers

= °.

The mean BID dose given to patients with an indication of positive control was 5.1 ± 1.7 mg/kg (95% CI = 4.9 – 5.2 mg/kg). The median and mode were both 5.0 mg/kg. The minimum dose was 0.5 mg/kg and the maximum dose was 10 mg/kg. The mean QD dose given to patients with an indication of positive control was 4.9 ± 2.0 mg/kg (95% CI = 4.4 – 5.4 mg/kg). The median and mode were both 5.0 mg/kg. The minimum dose was 1.0 mg/kg and the maximum dose was 11 mg/kg.

The proportion of responders versus non-responders was not significantly different ($p > 0.05$) between patients on BID versus QD dosing (see Table 3.2).

Dose Interval	Non-responder	Responder	Total
BID	151	351	502
QD	34	60	94
Total	185	411	596

Table 3.2: Proportion of responders versus non-responders on BID versus QD dosing.

Covariate Analysis

Age and gender were not significant covariates of PDC adjusted for dose. IMHA (F-value = 8.35, $p = 0.0041$), ketoconazole (F-value = 31.6, $p < 0.0001$), and generic preparations (F-value = 6.15, $p = 0.014$) were significant covariates of PDC adjusted for dose at BID dosing, peak samples (less than 4 hours post dose). Ketoconazole (F-value = 132.6, $p < 0.0001$) were significant covariates of PDC adjusted for dose at BID dosing, trough samples (between 10 and 14 hours post dose). Breed (F-value = 2.68, $p = 0.015$), and generic preparations (F-value = 19.58, $p < 0.0001$) were significant covariates of PCD adjusted for dose at QD dosing, trough samples (20 to 28 hours post dose) (Table 3.3).

Covariate	Dose Interval	Timing	Mean PDC ± SD (95% CI) with covariate (ng/mL)	Mean PDC ± SD (95% CI) w/o covariate (ng/mL)	p-value
IMHA	BID	Peak	283.8 ± 143.3 (229.3 – 338)	167.6 ± 211.9 (143.9 – 191.3)	0.0041
Ketoconazole	BID	Peak	528.7 ± 466.6 (194.9 – 862.5)	166.9 ± 187.8 (146.5 – 187.3)	<0.0001
Generic preparation	BID	Peak	216.1 ± 186.8 (161.3 – 271.0)	171.4 ± 212.4 (146.9 – 195.9)	0.014
Ketoconazole	BID	Trough	405.9 ± 333.4 (221.3 – 590.5)	71.1 ± 85.3 (61.6 – 80.6)	<0.0001
Generic preparation	QD	Trough	15.1 ± 9.0 (9.7 – 20.6)	51.4 ± 73.6 (28.5 – 74.3)	<0.0001

Table 3.3: Summary of significant covariates for difference in mean CsA PDC (ng/mL).

Concurrent therapy with pred (unspecified) (Point estimate = 2.8, p = 0.0011) led to an increased likelihood of being a non-responder. Treatment of arthritis (point estimate = 5.7, p = 0.047), inflammatory bowel disease (point estimate = 3.3, p = 0.0014), anemia (point estimate = 3.7, p <0.0001), and pure red cell aplasia (point estimate = 14.2, p = 0.016) also led to an increased likelihood of being a non-responder. The other diseases analyzed, age, and breed were not significant covariates (p > 0.05) for control of clinical signs.

3.4: Discussion and Conclusions

The mean CsA half-life of 11.1 hours is higher than reported mean half-lives (Allenspach, 2006; Steffan, 2004). This elevated value, combined with the substantial variability (standard deviation = 12.9), and a median and mode of 4.8 hours and 3.7 hours respectively, suggests a few high leverage values are pulling the value up. No tested covariates (concurrent

medications, diseases, age, or gender) were significant with CsA half-life. However, the lack of significant covariates may simply be due to lack of power of this study to detect the relationship.

Based on poor correlation (r) between CsA dose and PDC ($0.0007 - 0.32$; $p = 0.99 - 0.03$), prediction of PDC based on CsA dose is difficult. Therefore, monitoring of concentrations is recommended. Correlation (r) between logged PDC adjusted for dose was between 0.24 and 0.33 demonstrates that prediction of PDC based on time is also difficult. Concurrent ketoconazole therapy was the most consistent significant covariate for PDC adjusted for dose ($p < 0.05$). Prednisone/prednisolone, IMHA, atopy, and breed showed limited covariance with PDC adjusted for dose.

Patients receiving pred (unspecified) or azathioprine concurrently with CsA showed an increased likelihood of being non-responders. Rather than suggesting these concurrent therapies decreased efficacy, patients needing additional therapies probably are more likely to not respond. Patients being treated for arthritis, IBD, anemia, or PRCA also showed an increased likelihood of being non-responders.

The mean CsA dose given to patients where the clinical signs were considered controlled was 5.1 ± 1.7 mg/kg BID and 4.9 ± 2.0 mg/kg QD. This is consistent with the doses reported in the literature (Allenspach, 2006; Steffan, 2004).

Based on 95% CsA PDC distributions of patients with positive control of clinical signs, the recommend therapeutic ranges for cyclosporine are 43.4 – 1841.5 ng/mL for BID peak samples (less than 4 hours post dose), 52.0 – 1151.6 ng/mL for BID trough samples (10 – 14 hours post dose), 40.1 – 3050.6 ng/mL for QD peak samples (less than 4 hours post dose), 26.4 – 338.2 ng/mL for QD mid-interval samples (10 – 14 hours post dose), and 26.8 – 326.5 ng/mL for

QD trough samples (20 – 28 hours post dose). These ranges suggest that prolonged exposure may play a larger role in controlling clinical signs rather than reaching a maximum PDC.

Chapter 4: Summary Discussion and Conclusions

This study is the first of its kind to examine the role that TDM has in the treatment of immune-mediated disease in dogs. The investigation was based in part on a prospective approach to establishing conditions for monitoring leflunomide, and an entirely retrospective approach for cyclosporine.

The following conditions for leflunomide use and monitoring were determined: Approximately 77% of patients receiving leflunomide were considered responders. Although concentrations which discriminated responders from non-responders ranged from 5.0 mcg/mL to 59.3 mcg/mL, teriflunomide concentrations that exceed 25 mcg/mL at any time during the dosing interval are considered the minimum end of the therapeutic range. Although a concentration associated with toxicity could not be determined, concentrations higher than 45 mcg/mL do not appear to increase the likelihood of response. Thus, we propose an initial therapeutic range of 25 to 45 mcg/mL of teriflunomide. An initial oral dose of 3 to 4 mg/kg one daily is recommended in order to achieve teriflunomide concentrations considered therapeutic.

Monitoring can be based on either plasma or serum samples, and on the active metabolite, teriflunomide. Further, samples can be collected at any time during a dosing interval, although a trough sampling time will be recommended. Monitoring can be initiated in approximately 5 days (we recommend a week) after therapy is initiated or changed. Unfortunately, for leflunomide, the number of clinical samples was not sufficient to provide even a limited covariate analysis suggesting that the number should be increased and then analyzed.

For cyclosporine, disposition among the population is markedly variable. This variability could not be attributed to dose alone since the variability persisted when concentrations were adjusted for dose. Further, dose was poorly correlated to concentrations regardless of sampling time. The elimination half-life for all treated animals was longer than that reported and also is characterized by marked variability. The range in elimination half-life, and thus time to steady-state, is dramatically different. For some patients, therapeutic failure may be anticipated because of a very short half-life; for others, care must be taken to avoid marked accumulation because of a long half-life. Clinicians should consider collecting both a peak and trough in problematic patients.

Covariate analysis indicated the following potentially significant ($p < 0.05$) relationships with cyclosporine plasma drug concentrations: breed, disease, ketoconazole, and generic preparations. Covariates with a significant relationship ($p < 0.05$) with response were co-treatment of cyclosporine and prednisone or prednisolone, or disease (arthritis, anemia, and inflammatory bowel disease).

Approximately 60% of patients receiving cyclosporine were considered responders. Concentrations which discriminated between responders and non-responders could not be identified. A difference also could not be detected with once versus twice daily administration, although the proportion of animals receiving bid versus once daily dosing was not the same for each disease. One of our goals was to explore whether or not evidence existed that generic vs compounded cyclosporine concentrations were more or less predictable compared to Atopica®. Further, we sought to determine whether peak or trough concentrations were better predictors of PDC or response. Finally, we also hoped to identify patient conditions under which monitoring was particularly important because of the impact on PDC (eg, co-drug administration). Further

study clearly is needed to identify reasons for variability in the disposition of cyclosporine among patients. Variability reflects, in part, the limited information available through our TDM records. However, this study does suggest that monitoring is recommended.

