An Open-Label, First-in-human, Safety and Pharmacokinetic Study of 1-Month Sustained-Release Injectable Tacrolimus in Healthy Subjects (RTB-010)

SR Tacrolimus Safety and PK Study in Healthy Subjects

Version 2.0/July 2, 2018

Study Sponsor(s): The National Institute of Allergy and Infectious Diseases (NIAID)
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National Institute of Allergy and Infectious Diseases

INVESTIGATOR SIGNATURE PAGE	
Protocol: An Open-Label, First-in-human, Safety and Pharmacokinetic (PK) Study of 1-Month Sustained-Release (SR) Injectable Tacrolimus in Healthy Subjects	**Version/Date:** Version 2.0/July 2, 2018
Site Principal Investigator: George J. Atiee, MD	
Title: Vice President/Medical Director/CLIA Lab Director	
Study Sponsor: Auritec Pharmaceuticals, Inc.	
INSTRUCTIONS: *The site Principal Investigator should print, sign, and date at the indicated location below. The original page should be kept for your records and a copy mailed to:*	
I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements. As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB, Auritec Pharmaceuticals, Inc and DAIT/NIAID.	
Site Principal Investigator (Print)	
Site Principal Investigator (Signature)	**Date**

Protocol Synopsis

Title	An Open-Label, First-in-human, Safety and Pharmacokinetic Study of 1-Month Sustained-Release (SR) Injectable Tacrolimus in Healthy Subjects
Short Title	SR Tacrolimus Safety and PK in Healthy Subjects
Clinical Phase	First-in-human
Participating Site	Worldwide Clinical Trials Early Phase Services, LLC
IND Sponsor/Number	Auritec Pharmaceuticals, Inc./
Study Objectives	1) To evaluate the safety of a single dose of 1-month sustained-release subcutaneous injectable tacrolimus in healthy subjects. 2) To evaluate the pharmacokinetic profile after a single dose of sustained-release injectable tacrolimus for up to 60 days postdose.
Study Design	This is a single-center, nonrandomized, open-label, first-in-human study to assess the safety and pharmacokinetic (PK) profile of sustained-release (SR) tacrolimus, which will be administered as a single dose of 0.1 mg/kg by subcutaneous (SC) injection in 8 healthy subjects. Following a screening period, eligible subjects will be admitted to the clinical research unit (CRU). On Day 1 subjects will receive a single injection of SR tacrolimus. Subjects will remain at the CRU for at least 24-hours postdose for collection of serial blood samples for PK analysis and safety monitoring until discharge on Day 2. Subjects will return to the clinic for PK and safety evaluations for 60 days postdose.
Primary Endpoint(s)	1) Safety endpoints for this study include incidence, severity, and relatedness of adverse events (inclusive of injection site reactions), and changes from baseline (prior to dosing) in vital signs, clinical laboratory evaluations, physical examinations, and electrocardiograms. 2) Pharmacokinetic (PK) endpoints include calculation of the following PK parameters: maximum observed tacrolimus whole blood concentration (C_{max}), time to maximum observed tacrolimus whole blood concentration (t_{max}), area under the concentration-time curve (AUC) and absorption constant (k_{abs}).
Secondary Endpoint(s)	Not Applicable.

