CLINICAL STUDY PROTOCOL

A Single Dose, Open-Label, Randomized, Four-Way Crossover, Fully Replicate, Bioequivalence Study of Generic Tacrolimus and Prograf® Capsules in Healthy Volunteers under Fasting Conditions

BPSUSA Protocol Number:	2521
Protocol Version Number:	3.0
Protocol Version Date:	Jun 22, 2021
Sponsor:	U.S. Food and Drug Administration

PROTOCOL APPROVAL SIGNATORY PAGE:

I am aware of, and agree to comply with, all of the procedures contained within this protocol and requirements of applicable regulatory agencies:

[redacted]

Dr. Artan Markollari, M.D.

[redacted]

Date (Mmm dd, yyyy)

[redacted]

BioPharma SERVICES USA INC.

[redacted]

CLINICAL STUDY PROTOCOL

A Single Dose, Open-Label, Randomized, Four-Way Crossover, Fully Replicate, Bioequivalence Study of Generic Tacrolimus Capsules and Prograf® Capsules in Healthy Volunteers under Fasting Conditions

BPSUSA Protocol Number:	2521
Protocol Version Number:	3.0
Protocol Version Date:	Jun 22, 2021
Sponsor:	U.S. Food and Drug Administration

PROTOCOL APPROVAL SIGNATORY PAGE

I am aware of, and agree to comply with, all of the procedures contained within this protocol and requirements of applicable regulatory agencies:

[redacted] _____ _____

Sanjida Mahjabeen, Ph.D. Date (Mmm dd, yyyy)

[redacted]

REVISION HISTORY

Version # (Version Date)	Affected Sections
1.0 (Aug 07, 2020)	N/A
2.0 (Dec 23, 2020)	As per discussion between sponsor and BPSI/BPSUSA, the following sections were revised from the previously approved protocol: i) *Protocol Title Pages and Headers* – Protocol Version and Date revised to Version 2.0 and Dec 23, 2020, respectively. ii) Updated BPSUSA telephone and fax number. Following sections have been updated: • Title page • Section SPONSOR, INVESTIGATORS AND FACILITIES • Section INVESTIGATOR CREDENTIALS iii) Section Table of Contents – updated pagination iv) Tuberculosis (TB) Test and/or Questionnaire have been removed from the study. Following sections have been updated: • Section 2.0 TABLE OF STUDY PROCEDURES • Section 5.1.1 Screening (Visit 1) • Section 7.3 Exclusion Criteria • Section 8.6 Tuberculin Test (has been deleted) • APPENDIX A – SCHEDULE OF EVENTS v) Included light smokers to this study and removed cotinine test. Following sections have been updated: • Section 1.0 SYNOPSIS • Section 2.0 TABLE OF STUDY PROCEDURES • Section 4.1 Study Objective • Section 5.0 STUDY DESIGN • Section 6.0 STUDY POPULATION • Section 7.0 SUBJECT SELECTION AND WITHDRAWAL/TERMINATION • APPENDIX A – SCHEDULE OF EVENTS

- APPENDIX B – CLINICAL LABORATORY ASSESSMENT

vi) The test for SARS-CoV-2 at screening has been removed, and the test at check-in will be done if applicable, related wordings have been updated. Following sections have been updated:
- Section 1.0 SYNOPSIS
- Section 2.0 TABLE OF STUDY PROCEDURES
- Section 8.5.5 SARS-CoV-2 Test
- APPENDIX A – SCHEDULE OF EVENTS

vii) Section SPONSOR, INVESTIGATORS AND FACILITIES has been updated for the IRB official name.

viii) Section 7.3 Exclusion Criteria has been updated regarding Systolic blood pressure and temperature normal range, timeframe for the restriction of beverages containing grapefruit and/or pomelo consumption.

ix) Section 7.5 Study Restrictions has been updated regarding timeframe for the restriction of beverages containing grapefruit and/or pomelo consumption.

x) Section 8.5.4 Pulse Oximetry and Respiratory Rate has been updated to add normal range.

xi) Section 8.6 Vital Signs has been updated regarding vital sign normal ranges.

xii) End-of-Study (EOS) now has one-week ±3 days window. Following sections have been updated:
- Section 2.0 TABLE OF STUDY PROCEDURES
- Section 5.1.3 Post-Study [End-of-Study (EOS)]/Early Termination

xiii) Section 8.5.3 Temperature Check. Temperature will be measured using an oral thermometer instead of a non-contact thermometer.

xiv) Section 9.3 Drug Inventory has been updated regarding the purchase of study drug.

xv) Section 11.1.1 Treatment-emergent adverse events (TEAEs) added sentence "**The PI/sub-I will be responsible for determining whether a TEAE is possibly related to the SARS-CoV-2 and document accordingly.**"

	xvi) Section 11.3 Adverse Events and Serious Adverse Events Reporting has been updated regarding the responsibility of notifying the local IRB or regulatory agency and SAE contact. xvii) In Section 13.1 Safety Data Set, related sentences have been updated to: "In a case where SARS-CoV-2 related TEAEs lead to the withdrawal **or dismissal** of approximately 5 % or more of the dosed subjects, a subgroup analysis **related to Adverse Events (AE)** may be performed**. And the safety listings and tables for AE with subgroup and the total safety population will both be presented.**" xviii) Section 13.2.2 Statistical Analysis of Pharmacokinetic Data, updated statistical model regarding dosing in groups. xix) In Section 15.3 Study Completion/Termination, related sentences have been updated to "BioPharma Services USA Inc. and/or the Sponsor reserve the right to terminate the study at any time~~, and~~ for any reason **related to the safety of subjects or due to any other non-study specific reason**." xx) Minor administrative/grammatical revisions were also made. The study ICF was also revised to reflect applicable changes.
3.0 (Jun 22, 2021)	As per discussion between sponsor and BPSI/BPSUSA, the following sections were revised from the previously approved protocol: i) *Protocol Title Pages and Headers* – Protocol Version and Date revised to Version 3.0 and Jun 22, 2021, respectively. ii) Non or light smoking has been removed from Section 1.0. 4.1, 5.1.2.2 and 6.0. iii) Confinement has been updated to read as: "From at least 12 hours prior to dosing until at least 72 hours post dose, for a total of approximately 84 hours for each study period." And Footer in Section 2.0 and 5.2 has been revised accordingly. iv) Additional measurements of the heart rate and blood pressure have been added at 48- and 72-hour post-dose for

	additional safety monitoring. Therefore, Sections 1.0, 2.0, 8.6 and Appendix A have been updated.
v)	Section 7.1 sample size has been updated for the clarification.
vi)	MedWatch has been replaced with "FDA Adverse Event Reporting System (FAERS)" in Section 11.3
vii)	Section 12.1.1 and 13.1 has been updated to further clarify the samples to be assayed and data to be included in the pharmacokinetic analysis.
viii)	AUC_{72} is going to be measured as a primary endpoint in this study. Therefore, Sections 1.0, 13.2.1, 13.2.2, 13.4 and Appendix C have been updated.
ix)	Section 13.3.2 was updated to include the listing for magnesium, creatinine kinase and lipid panel test.
x)	Section 13.3.3 has been updated to include the following statements in line with the SAP: Descriptive statistics of all quantitative measurements from all clinical laboratory parameters will be presented by the type of laboratory test, laboratory parameter, and visit.

TABLE OF CONTENTS

SPONSOR, INVESTIGATORS AND FACILITIES

Principal Investigator:	Dr. Artan Markollari, M.D. BioPharma Services USA Inc. [redacted]	**Pharmacokinetic / Statistical Facility:**	[redacted]
Clinical Laboratory:	[redacted]	**Sponsor:**	OGD/CDER/FDA, [redacted]
Clinical Facility	[redacted]	**Designated, Local Institutional Review Board (IRB) Review and Oversight:**	[redacted]
Bioanalytical Facility:	[redacted]		

INVESTIGATOR CREDENTIALS

Dr. Artan Markollari, Principal Investigator

[redacted]

Dr. Artan Markollari, M.D.
BioPharma Services USA Inc.
[redacted]

Date (Mmm dd, yyyy)

1.0 SYNOPSIS

Title:	A Single Dose, Open-Label, Randomized, Four-Way Crossover, Fully Replicate, Bioequivalence Study of Generic Tacrolimus Capsules and Prograf® Capsules in Healthy Volunteers under Fasting Conditions.
Objective:	The objective of this study is to investigate the bioequivalence of Tacrolimus 1 mg capsules [redacted] and Prograf® 1 mg capsules [redacted] in healthy male and non-pregnant, non-lactating female volunteers under fasting conditions.
Experimental Design:	A single-dose, randomized, open-label, fully replicate crossover, four-period, two-treatment, two-sequence, bioequivalence study.
Study Rationale:	This is an *in-vivo* study to investigate the bioequivalence of generic tacrolimus and its reference listed drug (RLD). The outcome of this study will help further the Agency's understanding about pharmacokinetic (PK) performance of tacrolimus in a healthy volunteer population and improve review standards for bioequivalence of narrow therapeutic index (NTI) drugs.
Study Population:	72 subjects, including 8 standbys, will be enrolled to ensure dosing of approximately 64 subjects. The subjects may be dosed in more than one cohort. Healthy male and non-pregnant, non-lactating female volunteers, 18 - 59 years of age, inclusive, with a body mass index (BMI) within 18.5-33.0 kg/m^2, inclusive.
Test Drug (T):	Tacrolimus 1 mg capsules ([redacted]
Reference Drug (R):	Prograf® 1 mg capsules [redacted]
Treatment:	1 × 1 mg capsules in each study period
Duration of Confinement:	From at least 12 hours prior to dosing until at least 72 hours post-dose, for a total of approximately 84 hours for each study period.
Washout:	At least 14 days between each dosing.
Safety Monitoring:	• Vital signs (blood pressure [BP] and heart rate [HR]) will be obtained at pre-dose and at 1, 2, 4, 6, 12, 24, 48 and 72 hours after dosing in each study period. • All tests in Appendix B at screening, and selected tests at each period check-in and post-study. • The PI/Sub-Investigator will be present from approximately 30 minutes prior to dosing until at least 4 hours after the last subject is dosed in each study period. The PI/Sub-Investigator will remain on-call throughout the duration of the study.
COVID-19 prevention and control:	• Temperature, pulse oximetry and respiratory rate will be measured at screening, at each check-in, at pre-dose, at 6- and 12-hours post dose, then at every 12 hours until discharge at each study period, at return visits and at end of study (EOS) visit.

	• A COVID-19 symptom check and COVID-19 questionnaire will also be completed at screening, at each check-in, at return visits and at EOS visit. • If applicable, a locally available test for SARS-CoV-2 will be performed at each period check-in.
Type of Vacutainer	Pre-chilled K_2EDTA Vacutainer®, 4 mL
Blood Sampling Time points:	28 PK Timepoints: Pre-dose (0 hour) and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 72.00, 96.00, 120.00 and 144.00 hours after study drug dosing in each study period.
Total Blood Volume:	Approximately 520 mL of blood, including blood samples for PK time points, pre- and post- study procedures and the selected laboratory testing at each study period.
Analyte(s) to be measured:	Whole blood samples will be assayed for tacrolimus using a validated analytical method according to the principles of Good Laboratory Practice.
Statistical Analysis:	The ln-transformed PK parameters AUC_{72}, AUC_t, AUC_{inf} and C_{max} will be analyzed using both an unscaled average bioequivalence procedure and a reference-scaled average bioequivalence procedure. **Determination of the Within-subject Standard Deviation of the Test and Reference Products (S_{WT} and S_{WR}) for the PK Parameters AUC_{72}, AUC_t, AUC_{inf} and C_{max}** **Reference Scaled Average Bioequivalence Procedure** The 95% upper confidence bound for $(\mu_T - \mu_R)^2 - \theta S_{WR}^2$ will be determined for the ln-transformed PK parameters AUC_{72}, AUC_t, AUC_{inf} and C_{max} using a PROC MIXED model in SAS. **Unscaled Average Bioequivalence Procedure** The 90% Confidence Intervals (CI) for the Test/Reference ratios of geometric means for the PK parameters AUC_{72}, AUC_t, AUC_{inf} and C_{max} will be calculated using a PROC MIXED model in SAS. T_{max}, Lambda and $T_{1/2}$ will be analyzed using an un-scaled procedure. **Calculation of the 90% CI for the Ratio of the Within-subject Standard Deviation of Test Product to Reference Product (σ_{WT}/σ_{WR})** The 90% confidence interval of the ratio of the within subject standard deviation of test product to reference product **(σ_{WT}/σ_{WR})** will be calculated for tacrolimus.
Bioequivalence Criteria:	To establish bioequivalence, all the following conditions for individual PK parameter(s) $AUC_{72}$and C_{max} must be met: • The 90% CI for the Test/Reference ratios of geometric means are completely contained within 80.00%-125.00%.

- The 95% upper confidence bound for $(\mu_T - \mu_R)^2 - \theta S_{WR}^2$ should be less than or equal to 0.
- The upper limit of the 90% confidence interval for the Test/Reference ratio of the within-subject standard deviation $(\sigma_{WT}/\sigma_{WR})$ is less than or equal to 2.5.