Further limitations of this study are those common with retrospective studies, such as unknown patient information and difficulty confirming validity of medical history. As covariates were added, the number of samples in each group dropped and decreased the power of the statistical analysis. However, a framework has been laid for future retrospective or prospective analysis based on TDM.

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Appendix 1: Leflunomide SAS® Code

```
*Set output location of SAS Datasets 'Lef.';
LIBNAME Lef 'C:\Users\jss0012\Dropbox\Thesis\SAS datasets\Leflunomide\';

*Import excel file (must be xls notxlsx);
PROC IMPORT OUT= Lef.Leflunomide DATAFILE=
"C:\Users\jss0012\Dropbox\Thesis\Leflunomide 1-1-12 thru 5-31-14.xls"
    DBMS=xls REPLACE;
    SHEET="Leflunomide";
    GETNAMES=YES;
RUN;Quit;

Data Lef.Leflunomide;
Set Lef.Leflunomide;
If Index(lowercase(Value), "<") NE 0 Then Value = '!';
If Index(lowercase(Value), ">") NE 0 Then Value = '!';
If Index(lowercase(Value), ".") = 0 Then Value = Catt(Value, '!');
Conc = Input (Value, 5.2);
LogDose = Log (Dose);
LogConc = Log (Conc);
If Controlled = 'Yes' Then ControlledInt = 1;
If Controlled = 'No' Then ControlledInt = 0;
If Controlled = 'Unspecified' Then ControlledInt = .;
Run;Quit;

*Limit to K9s, Steady State (>=5d), Calculated Dose (mg/kg) and Removed those lacking a
result;
Data Lef.Leflunomide;
Set Lef.Leflunomide;
Where Species_Name = 'Canine' And SteadyState = 1 And DoseUnits = 'mg/kg' And
DoseInterval="QD" And Conc GE 2.5;
Run;Quit;

*Number of accessions for each patient;
Proc Freq Data=Lef.Leflunomide Noprint;
Tables Clinic_ID*Animal_Name*Owner_Name / out=Lef.AccFreq;
Run;Quit;

*Sort the dataset prior to merge statement;
Proc Sort Data=Lef.Leflunomide;
By Clinic_ID Animal_Name Owner_Name;
Run;Quit;
```

*Add number of accessions for each patient to drop scanned drugs;

```
Data Lef.Leflunomide;  
Merge Lef.Leflunomide Lef.AccFreq;  
By Clinic_ID Animal_Name Owner_Name;  
Drop Percent;  
Rename COUNT=Patient_Accessions;  
Run;Quit;
```

```
Data Lef.MultAcc;  
Set Lef.Leflunomide;  
Where Patient_Accessions > 1;  
Run;Quit;
```

```
Proc Sort Data=Lef.Leflunomide;  
By Descending Dose;  
Run;Quit;
```

*Drop duplicate patients;

```
Proc Sort Data=Lef.Leflunomide Nodupkey;  
By Clinic_ID Animal_Name Owner_Name;  
Run;Quit;
```

*MDR1 Breeds;

```
Data Lef.Leflunomide;  
Set Lef.Leflunomide;  
If Breed_Name NE 'Australian Shepherd' And Breed_Name NE 'Collie' And Breed_Name NE  
'Whippet' Then MDR1 = 0;  
If Breed_Name = 'Australian Shepherd' Or Breed_Name = 'Collie' Or Breed_Name = 'Whippet'  
Then MDR1 = 1;  
Run;Quit;
```

*Scan patient history (Report_Comments) for drug keywords and assign boolean value to a new variable;

```
Data Lef.Leflunomide;  
Set Lef.Leflunomide;  
If Index(Lowcase(Report_Comments), "pred") = 0 Then Pred = 0;  
If Index(Lowcase(Report_Comments), "pred") NE 0 Then Pred = 1;  
If Index(Lowcase(Report_Comments), "azith") = 0 Then Azithro = 0;  
If Index(Lowcase(Report_Comments), "azith") NE 0 Then Azithro = 1;  
If Index(Lowcase(Report_Comments), "cyclo") = 0 And Index(Lowcase(Report_Comments),  
"csa") = 0 Then Cyclo = 0;  
If Index(Lowcase(Report_Comments), "cyclo") NE 0 Or Index(Lowcase(Report_Comments),  
"csa") NE 0 Then Cyclo = 1;  
If Index(Lowcase(Report_Comments), "omep") = 0 Then Omeprazole = 0;  
If Index(Lowcase(Report_Comments), "omep") NE 0 Then Omeprazole = 1;
```

```
If Index(Lowcase(Report_Comments), "aza") = 0 Then Azathioprine = 0;
If Index(Lowcase(Report_Comments), "aza") NE 0 Then Azathioprine = 1;
Run;Quit;
```

*Scan patient history (Report_Comments) for disease keywords and assign boolean value to a new variable;

```
Data Lef.Leflunomide;
Set Lef.Leflunomide;
If Index(Lowcase(Report_Comments), "itp") = 0 Then ITP = 0;
If Index(Lowcase(Report_Comments), "itp") NE 0 Then ITP = 1;
If Index(Lowcase(Report_Comments), "histocytosis") = 0 Then Histocytosis = 0;
If Index(Lowcase(Report_Comments), "histocytosis") NE 0 Then Histocytosis = 1;
If Index(Lowcase(Report_Comments), "arthritis") = 0 Then Arthritis = 0;
If Index(Lowcase(Report_Comments), "arthritis") NE 0 Then Arthritis = 1;
If Index(Lowcase(Report_Comments), "gme") = 0 And Index(Lowcase(Report_Comments),
"meningo") = 0 And Index(Lowcase(Report_Comments), "menengio") = 0 Then GME = 0;
If Index(Lowcase(Report_Comments), "gme") NE 0 Or Index(Lowcase(Report_Comments),
"meningo") NE 0 Or Index(Lowcase(Report_Comments), "menengio") NE 0 Then GME = 1;
If Index(Lowcase(Report_Comments), "ibd") = 0 Then IBD = 0;
If Index(Lowcase(Report_Comments), "ibd") NE 0 Then IBD = 1;
If Index(Lowcase(Report_Comments), "anemia") = 0 Then Anemia = 0;
If Index(Lowcase(Report_Comments), "anemia") NE 0 Then Anemia = 1;
If Index(Lowcase(Report_Comments), "imha") = 0 Then IMHA = 0;
If Index(Lowcase(Report_Comments), "imha") NE 0 Then IMHA = 1;
Run;Quit;
```

```
*Age Summary;
TITLE 'Age Summary';
Proc means data=Lef.Leflunomide;
Var Age;
Run;Quit;
```

```
*Count Frequency of each breed;
TITLE 'Breed Frequencies';
Proc freq data=Lef.Leflunomide;
Tables Breed_Name;
Run;Quit;
```

```
*Count Frequency of Controlled;
TITLE 'Controlled Frequencies';
Proc freq data=Lef.Leflunomide;
Tables Conc;
Where Controlled = 'Yes';
Run;Quit;
```

```
*Count Frequency of Controlled;
```

```
TITLE 'Controlled Frequencies';  
Proc freq data=Lef.Leflunomide;  
Tables Controlled;  
Run;Quit;
```

```
*Count Frequency of each gender;  
TITLE 'Gender Frequencies';  
Proc freq data=Lef.Leflunomide;  
Tables Gender;  
Run;Quit;
```

```
*Count Frequency of each disease being indicated;  
TITLE 'Disease Frequencies';  
Proc freq data=Lef.Leflunomide;  
Tables ITP Histocytosis Arthritis GME IBD Anemia IMHA;  
Run;Quit;
```

```
*Identify disease relationship to plasma drug concentration;  
TITLE 'Disease Covariance';  
Proc reg data=Lef.Leflunomide;  
Model Conc = ITP Histocytosis Arthritis GME IBD Anemia IMHA / selection=adjrsq;  
Run;Quit;
```

```
*Count Frequency of other drugs being indicated;  
TITLE 'Drug Frequencies';  
Proc freq data=Lef.Leflunomide;  
Tables Pred Azithro cyclo Omeprazole Azathioprine;  
Run;Quit;
```

```
*Identify other drug relationship to plasma drug concentration;  
TITLE 'Drug Covariance';  
Proc reg data=Lef.Leflunomide;  
Model Conc = Pred Azithro cyclo Omeprazole Azathioprine / selection=adjrsq;  
Run;Quit;
```

```
*Frequency controlled on pred vs not;  
TITLE 'Control with Pred';
```

```
Proc sort data=Lef.Leflunomide;  
By Pred;  
Run;Quit;
```

```
*Odds ratio: pred more likely to achieve control;  
Proc freq data=Lef.Leflunomide;  
Tables Pred*Controlled / chisq relrisk;  
Where Controlled NE 'Unspecified';
```

```

Run;Quit;

/* (alternative method)
Odds ratio: pred more likely to achieve control;
Proc logistic descending data = Lef.Leflunomide;
Model ControlledInt = pred;
Where ControlledInt NE .;
Run;Quit;
*/

*Frequency controlled on cyclo vs not;
TITLE 'Control with Cyclo';

Proc sort data=Lef.Leflunomide;
By Cyclo;
Run;Quit;

*Odds ratio: cyclo more likely to achieve control;
Proc freq data=Lef.Leflunomide;
Tables Cyclo*Controlled / chisq relrisk;
Where Controlled NE 'Unspecified';
Run;Quit;

*Frequency GME controlled on pred vs not;
TITLE 'Control GME with Pred';

Proc sort data=Lef.Leflunomide;
By Pred;
Run;Quit;

Proc freq data=Lef.Leflunomide;
Where GME = 1;
By Pred;
Tables Controlled;
Run;Quit;

*Frequency GME controlled on cyclo vs not;
TITLE 'Control GME with Cyclo';

Proc sort data=Lef.Leflunomide;
By Cyclo;
Run;Quit;

Proc freq data=Lef.Leflunomide;
Where GME = 1;
By Cyclo;