Accrual Objective	The number of subjects is not hypothesis driven; rather, a sample size of at least 6 pharmacokinetically evaluable subjects will provide sufficient PK and safety data. Additional subjects may be added as necessary to achieve this goal.
Study Duration	The study duration is approximately 88 days, inclusive of Screening (up to 28 days); Inpatient Period (Day -1 to discharge on Day 2), with follow-up visits for 60 days.
Treatment Description	SR Tacrolimus, single dose, 0.1 mg/kg, SC injectable
Inclusion Criteria	Subjects who meet the following criteria may be included in the study: 1. Must be willing and able to provide signed informed consent. A signed informed consent form must be provided before any study assessments are done; 2. Males or females, between 18 and 45 years of age, inclusive; 3. Body mass index must be within the range 18.5 to 32.0 kg/m2, inclusive; 4. Must be in good health, as determined by no clinically significant findings from medical history, vital signs, and 12-lead electrocardiogram (ECG), inclusive of documented absence of QT prolongation; 5. Clinical laboratory evaluations (including clinical chemistry panel [fasted at least 10 hours], complete blood count [CBC], and urinalysis [UA]) must be within the reference range for the test laboratory, unless deemed not clinically significant by the Investigator; 6. Must be negative for autoimmune disorders in the past 3 months and at Screening - participant's medical history will be used for this evaluation; 7. Must not use any immunosuppressant calcineurin inhibitor product other than SR Injectable tacrolimus throughout the dosing period and until after the final visit; 8. Must agree to blood draws throughout the course of the study and have venous access sufficient to allow for blood sampling as per the protocol; 9. Must be negative for selected drugs of abuse at Screening and at Check-in (Day -1); 10. Must have a negative hepatitis panel (including hepatitis B surface antigen and hepatitis C virus antibody) and negative human immunodeficiency virus antibody screens; 11. Females will be nonpregnant, nonlactating, and either postmenopausal, defined as amenorrhea for at least 1 year and follicle-stimulating hormone levels of 40 mIU/mL or higher; surgically sterile (eg, tubal ligation, hysterectomy, oophorectomy) for at least 90 days prior to Screening; or agree to use, from the time of signing the

	informed consent or 10 days prior to Check-in on Day -1 of the Inpatient Period until 30 days after Study Discharge, one of the following forms of contraception: nonhormonal intrauterine device (IUD) with spermicide, female condom with spermicide, contraceptive sponge with spermicide, diaphragm with spermicide, cervical cap with spermicide, male sexual partner who agrees to use a male condom with spermicide, or sterile sexual partner. For all females of childbearing potential, the pregnancy test result must be negative at Screening; Check-in on Day -1 of the Inpatient period; alternatively, women must agree to maintain abstinence (must agree to use a double barrier method if they become sexually active during the study). Women must also agree not to douche throughout the dosing period and until after the final visit; 12. Males will either be sterile or agree to use, from Check-in on Day -1 of the Inpatient Period until 90 days following Study Discharge, one of the following approved methods of contraception: male condom with spermicide; sterile sexual partner; or use by female sexual partner of an IUD with spermicide; a female condom with spermicide; a contraceptive sponge with spermicide; an intravaginal system (eg, NuvaRing®); a diaphragm with spermicide; a cervical cap with spermicide; or oral, implantable, transdermal, or injectable contraceptives. Subjects will refrain from sperm donation from Check-in on Day -1 of the Inpatient Period until 90 days following Study Discharge.
Exclusion Criteria	Subjects will be excluded from the study if they meet the following criteria: 1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol; 2. Prisoners or patients who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (eg, infectious disease) illness must not be enrolled into this trial; 3. Females of childbearing potential, or who are breast-feeding, or who have a positive urine or serum pregnancy test result prior to receiving study drug, or at any time during the study; 4. Presence of an uncontrolled, unstable clinically significant medical condition that in the opinion of the Investigator may increase the risk to the subject or may interfere with the interpretation of safety and PK evaluations, or could impair the subject's ability to complete the trial, or could impair the decisional capacity of the subject; 5. Presence of clinically significant vital signs or a physical examination finding that, in the opinion of the Investigator, could increase the risk to the subject or may potentially

	interfere with the ability to evaluate safety and tolerability in the trial; 6. History of tacrolimus use, or hypersensitivity and/or adverse reaction to calcineurin inhibitor drugs; 7. History of toxic shock syndrome; 8. Currently receiving chemotherapy or immunosuppressive agents; 9. Use of investigative drugs within 30 days or 5 half-lives prior to study participation; 10. Currently using sirolimus; 11. Currently using live vaccines; 12. Currently on concomitant substrates and/or inhibitors of CYP3A4; 13. Requires the use of any concomitant medication, except for treatment of an adverse event (AE) during the study; 14. Any abnormality on clinical laboratory tests, or ECG finding that is considered to be clinically significant by the Investigator. 15. Known or suspected (non-febrile) seizure disorder; 16. Use of any other depot medications within the last three months. 17. Unwilling to commit to avoid eating grapefruit or drinking grapefruit juice during the first 30 days of this exploratory study 18. Grade ≥ 1 finding as described in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials
Study Stopping Rules	The entire study may be discontinued at the discretion of the Investigator, Study Sponsor, IND Sponsor or Sponsor's Medical Monitor based on the occurrence of the following: • AEs unknown to date with respect to their nature, severity, and duration; • increased frequency and/or severity and/or duration of known AEs; • medical or ethical reasons affecting the continued performance of the study; • difficulties in the recruitment of subjects; • cancellation of drug development.