2.0 TABLE OF STUDY PROCEDURES

Procedure/Activity	Time points						
	Visit 1	Visit 2 to 17					
	Screening	Each Period Check-in	Period 1	Period 2	Period 3	Period 4	Post-Study/ EOS^
Written informed consent	X						
Drugs of Abuse	X	X					
Alcohol Test	X	X					
Pregnancy (hCG) Test*	X	X					
Vital Signs (BP & HR)	X	X	X[a]	X[a]	X[a]	X[a]	X
Safety measures for COVID-19[#] (Symptom Check, Temperature, Pulse Oximetry and RR)	X	X	X	X	X	X	X
SARS-CoV-2 Test[c]		X					
BMI	X						
Laboratory Testing[+] (Appendix B)	X	X					X
ECG	X						
Inclusion/Exclusion Assessment	X	X[b]					
Restrictions Compliance Check		X[d]	X[d]	X[d]	X[d]	X[d]	
Physical Exam	X						X
Tacrolimus Capsule Administration			X	X	X	X	
PK Sampling			X[e]	X[e]	X[e]	X[e]	
Adverse Event Reporting		X[f]	X[f]	X[f]	X[f]	X[f]	X

Meals		X[g]	X[g]	X[g]	X[g]	X[g]	

^ EOS with one-week ±3 days window.

* Serum pregnancy test at screening and urine pregnancy test at each period check-in.

Temperature, pulse oximetry and respiratory rate will be measured at screening, at each check-in, at pre-dose, at 6- and 12-hours post dose, then at every 12 hours until discharge at each study period, at return visits and at EOS visit. A COVID-19 symptom check, and COVID-19 questionnaire will also be completed at screening, at each check-in, at return visits and at EOS visit.

ǂ All tests in Appendix B at screening, and selected tests at each check-in and at EOS (details in Appendix A and Appendix B).

[a]- Vital signs measurements (BP and HR) to be obtained at pre-dose and at 1, 2, 4, 6, 12,24, 48 and 72 hours after dosing in each study period.

b Only at check-in for the period 1.

c- At each period check-in, if applicable.

d- Confirmed at check-in and each return visits, if applicable.

e- PK sampling: Pre-dose (0 hour) and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 72.00, 96.00, 120.00 and 144.00 hours after study drug dosing in each study period. PK sampling at 96, 120 and 144 hours will be collected during outpatient return visits.

f- Pre-dose conditions at Period 1 check-in and each return visit, if applicable. AEs will be monitored throughout the study.

g- Meals will be served at the scheduled times during confinement prior to dosing and at approximately 4.5, 9.5, 13.5, 24.5, 28.5, 33.5, 37.5, 48.5, 52.5, 57.5 and 61.5 hours after dosing.

3.0 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

3.1 Background Information

Tacrolimus, a calcineurin inhibitor (CNI), has been widely used in solid organ and bone marrow transplants for more than two decades. When an organ is transplanted between a donor and a genetically non identical recipient, the recipient's immune system may recognize the transplant "foreign" and can trigger humoral immune response by activating immune cells such as T cells, B cells, and macrophages causing transplant rejection. Calcineurin, a calcium-dependent phosphatase, is instrumental for signal transduction for activation of T cell and B cell, which in turn cause production of autoinflammatory cytokines such as such as interleukin 2 (IL-2), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ). CNIs prevents the dephosphorylation and translocation of various factors such as the nuclear factor of activated T-cells (NF-AT), and nuclear factor kappa-light-chain enhancer of activated B-cells (NF-κB), thereby inhibiting the signal transduction responsible for growth and proliferation of activated T cells and expression of autoinflammatory cytokines. This mechanism of action results in immunosuppression and prevents organ rejection. Tacrolimus is one of the widely used CNI immunosuppressants in liver and kidney transplant patients [1].

Oral bioavailability of tacrolimus is low, ranged from 11.2 to 19.1% in previous studies in adult kidney recipients. The limited oral bioavailability of tacrolimus could be attributed to-

- Extensive metabolism through CYP3A4 and 3A5 enzymes which are abundantly expressed in gut [1],
- Poor oral absorption of the drug resulting from poor aqueous solubility [1].

Optimum tacrolimus bioavailability is critical for -

i. Achieving desired clinical endpoint: The trough blood concentrations are significantly related to clinical endpoints, i.e., there was a significant correlation between increased trough concentration and decreased risk of acute rejection and vice versa [2].
ii. Minimizing acute toxicity: Long term use of tacrolimus is associated with nephrotoxicity. Study showed monitoring trough tacrolimus whole blood concentrations below 15 ng/mL decreased the occurrence of nephrotoxicity [3].

To enhance the oral bioavailability intricate formulation strategies have been studied. One such strategy in formulating tacrolimus into amorphous solid dispersions (ASD). In ASDs, the amorphous form of the drug, which is believed to have increased solubility compared to crystalline form, is stabilized with the aid of a polymer. Currently, Prograf® and the six approved generic products referencing Prograf® are being formulated using ASD strategy. There are different

manufacturing processes to produce ASDs such as spray-freeze drying, hot melt extrusion etc. However, the interplay of manufacturing, processing, and storage conditions on the critical quality attributes of ASDs that impact dissolution, precipitation, and ultimately in vivo performances are poorly understood. For example, amorphous tacrolimus can be converted to crystalline tacrolimus during manufacture and/or storage, which may lead to decreased solubility and thereby suboptimal therapeutic level. This is undesirable as blood concentration is tied to therapeutic performance [1].

There are concerns in the transplant community that generic ASDs may not perform similarly to the brand ASD. To this end, the Office of Generic Drugs aims to evaluate BE of one of the generic products (generic from Acord) which showed likely inequivalent performance in vitro and in vivo in a preclinical setting [4, 5].

3.2 Rationale

This is an in-vivo study to investigate the bioequivalence of a generic tacrolimus product in comparison with that of its reference listed drug (RLD). The outcome of this study will help advance the Agency's understanding about the generic tacrolimus product's pharmacokinetic (PK) performance in healthy volunteer population and improve review standards for bioequivalence of NTI drugs.

3.3 Product Information

PROGRAF® [6]

Prograf® (tacrolimus) capsule is indicated for the prophylaxis of organ rejection in adult and pediatric patients receiving allogeneic kidney, liver, and heart transplants, in combination with other immunosuppressants.

Mechanism of Action

Tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin (a ubiquitous mammalian intracellular enzyme) is then formed, after which the phosphatase activity of calcineurin is inhibited. Such inhibition prevents the dephosphorylation \ of various factors such as the nuclear factor of activated T-cells (NF-AT), and nuclear factor kappa-light-chain enhancer of activated B-cells (NF-κB). This dephosphorylation, in turn, prevents the translocation of these factors to the nucleus, leading to decreased activity and of genes coding for many cytokines. Tacrolimus inhibits the expression and/or production of several cytokines that include interleukin (IL)-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, gamma interferon, tumor necrosis factor-alpha, and granulocyte macrophage colony-stimulating factor. Tacrolimus also inhibits IL-2 receptor expression and nitric oxide release, induces apoptosis and production of transforming growth factor beta that can lead to immunosuppressive

activity. The net result is the inhibition of T-lymphocyte activation and proliferation, as well as T-helper-cell-dependent B-cell response (i.e., immunosuppression).

Pharmacokinetics

Tacrolimus activity is primarily due to the parent drug. The pharmacokinetic parameters (mean ± S.D.) of tacrolimus have been determined following intravenous (IV) and/or oral (PO) administration in healthy volunteers, and also in kidney, liver, and heart transplant patients.

Population	N	Route (Dose)	Parameters					
			C_{max} (ng/mL)	T_{max} (hr)	AUC (ng•hr/mL)	$t_{1/2}$ (hr)	CL (L/hr/kg)	V (L/kg)
Healthy Volunteers	8	IV (0.025 mg/kg/4 hr)	*	*	652† ± 156	34.2 ± 7.7	0.040 ± 0.009	1.91 ± 0.31
	30	PO (5 mg) (granules)	35.6 ± 10.9	1.3 ± 0.5	320† ± 164	32.1 ± 5.9	‡	‡
		PO (5 mg) (capsules)	28.8 ± 8.9	1.5 ± 0.7	266† ± 95	32.3 ± 8.8	‡	‡

Due to inter-subject variability in tacrolimus pharmacokinetics, individualization of the dosing regimen is necessary for optimal therapy. Pharmacokinetic data indicate that whole blood concentrations rather than plasma concentrations serve as the more appropriate sampling compartment to describe tacrolimus pharmacokinetics.

Absorption

Absorption of tacrolimus from the gastrointestinal tract after oral administration is incomplete and variable. The absolute bioavailability of tacrolimus was 17 ± 10% in adult kidney transplant patients (N = 26), 22 ± 6% in adult liver transplant patients (N = 17), 23 ± 9% in adult heart transplant patients (N = 11) and 18 ± 5% in healthy volunteers (N = 16).

A single dose trial conducted in 32 healthy volunteers established the bioequivalence of the 1 mg and 5 mg capsules. Another single dose trial in 32 healthy volunteers established the bioequivalence of the 0.5 mg and 1 mg capsules. Tacrolimus maximum blood concentrations (C_{max}) and area under the curve (AUC) appeared to increase in a dose-proportional fashion in 18 fasted healthy volunteers receiving a single oral dose of 3, 7, and 10 mg.

In 18 kidney transplant patients, tacrolimus trough concentrations from 3 to 30 ng/mL measured at 10-12 hours post-dose (C_{min}) correlated well with the AUC (correlation coefficient 0.93). In 24 liver transplant patients over a concentration range of 10 to 60 ng/mL, the correlation coefficient was 0.94. In 25 heart transplant patients over a concentration range of 2 to 24 ng/mL, the correlation coefficient was 0.89 after an oral dose of 0.075 or 0.15 mg/kg/day at steady state.

Prograf® granules are indicated for pediatric patients who have difficulty swallowing Prograf® capsules. In a healthy volunteer adult study, the systemic exposure to tacrolimus (AUC) for Prograf® Granules was approximately 16% higher than that for Prograf® capsules when administered as single doses. Hence, if pediatric patients are converted between formulations (capsules to granules or granules to capsules), therapeutic drug monitoring must be performed, and dose adjustments made to ensure that systemic exposure to tacrolimus is maintained.

Food Effects

The rate and extent of tacrolimus absorption were greatest under fasted conditions. The presence and composition of food decreased both the rate and extent of tacrolimus absorption when administered to 15 healthy volunteers.

The effect was most pronounced with a high-fat meal (848 kcal, 46% fat): mean AUC and C_{max} were decreased 37% and 77%, respectively; T_{max} was lengthened 5-fold. A high-carbohydrate meal (668 kcal, 85% carbohydrate) decreased mean AUC and mean C_{max} by 28% and 65%, respectively.

In healthy volunteers (N = 16), the time of the meal also affected tacrolimus bioavailability. When given immediately following the meal, mean C_{max} was reduced 71%, and mean AUC was reduced 39%, relative to the fasted condition. When administered 1.5 hours following the meal, mean C_{max} was reduced 63%, and mean AUC was reduced 39%, relative to the fasted condition.

In 11 liver transplant patients, Prograf® administered 15 minutes after a high fat (400 kcal, 34% fat) breakfast, resulted in decreased AUC (27 ± 18%) and Cmax (50 ± 19%), as compared to a fasted state.

Prograf® capsules should be taken consistently every day either with or without food because the presence and composition of food decreases the bioavailability of Prograf®.

Distribution

The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein and has a high level of association with erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors, such as hematocrit, temperature at the time of plasma separation, drug concentration, and plasma protein concentration. In a U.S. trial, the ratio of whole blood concentration to plasma concentration averaged 35 (range 12 to 67).

Metabolism

Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A). A metabolic pathway leading to the formation of 8 possible metabolites has been proposed. Demethylation and

hydroxylation were identified as the primary mechanisms of biotransformation in vitro. The major metabolite identified in incubations with human liver microsomes is 13-demethyl tacrolimus. In in vitro studies, a 31-demethyl metabolite has been reported to have the same activity as tacrolimus.

Excretion
The mean clearance following IV administration of tacrolimus is 0.040, 0.083, 0.053, and 0.051 L/hr/kg in healthy volunteers, adult kidney transplant patients, adult liver transplant patients, and adult heart transplant patients, respectively. In man, less than 1% of the dose administered is excreted unchanged in urine.

In a mass balance study of IV-administered radiolabeled tacrolimus to 6 healthy volunteers, the mean recovery of radiolabel was 77.8 ± 12.7%. Fecal elimination accounted for 92.4 ± 1.0% and the elimination half-life based on radioactivity was 48.1 ± 15.9 hours whereas it was 43.5 ± 11.6 hours based on tacrolimus concentrations. The mean clearance of radiolabel was 0.029 ± 0.015 L/hr/kg and clearance of tacrolimus was 0.029 ± 0.009 L/hr/kg. When administered PO, the mean recovery of the radiolabel was 94.9 ± 30.7%. Fecal elimination accounted for 92.6 ± 30.7%, urinary elimination accounted for 2.3 ± 1.1% and the elimination half-life based on radioactivity was 31.9 ± 10.5 hours whereas it was 48.4 ± 12.3 hours based on tacrolimus concentrations. The mean clearance of radiolabel was 0.226 ± 0.116 L/hr/kg and clearance of tacrolimus was 0.172 ± 0.088 L/hr/kg.

4.0 STUDY OBJECTIVE

4.1 Study Objective

The objective of this study is to investigate the bioequivalence of Tacrolimus 1 mg capsules [redacted] and Prograf® 1 mg capsules [redacted] in healthy male and non-pregnant, non-lactating female volunteers under fasting condition.