```

Tables Controlled;
Run;Quit;

/*Timing breakdowns ruled irrelevant

*ANOVA comparing Concentrations across Controlled responses for 20-25hr samples;
TITLE 'Conc 20-25hrs';
Proc glm data=Lef.Leflunomide;
Class Controlled;
Where Timing GE 20 and Timing LE 25;
Model Conc = Controlled;
Means Controlled / clm t tukey;
Run;Quit;

*ANOVA comparing Doses (mg/kg) across Controlled responses for 20-25hr samples;
TITLE 'Dose 20-25hrs';
Proc glm data=Lef.Leflunomide;
Class Controlled;
Where DoseUnits = 'mg/kg' and Timing GE 20 and Timing LE 25;
Model Dose = Controlled;
Means Controlled / clm t tukey;
Run;Quit;

*Correlation of Dose (mg/kg) vs Conc for 20-25hr samples, nothing logged;
TITLE 'Correlation 20-25hrs (Regular)';
Proc corr data=Lef.Leflunomide plots=matrix;
Var Dose Conc;
Where DoseUnits = 'mg/kg' and Timing GE 20 and Timing LE 25;
Run;Quit;

*Correlation of Dose (mg/kg) vs Conc for 20-25hr samples, dose logged;
TITLE 'Correlation 20-25hrs (LogDose)';
Proc corr data=Lef.Leflunomide plots=matrix;
Var LogDose Conc;
Where DoseUnits = 'mg/kg' and Timing GE 20 and Timing LE 25;
Run;Quit;

*Correlation of Dose (mg/kg) vs Conc for 20-25hr samples, conc logged;
TITLE 'Correlation 20-25hrs (LogConc)';
Proc corr data=Lef.Leflunomide plots=matrix;
Var Dose LogConc;
Where DoseUnits = 'mg/kg' and Timing GE 20 and Timing LE 25;
Run;Quit;

*Correlation of Dose (mg/kg) vs Conc for 20-25hr samples, both logged;
TITLE 'Correlation 20-25hrs (LogBoth)';

```
Proc corr data=Lef.Leflunomide plots=matrix;  
Var LogDose LogConc;  
Where DoseUnits = 'mg/kg' and Timing GE 20 and Timing LE 25;  
Run;Quit;
```

```
*ANOVA comparing Concentrations accross Controlled responses for 12-25hr samples;  
TITLE 'Conc 12-25hrs';  
Proc glm data=Lef.Leflunomide;  
Class Controlled;  
Where Timing GE 12 and Timing LE 25;  
Model Conc = Controlled;  
Means Controlled / clm t tukey;  
Run;Quit;
```

```
*ANOVA comparing Doses (mg/kg) accross Controlled responses for 12-25hr samples;  
TITLE 'Dose 12-25hrs';  
Proc glm data=Lef.Leflunomide;  
Class Controlled;  
Where DoseUnits = 'mg/kg' and Timing GE 12 and Timing LE 25;  
Model Dose = Controlled;  
Means Controlled / clm t tukey;  
Run;Quit;
```

```
*Correlation of Dose (mg/kg) for 12-25hr samples, nothing logged;  
TITLE 'Correlation 12-25hrs (Regular)';  
Proc corr data=Lef.Leflunomide plots=matrix;  
Var Dose Conc;  
Where DoseUnits = 'mg/kg' and Timing GE 12 and Timing LE 25;  
Run;Quit;
```

```
*Correlation of Dose (mg/kg) for 12-25hr samples, dose logged;  
TITLE 'Correlation 12-25hrs (LogDose)';  
Proc corr data=Lef.Leflunomide plots=matrix;  
Var LogDose Conc;  
Where DoseUnits = 'mg/kg' and Timing GE 12 and Timing LE 25;  
Run;Quit;
```

```
*Correlation of Dose (mg/kg) for 12-25hr samples, conc logged;  
TITLE 'Correlation 12-25hrs (LogConc)';  
Proc corr data=Lef.Leflunomide plots=matrix;  
Var Dose LogConc;  
Where DoseUnits = 'mg/kg' and Timing GE 12 and Timing LE 25;  
Run;Quit;
```

```
*Correlation of Dose (mg/kg) for 12-25hr samples, both logged;  
TITLE 'Correlation 12-25hrs (LogBoth)';
```

```
Proc corr data=Lef.Leflunomide plots=matrix;
Var LogDose LogConc;
Where DoseUnits = 'mg/kg' and Timing GE 12 and Timing LE 25;
Run;Quit;
```

```
*/
```

```
*ANOVA comparing Concentrations accross Controlled responses for all samples;
TITLE 'Conc All';
Proc glm data=Lef.Leflunomide;
Class Controlled;
Model Conc = Controlled / cli p;
Means Controlled / clm t tukey;
Where Controlled NE 'Unspecified';
Run;Quit;
```

```
Proc means data=Lef.Leflunomide n mean median mode std max min range clm;
Where Controlled = "Yes";
Var Conc;
run;Quit;
```

```
Proc means data=Lef.Leflunomide n mean median mode std max min range clm;
Where Controlled = "No";
Var Conc;
run;Quit;
```

```
TITLE 'Conc Pred';
Proc glm data=Lef.Leflunomide;
Where Controlled = "Yes";
Class Pred;
Model Conc = Pred / cli p;
Means Pred / clm t tukey;
Run;Quit;
```

```
*ANOVA comparing Doses (mg/kg) accross Controlled responses for all samples;
TITLE 'Dose All';
Proc glm data=Lef.Leflunomide;
Class Controlled;
Model Dose = Controlled / cli p;
Means Controlled / clm t tukey;
Run;Quit;
```

```
TITLE 'Dose Pred';
Proc glm data=Lef.Leflunomide;
Where Controlled = "Yes";
Class Pred;
```

```
Model Dose = Pred / cli p;  
Means Pred / clm t tukey;  
Run;Quit;
```

```
Proc means data=Lef.Leflunomide n mean median mode max min range clm;  
Where Controlled = "Yes";  
Var Dose;  
run;Quit;
```

```
*Correlation of Dose (mg/kg) for all samples, nothing logged;  
TITLE 'Correlation All (Regular)';  
Proc corr data=Lef.Leflunomide plots=matrix;  
Var Dose Conc;  
Run;Quit;
```

```
/*Logging variables did not improve correlation
```

```
*Correlation of Dose (mg/kg) for all samples, dose logged;  
TITLE 'Correlation All (LogDose)';  
Proc corr data=Lef.Leflunomide plots=matrix;  
Var LogDose Conc;  
Where DoseUnits = 'mg/kg';  
Run;Quit;
```

```
*Correlation of Dose (mg/kg) for all samples, conc logged;  
TITLE 'Correlation All (LogConc)';  
Proc corr data=Lef.Leflunomide plots=matrix;  
Var Dose LogConc;  
Where DoseUnits = 'mg/kg';  
Run;Quit;
```

```
*Correlation of Dose (mg/kg) for all samples, both logged;  
TITLE 'Correlation All (LogBoth)';  
Proc corr data=Lef.Leflunomide plots=matrix;  
Var LogDose LogConc;  
Where DoseUnits = 'mg/kg';  
Run;Quit;
```

```
*/
```

```
*Regression plot of A77-1726 vs Dose for all samples;  
TITLE 'A77-1726 in Canine Plasma';  
proc sgplot data=Lef.leflunomide;  
reg y=dose x=conc / NOLEGFIT LINEATTRS= (THICKNESS= 2);  
run;quit;
```