Study Contact: Bioanalytical Laboratory

BIOANALYTICAL LABORATORY

Worldwide Clinical Trials Early Phase Services LLC

Table of Contents

Glossary of Abbreviations

CFR	Code of Federal Regulations
CRF	Case Report Form
CRU	Clinical Research Unit
CYP450	Cytochrome P450
DAIT	Division of Allergy, Immunology, and Transplantation
DSMB	Data Safety Monitoring Board
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HDYF	How Do You Feel
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
MOP	Manual of Procedures
NF-AT	Nuclear factor of activated T cells
NIAID	National Institute of Allergy and Infectious Diseases
OPTN	Organ Procurement and Transplantation Network
PI	[Site] Principal Investigator
PK	Pharmacokinetic
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SC	Subcutaneous
SOP	Standard Operating Procedure
SR	Sustained Release
SUSAR	Serious Unexpected Suspected Adverse Reaction
US	United States
WCT	Worldwide Clinical Trials Early Phase Services, LLC

1. Background and Rationale

1.1. Background and Scientific Rationale

Tacrolimus is a calcineurin inhibitor that is routinely used in the prophylaxis of kidney, liver and heart transplant rejection.

Over 1 million people receive medical assistance from tissue transplant procedures each year. Of the five major solid organ transplants which include heart transplant, pancreas transplant, lung transplant, kidney transplant and liver transplant, tacrolimus is routinely used for prophylaxis of organ rejection in patients receiving allogeneic liver, kidney or heart transplants. In 2012, greater than 50,000 organ transplants were conducted in the seven major markets - US, Japan, the United Kingdom, France, Germany, Italy and Spain.[1] In 2013, organ transplant procedures saved more than 28,900 lives.[2] According to a 2010 IMS Health report, the immunosuppression market for transplant patients in seven major markets added up to $5 billion.[3] Per the Organ Procurement and Transplantation Network (OPTN), as of April 2015, 123,193 patients are currently waiting for lifesaving organ transplants in the U.S. Of these, 83% (101,662) are waiting for kidney transplants. On average, more than 3,000 new people are added to the kidney waiting list each month.[4] Given these statistics, some analysts forecast the global transplantation market to grow at a CAGR of 9.36% over the period 2014-2019.[5]

Tacrolimus is an FDA-approved immunosuppressive calcineurin inhibitor commonly used for prevention of transplant rejection. However, the calcineurin inhibitors have narrow therapeutic indices and poor bioavailability. This is partly due to inadequate uptake across the gastrointestinal epithelium and partly due to variable hepatic first pass metabolism.[6, 7-9] Attempts to improve bioavailability by modifying oral formulations have met with modest success.[10-15] For this reason, there are continued efforts to improve the formulations of these drugs.[16-19]

Auritec Pharmaceuticals has developed a sustained-release (SR) formulation of tacrolimus as a subcutaneous (SC) injectable. The short-term general investigational plan is to evaluate SR tacrolimus in healthy subjects for up to 30 days in an open-label pilot study designed to assess safety and determine the pharmacokinetic (PK) profile of this new formulation following a single proposed 0.1 mg/kg dose. Sustained release (SR) injectables provide a unique and promising system capable of delivering safe, yet potent concentrations over long durations, and immunosuppressive calcineurin inhibitor injectables could potentially be useful in prevention of organ transplant rejection. Importantly, compliance has been poor with the current formulation of tacrolimus due to the frequency of dosing.[20,21] Improved delivery characteristics of an injectable formulation could decrease the dosing frequency, thereby improving compliance and treatment outcomes in prophylaxis of organ (kidney, heart and liver) transplant rejection.

The results from this study will inform the long-term goal of this program, which is to provide an improved treatment modality for prophylaxis of organ (kidney, liver and heart) transplant rejection with the additional benefit of enhancing medication compliance. These improvements have the potential to mitigate both the personal and economic burden of this disease.