5.0 STUDY DESIGN

5.1 Discussion of Study Design

This is a single-dose, randomized, open-label, four-period, two-sequence, two-treatment, single-center, crossover, bioequivalence study of tacrolimus 1 mg capsules [redacted] and Prograf® 1 mg capsules [redacted] under fasting conditions.

FDA has concluded that tacrolimus is a NTI drug based on the following evidence:

- The range between tacrolimus therapeutic and toxic tacrolimus whole blood concentrations is narrow;

- Some tacrolimus toxicities are serious and/or irreversible;
- Subtherapeutic tacrolimus concentrations may lead to morbidity/mortality associated with graft rejection;
- Tacrolimus requires individual dose titration to achieve a satisfactory balance between maximizing efficacy and minimizing serious dose-related toxicity; Therapeutic drug monitoring is routinely employed to facilitate tacrolimus dose titration; and
- Tacrolimus has small to medium within subject variability.

This study is designed based on FDA draft guidance on Tacrolimus capsule (revised on Dec 2012) [7]. The bioequivalence assessment will be based on the recommendation from FDA for NTI drug.

5.1.1 Screening (Visit 1)

Subjects will review ICF and document their consent by signing the ICF prior to any study related procedures.

Screening procedures will be conducted within thirty (30) days prior to dosing in Period 1. The Principal Investigator (PI)/Sub-Investigator will review all screening results/data to assess eligibility of each potential subject. In case of an unforeseeable delay, a selected safety screening test may be repeated at the PI's discretion.
The Screening procedures will include (but not limited to): COVID-19 prevention and control measures, collection of demographic data, medical and medication histories, physical examinations, body measurements (height and weight for Body Mass Index [BMI] calculation), vital signs (seated blood pressure [BP], heart rate [HR], temperature, respiratory rate [RR] and pulse oximetry), electrocardiogram (ECG), hematology, biochemistry, serology, urinalysis, and urine screening for drugs of abuse, alcohol test and serum pregnancy test (female subjects only). For a complete listing of all tests to be performed, please refer to Appendix B - Clinical Laboratory Assessment.

5.1.2 Treatment Phase (Visit 2 to 14)

5.1.2.1 Study Period Check-in Procedures (Visit 2, 6, 10 and 14)

At check-in for each study period, subjects will be questioned about whether they have complied with the study restrictions and will be asked about the COVID-19 related questionnaire. Temperature, pulse oximetry and respiratory rate will be measured at each check-in and at pre-dose at each study period. Subjects will be asked to come to the study site approximately 24 hours prior to dosing and will be discharged after 72-hour blood draw.

If drug therapy other than that specified in the protocol was used, a decision to continue or discontinue the subject's participation will be made by the PI/Sub-Investigator and will be reported to the Sponsor.

Urine tests for drugs of abuse and a breath alcohol test will be performed on all subjects at each study period check-in. In addition, urine hCG testing will be performed on all female subjects at each study period check-in.

The subject's hemoglobin and hematocrit levels will be assessed by the PI/Sub-Investigator regarding the subject's continued participation. Male subjects having a hemoglobin level below 12.0 g/dL and/or hematocrit level below 36.0% or a female subjects having hemoglobin level below 12.0 g/dL and/or hematocrit level below 34.0%, the subjects will not be permitted to continue in the study unless deemed non-clinically significant (NCS) by PI/Sub-Investigator.

Clinical staff reserves the right to conduct random testing (urine drugs of abuse, urine hCG [females only], or alcohol) on any subject at any time during the study to ensure subject compliance and/or safety. Any subject with a positive test for urine drugs of abuse, alcohol or urine hCG (females only) will be dismissed immediately from the study.

5.1.2.2 Study Treatment

Tacrolimus capsules will be studied using a fully replicate crossover design in 72 healthy male and non-pregnant, non-lactating female volunteers, who will receive a single oral dose of 1 × 1 mg capsule of each tacrolimus under fasting conditions. For each treatment, safety assessments and monitoring will be performed for the duration of confinement and PK assessments for up to 144 hours after each tacrolimus administration, as listed Section 2 – Table of Study Procedures. Adverse event(s) (AEs) will be monitored throughout the study. There will be at least a 14-day washout period between each study period. Based on the half-life of tacrolimus capsule, this time-period is adequate to avoid drug carry-over from the preceding treatments. Blood samples will be collected at pre-dose (0 hour) and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 72.00, 96.00, 120.00 and 144.00 hours after study drug dosing in each study period.

5.1.3 Post-Study [End-of-Study (EOS)]/Early Termination

Clinical laboratory tests (hematology, serum chemistry and urinalysis), as per Appendix B – Clinical Laboratory Assessment, will be performed at the end of the study or after withdrawal/dismissal of a subject from the study.

At the end of the study or after withdrawal/dismissal of a subject from the study, a symptom-directed physical exam with the possibility for a full physical exam if deemed necessary by the PI/Sub-Investigator including vital signs (BP, HR, temperature, pulse oximetry and RR) will be conducted unless the subject is lost to follow up.

A follow up phone call will be made about a week ±3 days after study participants have been discharged from the study i.e., End-of-study. Clinic will make two additional attempts to contact study participants. If all three attempts are unsuccessful, clinic will close the file and will be deemed lost to follow up. If the first attempted phone call is unsuccessful, the second phone call will be attempted 5 ±3 days from the first phone call. If second attempt to contact study participant via phone is unsuccessful, a registered letter will be sent 5 ±3 days from the second phone call.

5.2 Study Duration and Confinement

Subjects will be confined to the clinic from at least 12 hours prior to dosing until at least 72-hours post-dose, for a total of approximately 84 hours in each study period. Subjects will be required to return to the clinical facility for the 96, 120 and 144-hour blood draws in each study period.

This study will consist of four study periods with a washout period of at least 14 days between each tacrolimus dosing. The typical, total anticipated duration of the clinical part of the study for each subject will be approximately 12 weeks, including an approximately 30-day screening period and approximately 50 days for the treatment periods with a follow-up phone call.

5.3 Randomization and Blinding

In this study, the assignment of treatment groups (simple randomization scheme) will be generated by a computer program designed and run in SAS® Version 9.4 [redacted]

Subjects who meet the eligibility criteria will be randomly assigned equally into one of the following two sequence groups:

Table 5-1 Randomization Scheme

	Period 1	Period 2	Period 3	Period 4
Sequence 1	T	R	T	R
Sequence 2	R	T	R	T

Each subject is scheduled to receive each treatment twice by the end of the study.

Treatment T (Test): 1 × 1mg tacrolimus capsules [redacted]

Treatment R (Reference): 1 × 1mg Prograf® 1 mg capsules [redacted]

Approximately 32 subjects are planned to receive treatment sequence T-R-T-R and approximately 32 subjects are planned to receive treatment sequence R-T-R-T.

The trial will be performed as an open-label study as the pharmacokinetic profile should not be expected to be affected by having the knowledge of whether reference or generic tacrolimus capsules were administered. Blinding of the subjects as well as clinic staff is not considered necessary. Only the bioanalytical laboratory staff will not have access to the randomization scheme until the bioanalytical analysis is complete.

6.0 STUDY POPULATION

A required number of healthy male and non-pregnant, non-lactating female volunteers, as intended for general population [9], aged between 18- 59 years of age, inclusive will be screened according to the inclusion and exclusion criteria listed in Sections 7.2 and 7.3 to be sure that about 64 are randomized.

To assure subjects' safety, eligibility will be assessed at the participating clinical site at the time of screening and upon entry into the clinic prior to first drug administration.

7.0 SUBJECT SELECTION AND WITHDRAWAL/TERMINATION

7.1 Sample Size

Up to a total of 72 subjects will be enrolled in the study, including the targeted sample size of approximately 64 subjects and 8 standbys to ensure the completion of at least 48 subjects. Subjects who are dosed will not be replaced. The subjects may be dosed in more than one cohort to achieve the total required sample size.

7.2 Inclusion Criteria

Potential subjects meeting all of the following criteria may be included in the study: (medical/operative reports may be requested to support assessment, if necessary):

1. Males and female volunteers, 18 -59 years of age (inclusive).
2. BMI that is within 18.5-33.0 kg/m², inclusive.
3. Healthy, according to medical history, ECG, vital signs, laboratory results and physical examination as determined by the PI/Sub-Investigator.
4. Capable of giving written informed consent prior to receiving any study medication.
5. Availability to volunteer for the entire study duration and willing to adhere to all protocol requirements.
6. Smokers: Capable of refraining from smoking for the duration of the confinement.
7. Female subjects must be non-pregnant, non-lactating and fulfill at least one of the following:
    - Be surgically sterile for a minimum of 6 months
    - Post-menopausal for a minimum of 12 months; Menopause defined as 12 months of amenorrhea without any other obvious pathological or physiological cause.
    - Agree to avoid pregnancy and use medically acceptable method of contraception from at least 30 days prior to the study until 30 days after the study has ended (last study procedure).

    Medically acceptable methods of contraception include non-hormonal intrauterine device or double barrier method (foam or vaginal spermicidal suppository in conjunction with a male condom, diaphragm with spermicide in conjunction with a male condom). Abstinence as a method of contraception is acceptable if it is in line with the preferred and usual lifestyle of the study participant.

    If a female subject becomes pregnant during participation in the study or within 30 days after she has completed her last tacrolimus administration (whichever was administered last), she must inform BPSUSA staff immediately.
8. Males who are able to father children must agree to use medically acceptable methods of contraception and not to donate sperm during the study and for 30 days after the end of the study.

    Medically acceptable methods of contraception include using a condom with a female partner of child-bearing potential who is using: oral contraceptives,

hormonal patch, implant or injection, intrauterine device, or diaphragm with spermicide.

If a male subject's partner becomes pregnant during his participation in the study or within 30 days after he has completed his last tacrolimus administration (whichever was administered last), he must inform BPSUSA staff immediately.

7.3 Exclusion Criteria

Potential subjects meeting any of the following criteria will be excluded:

1. Known history and/or presence of any clinically significant hepatic (e.g., hepatitis, jaundice, hepatic failure, hepatic necrosis, hepatic encephalopathy, biliary tract diseases, cirrhosis), renal/genitourinary (e.g., urethral stricture, any renal impairment), gastrointestinal, cardiovascular (e.g., hypotension including orthostatic hypotension, cor pulmonale, congenital long QT, congestive heart failure, brady arrhythmias), cerebrovascular, pulmonary (e.g., chronic obstructive pulmonary disease, decreased respiratory reserve, hypoxia, pre-existing respiratory depression), endocrine (e.g., myxedema, hypothyroidism, adrenal cortical insufficiency), immunological, musculoskeletal (e.g., kyphoscoliosis), neurological (e.g., CNS depression or coma, increased cerebrospinal pressure), psychiatric (e.g., psychosis, depression, hallucinations, delirium tremens, suicidal thoughts or behavior), dermatological or hematological (e.g., thrombocytopenic purpura) disease or condition unless determined as not clinically significant by the PI/Sub-Investigator.
2. History or presence of any clinically significant gastrointestinal pathology (e.g., chronic diarrhea, inflammatory bowel disease), unresolved gastrointestinal symptoms (e.g., diarrhea, vomiting), or other conditions known to interfere with the absorption, distribution, metabolism or excretion of the drug experienced within 7 days prior to first tacrolimus administration, as determined by the PI/Sub-Investigator.
3. Systolic blood pressure outside 90-130 mmHg, inclusive, and diastolic blood pressure outside 55-80 mmHg, inclusive, and heart rate between 50-100 bpm, inclusive, unless deemed otherwise by the PI/Sub-Investigator.
4. QTc interval $\geq$ 440 milliseconds for males and $\geq$ 460 milliseconds for females, unless deemed otherwise by the PI/Sub-Investigator.
5. Abnormal clinical laboratory values unless values are deemed by the PI/Sub-Investigator as "Not Clinically Significant".
6. Abnormal vital signs (blood pressure [BP], heart rate [HR], respiratory rate [RR], pulse oximetry and temperature) measurements, unless deemed otherwise by the PI/Sub-Investigator.

7. Presence of any clinically significant illness within 30 days prior to first dosing, as determined by the PI/Sub-Investigator.
8. Presence of any significant physical or organ abnormality as determined by the PI/Sub-Investigator.
9. A positive test result for any of the following: HIV, Hepatitis B surface antigen, Hepatitis C, drugs of abuse (marijuana, amphetamines, barbiturates, cocaine, opiates, phencyclidine and benzodiazepines), and alcohol test. Positive serum or urine pregnancy test for female subjects.
10. Known history or presence of:
    - Alcohol abuse or dependence within one year prior to first study drug administration;
    - Drug abuse or dependence;
    - Hypersensitivity or idiosyncratic reaction to tacrolimus, its excipients, and/or related substances;
    - Lymphoma and other malignancies (particularly of the skin);
    - Bacterial, viral, fungal and protozoal infections (e.g., polyoma virus-associated nephropathy [PVAN] due to BK virus infection, JC virus associated progressive multifocal leukoencephalopathy [PML], Epstein Barr Virus (EBC) associated Post transplant lymphoproliferative disorder [PTLD], cytomegalovirus (CMV) infections associated CMV viremia and CMV disease);
    - Tuberculosis;
    - Posterior reversible encephalopathy syndrome (PRES);
    - Pure red cell aplasia (PRCA);
    - Gastrointestinal perforation;
    - Hyperkalemia;
    - Hypertension;
    - Lactose intolerance, galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption;
    - Food allergies and/or presence of any dietary restrictions unless deemed by the PI/Sub-I as “Not Clinically Significant”.
    - Severe allergic reactions (e.g. anaphylactic reactions, angioedema).
11. History of intolerance to and/or difficulty with blood sampling through venipuncture.