Appendix 2: Cyclosporine SAS® Code

*Begin File Operations;

*Set output location of SAS Datasets 'Cyclo.';

LIBNAME Cyclo 'C:\Users\jss0012\Dropbox\Thesis\SAS datasets\Cyclosporine\';

*Import excel file (must be xls not xlsx);

PROC IMPORT OUT= Cyclo.Cyclosporine DATAFILE=

"C:\Users\jss0012\Dropbox\Thesis\Cyclosporine 1-2-03 thru 5-31-14.xls"

DBMS=xls REPLACE;

SHEET="Cyclosporine";

GETNAMES=YES;

RUN;Quit;

*End File Operations;

*Begin Dataset Operations;

*Initial data clean-up;

Data Cyclo.Cyclosporine;

Set Cyclo.Cyclosporine;

If Value = '0' Then Value = '!';

If Index(lowercase(Value), ".") = 0 Then Value = Catt(Value, '!');

If Index(lowercase(Value), ">") = 0 Then AboveRange = 0;

If Index(lowercase(Value), ">") NE 0 Then AboveRange = 1;

If Index(lowercase(Value), "<") = 0 Then NonDetectable = 0;

If Index(lowercase(Value), "<") NE 0 Then NonDetectable = 1;

If Test_Name = 'Cyclosporine Dilution' Then Test_Name = 'Cyclosporine';

Conc = Input (Value, 7.2);

Half_Life = Input (Value, 7.2);

AdjConc = Conc/Dose;

If Controlled = 'Yes' Then ControlledInt = 1;

If Controlled = 'No' Then ControlledInt = 0;

If Controlled = 'Unspecified' Then ControlledInt = .;

If Dose = . And OtherDose NE . And OtherDose NE 0 Then Dose = OtherDose And DoseUnits = 'mg/kg';

If DoseInterval = " And OtherInterval NE " Then DoseInterval = OtherInterval;

Run;Quit;

```

*Gender categorization;
Data Cyclo.Cyclosporine;
Set Cyclo.Cyclosporine;
If Gender = 'Female Spayed' Then Do; Neutered = 'Yes'; Sex='Female'; End;
If Gender = 'Female Intact' Then Do; Neutered = 'No'; Sex='Female'; End;
If Gender = 'Female' Then Do; Neutered = "; Sex = 'Female'; End;
If Gender = 'Male Neutered' Then Do; Neutered = 'Yes'; Sex='Male'; End;
If Gender = 'Male Intact' Then Do; Neutered = 'No'; Sex='Male'; End;
If Gender = 'Male' Then Do; Neutered = "; Sex='Male'; End;
Run;Quit;

```

```

*Limit to K9s, Calculated Dose (mg/kg) and Removed those lacking a result or dose;
Data Cyclo.Cyclosporine;
Set Cyclo.Cyclosporine;
Where Species_Name = 'Canine' And DoseUnits = 'mg/kg' And Timing NE . And Dose NE .
And Controlled NE ";
Run;Quit;

```

```

*Limit to BID or QD and log dose and conc;
Data Cyclo.Cyclosporine;
Set Cyclo.Cyclosporine;
Where DoseInterval = 'BID' Or DoseInterval = 'QD';
LogDose = Log (Dose);
LogConc = Log (Conc);
LogAdjConc = Log (AdjConc);
Run;Quit;

```

/*This coded limited to just accessions with HL value. No longer necessary

```

*Create list of accessions with half-life test value;
Data Cyclo.Acc;
Set Cyclo.Cyclosporine;
Where Test_Name = 'Cyclosporine Half-Life';
HL = 1;
Keep Accession_Number HL;
Run;Quit;

```

```

*Sort all datasets by accession number so can merge;
Proc Sort Data=Cyclo.Cyclosporine; By Accession_Number; Run; Quit;
Proc Sort Data=Cyclo.Acc; By Accession_Number; Run; Quit;

```

```

*Merge datasets;
Data Cyclo.Cyclosporine;
Merge Cyclo.Cyclosporine Cyclo.Acc;
By Accession_Number;
Run;Quit;

```

```

*Only keep results where we had a half-life test value;
Data Cyclo.Cyclosporine;
Set Cyclo.Cyclosporine;
Where HL = 1;
Run;Quit;

*/

*Create fields to categorize Peak/Trough;
Data Cyclo.Cyclosporine;
Set Cyclo.Cyclosporine;
If Timing LE 4 And DoseInterval = 'BID' Then PeakBID = 1;
If Timing GE 10 And Timing LE 14 And DoseInterval = 'BID' Then TroughBID = 1;
If Timing LE 4 And DoseInterval = 'QD' Then PeakQD = 1;
If Timing GE 10 And Timing LE 14 And DoseInterval = 'QD' Then MidQD = 1;
If Timing GE 20 And DoseInterval = 'QD' Then TroughQD = 1;
Run;Quit;

*Drop Entries with potential outlier doses (BID >10, QD >15);
Data Cyclo.Cyclosporine;
Set Cyclo.Cyclosporine;
If DoseInterval = 'BID' And Dose >10 Then Delete;
If DoseInterval = 'QD' And Dose >15 Then Delete;
Run;Quit;

*Sort prior to proc freq;
Proc Sort Data=Cyclo.Cyclosporine;
By Test_Name descending PeakBID descending TroughBID descending PeakQD descending
MidQD descending TroughQD Controlled;
Run;Quit;

*Drop duplicate accessions;
Proc Sort Data=Cyclo.Cyclosporine NoDupkey Out=Cyclo.PatientAcc;
By Clinic_ID Animal_Name Owner_Name Accession_Number;
Run;Quit;

*Number of accessions for each patient;
Proc Freq Data=Cyclo.PatientAcc Noprint;
Tables Clinic_ID*Animal_Name*Owner_Name / out=Cyclo.PatientAccFreq;
Run;Quit;

*Sort the dataset prior to merge statement;
Proc Sort Data=Cyclo.Cyclosporine;
By Clinic_ID Animal_Name Owner_Name;
Run;Quit;

```

*Add number of accessions for each patient to main dataset;

```
Data Cyclo.Cyclosporine;  
Merge Cyclo.Cyclosporine Cyclo.PatientAccFreq;  
By Clinic_ID Animal_Name Owner_Name;  
Drop Percent;  
Rename COUNT=Patient_Accessions;  
Run;Quit;
```

*Sort high-low patients by number of accessions;

```
Proc Sort Data=Cyclo.Cyclosporine;  
By descending Patient_Accessions Clinic_ID Animal_Name Owner_Name Accession_Number;  
Run;Quit;
```

*Create a new set of patients that have more than one accession;

```
Data Cyclo.MultAcc;  
Set Cyclo.Cyclosporine;  
Where Patient_Accessions >1;  
Run;Quit;
```

*Create a new set of patients that have only one accession;

```
Data Cyclo.SingleAcc;  
Set Cyclo.Cyclosporine;  
Where Patient_Accessions = 1;  
Run;Quit;
```

*Sort multiple accession set by Patient, descending control (1=yes, 0=no, . = unspecified), and ascending received date;

```
Proc Sort Data=Cyclo.MultAcc;  
By Clinic_ID Animal_Name Owner_Name descending ControlledInt Received_Date;  
Run;Quit;
```

*Output a list of accessions by dropping duplicates;

```
Proc Sort Data=Cyclo.MultAcc Nodupkey Out=Cyclo.MultAccList;  
By Clinic_ID Animal_Name Owner_Name;  
Run;Quit;
```

*Assign value to new field so we can use as criteria later. output only Accession number and new field;

```
Data Cyclo.MultAccList;  
Set Cyclo.MultAccList;  
MultKeep = 1;  
Keep Accession_Number MultKeep;  
Run;Quit;
```