12. Abnormal diet patterns (for any reason) during the four weeks preceding the study, including fasting, high protein diets, vegan, etc.
13. Individuals who have donated, in the days prior to first tacrolimus administration:
    - 50-499 mL of blood in the previous 30 days;
    - 500 mL or more or double red blood cell ("Power red") donation in the previous 56 days.
14. Donation of plasma by plasmapheresis within 7 days prior to first tacrolimus administration.
15. Hemoglobin level of 12.0 g/dL or lower.
16. Individuals who have participated in another clinical trial or who received an investigational drug within 30 days prior to first tacrolimus administration.
17. Not being able to fast for at least 14 hours.
18. Consumption of food or beverages containing grapefruit and/or pomelo within 3 days prior to first study drug administration.
19. Consumption of food or beverages containing caffeine/methylxanthines, poppy seeds and/or alcohol within 48 hours before dosing in each study period.
20. Use of any prescription medication within 14 days prior to first tacrolimus administration (except for allowed contraceptive products).
21. Use of any over-the-counter medications (including low-dose aspirin, oral multivitamins, allergy medications, herbal and/or dietary supplements) within 14 days prior to first tacrolimus administration (except for spermicidal/barrier contraceptive products).
22. Use of any enzyme-modifying drugs and/or other products, including inhibitors of cytochrome P450 (CYP) enzymes such as antifungals (e.g., ketoconazole, itraconazole, fluconazole, voriconazole, posaconazole, clotrimazole), macrolide antibiotics (e.g., erythromycin, clarithromycin, josamycin), protease inhibitors (e.g., ritonavir, telaprevir [Incivek™], boceprevir [Victrelis™], nelfinavir [Viracept®], saquinavir), calcium channel blockers (e.g., verapamil, diltiazem, nifedipine, nicardipine), nucleotide reverse transcriptase inhibitors (e.g., tenofovir), gastric acid suppressors/neturalizers (e.g., lansoprazole, omeprazole, cimetidine, cisapride, magnesium-aluminum hydroxide antacids), bromocriptine, cortisone, dapsone, ergotamine, gestodene, lidocaine, mephenytoin, miconazole, midazolam, nilvadipine, quinidine, tamoxifen, and triacetyl-oleandomycin and inducers of CYP enzymes such as anti-myobacterials (e.g., rifampin, rifabutin), antifungal agent (e.g., caspofungin), anticonvulsants (e.g. phenytoin, carbamazepine, phenobarbital),

corticosteroids (e.g. prednisolone or methylprednisolone), metamizole, isoniazid and products containing St. John's Wort in the previous 30 days before first study drug administration.

23. Use of medicines such as, mycophenolic acid (MPA) products, aminoglycosides, gyrase inhibitors, vancomycin, cotrimoxazole, NSAIDs, oral anticoagulants, or oral antidiabetics, ganciclovir, acyclovir, amphotericin B, ibuprofen, cisplatin, potassium supplements, potassium-sparing diuretics (e.g., amiloride, triamterene or spironolactone), ACE inhibitors (for example, benazepril, enalapril, quinapril), statins (for example, atorvastatin, fluvastatin, simvastatin), angiotensin receptor blockers (for example, irbesartan, valsartan, azilsartan), nefazodone, metoclopramide, danazol, and herbal products containing *Schisandra sphenanthera* extracts should be avoided during treatment with tacrolimus in the previous 30 days before first study drug administration.
24. Use of any QT prolonging drugs (e.g., citalopram, chlorpromazine, haloperidol, methadone), amiodarone [Cordarone™, Nexterone™, Pacerone™], immunosuppressive or immunomodulating therapies (e.g. sirolimus [Rapamune®], cyclosporins [Gengraf®, Neoral®, and Sandimune®], antilymphocytic antibodies [e.g., basiliximab, daclizumab]) should be avoided during treatment with tacrolimus in the previous 30 days before first study drug administration.
25. Individuals having undergone any major surgery within 6 months prior to the start of the study, unless deemed otherwise by PI/Sub-Investigator.
26. Does not agree, to refrain from driving or operating heavy machinery if feeling dizzy or drowsy following tacrolimus administration until full mental alertness is regained.
27. Does not have an ability to fast for at least 14 hours.
28. Recent history (within 8 weeks prior to screening) of travel to or emigration from any country with high incidence of tuberculosis.
29. Temperature at visit is ≥ 100.4 F (38.0° C).
30. Test positive for SARS-CoV-2 by locally available, verified test.
31. Have had common symptoms of COVID-19 currently or in the past 14 days as documented in the COVID-19 screening questionnaire and symptom checklist
32. Have had an exposure to suspected or confirmed cases of COVID-19 in the past two weeks as documented in the COVID-19 screening questionnaire

33. Recent live attenuated vaccine[1] recipients in the past month.

7.4 Informed Consent Procedure

The Investigator will be responsible for ensuring that the informed consent is obtained before any protocol specific procedures are carried out. The decision of a subject to participate in clinical research is voluntary and will be based on a clear understanding of what is involved.

Subjects will receive adequate oral and written information. The oral explanation to the subject will be performed by the Investigator or a designated person and will cover all the elements specified in the informed consent form.

The subject will be given every opportunity to clarify any points they do not understand and, if necessary, to ask for more information. The subject will be given sufficient time to consider the provided information. It will be emphasized that the subjects may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The subjects will be informed that all attempts to maintain confidentiality will be made. Their names or personally identifiable information will not be disclosed outside the site. The Investigator or delegated member of the trial team and the subject will sign and date the Informed Consent Form (ICF) to confirm that consent has been obtained. The subject will receive a copy of this document and the original will be filed in the subject binder. In case of a screen failure, it will be filed separately in the designated binder.

7.5 Study Restrictions

If at any time prior to or during the study, any subject does not comply with the restrictions mentioned below, the subject's continued participation will be re-assessed by the PI/Sub-Investigator, Pharmacokinetic staff, and reported to the Sponsor.

1. No food will be allowed from at least 10 hours before dosing until at least 4 hours after dosing. Except for the 8 ± 0.1 fl. oz. water given with study medication, no fluid will be allowed from 1 hour before dosing until 1-hour post-dose. Water will be allowed/provided *ad libitum* at all other times.
2. No smoking will be permitted during confinement.
3. Subjects will be required not to drive or operate heavy machinery as indicated in Section 7.3.

[1] Adenovirus Type 4 and Type 7 vaccine (Oral), Cholera vaccine, Ebola Zaire vaccine (Live), Influenza vaccine, Measles, Mumps, and Rubella Virus Vaccine (Live), Smallpox (Vaccinia) Vaccine (Live), Smallpox and Monkeypox vaccine (Live, Non-replicating), Typhoid Vaccine (Live Oral Ty21a), Yellow Fever Vaccine and Zoster Vaccine (Live).

4. Subjects will be required to limit exposure to sunlight and UV light by wearing protective clothing and using a sunscreen with a high protection factor during study participation until the study completion.
5. Subjects will be required not to receive a vaccination (live attenuated vaccine). Examples include (but are not limited to): Adenovirus Type 4 and Type 7 vaccine (Oral), Cholera vaccine, Ebola Zaire vaccine (Live), Influenza vaccine, Measles, Mumps, and Rubella Virus Vaccine (Live), Smallpox (Vaccinia) Vaccine (Live), Smallpox and Monkeypox vaccine (Live, Non-replicating), Typhoid Vaccine (Live Oral Ty21a), Yellow Fever Vaccine and Zoster Vaccine (Live) during the study and until 30 days after the study has ended (last study procedure).
6. The consumption of alcohol of any kind (e.g., wine, beer, liquor, cocktails), products containing caffeine/methylxanthines (e.g., coffee, tea, chocolate, caffeine-containing soft drinks [e.g. Coke, Pepsi, Red Bull]) and poppy seeds containing products (poppy seed cake, cookies, bagels) will be prohibited for 48 hours prior to tacrolimus administration until after the last blood draw in each study period.
7. The consumption of foods and/or beverages containing grapefruit and/or pomelo (e.g., grapefruit, grapefruit juice, grapefruit candies, pomelo, etc.) will be prohibited for 3 days prior to first tacrolimus administration until the last blood draw in the final study period.
8. Any concomitant medication (prescription medications [e.g., prescription pills, topical systemic creams, inhalants, sprays] or over-the-counter such as low-dose aspirin, allergy medications and oral multivitamins), any herbal and/or dietary supplements will not be permitted for 14 days prior to first drug administration.
9. Any concomitant medication (prescription medications [e.g., prescription pills, topical systemic creams, inhalants, sprays] or over-the-counter), any herbal and/or dietary supplements from the start of dosing of the study until the last blood draw in the final study period unless deemed otherwise by the PI/Sub-Investigator for treatment of any adverse events (AEs).

   Any use of concomitant medication or herbal/dietary supplement during the restriction period for the restriction # 9 will be reported as soon as possible to the PI/Sub-Investigator, Pharmacokinetic staff and reported to the Sponsor. In each case, the decision whether to continue or discontinue the subject's participation in the study will be made by the PI/Sub-Investigator and/or by the Pharmacokinetic staff and reported to the Sponsor.
10. Use of any enzyme-modifying drugs and/or other products, including inhibitors of cytochrome P450 (CYP) enzymes such as antifungals (e.g., ketoconazole, itraconazole, fluconazole, voriconazole, posaconazole, clotrimazole), macrolide antibiotics (e.g., erythromycin, clarithromycin, josamycin), protease inhibitors (e.g., ritonavir, telaprevir [Incivek™], boceprevir [Victrelis™], nelfinavir [Viracept®], saquinavir), calcium channel blockers (e.g., verapamil, diltiazem, nifedipine, nicardipine), nucleotide reverse transcriptase inhibitors (e.g.,

tenofovir), gastric acid suppressors/neturalizers (e.g., lansoprazole, omeprazole, cimetidine, cisapride, magnesium-aluminum hydroxide antacids), bromocriptine, cortisone, dapsone, ergotamine, gestodene, lidocaine, mephenytoin, miconazole, midazolam, nilvadipine, quinidine, tamoxifen, and triacetyl-oleandomycin and inducers of CYP enzymes such as anti-myobacterials (e.g., rifampin, rifabutin), antifungal agent (e.g., caspofungin), anticonvulsants (e.g., phenytoin, carbamazepine, phenobarbital), corticosteroids (e.g., prednisolone or methylprednisolone), metamizole, isoniazid and products containing St. John's Wort within 30 days prior to first study drug administration until the last blood draw in the final study period.

11. Use of medicines such as, mycophenolic acid (MPA) products, aminoglycosides, gyrase inhibitors, vancomycin, cotrimoxazole, NSAIDs, oral anticoagulants, or oral antidiabetics, ganciclovir, acyclovir, amphotericin B, ibuprofen, cisplatin, potassium supplements, potassium-sparing diuretics (e.g., amiloride, triamterene or spironolactone), ACE inhibitors (for example, benazepril, enalapril, quinapril), statins (for example, atorvastatin, fluvastatin, simvastatin), angiotensin receptor blockers (for example, irbesartan, valsartan, azilsartan), nefazodone, metoclopramide, danazol, and herbal products containing *Schisandra sphenanthera* extracts should be avoided during treatment with tacrolimus within 30 days prior to first study drug administration until the last blood draw in the final study period.
12. Use of any QT prolonging drugs (e.g., citalopram, chlorpromazine, haloperidol, methadone), amiodarone [Cordarone™, Nexterone™, Pacerone™], immunosuppressive or immunomodulating therapies (e.g., sirolimus [Rapamune®], ciclosporins [Gengraf®, Neoral®, and Sandimune®], antilymphocytic antibodies [e.g., basiliximab, daclizumab]) should be avoided during treatment with tacrolimus within 30 days prior to first study drug administration until the last blood draw in the final study period.
13. Physical activity: Subjects will remain awake for the first 4 hours following tacrolimus administration. If a medical event (i.e., AE) occurs, subjects may be placed in an appropriate position at that time. Subjects will be required to abstain from strenuous/vigorous activities for the duration of the study period(s). When the subjects leave the clinic, they will be requested to refrain from strenuous physical activity until the completion of the 144-hour blood draw in each study period.
14. For 3 hour after each study drug administration, subject visits to the washroom will be monitored and recorded by clinic staff. Subjects will be restricted from flushing the toilet to allow staff to inspect the content during this time.
15. Additional study restriction for COVID-19 prevention and control:
    - Subjects must be willing to social distance, wear masks and practice hand hygiene while enrolled in the study

- Report to study staff in case of exposures to known or possible COVID-19 positive cases during the study

7.6 Withdrawal and Dismissal

A subject is free to withdraw from participation in the study at any time, for any reason. An Investigator may terminate a study subject's participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

The cause, date and time of withdrawal or termination will be documented in the source documents and in the final study report. If a subject's participation is terminated prematurely, the cause for the early termination date and time of the termination will be documented on the source documents and in the final study report.

Subjects experiencing emesis within 3 hours (2 times median $T_{max,}$ 1.5 hour, reported in the label [6]) after dosing will be dismissed from the affected period in the study.

Subjects experiencing 3 or more episodes of loose stool (diarrhea) after dosing will be allowed to stay in the study unless the PI/Sub-Investigator recommends the subject to be dismissed from the study due to safety reasons. All decisions will be made prior to the bioanalytical laboratory commencing bioanalysis.