*Sort prior to merge;

```
Proc Sort Data = Cyclo.MultAcc; By Accession_Number; Run;Quit;  
Proc Sort Data = Cyclo.MultAccList; By Accession_Number; Run;Quit;
```

```
*Added field for keep criteria to multiple accessions;  
Data Cyclo.MultAcc;  
Merge Cyclo.MultAcc Cyclo.MultAccList;  
By Accession_Number;  
Run;Quit;
```

```
*Drop all but accession that was identified;  
Data Cyclo.MultAcc;  
Set Cyclo.MultAcc;  
Where MultKeep = 1;  
Run;Quit;
```

```
*Combine single accessions and kept accessions from multi;  
Proc Datasets Force Nolist;  
Append Base = Cyclo.SingleAcc Data= Cyclo.MultAcc;  
Run;Quit;
```

```
*Rename combined set back as main set;  
Data Cyclo.Cyclosporine;  
Set Cyclo.SingleAcc;  
Run;Quit;
```

```
*Resort by patients with descending accession numbers to check function of previous steps;  
Proc Sort Data=Cyclo.Cyclosporine;  
By descending Patient_Accessions Clinic_ID Animal_Name Owner_Name Accession_Number;  
Run;Quit;
```

```
*MDR1 Breeds;  
Data Cyclo.Cyclosporine;  
Set Cyclo.Cyclosporine;  
If Breed_Name NE 'Australian Shepherd' And Breed_Name NE 'Collie' And Breed_Name NE  
'Whippet' Then MDR1 = 0;  
If Breed_Name = 'Australian Shepherd' Or Breed_Name = 'Collie' Or Breed_Name = 'Whippet'  
Then MDR1 = 1;  
Run;Quit;
```

```
*Scan patient history (Report_Comments) for drug keywords and assign boolean value to a new  
variable;  
Data Cyclo.Cyclosporine;  
Set Cyclo.Cyclosporine;  
If Index(Lowcase(Report_Comments), "pred") = 0 Then Pred = 0;  
If Index(Lowcase(Report_Comments), "pred") NE 0 And Index(Lowcase(Report_Comments),  
"prednisone") = 0
```

```

And Index(Lowcase(Report_Comments), "prednisolone") = 0 Then Pred = 1;
If Index(Lowcase(Report_Comments), "prednisone") = 0 Then Prednisone = 0;
If Index(Lowcase(Report_Comments), "prednisone") NE 0 Then Prednisone = 1;
If Index(Lowcase(Report_Comments), "prednisolone") = 0 Then Prednisolone = 0;
If Index(Lowcase(Report_Comments), "prednisolone") NE 0 Then Prednisolone = 1;
If Index(Lowcase(Report_Comments), "azith") = 0 Then Azithro = 0;
If Index(Lowcase(Report_Comments), "azith") NE 0 Then Azithro = 1;
If Index(Lowcase(Report_Comments), "leflun") = 0 Then Leflunomide = 0;
If Index(Lowcase(Report_Comments), "leflun") NE 0 Then Leflunomide = 1;
If Index(Lowcase(Report_Comments), "omep") = 0 Then Omeprazole = 0;
If Index(Lowcase(Report_Comments), "omep") NE 0 Then Omeprazole = 1;
If Index(Lowcase(Report_Comments), "aza") = 0 Then Azathioprine = 0;
If Index(Lowcase(Report_Comments), "aza") NE 0 Then Azathioprine = 1;
If Index(Lowcase(Report_Comments), "ketacon") = 0 And Index(Lowcase(Report_Comments),
"ketocon") = 0 Then Ketoconazole = 0;
If Index(Lowcase(Report_Comments), "ketacon") NE 0 Or Index(Lowcase(Report_Comments),
"ketocon") NE 0 Then Ketoconazole = 1;
Run;Quit;

```

*Scan patient history (Report_Comments) for disease keywords and assign boolean value to a new variable;

```

Data Cyclo.Cyclosporine;
Set Cyclo.Cyclosporine;
If Index(Lowcase(Report_Comments), "itp") = 0 Then ITP = 0;
If Index(Lowcase(Report_Comments), "itp") NE 0 Then ITP = 1;
If Index(Lowcase(Report_Comments), "histocytosis") = 0 Then Histocytosis = 0;
If Index(Lowcase(Report_Comments), "histocytosis") NE 0 Then Histocytosis = 1;
If Index(Lowcase(Report_Comments), "arthritis") = 0 Then Arthritis = 0;
If Index(Lowcase(Report_Comments), "arthritis") NE 0 Then Arthritis = 1;
If Index(Lowcase(Report_Comments), "gme") = 0 And Index(Lowcase(Report_Comments),
"meningo") = 0 And Index(Lowcase(Report_Comments), "menengio") = 0 Then GME = 0;
If Index(Lowcase(Report_Comments), "gme") NE 0 Or Index(Lowcase(Report_Comments),
"meningo") NE 0 Or Index(Lowcase(Report_Comments), "menengio") NE 0 Then GME = 1;
If Index(Lowcase(Report_Comments), "ibd") = 0 Then IBD = 0;
If Index(Lowcase(Report_Comments), "ibd") NE 0 Then IBD = 1;
If Index(Lowcase(Report_Comments), "anemia") = 0 Then Anemia = 0;
If Index(Lowcase(Report_Comments), "anemia") NE 0 Then Anemia = 1;
If Index(Lowcase(Report_Comments), "aplasia") = 0 Then RPA = 0;
If Index(Lowcase(Report_Comments), "aplasia") NE 0 Then RPA = 1;
If Index(Lowcase(Report_Comments), "imha") = 0 Then IMHA = 0;
If Index(Lowcase(Report_Comments), "imha") NE 0 Then IMHA = 1;
If Index(Lowcase(Report_Comments), "atopy") = 0 Then Atopy = 0;
If Index(Lowcase(Report_Comments), "atopy") NE 0 Then Atopy = 1;
Run;Quit;

```

*Scan patient history (Report_Comments) for drug keywords and assign boolean value to a new variable;

```
Data Cyclo.Cyclosporine;  
Set Cyclo.Cyclosporine;  
If Index(Lowcase(Report_Comments), "atopica") = 0 Then Atopica = 0;  
If Index(Lowcase(Report_Comments), "atopica") NE 0 Then Atopica = 1;  
If Index(Lowcase(Report_Comments), "neoral") = 0 Then Neoral = 0;  
If Index(Lowcase(Report_Comments), "neoral") NE 0 Then Neoral = 1;  
If Index(Lowcase(Report_Comments), "generic") = 0 Then Generic = 0;  
If Index(Lowcase(Report_Comments), "generic") NE 0 Then Generic = 1;  
If Index(Lowcase(Report_Comments), "compound") = 0 Then Compounded = 0;  
If Index(Lowcase(Report_Comments), "compound") NE 0 Then Compounded = 1;  
Run;Quit;
```

*Split into datasets (one with full data, one with each patient only entered once);

```
Data Cyclo.Full;  
Set Cyclo.Cyclosporine;  
Run;Quit;
```

```
Proc Sort Data = Cyclo.Cyclosporine Nodupkey Out= Cyclo.Cases;  
By Clinic_ID Animal_Name Owner_Name;  
Run;Quit;
```

**Population Descriptions;

```
*Count N's;  
Title 'Group Ns-Full';  
Proc Freq Data=Cyclo.Full;  
Tables Test_Name*Controlled PeakBID*Controlled TroughBID*Controlled  
PeakQD*Controlled MidQD*Controlled TroughQD*Controlled  
DoseInterval*Test_Name*Controlled;  
Run;Quit;
```

```
*Count N's;  
Title 'Group Ns- Cases';  
Proc Freq Data=Cyclo.Cases;  
Tables Test_Name*Controlled PeakBID*Controlled TroughBID*Controlled  
PeakQD*Controlled MidQD*Controlled TroughQD*Controlled  
DoseInterval*Test_Name*Controlled;  
Run;Quit;
```

```
*Export to see date range;  
Proc sort Data=Cyclo.Full out=Cyclo.DateSort;  
By Received_Date;  
Run;Quit;
```

```
*Percent ND/Above range;  
Title 'ND/Above assay range by group';  
Proc Freq Data = Cyclo.Full;  
Tables PeakBID*AboveRange*NonDetectable TroughBID*AboveRange*NonDetectable  
PeakQD*AboveRange*NonDetectable MidQD*AboveRange*NonDetectable  
TroughQD*AboveRange*NonDetectable;  
Run;Quit;
```

```
*Age Summary;  
TITLE 'Age Summary';  
Proc means data=Cyclo.Cases;  
Var Age;  
Where Age NE 0 and Age NE .;  
Run;Quit;
```

```
*Count Frequency of each breed;  
TITLE 'Breed Frequencies';  
Proc freq data=Cyclo.Cases;  
Tables Breed_Name / out=Cyclo.Breeds;  
Run;Quit;
```

```
Proc Sort Data=Cyclo.Breeds;  
By descending COUNT;  
Run;Quit;
```

```
*Count Frequency of each gender;  
TITLE 'Gender Frequencies';  
Proc freq data=Cyclo.Cases;  
Tables Gender;  
Run;Quit;
```

```
*Count Frequency of other drugs being indicated;  
TITLE 'Drug Frequencies';  
Proc freq data=Cyclo.Cases;  
Tables Pred Prednisone Prednisolone Azithro Leflunomide Omeprazole Azathioprine  
Ketoconazole;  
Run;Quit;
```

```
*Count Frequency of each disease being indicated;  
TITLE 'Disease Frequencies';  
Proc freq data=Cyclo.Cases;  
Tables ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA Atopy MDR1;  
Run;Quit;
```

```
**Begin Analysis;
```