Subjects who are dismissed or withdrawn from Period 1, may be eligible to return for Period 2 and/or Period 3 and/or Period 4, if deemed safe and appropriate by the PI/Sub-Investigator. All subjects will be reviewed by the PI and the sponsor prior to re-entry. Subjects who are dismissed due to a Treatment-Emergent Adverse Event (TEAE) or non-compliance will not be eligible to return for Period 2 and/or Period 3 and/or Period 4.

Withdrawn and dismissed subjects who are returning for subsequent periods are required to adhere to the study specific procedures (e.g., study restrictions). If a subject is withdrawn or dismissed following tacrolimus administration and will not be returning in subsequent periods (i.e., for Period 2 and/or Period 3 and/or Period 4), these subjects are still required to adhere to the study restrictions with regards to the safety, prescription medication, over-the-counter medication, dietary and/or herbal supplements for the expected duration of the restriction relevant to the study period the subject withdrew or was dismissed from (where that period becomes the subject's last study period).

Female subjects will be asked to adhere to the requirements of not becoming pregnant and using a medically acceptable method of contraception for 30 days after

discontinuation from the study (if applicable). Male subjects who are able to father children will be asked to adhere to the requirements of using medically acceptable methods of contraception for 30 days after discontinuation in the study.

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the investigator, sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination of futility.

Subjects whose participation in the study is discontinued (for any reason) will not be replaced. If a subject withdraws or is dismissed from the study, a post-study symptom-directed physical exam with the possibility for a full physical exam if deemed necessary by the PI/Sub-Investigator will be conducted and post-study testing will be completed, where possible.

8.0 STUDY PROCEDURES

All study assessments will be performed at the visits and time points outlined in the Table of Study Procedures (Section 2.0).

8.1 Demographics and Other Baseline Characteristics

8.1.1 Demographics

Demographics including age (date of birth and years), sex, race, and ethnicity will be recorded at screening.

8.1.2 Medical History

The complete medical history will include history of acute, chronic, or infectious diseases; surgical or oncologic histories; and any reported conditions affecting major body systems. All findings on medical history will be evaluated by the PI/Sub-Investigator for clinical significance.

8.1.3 Medication History

All medications (prescription and non-prescription/OTC, herbal medications/natural health products, or investigational drugs) taken by the subjects during the 30 days prior to Screening will be recorded in the source documentation as medication history.

8.1.4 Clinical Laboratory Assessments

Clinical laboratory assessments will be conducted at Screening, each period check-in and at the EOS. All laboratory safety data will be reviewed by the PI/Sub-Investigator for clinical significance. Additional laboratory samples may be taken at the discretion of the PI/Sub-Investigator (e.g., if the results of any tests fall outside reference ranges or clinical symptoms necessitate testing to ensure safety).

Blood and urine samples collected for clinical laboratory assessments will be processed and shipped according to the research site's SOPs and instructions from the clinical laboratory.

For a complete listing of all tests to be performed, please refer to Appendix B - Clinical Laboratory Assessment.

8.2 Food and Fluid Intake

Following at least a 10-hour overnight fasting period, subjects will be dosed with tacrolimus and will be required to continue to fast for at least 4 hours after dosing.

Following the fasting period of at least 4-hours after dosing, subjects will be given standardized meals and caffeine/methylxanthine-free beverages at approximately 4.5, 9.5, 13.5, 24.5, 28.5, 33.5, 37.5, 48.5, 52.5, 57.5 and 61.5 hours after dosing. Meals and beverages during confinement will be identical between each study period.

Except for water given with study medication, no fluid will be allowed from 1 hour before dosing until 1-hour post-dose. Water will be allowed/provided *ad libitum* at all other times.

8.3 Dosing

After a fast of at least 10 hours, subjects will take their assigned formulation with 8 ± 0.1 fl. oz. of ambient temperature water at their scheduled time point in each study period

Subjects will be instructed not to touch, chew, bite, or break tacrolimus capsules. If a subject chews, bites, or breaks the capsules, that subject will be removed from the study. If the subject touches the capsules a replacement dose will be administered. Subjects must swallow the capsules and finish the water within 60 seconds.

Clinical staff will conduct a hand and mouth check immediately following dosing.

8.4 Safety Monitoring

In the interest of subjects' safety, the following safety parameters will be assessed in all subjects.

8.5 COVID-19 prevention and control

Additional procedures will be performed to prevent, and control spread of COVID-19 among the study subjects

8.5.1 COVID-19 questionnaire

The COVID-19 questionnaire for each subject will be completed by a clinic staff to document all history of exposure and history of symptoms related to COVID-19. The COVID-19 questionnaire is provided in the site's COVID-19 related procedural SOP.

8.5.2 COVID-19 symptom check

The medical staff will check for any common symptoms for COVID-19 at screening, at each check-in, discharge at each study period, at return visits, and at EOS. Additional symptom checks might be performed during the study as deemed necessary by the PI or study staff. The COVID-19 symptom checklist is provided in the site's COVID-19 related procedural SOP.

8.5.3 Temperature check

Temperature will be measured using an oral thermometer at screening, each check-in, at 6- and 12-hours post dose, then at every 12 hours until discharge at each study period, at return visits and at EOS. Additional temperature checks might be performed during the study as deemed necessary by the PI or study staff.

8.5.4 Pulse Oximetry and Respiratory Rate

Pulse oximetry and respiratory rate will be performed at screening, each check-in, at pre-dose, at 6- and 12-hours post dose, then at every 12 hours until discharge at each study period, at return visits and at EOS. Additional pulse oximetry and respirator rate measurements might be taken during the study as deemed necessary by the PI or study staff. The normal ranges of pulse oximetry and respiratory rate are:

- a pulse oximeter reading of: $\geq 95\%$
- a range of resting respiratory rate as 12 to 20 per minute

8.5.5 SARS-CoV-2 Test

If applicable, a nasal, oral swab or blood might be collected from each subject at each check-in for detection of SARS-CoV-2. Additional tests might be performed during the study as deemed necessary by the PI or study staff. The swabs or blood will be sent to a local CLIA certified laboratory for analysis using a verified, locally acceptable test. The sample collection and shipment will follow a specific procedure

provided by the laboratory.as described. The subjects who test positive for SARS-CoV-2 will be excluded from any subsequent study procedures.

8.6 Vital Signs

Vital signs (BP and HR) will be monitored at pre-dose and at 1, 2, 4, 6, 12-,24-, 48- and 72-hours post-dose in each study period.

Pre-Dose and Post-Dose (all periods):
Acceptable Range - systolic blood pressure between 90-130 mmHg, diastolic blood pressure between 55-80 mmHg, inclusive, and heart rate between 50-100 bpm, inclusive.

If the Investigator is not immediately available on site to assess out of range vital signs, the vital signs will be repeated up to two times. If vital signs are still outside of the acceptable range, the PI/Sub-Investigator will be informed, and PI/Sub-Investigator will determine the appropriate course of action.

Additional vital signs measurements will be taken if deemed necessary by the PI/Sub-Investigator. Blood draws will take precedence over vital signs measurements and other scheduled activities if the scheduled time of several activities is overlapped to each other unless deemed necessary by the PI/Sub-Investigator or due to subject's safety.

8.7 ECG

A 12-lead ECG with rhythm strip will be performed at screening, Additional measurements are not required during this study unless deemed necessary by the PI/Sub-Investigator.

8.8 Health Monitoring

The PI/Sub-Investigator will be present approximately 30 minutes prior to dosing until at least 4 hours after the last subject is dosed in each study period. The PI/Sub-Investigator will remain on-call throughout the duration of the study.

Health monitoring will be conducted throughout the study or as needed. Adverse events will be monitored throughout the study.

8.9 Sample Collection and Processing

Blood will be obtained by direct venipuncture in the arm. Blood sample collection times will be recorded on the appropriate source documents and reported for each subject.

Number of Samples	28 samples from 28 time points in each study period.
Total Volume of Blood including blood samples for PK time points, pre- and post- study procedures and the selected laboratory testing at each study period.	Approximately 520 mL of blood
Type of Vacutainer	Pre-chilled K_2EDTA Vacutainer®, 4 mL
Blood Sampling Time Points[2,3]	Pre-dose (0 hour) and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.000, 24.000, 36.00, 48.00, 72.00, 96.00, 120.00 and 144.00 hours post study in each study period. The actual blood sample collection time will be documented. For all blood sample time points, any sample collected outside of the scheduled time will not be considered as protocol deviation.

Whole blood will be aspirated and aliquoted into 2 pre-chilled clear polypropylene tubes. A minimum of 2 mL whole blood will be transferred to the first tube and the remaining whole blood (if any) will be aliquoted into a second tube. Polypropylene tubes will be pre-chilled and pre-labeled with at least the following information: Time-point, Protocol Number, Study Period, Aliquot Number, Matrix and Subject Number. The samples will be stored at -20ºC (-15ºC or colder) in a freezer pending shipment.

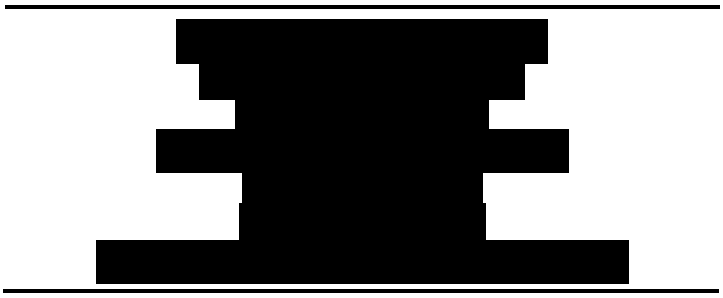
Throughout sample collection, the samples will be maintained in an ice-bath until stored in the freezer.

8.10 Sample Shipment

One set of aliquots from all subjects will be delivered to the bioanalytical facility packed on dry ice. Once the initial set has been confirmed to have been received by the bioanalytical facility, the second set of aliquots will then be shipped/delivered.

All shipments will be accompanied by an inventory list and delivered to the bioanalytical facility:

[2] - Pre-dose samples to be obtained within 2 hours prior to study drug administration.
[3] - The blood sample will be collected within ± 1-hour window for the 96, 120 and 144-hours time points.



Clinic personnel will notify the bioanalytical laboratory prior to shipment by phone, fax or e-mail. The bioanalytical laboratory will notify BPSUSA by either e-mail or fax of confirmation of receipt of each aliquot.

9.0 DRUG PRODUCTS

9.1 Drug Information

Table 9-1 Study Drugs Information

	T (Test)	R (Reference)
Drug Name:	Tacrolimus	Prograf®
Strength:	1 mg	1 mg
Dosage Form:	Capsules	Capsules
Manufactured by/for:	[redacted]	[redacted]
Dose:	1 × 1 mg	1 × 1 mg

9.2 Labeling, Maintenance, and Retention

Each investigational study drug will be labeled (in English) with a statement indicating that the drug is an investigational drug to be used only by a Qualified Investigator and will include but is not limited to: Drug Name, Strength, Protocol Number, Sponsor's Name and Address, the recommended storage conditions for the drug, Expiry/Retest Date (when available), and Lot/Batch Number.

9.3 Drug Inventory

The BPSUSA will supply sufficient quantities of the study drugs for the following: (1) completion of this study and (2) retention, as per applicable regulations. All drug supplies provided for this study will be stored in a secure area with restricted access, under controlled storage conditions described in the product package labelling, unless otherwise instructed per protocol.

Records will be made of receipt and dispensing of tacrolimus supplied.

9.4 Disposition of Unused Tacrolimus

Upon completion or termination of the study, all remaining study supplies will be retained according to applicable regulations. Once the retention period has elapsed, any remaining unused drug will be returned to the Sponsor in the original containers, or destroyed, as directed in writing by the Sponsor.

10.0POTENTIAL RISKS AND BENEFITS

10.1.1 Potential Risks

In clinical studies, the most common adverse reactions (≥ 15%) were abnormal renal function, hypertension, diabetes mellitus, fever, CMV infection, tremor, hyperglycemia, leukopenia, infection, anemia, bronchitis, pericardial effusion, urinary tract infection, constipation, diarrhea, headache, abdominal pain, insomnia, paresthesia, peripheral edema, nausea, hyperkalemia, hypomagnesemia, and hyperlipemia. [6].

The following serious adverse reactions were also reported in the patients who were tacrolimus users [6]:

- Lymphoma and Other Malignancies
- Serious Infections
- New Onset Diabetes After Transplant
- Nephrotoxicity
- Neurotoxicity
- Hyperkalemia
- Hypertension
- QT Prolongation
- Myocardial Hypertrophy
- Immunizations
- Pure Red Cell Aplasia

COVID-19 related risks

BPSUSA is taking significant steps to minimize subject exposure to COVID-19. However, risk of exposure of the subjects to COVID-19 due to close contact with study staff or other study subjects does exist during the conduct of the study.

10.1.2 Potential Benefits

While it may not provide direct benefit to subjects, the knowledge that may result from the study may help with the development of generic formulations of tacrolimus and may enhance the regulatory review standards for equivalence of this category.

11.0 SAFETY ASSESSMENT

BioPharma Services USA Inc. (BPSUSA) ensure the timely, accurate and complete reporting of safety information in conformity with regulatory requirements.

PI/Sub-Investigators at BPSUSA are responsible for monitoring the safety and for providing appropriate medical care for subjects who have entered this trial (i.e., from the signing of ICF onwards). In addition, the PI/Sub-Investigator remains responsible for following-up all AEs.