****Half-Life;**

*Half-life descriptive statistics;

Title 'Half-Life Statistics';

Proc Means Data=Cyclo.Full n mean median mode std max min range clm;

Var Half_Life;

Where Test_Name = 'Cyclosporine Half-Life';

Run;Quit;

*Drugs affect half-life?;

TITLE 'GLM-Halflife-Drugs';

Proc reg data=Cyclo.Full;

Where Test_Name = 'Cyclosporine Half-Life';

Model Half_Life = Pred Prednisone Prednisolone Azithro Leflunomide Omeprazole

Azathioprine Ketoconazole / selection=adjrsq;

Run;Quit;

*Drugs affect half-life?;

TITLE 'GLM-Halflife-Diseases';

Proc reg data=Cyclo.Full;

Where Test_Name = 'Cyclosporine Half-Life';

Model Half_Life = ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA Atopy MDR1 /

selection=adjrsq;

Run;Quit;

*Age affect half-life?;

TITLE 'Age Effect on Half-life';

Proc reg data=Cyclo.Full;

Where Test_Name = 'Cyclosporine Half-Life';

Model Half_Life = Age;

Run;Quit;

*Gender affect half-life?;

TITLE 'Gender Effect on Half-life';

Proc glm data=Cyclo.Full;

Class Gender Sex Neutered;

model Half_Life = Gender Sex Neutered;

Means Gender Sex Neutered/ clm t tukey;

Where Test_Name = 'Cyclosporine Half-Life';

Run;Quit;

****BID PEAK;**

*ANOVA comparing Concentrations accross Controlled for BID Peaks;

TITLE 'GLM-BID-Peak';

Proc glm data=Cyclo.Full;

```
Where Controlled NE 'Unspecified' And Test_Name = 'Cyclosporine' And PeakBID = 1;
Class Controlled;
Model Conc = Controlled / cli p clm;
Means Controlled / clm t tukey;
Run;Quit;
```

```
*Correlation of Dose (mg/kg) vs PDC for BID Peaks;
TITLE 'Correlation Dose vs Conc BID Peak';
Proc corr data=Cyclo.Full plots=matrix;
Var Dose Conc;
where Test_Name = 'Cyclosporine' And PeakBID = 1;
Run;Quit;
```

```
*Regression plot of Cyclosporine PDC vs Dose for BID Peaks;
TITLE 'Cyclosporine Dose vs Conc BID Peak';
proc sgplot data=Cyclo.Full;
reg y=Dose x=Conc / NOLEGFIT LINEATTRS= (THICKNESS= 2);
Where Test_Name = 'Cyclosporine' And PeakBID = 1;
run;quit;
```

```
*Generate frequency and find double sided 95% to find "therapeutic range" for BID Peaks;
TITLE 'Therapeutic Range BID Peak';
Proc Freq Data=Cyclo.Full;
Tables Conc;
Where Test_Name = 'Cyclosporine' And PeakBID = 1 And Controlled = 'Yes';
Run;Quit;
```

```
**BID TROUGH;
```

```
*ANOVA comparing Concentrations accross Controlled responses for BID Troughs;
TITLE 'GLM-BID-Trough';
Proc glm data=Cyclo.Full;
Where Controlled NE 'Unspecified' And Test_Name = 'Cyclosporine' And TroughBID = 1;
Class Controlled;
Model Conc = Controlled / cli p clm;
Means Controlled / clm t tukey;
Run;Quit;
```

```
*Correlation of Dose (mg/kg) vs PDC for BID Troughs;
TITLE 'Correlation Dose vs Conc BID Trough';
Proc corr data=Cyclo.Full plots=matrix;
Var Dose Conc;
where Test_Name = 'Cyclosporine' And TroughBID = 1;
Run;Quit;
```

```
*Regression plot of Cyclosporine PDC vs Dose for BID Troughs;
```

```

TITLE 'Cyclosporine Dose vs Conc BID Trough';
proc sgplot data=Cyclo.Full;
reg y=Dose x=Conc / NOLEGFIT LINEATTRS= (THICKNESS= 2);
Where Test_Name = 'Cyclosporine' And TroughBID = 1;
run;quit;

*Generate frequency and find double sided 95% to find "therapuetic range" for BID Troughs;
TITLE 'Therapeutic Range BID Trough';
Proc Freq Data=Cyclo.Full;
Tables Conc;
Where Test_Name = 'Cyclosporine' And TroughBID = 1 And Controlled = 'Yes';
Run;Quit;

**QD PEAK;

*ANOVA comparing Concentrations accross Controlled responses for QD Peaks;
TITLE 'GLM-QD-Peak';
Proc glm data=Cyclo.Full;
Where Controlled NE 'Unspecified' And Test_Name = 'Cyclosporine' And PeakQD = 1;
Class Controlled;
Model Conc = Controlled / cli p clm;
Means Controlled / clm t tukey;
Run;Quit;

*Correlation of Dose (mg/kg) vs PDC for QD Peaks;
TITLE 'Correlation Dose vs Conc QD Peak';
Proc corr data=Cyclo.Full plots=matrix;
Var Dose Conc;
where Test_Name = 'Cyclosporine' And PeakQD = 1;
Run;Quit;

*Regression plot of Cyclosporine PDC vs Dose for QD Peaks;
TITLE 'Cyclosporine Dose vs Conc QD Peak';
proc sgplot data=Cyclo.Full;
reg y=Dose x=Conc / NOLEGFIT LINEATTRS= (THICKNESS= 2);
Where Test_Name = 'Cyclosporine' And PeakQD = 1;
run;quit;

*Generate frequency and find double sided 95% to find "therapuetic range" for QD Peaks;
TITLE 'Therapeutic Range QD Peak';
Proc Freq Data=Cyclo.Full;
Tables Conc;
Where Test_Name = 'Cyclosporine' And PeakQD = 1 And Controlled = 'Yes';
Run;Quit;

**QD Mid;

```

```
*ANOVA comparing Concentrations accross Controlled responses for QD Mid;
TITLE 'GLM-QD-Mid';
Proc glm data=Cyclo.Full;
Where Controlled NE 'Unspecified' And Test_Name = 'Cyclosporine' And MidQD = 1;
Class Controlled;
Model Conc = Controlled / cli p clm;
Means Controlled / clm t tukey;
Run;Quit;
```

```
*Correlation of Dose (mg/kg) vs PDC for QD Mid;
TITLE 'Correlation Dose vs Conc QD Mid';
Proc corr data=Cyclo.Full plots=matrix;
Var Dose Conc;
where DoseInterval='QD' And Test_Name = 'Cyclosporine' And MidQD = 1;
Run;Quit;
```

```
*Regression plot of Cyclosporine PDC vs Dose for QD Mid;
TITLE 'Cyclosporine Dose vs Conc QD Mid';
proc sgplot data=Cyclo.Full;
reg y=Dose x=Conc / NOLEGFIT LINEATTRS= (THICKNESS= 2);
Where DoseInterval = 'QD' And MidQD = 1;
run;quit;
```

```
*Generate frequency and find double sided 95% to find "therapeutic range" for QD Mid;
TITLE 'Therapeutic Range QD Mid';
Proc Freq Data=Cyclo.Full;
Tables Conc;
Where Test_Name = 'Cyclosporine' And MidQD = 1 And Controlled = 'Yes';
Run;Quit;
```