Each subject will be carefully questioned and/or examined by the PI/Sub-Investigator or a medically qualified delegate (i.e., authorized by the Investigator, in a separate form, to record AEs) to obtain information regarding AEs. All AEs will be reported and documented as stated below.

Any symptoms or positive tests related to COVID-19 will be documented as part of AEs reporting. This documentation applies from the time that volunteers receive the study drugs until the completion of the end-of-study procedures.

11.1 Definitions

Adverse Event (AE) – is any untoward medical occurrence in clinical trial subject who are administered with any of study drugs and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not considered related to the study drug.

Adverse Drug Reaction (ADR) of study drug any of study drugs is any untoward and unintended response to any of study drugs related to any dose administered.

Unexpected Adverse (Drug) Reaction – is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Prescribing Information for an authorized product).

Serious Adverse Event (SAE) / Reaction – a serious adverse event (experience) or reaction is any untoward medical occurrence or effect that at any dose can:

- result in death,
- be life-threatening,
- require hospitalization or prolongation of existing inpatient hospitalization,
- result in persistent or significant disability or incapacity,
- cause a congenital anomaly/birth defect,
- be other medically important event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the above-listed outcomes.

Suspected Unexpected Serious Adverse Reaction (SUSAR) – is all suspected adverse reactions related to Prograf® and generic tacrolimus capsules which can occur in the concerned trial, and that are both unexpected and serious.

Unanticipated Problems – The unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Subjects will be instructed to inform clinic personnel of any untoward medical symptoms and/or events that may arise during the study.

11.1.1 Treatment-emergent adverse events (TEAEs)

Treatment emergent adverse events (TEAEs) are undesirable events, not present prior to medical treatment, or an already present event that worsens either in intensity or frequency following the treatment. A TEAE is an adverse event that occurs only once treatment has started. All TEAEs and all treatment emergent SAEs will be summarized.

The PI/sub-I will be responsible for determining whether a TEAE is possibly related to the SARS-CoV-2 and document accordingly.

11.2 Characteristics of an Adverse Event

11.2.1 Relationship to Study Drug

The PI/Sub-Investigator will assess the relationship of all adverse reactions to the study drug, using the following scale:

Probable	A clinical event, including laboratory test abnormality, with a reasonable time sequence to drug administration, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to drug administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Unlikely	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and which other drugs, chemicals or underlying disease provide plausible explanation.
Unrelated	This category is applicable to AEs which are judged to be clearly and incontrovertibly due to extraneous causes (diseases, environment, etc.) and do not meet the criteria for drug relationship listed for the above-mentioned conditions.

All AEs will be evaluated by the PI/Sub-Investigator, who must approve the subject for subsequent dosing.

Any AEs, whether serious or non-serious, will be monitored throughout the study and followed to resolution, when possible, regardless of whether the subject is still participating in the study.

Adverse Event monitoring and reporting will be followed-up until resolution. If resolution cannot be achieved within 1 week of study completion, the PI/Sub-investigator will decide the course of action.

11.2.2 Expectedness of SAE

The Study PI will be responsible for determining whether an SAE is expected or unexpected as per drug labels. An adverse event will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described in the Investigator's Brochure, Product Monograph, Product Label, or on the label of the drug.

11.2.3 Severity Assessment

The term "severe" describes the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as severe headache). This means it is not the same as "serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning

The severity of all AEs will be graded by the PI/Sub-Investigator or a medical qualified delegate according to the following definitions:

Mild	Adverse event resulting in discomfort, but not sufficient to cause interference in normal daily activities.
Moderate	Adverse event resulting in discomfort that is sufficient to cause interference in daily activities.
Severe	Adverse event resulting in discomfort causing an inability to carry out normal daily activities.

Every effort will be made to obtain an adequate evaluation of the severity.

11.3 Adverse Events and Serious Adverse Events Reporting

An Adverse Event as defined above generally includes any condition that was:

1) Not present prior to administration of study drugs but appeared after initiation of administration of study drug; or
2) Present prior to administration of study drugs but worsened after administration of study drug; or
3) Reported as part of the subject's history, and while not present immediately prior to initiating administration of study drugs, reappeared after administration of study drug.

During the study, an AE can also occur outside the time that the investigational product(s) was given (e.g., during a washout period).

Pre-existing conditions, diseases, or disorders are not considered AEs unless there is a change in intensity, frequency, or quality. Planned hospital admissions and/or surgical operations/procedures for an illness or disease that existed before the investigational product was given or the subject was enrolled in a clinical trial are not to be considered AEs.

Clinical laboratory data collected during the course of the study, which exceeds or drops below the acceptable limits for the subject population and which, based on baseline values, are considered by the Investigator to be clinically significant, will be reported as an AE. If clinically significant abnormal ECGs and/or laboratory

values lead to, or are associated with clinical symptom(s), the diagnosis should be reported as an AE.

If a subject discontinues from the study because of an AE, study site personnel must clearly document the circumstances and data leading to the reason for discontinuation.

The investigator/designee is responsible for recording all AEs which have occurred during the study (including clinically important deviations of laboratory values from normal ranges), regardless of their relationship to the study drugs. This includes AEs spontaneously reported by the patient, observed by the investigator/designee or elicited by general questioning. All AEs will be determined as Serious or Non-Serious.

Subjects will be instructed to inform clinic personnel of AEs that may arise during the study.

Treatment of any AEs will be administered under the direction of a physician, either at BPSUSA or at a nearby hospital emergency room. First-aid treatment will be provided on site, but if additional treatment is required, subjects will be transferred to a hospital.

All symptoms will be recorded by clinic staff and will be reviewed by the PI/Sub-Investigator prior to any subsequent dosing. When appropriate, medical tests and examinations will be performed to document resolution of the event(s).

All reported adverse events will be documented in the Clinical Report.

The Investigator will monitor the subject's condition until recovery to a satisfactory state or stabilization. Thus, follow-up visits may be required even after the administration of the study drugs has been discontinued.

All SAE(s), whether or not the event is deemed study drug-related, will be reported to the Sponsor by telephone and email within 24 hours of BPSUSA being aware of SAE, followed by a written report within five business days.

Reports of all SAEs must be communicated as soon as possible to the appropriate Institutional Review Board (IRB) or Independent Ethics Committee (IEC) and/or reported in accordance with local laws and regulations.

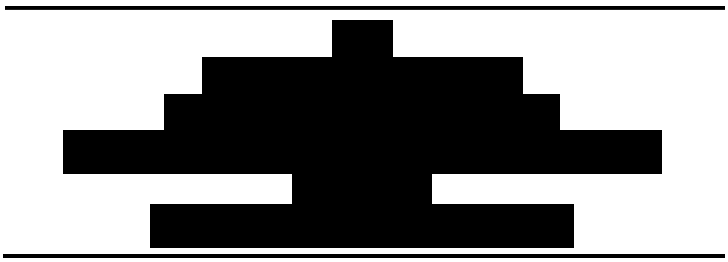
Adverse events will be coded into the Preferred Term (PT), classified according to the Medical Dictionary for Regulatory Activities (MedDRA) with System Organ Classification (SOC) and reported with severity, duration, onset time and relationship to study drugs and action taken.

BPSUSA will be responsible for notifying the local IRB. The Sponsor will be responsible for notifying the regulatory agencies, if applicable. BPSUSA will also report SAEs to FDA Adverse Event Reporting System (FAERS) per their procedures. The Principal Investigator will report to the local IRB and to the FDA

Sponsor any unanticipated problems involving risk to human subjects or others 45 CFR 46.103(b)(5)(i). Certain adverse events must be reported to the IRB within a specified period of time after the discovery of the event. The Office for Human Research Protections guidance defines these adverse events as including, but not limited to the following:

- An adverse event that is not expected, i.e., not listed in the informed consent document or the investigator's brochure;
- An expected adverse event that occurs at a greater frequency or duration than expected;
- Any adverse event that would require modification of the protocol and/or informed consent document.

All SAEs need to be emailed with SAE in a subject line to:



11.4 Procedures for Reporting Pregnancy

A positive urine pregnancy test or any hCG level ≥ 5 IU/L for females of child-bearing potential and ≥ 8 IU/L for post-menopausal females will be assessed as clinically significant and will result in the immediate dismissal of the subject from the study, unless deemed otherwise by the Investigator. The elevated hCG will be investigated as a potential pregnancy and will be followed up in the same manner as an AE. A confirmatory hCG blood test will be required for any elevated values. If pregnancy is confirmed, any out of range hCG levels will not be considered an adverse event.

Once notified that pregnancy has occurred during the course of the study or within 30 days of study completion, clinic personnel will report the pregnancy within 24 hours of the confirmatory hCG blood test, to the PI/Sub-Investigator, the IRB, and appropriate Sponsor representative. A Pregnancy Notification Form will be completed as per BPSUSA internal Standard Operating Procedures and will be included alongside any pertinent information at the time of pregnancy reporting. The confirmed pregnancy will be followed to birth of the child or elective/spontaneous termination of the pregnancy. Follow up will occur at minimum once every three months (i.e., once per trimester) until the end of the pregnancy (birth, termination, etc.).

Elective termination will not be considered an adverse event, however supporting documentation detailing the outcome of the procedure will be required. Spontaneous

termination of the pregnancy will be considered a Serious Adverse Event and will be followed up accordingly (as per Section 11.3). Pregnancy will be treated as AEs for this study.

12.0 BIOANALYTICAL ANALYSIS

12.1 Analytical Procedures

Data management, quality review and reporting of study data pertaining to laboratory analysis of study data will be the responsibility of the bioanalytical facility.

12.1.1 Samples to be Assayed

Sample from all subjects who complete at least 1 study period will be analyzed for drug concentration.

12.1.2 Analyte(s) in Biological Matrix

Whole blood samples will be assayed for tacrolimus using a validated analytical method according to the principles of Good Laboratory Practice.

12.1.3 Incurred sample reproducibility

Incurred Sample Reanalysis (ISR) shows repeatability and stability of an analytical method in real sample matrix. Approximately 10% of the samples will be reanalyzed in case the number of samples is less than 1000 samples. If the number of samples is greater than 1000, 10% of the first 1000 samples and 5% of the remaining samples will be reanalyzed. The ISR test will contain samples from all analyzed subjects. For each subject, at least two samples should be chosen, one at or near C_{max} and the other one in the elimination phase.

If an ISR sample result is not valid for analytical reasons (e.g. low area of internal standard response, sample processing error, instrument failure etc.), this sample will be excluded from the statistical evaluation of the ISR test and will not be reanalyzed. Acceptance criterion of the ISR test: the concentration obtained for the initial analysis and the concentration obtained by reanalysis should be within 20% of their mean for at least 67% of the repeats. If acceptance criterion of the ISR test is not met, an investigation will be conducted to determine the reason for differences, and adequate steps will be taken to minimize inaccuracy (and imprecision).

The ISR test results will be presented in the Bioanalytical Report; they will not be used for any correction of the concentration results for pharmacokinetic and statistical evaluation of the study.

13.0 PHARMACOKINETIC AND STATISTICAL ANALYSIS

13.1 Pharmacokinetic and Statistical Analysis Data Set

The data from at least one of the following subjects will be included in the PK and statistical analysis:

1. Subjects who complete all study periods.
2. Subjects who have missed samples but for whom it has been predicted prior to the start of bioanalytical analysis that reliable estimates of the PK parameters should be possible.
3. Subjects who drop out from the study but have completed at least 2 periods.

If a subject's pre-dose concentration is less than or equal to 5% of the C_{max} value for that subject in the given period, then the subject's data in that period without any adjustments can be included in all PK measurements and calculations. If the pre-dose value is greater than 5% of the C_{max}, data from that subject in that period will be dropped from the PK and statistical analysis. Data for subjects dropped due to higher than 5% of C_{max} pre-dose concentrations will be included in a separate appendix in the final study report.

For subjects who are dismissed from the study due to non-compliance or any reason other than AEs, other data of non-impacted period(s) for that subject, which fulfills the PK analysis data set requirements, as above, will be included in PK stats analysis. Data does not fulfill the requirements will be presented separately.

If a subject is dismissed/withdrawn due to an AE from a study period, the data from that period will not be included in the PK stats analysis and will be presented separately. All other data for that subject, which fulfills the PK analysis data set requirement, as above, will be included in PK stats analysis. Data does not fulfill the requirements will be presented separately.

Data from other subjects who were dismissed or withdrew due to elevated hCG levels or are confirmed to be pregnant will not be included in the PK and statistical analysis. The data for these subjects will be presented separately.

Any decision for excluding data prior to start of sample bioanalysis (e.g Major protocol deviation) will be provided with a detailed explanation and will be properly recorded and dated.

13.2 Analysis of Pharmacokinetic Data

The PK and statistical analysis will be performed at BPSI using SAS® Version 9.4.

The PK and/or statistical analyses outlined in this protocol may be altered with appropriate justification.

13.2.1 Pharmacokinetic Analysis

Pharmacokinetic parameters will be calculated using non-compartmental analysis (NCA) method. The following PK parameters will be estimated (where possible) for tacrolimus and included in the PK and statistical analysis:

Primary endpoint	
AUC_{72}:	Area under the concentration-time curve from time zero until time point 72 hours post dose. AUC_{72} is estimated using the linear trapezoidal method.
C_{max}:	The maximal observed whole blood concentration.