****QD TROUGH;**

```
*ANOVA comparing Concentrations accross Controlled responses for QD Troughs;
TITLE 'GLM-QD-Trough';
Proc glm data=Cyclo.Full;
Where Controlled NE 'Unspecified' And Test_Name = 'Cyclosporine' And TroughQD = 1;
Class Controlled;
Model Conc = Controlled / cli p clm;
Means Controlled / clm t tukey;
Run;Quit;
```

```
*Correlation of Dose (mg/kg) vs PDC for QD Troughs;
TITLE 'Correlation Dose vs Conc QD Trough';
Proc corr data=Cyclo.Full plots=matrix;
```

```
Var Dose Conc;
where DoseInterval='QD' And Test_Name = 'Cyclosporine' And TroughQD = 1;
Run;Quit;
```

```
*Regression plot of Cyclosporine PDC vs Dose for QD Troughs;
TITLE 'Cyclosporine Dose vs Conc QD Trough';
proc sgplot data=Cyclo.Full;
reg y=Dose x=Conc / NOLEGFIT LINEATTRS= (THICKNESS= 2);
Where DoseInterval = 'QD' And TroughQD = 1;
run;quit;
```

```
*Generate frequency and find double sided 95% to find "therapeutic range" for QD Trough;
TITLE 'Therapeutic Range QD Trough';
Proc Freq Data=Cyclo.Full;
Tables Conc;
Where Test_Name = 'Cyclosporine' And TroughQD = 1 And Controlled = 'Yes';
Run;Quit;
```

```
**Log Conc over Time;
```

```
*Correlation of pdc adjusted for dose over time;
TITLE 'Cyclosporine LogAdjConc vs Time';
Proc Corr Data = Cyclo.Full;
Var Logadjconc Dose;
Where Test_Name = 'Cyclosporine' And Timing LE 28;
Run;Quit;
```

```
*Regression plot of Cyclosporine Log(PDC) over time;
TITLE 'Cyclosporine LogAdjConc vs Time';
proc sgplot data=Cyclo.Full;
reg y=logadjconc x=Timing / NOLEGFIT LINEATTRS= (THICKNESS= 2);
where Test_Name='Cyclosporine' And Timing LE 28;
run;quit;
```

```
*Correlation of pdc adjusted for dose over time BID;
TITLE 'Cyclosporine LogAdjConc vs Time BID';
Proc Corr Data = Cyclo.Full;
Var Logadjconc Dose;
Where Test_Name = 'Cyclosporine' And DoseInterval = 'BID' And Timing LE 14;
Run;Quit;
```

```
*Regression plot of Cyclosporine Log(PDC) over time BID;
TITLE 'Cyclosporine LogAdjConc vs Time BID';
proc sgplot data=Cyclo.Full;
reg y=logadjconc x=Timing / NOLEGFIT LINEATTRS= (THICKNESS= 2);
where Test_Name='Cyclosporine' And DoseInterval = 'BID' And Timing LE 14;
```

```
run;quit;
```

```
*Correlation of pdc adjusted for dose over time BID;  
TITLE 'Cyclosporine LogAdjConc vs Time QD';  
Proc Corr Data = Cyclo.Full;  
Var Logadjconc Dose;  
Where Test_Name = 'Cyclosporine' And DoseInterval = 'QD' And Timing LE 28;  
Run;Quit;
```

```
*Regression plot of Cyclosporine Log(PDC) over time QD;  
TITLE 'Cyclosporine LogAdjConc vs Time QD';  
proc sgplot data=Cyclo.Full;  
reg y=logadjconc x=Timing / NOLEGFIT LINEATTRS= (THICKNESS= 2);  
where Test_Name='Cyclosporine' And DoseInterval = 'QD' And Timing LE 28;  
run;quit;
```

```
**Identify relationships;
```

```
*Identify disease relationship to plasma drug concentration;  
TITLE 'Disease Covariance BID Peak';  
Proc reg data=Cyclo.Full;  
Model AdjConc = ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA Atopy MDR1 /  
selection=backward slstay=0.05 clb p;  
Where Test_Name = 'Cyclosporine' And PeakBID = 1;  
Run;Quit;
```

```
TITLE 'Disease Covariance BID Trough';  
Proc reg data=Cyclo.Full;  
Model AdjConc = ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA Atopy MDR1 /  
selection=backward slstay=0.05 clb p;  
Where Test_Name = 'Cyclosporine' And TroughBID = 1;  
Run;Quit;
```

```
TITLE 'Disease Covariance QD Peak';  
Proc reg data=Cyclo.Full;  
Model AdjConc = ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA Atopy MDR1 /  
selection=backward slstay=0.05 clb p;  
Where Test_Name = 'Cyclosporine' And PeakQD = 1;  
Run;Quit;
```

```
TITLE 'Disease Covariance QD Mid';  
Proc reg data=Cyclo.Full;  
Model AdjConc = ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA Atopy MDR1 /  
selection=backward slstay=0.05 clb p;  
Where Test_Name = 'Cyclosporine' And MidQD = 1;  
Run;Quit;
```

```
TITLE 'Disease Covariance QD Trough';
Proc reg data=Cyclo.Full;
Model AdjConc = ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA Atopy MDR1 /
selection=backward slstay=0.05 clb p;
Where Test_Name = 'Cyclosporine' And TroughQD = 1;
Run;Quit;
```

```
*Identify other drug relationship to plasma drug concentration;
TITLE 'Drug Covariance BID Peak';
Proc reg data=Cyclo.Full;
Model AdjConc = Pred Azithro Leflunomide Omeprazole Azathioprine Ketoconazole /
selection=backward slstay=0.05 clb p;
Where Test_Name = 'Cyclosporine' And PeakBID = 1;
Run;Quit;
```

```
TITLE 'Drug Covariance BID Trough';
Proc reg data=Cyclo.Full;
Model AdjConc = Pred Prednisone Prednisolone Azithro Leflunomide Omeprazole Azathioprine
Ketoconazole / selection=backward slstay=0.05 clb p;
Where Test_Name = 'Cyclosporine' And TroughBID = 1;
Run;Quit;
```

```
TITLE 'Drug Covariance QD Peak';
Proc reg data=Cyclo.Full;
Model AdjConc = Pred Prednisone Prednisolone Azithro Leflunomide Omeprazole Azathioprine
Ketoconazole / selection=backward slstay=0.05 clb p;
Where Test_Name = 'Cyclosporine' And PeakQD = 1;
Run;Quit;
```

```
TITLE 'Drug Covariance QD Mid';
Proc reg data=Cyclo.Full;
Model AdjConc = Pred Prednisone Prednisolone Azithro Leflunomide Omeprazole Azathioprine
Ketoconazole / selection=backward slstay=0.05 clb p;
Where Test_Name = 'Cyclosporine' And MidQD = 1;
Run;Quit;
```

```
TITLE 'Drug Covariance QD Trough';
Proc reg data=Cyclo.Full;
Model AdjConc = Pred Prednisone Prednisolone Azithro Leflunomide Omeprazole Azathioprine
Ketoconazole / selection=backward slstay=0.05 clb p;
Where Test_Name = 'Cyclosporine' And TroughQD = 1;
Run;Quit;
```

```
*Identify age relationship to plasma drug concentration;
TITLE 'Age Covariance BID Peak';
```

```
Proc reg data=Cyclo.Full;
Model AdjConc = Age / clb p;
Where Test_Name = 'Cyclosporine' And PeakBID = 1;
Run;Quit;
```

```
TITLE 'Age Covariance BID Trough';
Proc reg data=Cyclo.Full;
Model AdjConc = Age / clb p;
Where Test_Name = 'Cyclosporine' And TroughBID = 1;
Run;Quit;
```

```
TITLE 'Age Covariance QD Peak';
Proc reg data=Cyclo.Full;
Model AdjConc = Age / clb p;
Where Test_Name = 'Cyclosporine' And PeakQD = 1;
Run;Quit;
```

```
TITLE 'Age Covariance QD Mid';
Proc reg data=Cyclo.Full;
Model AdjConc = Age / clb p;
Where Test_Name = 'Cyclosporine' And MidQD = 1;
Run;Quit;
```

```
TITLE 'Age Covariance QD Trough';
Proc reg data=Cyclo.Full;
Model AdjConc = Age / clb p;
Where Test_Name = 'Cyclosporine' And TroughQD = 1;
Run;Quit;
```

```
*Identify Breed/Gender relationship to plasma drug concentration;
TITLE 'Breed/Gender Covariance BID Peak';
Proc glm data=Cyclo.Full;
Class Breed_Name Gender Sex Neutered;
Model AdjConc = Breed_Name Gender Sex Neutered;
Where Test_Name = 'Cyclosporine' And PeakBID = 1;
Run;Quit;
```

```
TITLE 'Breed/Gender Covariance BID Trough';
Proc glm data=Cyclo.Full;
Class Breed_Name Gender Sex Neutered;
Model AdjConc = Breed_Name Gender Sex Neutered;
Where Test_Name = 'Cyclosporine' And TroughBID = 1;
Run;Quit;
```

```
TITLE 'Breed/Gender Covariance QD Peak';
Proc glm data=Cyclo.Full;
```

```
Class Breed_Name Gender Sex Neutered;
Model AdjConc = Breed_Name Gender Sex Neutered;
Where Test_Name = 'Cyclosporine' And PeakQD = 1;
Run;Quit;
```

```
TITLE 'Breed/Gender Covariance QD Mid';
Proc glm data=Cyclo.Full;
Class Breed_Name Gender Sex Neutered;
Model AdjConc = Breed_Name Gender Sex Neutered;
Where Test_Name = 'Cyclosporine' And MidQD = 1;
Run;Quit;
```

```
TITLE 'Breed/Gender Covariance QD Trough';
Proc glm data=Cyclo.Full;
Class Breed_Name Gender Sex Neutered;
Model AdjConc = Breed_Name Gender Sex Neutered;
Where Test_Name = 'Cyclosporine' And TroughQD = 1;
Run;Quit;
```

**Any drug show better control with than without?;

/*Alternative Way to do Odds ratio, proc logistic more efficient for multiple variables

```
TITLE 'Control with Pred';
Proc sort data=Cyclo.Cyclosporine;
By Pred;
Run;Quit;
```

```
*Odds ratio: pred more likely to achieve control;
Proc freq data=Cyclo.Cyclosporine;
Tables Pred*Controlled / chisq relrisk;
Where Controlled NE 'Unspecified';
Run;Quit;
```

*/

```
TITLE 'Drug more likely controlled?';
Proc logistic data=Cyclo.Cases;
Class Pred Azithro Leflunomide Omeprazole Azathioprine Ketoconazole;
model Controlled (event='Yes') = Pred Prednisone Prednisolone Azithro Leflunomide
Omeprazole Azathioprine Ketoconazole / selection=backward;
Where Controlled NE 'Unspecified';
Run;Quit;
```

*Any disease more likely controlled?;
TITLE 'Disease more likely controlled?';

```
Proc logistic data=Cyclo.Cases;
Class ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA Atopy;
model Controlled (event='Yes') = ITP Histocytosis Arthritis GME IBD Anemia IMHA RPA
Atopy / selection=backward;
Where Controlled NE 'Unspecified';
Run;Quit;
```

```
*Breed, Gender more likely controlled?;
TITLE 'Breed/Gender more likely controlled?';
Proc logistic data=Cyclo.Cases;
Class Breed_Name Gender Sex Neutered;
model Controlled (event='Yes') = Breed_Name Gender Sex Neutered / selection=backward;
Where Controlled NE 'Unspecified';
Run;Quit;
```

```
*Preparation more likely controlled?;
TITLE 'Preparation more likely controlled?';
Proc logistic data=Cyclo.Cases;
Class Atopica Neoral Generic Compounded;
model Controlled (event='Yes') = Atopica Neoral Generic Compounded / selection=backward;
Where Controlled NE 'Unspecified';
Run;Quit;
```

```
*Age affect controlled?;
TITLE 'Age Effect on Controlled';
Proc glm data=Cyclo.Cases;
Class Controlled;
Model Age = Controlled;
Where Controlled NE 'Unspecified';
Means Controlled / clm t tukey;
Run;Quit;
```

****Dose Comparisons;**

```
*ANOVA comparing Doses (mg/kg) accross Controlled responses for BID;
TITLE 'GLM-Dose-BID';
Proc glm data=Cyclo.Cases;
Class Controlled;
Model Dose = Controlled;
Means Controlled / clm t tukey;
Where DoseInterval = 'BID' And Controlled NE 'Unspecified';
Run;Quit;
```

```
Proc sort data=Cyclo.Cases;
By Controlled;
Run;Quit;
```

```
Proc means data=Cyclo.Cases n mean median mode std max min range clm;  
Var Dose;  
By Controlled;  
Where DoseInterval = 'BID' And Controlled NE 'Unspecified';  
Run;Quit;
```

```
*ANOVA comparing Doses (mg/kg) across Controlled responses for QD;  
TITLE 'GLM-Dose-QD';  
Proc glm data=Cyclo.Cases;  
Class Controlled;  
Model Dose = Controlled;  
Means Controlled / clm t tukey;  
Where DoseInterval = 'QD' And Controlled NE 'Unspecified';  
Run;Quit;
```

```
Proc sort data=Cyclo.Cases;  
By Controlled;  
Run;Quit;
```

```
Proc means data=Cyclo.Cases n mean median mode std max min range clm;  
Var Dose;  
By Controlled;  
Where DoseInterval = 'QD' And Controlled NE 'Unspecified';  
Run;Quit;
```

```
*DoseInterval more likely controlled?;  
Proc freq data=Cyclo.Cases;  
Tables DoseInterval*Controlled;  
Where Controlled NE 'Unspecified';  
Run;Quit;
```

```
TITLE 'BID vs QD more likely controlled?';  
Proc logistic data=Cyclo.Cases;  
Class DoseInterval;  
model Controlled (event='Yes') = DoseInterval;  
Where Controlled NE 'Unspecified';  
Run;Quit;
```

```
*Identify Preparation relationship to plasma drug concentration;  
TITLE 'Preparation Covariance BID Peak';  
Proc glm data=Cyclo.Full;  
Class Atopica Neoral Generic Compounded;  
Model AdjConc = Atopica Neoral Generic Compounded;  
Where Test_Name = 'Cyclosporine' And PeakBID = 1;  
Run;Quit;
```

```
TITLE 'Preparation Covariance BID Trough';
Proc glm data=Cyclo.Full;
Class Atopica Neoral Generic Compounded;
Model AdjConc = Atopica Neoral Generic Compounded;
Where Test_Name = 'Cyclosporine' And TroughBID = 1;
Run;Quit;
```

```
TITLE 'Preparation Covariance QD Peak';
Proc glm data=Cyclo.Full;
Class Atopica Neoral Generic Compounded;
Model AdjConc = Atopica Neoral Generic Compounded;
Where Test_Name = 'Cyclosporine' And PeakQD = 1;
Run;Quit;
```

```
TITLE 'Preparation Covariance QD Mid';
Proc glm data=Cyclo.Full;
Class Atopica Neoral Generic Compounded;
Model AdjConc = Atopica Neoral Generic Compounded;
Where Test_Name = 'Cyclosporine' And MidQD = 1;
Run;Quit;
```

```
TITLE 'Preparation Covariance QD Trough';
Proc glm data=Cyclo.Full;
Class Atopica Neoral Generic Compounded;
Model AdjConc = Atopica Neoral Generic Compounded;
Where Test_Name = 'Cyclosporine' And TroughQD = 1;
Run;Quit;
```

```
*Disease treatment frequency;
Proc freq data=Cyclo.Cases;
Tables ITP*DoseInterval Histocytosis*DoseInterval Arthritis*DoseInterval GME*DoseInterval
IBD*DoseInterval Anemia*DoseInterval IMHA*DoseInterval RPA *DoseInterval
Atopy*DoseInterval;
Run;Quit;
```

```
Proc sort data = Cyclo.Full;
By IMHA;
Run;Quit;
```

```
Proc means data = Cyclo.Full n mean median mode std clm;
Var AdjConc;
Where Test_Name = 'Cyclosporine' And PeakBID = 1;
By IMHA;
Run;Quit;
```

```
Proc sort data = Cyclo.Full;  
By Ketoconazole;  
Run;Quit;
```

```
Proc means data = Cyclo.Full n mean median mode std clm;  
Var AdjConc;  
Where Test_Name = 'Cyclosporine' And PeakBID = 1;  
By Ketoconazole;  
Run;Quit;
```

```
Proc sort data = Cyclo.Full;  
By Generic;  
Run;Quit;
```

```
Proc means data = Cyclo.Full n mean median mode std clm;  
Var AdjConc;  
Where Test_Name = 'Cyclosporine' And PeakBID = 1;  
By Generic;  
Run;Quit;
```

```
Proc sort data = Cyclo.Full;  
By Ketoconazole;  
Run;Quit;
```

```
Proc means data = Cyclo.Full n mean median mode std clm;  
Var AdjConc;  
Where Test_Name = 'Cyclosporine' And TroughBID = 1;  
By Ketoconazole;  
Run;Quit;
```

```
Proc sort data = Cyclo.Full;  
By Generic;  
Run;Quit;
```

```
Proc means data = Cyclo.Full n mean median mode std clm;  
Var AdjConc;  
Where Test_Name = 'Cyclosporine' And TroughQD = 1;  
By Generic;  
Run;Quit;
```

```
Proc sort data = Cyclo.Full;  
By Compounded;  
Run;Quit;
```

```
Proc means data = Cyclo.Full n mean median mode std clm;  
Var AdjConc;
```

```
Where Test_Name = 'Cyclosporine' And TroughQD = 1;  
By Compounded;  
Run;Quit;
```