Secondary endpoint	
AUC_{inf}:	Area under the concentration-time curve from time zero to infinity, calculated as $AUC_t + C_{last}/\lambda$, where C_{last} is the last measurable concentration.
AUC_t:	Area under the concentration-time curve from time zero until the last measurable concentration or last sampling time t, whichever occurs first. AUC_t is estimated using the linear trapezoidal method.
$T_{1/2}$:	Terminal elimination half-life, estimated as $\ln(2)/\lambda$.
T_{max}:	Time when the maximal whole blood concentration is observed.
λ:	Terminal elimination rate constant, estimated by linear regression analysis of the terminal portion of the ln-concentration vs. time plot.

During PK and statistical analyses, drug concentrations below the lower limit of quantitation (BLQ) of an assay will be considered as zero except when they occur between two non-BLQ concentrations where they will be considered as missing during PK calculations and estimations.

The actual time of blood samples collection will be used for pharmacokinetic and statistical analysis.

Missed samples and non-reportable concentrations (e.g. quantity not sufficient) from the analytical laboratory will be treated in the pharmacokinetic analysis as if they have not been scheduled for collection.

For subjects with missing or non-reportable tacrolimus concentration at time point 72-hour or the sampling time deviation at 72-hours is greater than 2 hours, the AUC_{72} will not be calculated. If concentration(s) at 72 hours or at the time point(s) immediately preceding 72 hours is/are BLQ(s), this/these BLQ value(s) will be set as zero and the calculated AUC will be treated as AUC_{72}.

The λ, $T_{1/2}$, and AUC_{inf} parameters will not be estimated for whole blood concentration-time profiles where the terminal linear phase is not clearly defined.

For subjects with missing or non-reportable tacrolimus concentrations for three or more of the last samples, only the C_{max} and T_{max} will be presented and included in the statistical analysis. Other PK parameters will not be reported. The AUC_{72} could also be estimated for these subjects if data allows.

13.2.2 Statistical Analysis of Pharmacokinetic Data

Step 1: Determine the Within-subject Standard Deviation of the Test and Reference Products (S_{WT} and S_{WR}) for the PK Parameters AUC_{72}, AUC_t, AUC_{inf} and C_{max}:

The within-subject standard deviation of the test and reference products will be determined for the ln-transformed PK parameters AUC_{72}, AUC_t, AUC_{inf} and C_{max} for tacrolimus.

For a fully replicated 4-way design with 2 sequences TRTR, RTRT, the within subject variance of the reference product, S_{WR}^2 can be determined as follows:

$$S_{WR}^2 = \frac{\sum_{i=1}^{2}\sum_{j=1}^{n_i}(D_{ij} - \overline{D}_{i.})^2}{2(n-2)}$$

Where,

$i =$ Number of sequences (2 used in the study),

$j =$ Number of subjects within each sequence,

$D_{ij} = R_{ij1} - R_{ij2}$ (where R_{ij1} represents the first administered (early) reference and R_{ij2} represents the second administered (late) reference.)

$\overline{D}_{i.} = \frac{\sum_{j=1}^{n_i} D_{ij}}{n_i}$ (Mean within sequence i.)

$n = \sum_{i=1}^{2} n_i$ (total number of subjects used in the study, while n_i is the number of subjects used in sequence i.)

S_{WR} will be calculated as follows:

$$S_{WR} = \sqrt{S_{WR}^2}$$

The S_{WR} calculation will include subjects with estimated PK parameters for both reference administrations.

For a fully replicated 4-way design with 2 sequences TRTR, RTRT, the above method can be applied for the calculation of the within subject variance of the test product S_{WT} as well.

Step 2: Reference Scaled Average Bioequivalence Procedure

The 95% upper confidence bound for $(\mu_T - \mu_R)^2 - \theta S_{WR}^2$ will be determined for the ln-transformed PK parameters AUC_{72}, AUC_t, AUC_{inf} and C_{max} using a PROC MIXED model in SAS®, where μ_T and μ_R are the means of the ln-transformed PK parameters obtained from the BE study for the test and reference products, respectively. θ is the scaled average bioequivalence limit defined as $\theta = \left(\frac{\ln(1/0.9)}{\sigma_{w0}}\right)^2$, where σ_{w0} is a regulatory constant which is set at 0.10.

The method of obtaining the upper confidence bound is based on *Howe's Approximation* [8]. The following steps will be followed to construct the 95% upper confidence bound.

1. Let T_{ij1}, T_{ij2}, R_{ij1} and R_{ij2} be the log transformed observations on early test, late test, early reference and late reference for subject j within sequence i. Then we will calculate the following two terms:

   i. $I_{ij} = \frac{T_{ij1} + T_{ij2}}{2} - \frac{R_{ij1} + R_{ij2}}{2}$, and

   ii. $D_{ij} = R_{ij1} - R_{ij2}$.

2. Fit the Generalized linear mixed model (GLMM) with I_{ij} values as dependent variable. Sequence is the explanatory variable of the model. The confidence interval for the least squares mean of I will be estimated at 10% significance level. Let L and U be the lower and upper bounds of the confidence interval. Also the least squares mean of I will be calculated by determining the average of the 2 sequence means, using the ESTIMATE function of GLMM. Suppose Φ is the estimated least squares mean of I and $SE(\Phi)$ is the standard error of this estimate.

   Calculate the following estimates:

   $\Delta_1 = \Phi^2 - SE(\Phi)^2$

   $B_1 = (\max((\text{abs}(L)), (\text{abs}(U))))^2$

3. Fit the GLMM with D_{ij} values as the dependent variable. Sequence is the explanatory variable of the model. The within subject variance of the reference product, S_{WR}^2, will be calculated as,

$$S_{WR}^2 = \frac{MSE}{2}$$

Where MSE is the mean square error from the model and let κ be the corresponding degrees of freedom.

4. Now the 95% upper confidence bound for $(\mu_T - \mu_R)^2 - \theta S_{WR}^2$ will be calculated as follows:

$$(\Delta_1 + \Delta_2) + \sqrt{(B_1 - \Delta_1)^2 + (B_2 - \Delta_2)^2}$$

Where,

$\Delta_2 = -\theta S_{WR}^2$

$B_2 = \frac{\Delta_2 \kappa}{\chi^2_{\kappa,0.95}}$

$\chi^2_{\kappa,0.95}$ is the chi-square tabulated values at 5% significance level.

Step 3: Unscaled Average Bioequivalence Procedure

The unscaled average bioequivalence analysis was performed using PROC MIXED in SAS@, with SEQUENCE, PERIOD, and TREATMENT as fixed effects, TREATMENT and SUBJECT as random effects on the logarithm of the pharmacokinetic parameters (AUC_{72}, AUC_t, AUC_{inf} or C_{max}).

The following illustrates the program statements to run the ANOVA using PROC MIXED in SAS, with SEQ, SUBJ, PER, and TRT identifying sequence, subject, period, and treatment variables, respectively, and Y denoting the response measure (e.g.,ln(AUC_{72}), ln(AUC_t), ln(AUC_{inf}), ln(C_{max})) being analyzed:

```
PROC MIXED;
CLASSES SEQ SUBJ PER TRT;
MODEL Y = SEQ PERTRT / DDFM=SATTERTH;
RANDOM TRT/TYPE=FA0(2) SUB=SUBJ G;
REPEATED/GRP=TRT SUB=SUBJ;
 ESTIMATE 'T vs. R' TRT 1 -1/CL ALPHA=0.1;
```

All the fixed effects will be tested using the Type 3 Tests of Fixed Effects in the ANOVA to detect statistically significant differences (α=0.05).

The REPEATED statement in PROC MIXED is used to specify covariance structures for repeated measurements on subjects. Least squares means for the treatments and the adjusted difference between treatment means together with the associated standard error (σ_D) will be estimated in this procedure.

The two one-sided hypotheses [9] at the alpha level (α=0.05) of significance will be tested by constructing the 90% confidence interval for the ratio between the test and reference means.

The same ANOVA using the linear mixed-effects model will be performed on the raw data for T_{max}, Lambda (or λ), and $T_{1/2}$ parameters.

Step 4: Calculate the 90% CI of the Ratio of the Within-subject Standard Deviation of Test Product to Reference Product (σ_{WT}/σ_{WR}).

The 90% CI for σ_{WT}/σ_{WR} is given by

$$\left(\frac{S_{WT}/S_{WR}}{F_{\alpha/2}(v_1, v_2)}, \frac{S_{WT}/S_{WR}}{F_{1-\alpha/2}(v_1, v_2)}\right)$$

where

- S_{WT} is the estimate of σ_{WT} with v_1 as the degree of freedom;
- S_{WR} is the estimate of σ_{WR} with v_2 as the degree of freedom;
- $F_{\alpha/2}(v_1, v_2)$ is the value of the F-distribution with v_1 (numerator) and v_2 (denominator) degrees of freedom that has the probability of $\alpha/2$ to its right
- $F_{1-\alpha/2}(v_1, v_2)$ is the value of the F-distribution with v_1 (numerator) and v_2 (denominator) degrees of freedom that has the probability of $1-\alpha/2$ to its right
- here $\alpha = 0.1$.

Individual concentration at each scheduled time point and individual PK parameters will be listed by treatment. Two SAS transport files including xconc.xpt for individual concentration at each scheduled time point and xpara.xpt for individual PK parameters, as well as a Define.pdf file to describe each SAS transport file will be provided.

Descriptive statistics (including number of subjects (N), arithmetic mean, standard deviation (SD), minimum, maximum, median and coefficient of variation (CV%)) of concentrations will be presented by treatment at each scheduled time point.

Descriptive statistics (including N, arithmetic mean, SD, minimum, maximum, median and CV%) of all PK parameters will be presented by treatment.

The mean and individual concentration-time profiles will be plotted in linear and semi-logarithmic scales.

If the study is dosed in two or more groups of subjects, the statistical model will be modified to reflect the multi-group nature of the study.
Additional statistical and alternate tests will be performed if necessary.

Further details for statistical analysis of PK data will be described in the Statistical Analysis Plan (SAP).

13.3 Safety Analysis

13.3.1 Safety Data Set

Safety data set is defined as all subjects dosed in this study. In a case where SARS-CoV-2 related TEAEs lead to the withdrawal or dismissal of approximately 5 % or more of the dosed subjects, a subgroup analysis related to Adverse Events (AE) may be performed. And the safety listings and tables for AE with subgroup and the total safety population will both be presented.

13.3.2 Safety Listings

The safety analysis data set for Adverse Events (AE), demographic characteristics, vital signs and clinical laboratory will be listed by subject or by subject and treatment.

A complete listing of AE or (Serious Adverse Events) SAE (if applicable) including period, actual treatment received, primary System Organ Class (SOC) and Preferred Term (PT), reported term, AE severity, AE causality, start/end date and time, duration, action taken etc., will be presented by subject.

A complete listing of demographic characteristics including age, race, gender, ethnicity, BMI, weight and height will be presented by subject.

Vital signs parameters including Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Heart Rate (HR), Respiratory Rate (RR), temperature and oxygen saturation (if applicable) will be presented by subject.

A complete listing of clinical laboratory measurements (including hematology, serum chemistry, urinalysis, serology, breath alcohol test, urine tests for drugs of abuse, urine HCG test (for females), serum HCG test (for females), magnesium, creatinine kinase and lipid panel will be presented by subject.

13.3.3 Safety Tables

The safety data set for AE, demographic characteristics and vital signs will be summarized in frequency or using descriptive statistics (including N, arithmetic mean, SD, minimum, maximum and median) of quantitative measures by treatment, as appropriate.

A brief overall summary of AEs including number of AEs/TEAEs (Treatment Emergent Adverse Event) /SAEs and number and percentage of subjects experienced at least one AEs/TEAEs/SAEs will be presented by treatment and the overall population. TEAEs/SAEs will also be summarized using the number and percentage of subjects experiencing at least one TEAE/SAE (with the number of subjects who received study treatment as the denominator of percentage) by primary SOC, PT, AE severity and AE causality, for each treatment and the overall population.

Descriptive statistics of age, BMI, weight and height and frequencies for race, gender and ethnicity will be provided by treatment and the overall population.

Descriptive statistics of each vital sign's parameters will be presented by treatment at each visit.

Descriptive statistics of all quantitative measurements from all clinical laboratory parameters will be presented by the type of laboratory test, laboratory parameter, and visit.

Further details for safety analysis will be described in the Statistical Analysis Plan (SAP).

13.4 Bioequivalence Criteria [7, 10]

To establish bioequivalence, all the following conditions for individual PK parameter(s) AUC_{72}, and C_{max} must be met:

- The 90% CI for the Test/Reference ratios of geometric means are completely contained within 80.00%-125.00%.
- The 95% upper confidence bound for $(\mu_T - \mu_R)^2 - \theta S_{WR}^2$ should be less than or equal to 0.
- The upper limit of the 90% confidence interval for the Test/Reference ratio of the within-subject standard deviation $(\boldsymbol{\sigma_{WT}/\sigma_{WR}})$ is less than or equal to 2.5.

14.0 ETHICAL CONSIDERATIONS / PROTECTION OF HUMAN SUBJECTS

14.1 Basic Principles

This research will be carried out in accordance with Good Clinical Practice (GCP) as set out by the International Conference on Harmonization (ICH), the basic principles defined in the U.S. Code of Federal Regulations (21 CFR Part 312), the Belmont Report, Directive 2001/20/EC (Europe), and the principles enunciated in the most recent version of the World Medical Association Declaration of Helsinki.

14.2 Institutional Review Board

The local IRB will be the Institutional Review Board (IRB) for this study. This Protocol and the ICF will be reviewed and approved by the IRB prior to the initiation of the study. Termination / suspension of any prior approval / favorable opinion can be done by IEC (Institutional Ethics Committee)/IRB.

The board is constituted and operates in accordance with Division 5 of the Food and Drug Regulations, ICH Harmonized Tripartite Guideline (GCP Consolidated Guideline), and 21 CFR Parts 56 and 50.

14.3 Informed Consent Form

Subjects will sign a study ICF prior to start of any study related procedures. Each subject will be provided with verbal and written information, in non-technical terms, which will describe the nature of the screening procedures as well as conduct of the study. Prior to signing the ICF, subjects will be allowed adequate time to consider the potential benefits and risks associated with their participation in the study. Signed and dated ICF will be retained with the study records and a copy will be provided to the subject.

14.4 Confidentiality

The information in this study protocol is confidential. This document contains trade secrets and commercial information that is confidential and may not be disclosed to third parties (except to the IRB, FDA and/or relevant regulatory agencies). These restrictions will apply as well to all future communications if deemed privileged or confidential.

Sponsor, study monitors, auditors, the IRB, FDA and applicable regulatory authorities will be granted direct access to the subject's original medical records for verification of clinical trial procedure and/or data, without violating the confidentiality of the subject, to the extent permitted by applicable laws and regulations and that, by signing a written informed consent form, the subject or the subjects legally acceptable representative is authorizing such access.

All documentation collected by BPSI/BPSUSA Inc. will be kept confidential. The name and identity of the subjects will remain confidential. If documents containing

the subjects' names are photocopied, the name will be omitted from the photocopied version.

The Certificate of Confidentiality (CoC), issued by the FDA in accordance with new requirements under the 21st Century Cures Act, will be filed and maintained in the regulatory binder. As per the CoC, researchers cannot disclose a subject's name or other information that could identify them in any civil, criminal, administrative, legislative or other proceedings (such as a court trial), without their consent. Also, information collected for this research that could identify a subject cannot be used as evidence in a legal proceeding without their consent.

In addition, with the CoC, researchers involved in this study generally may not provide a subject's name, or any other information that could identify them, to anyone who is not connected with the research. However, in the following situations, the CoC does not prevent the researchers involved in this study from disclosing study information that could identify a subject:

- If the subject consents to someone receiving their information from this study, including situations where the information is necessary for their medical treatment
- When the subject's study information is used for other scientific research, as allowed by federal regulations protecting research subjects
- When information is needed by the FDA, which is funding this study, in order to audit or evaluate federally-funded studies
- When a law otherwise requires disclosure [such as requirements to 1) make certain reports to FDA, 2) report threats of harm to self/others, 3) report incidents of child abuse, or 4) report cases of contagious disease, such as HIV, to the state as mandated by state law]. This does not apply to disclosure in a legal proceeding.

The CoC does not prevent a subject from voluntarily providing information about themselves or their involvement in this research study to others.

The CoC will not be used to prevent disclosure for any purpose the subject has consented to in this ICF, such as sharing their participation and research data with their family doctor (if the subject has agreed to share this information).

14.5 Compensation for Participation

[redacted]:

[redacted]

15.0 ADMINISTRATIVE CONSIDERATIONS

15.1 Revisions and/or Amendments to the Protocol

Revisions and/or amendments to the protocol must be documented and approved by the Principal Investigator and discussed with sponsor prior to finalization. If the revision/amendment will affect subject safety and/or study design, the amendment will be re-submitted to the IRB for approval. Administrative changes (i.e., change of analytical facility, typographical errors, discrepancies, clarifications) will also be submitted to the IRB, but may not require approval. A copy of the IRB's approval or acknowledgement documents will be included in the final report.

For revisions or amendments to the protocol that substantially alter the study design after initiation of the study, a signed revised informed consent form will be needed for continued participation by the subject.

15.2 Investigator Responsibilities

The Principal Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, the current revision of the Declaration of Helsinki, ICH - GCP guidelines and applicable regulatory requirements.

15.3 Study Completion/Termination

BioPharma Services USA Inc. and/or the Sponsor reserve the right to terminate the study at any time for any reason related to the safety of subjects or due to any other non-study specific reason.

15.4 Sponsor Visits

The Sponsor is encouraged to visit BioPharma Services USA Inc., if desired, and at their convenience. The PI and staff will provide, if requested, all source documents and/or other study-related documents. The PI will maintain regular written and telephone communication with the Sponsor.

16.0 DATA MANAGEMENT/RECORD KEEPING

16.1 Source Data

All data will be recorded in accordance with GCP to ensure accuracy, completeness, legibility, and timeliness of the data reported.

All data will be recorded directly on the source documents and will be considered source data. Subjects will be identified using an assigned ID number and/or randomization number on source documents.

16.2 Quality of Data

All source documents and laboratory reports will be Quality Control reviewed to ensure accuracy and completeness. Adverse events will be reviewed and assessed for severity and causality by the PI/Sub-Investigator. Specific processes of the study, its source documentation and any reports (if applicable) will be audited by the Quality Assurance (QA) unit of BPSI/BPSUSA.

16.3 Retention of Documents

All records and documents pertaining to the study will be retained by BioPharma Services USA Inc. for at least 25 years from the completion of the study and will be available for inspection by the Sponsor and/or Regulatory Agencies.

17.0 REFERENCES

1. Zhang W et al., Recent Topics on The Mechanisms of Immunosuppressive Therapy-Related, Int J Mol Sci. 2019, 20(13):3210.
2. Gaynor J et al., Lower tacrolimus trough levels are associated with subsequently higher acute rejection risk during the first 12 months after kidney transplantation. Transplant International, 2016, 29: 216
3. Sikma M et al., Association of Whole Blood Tacrolimus Concentrations with Kidney Injury in Heart Transplantation Patients, Eur J Drug Metab Pharmacokinet. 2018; 43(3): 311.
4. Hitesh S. Purohit et al., Investigating the Impact of Drug Crystallinity in Amorphous Tacrolimus Capsules on Pharmacokinetics and Bioequivalence Using Discriminatory In Vitro Dissolution Testing and Physiologically Based Pharmacokinetic Modeling and Simulation, J Pharm Sci. 2018, 107(5):1330
5. Hitesh S. Purohit et al., Assessing the Impact of Endogenously Derived Crystalline Drug on the in Vivo Performance of Amorphous Formulations. Mol. Pharmaceutics 2019, 16 (8): 3617
6. PROGRAF® (tacrolimus) capsules, for oral use, [label]. [redacted]
7. FDA draft guidance on Tacrolimus capsule (revised on Dec 2012) https://www.accessdata.fda.gov/drugsatfda_docs/psg/Tacrolimus_cap_50708_RV12-12.pdf
8. W. G. Howe (1974), Approximate Confidence Limits on the Mean of X+Y Where X and Y are Two Tabled Independent Random Variables, *Journal of the American Statistical Association*, 69 (347): 789 – 794.
9. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J. Pharmacokinet BioPharm 1987; 15:657-80.
10. FDA draft guidance on Warfarin Sodium tablet (revised on Dec 2012) https://www.accessdata.fda.gov/drugsatfda_docs/psg/Warfarin_Sodium_tab_09218_RC12-12.pdf

APPENDIX A – SCHEDULE OF EVENTS

	Screening	Check -in	Period 1, 2, 3 and 4				Return Visit			EOS
Study Day	**-30 to 0**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Last visit of Period 4**
Confinement		**X**	**X**	**X**	**X**	**X[#]**				
ICF	X									
Demographics	X									
Medical/Medication History	X									
Physical Exam	X									X
Body weight	X									
Height	X									
BMI	X									
Vital Signs (BP & HR)	X	X	X[a]	X[b]	X[c]	X[d]				X
Safety measures for COVID-19[#] (Symptom Check, Temperature, Pulse Oximetry and RR)	X	X[e]	X[e]	X[e]	X[e]	X[e]	X	X	X	
SARS-CoV-2 Test (If applicable)		X								
Urine Drug Tests	X	X								
Alcohol Test	X	X								
Pregnancy Test	X[*]	X[*]								
12-Lead ECG	X									
Hematology	X	X								X
Serum Chemistry	X	X[f]								X
Urinalysis	X	X								X
Serology (HIV, Hepatitis B and C) Magnesium Creatinine Kinase Lipid panel	X									
Inclusion/Exclusion criteria	X	X[g]								
Compliance check		X					X	X	X	
Randomization			X							
Tacrolimus Capsule Administration			X							
PK Sampling			X[h]	X[h]	X[h]	X[h]	X[h]	X[h]	X[h]	
Questioning subjects if use of any concomitant medication	X	X					X	X	X	
Adverse Events			X	X	X	X	X	X	X	
Meals		X	X	X	X	X				

* Serum pregnancy test at screening and urine pregnancy test at each period check-in.
# Study exit at 72 hours post-dose tacrolimus
a: 1, 2, 4, 6- and 12-hours post-dose
b: 24hours post-dose
c: 48 hours post-dose
d: 72 hours post-dose
e: At pre-dose, at 6- and 12-hours post-dose and then at every 12 hours until discharge at each study period.
f: At check-in period 1 and 3.

g: Only at check-in for the period 1
h: Pre-dose (0 hour) and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.000, 24.000, 36.00, 48.00, 72.00, 96.00, 120.00 and 144.00 hours after study drug dosing in each study period

APPENDIX B – CLINICAL LABORATORY ASSESSMENT

TYPE OF TEST	COMPONENTS			
Hematology[4]	• Hemoglobin • Hematocrit	• RBC • Platelet count	• WBC and automated differential	
Serum[5] **Chemistry**	• Glucose • Calcium • Sodium • Chloride • Bicarbonate (Total CO_2)	• Albumin • Protein • Bilirubin	• AST • ALT • Potassium • Alkaline Phosphatase	• BUN • Urea • Uric Acid • Creatinine
Urinalysis	• Bilirubin • Blood • Glucose	• pH • Ketones • Leukocytes	• Nitrites • Protein	• Specific Gravity
Additional Tests	Serology (HIV, Hepatitis B surface antigen, Hepatitis C antibody)[6] Magnesium[6] Creatine Kinase[6] Lipid panel[6]		Alcohol test Serum hCG (only females, at Screening) Urine hCG (only females, at each period check-in)	
Urine Tests for Drugs of Abuse	Marijuana, Amphetamines, Phencyclidine, Barbiturates, Cocaine, Opiates, Benzodiazepines			

[4] At Screening, at each check-in and at EOS
[5] At screening, at check-in for period 1 and 3 and at EOS
[6] At screening only

APPENDIX C – LIST OF COMMON ABBREVIATIONS

AE:	Adverse Event	LSMEANS:	Least Square Means
ALT:	Alanine Aminotransferase	MedDRA:	Medical Dictionary for Regulatory Activities
ANOVA	Analysis of Variance	mg:	Milligram
AST:	Aspartate Aminotransferase	mL:	Milliliters
AUC_{72}	Area under the concentration-time curve from time zero until time point 72 hours post dose. AUC_{72} is estimated using the linear trapezoidal method.	mmHg:	Millimeter of Mercury
AUC_t:	Area under the concentration-time curve from time zero until the last measurable concentration or last sampling time t, whichever occurs first. AUC_t is estimated using the trapezoidal method	N/A or NA:	Not applicable, Not Available
AUC_{inf}:	Area under the concentration-time curve from time zero to infinity, calculated as $AUC_t + C_{last}/\lambda$, where C_{last} is the last measurable concentration	PI:	Principal Investigator
$AUC_{\tau,ss}$:	Area under the concentration-time curve from time 0 to τ (the length of the dosing interval) at steady state. AUC_τ is estimated using the trapezoidal method.	PK:	Pharmacokinetic
BLQ	Below the Limit of Quantitation / Below Quantitation Limit	PT:	Preferred Term
BMI:	Body Mass Index (kg/m^2)	QA:	Quality Assurance
BP:	Blood Pressure	RBC:	Red Blood Cell
BPM:	Beats Per Minute	RPM:	Revolutions Per Minute
BPSI:	BioPharma Services Inc.	RR:	Respiratory Rate
BPSUSA:	BioPharma Services USA Inc.	RLD:	Reference Listed Drug
cGMP	Current Good Manufacturing Practice	SAE:	Serious Adverse Event
CI:	Confidence Interval	SAS®:	Statistical Analysis System
C_{max}:	The maximal observed-whole blood concentration.	SD or STD:	Standard Deviation
CoC	Certificate of Confidentiality	SOC:	System Organ Classification
CYP:	Cytochrome P450	SOP:	Standard Operating Procedure
ECG:	Electrocardiogram	Sub-I	Sub-Investigator
FDA:	Food and Drug Administration	$T_{1/2}$:	Terminal elimination half-life, estimated as $\ln(2)/\lambda$
FSH:	Follicle-Stimulating Hormone	T_{max} / t_{max}:	Time when the maximal-whole blood concentration is observed
GCP:	Good Clinical Practice	WBC:	White Blood Cell
GLP:	Good Laboratory Practice		
hCG:	Human Chorionic Gonadotropin		
HIV:	Human Immunodeficiency Virus		
ICF:	Informed Consent Form		
ICH:	International Conference on Harmonization		
IEC	Institutional Ethic Committee		
IRB:	Institutional Review Board		

Lambda / λ:	Terminal elimination rate constant, estimated by linear regression analysis of the terminal portion of the ln-concentration vs. time plot		
kg/m^2:	Kilogram/Meter Squared		

APPENDIX D – PROGRAF® LABEL

(See attached)