1.2. Rationale for Selection of Investigational Product or Intervention

Auritec Pharmaceuticals has developed a membrane based, diffusion-driven release mechanism with drug particle sizing large enough to allow high drug loading, but small enough to be injected. This platform is used to develop SR formulations of the proposed 0.1 mg/kg injectable tacrolimus. Other investigators have proposed the SR formulation of calcineurin inhibitors through the use of polymer based delivery systems [22,23] Polymer

encapsulation has also been suggested in order to improve oral bioavailability[15,18-22] but, with the exception of the once-a-day formulation Astagraf XL®, none of these proposals has led to clinical trials or approval to date. In contrast, Auritec's Plexis® technology is well suited to the development of a SR formulation because the injectable depot is made up of thousands of biodegradable "mini-Norplants." This technology combines the benefits of linearization of release with those of injectability. The polymers chosen for the membrane are stable over the projected time of drug delivery[24] (in the proposed case, 1 month), which is essential for an optimal SR formulation, and yet ultimately biodegradable. Auritec has two broad patents pending for this technology, WIPO patents numbered 2004/058222 and 2004/058223, as well as Australian and Canadian applications which have been allowed (200329982 and 2,510,320 respectively), which is termed "Plexis™".

The Auritec SR injectable formulation has not been tested clinically. The short-term general investigational plan is to evaluate SR tacrolimus in a first-in-human study in healthy subjects as described above (Section 1.1). Additional exploratory studies will be considered and planned based in part on the results obtained in this study. The long-term investigational plan is to evaluate the safety and efficacy of SR tacrolimus injectable for its ability to provide an improved treatment modality for prophylaxis of organ (kidney, liver and heart) transplant rejection.

The mechanism of action of tacrolimus is well documented. Tacrolimus is a macrolide calcineurin inhibitor with immunosuppressive properties initially developed and used to prevent organ transplant rejection reactions. It acts by binding to specific intracellular proteins after entering T cells. The formed complex inhibits calcinuerin phosphatase, which prevents the activation of the nuclear factor of activated T cells (NF-AT), a transcription factor needed for the production of cytokines such as interleukin 2 (IL-2) and γ interferon.[25,26] Its mechanism of action is similar to that of cyclosporin A, but its immunosuppressive effect has been shown to be 30 to 100 times greater in vitro and 10 to 20 times greater in vivo.[27]

Current formulations of tacrolimus are effective, but, due to variable uptake and inter- and intra-individual variability in metabolism, require intense drug monitoring. Even elegant Bayesian approaches to modeling levels to guide dosing are unable to eliminate the problems of peaks that are too high and troughs that are too low.[28-30] Poor bioavailability requires more frequent dosing, and this usually leads to problems with medication compliance. It has been well documented across many disease states that adherence to therapy is inversely proportional to dosing frequency and it has been specifically demonstrated that parenteral depot administration can significantly increase adherence to therapy.[31-33] Improved delivery characteristics of an injectable formulation could decrease the dosing frequency, increase the bioavailability, reduce the first pass effects, and decrease the intra-patient variability in drug levels. It is well recognized that the peak-to trough ratio can be significantly reduced by sustained-release drug delivery.[34,35]
There are no SR formulations of tacrolimus. Therefore, SR tacrolimus injectable offers an improved treatment option for prophylaxis of organ (kidney, liver and heart) transplant rejection due to the following potential advantages

- Improved bioavailability with the opportunity to enhance efficacy;
- Less frequent dosing to improve medication compliance;
- Optimal treatment outcomes, which can decrease both disease and economic burden to the patient.

1.2.1. Major advantages of the novel Plexis®- based sustained release injectable tacrolimus formulation

Our formulation has demonstrated pseudo-zero order release in vitro and in vivo, suggesting that the formulation could be developed as weekly or monthly injectable, which could help improve treatment adherence and compliance in transplant patients, who are currently prescribed daily pills. Additionally, these pills are known to undergo first-pass metabolism, causing substantial inter-and intra-patient variability of tacrolimus due to genomic heterogeneity means that even frequent drug monitoring cannot eradicate rejection due to sub-therapeutic troughs, or toxicity due to unavoidable peak levels. The potency and the long elimination half-life of tacrolimus makes it suitable for depot formulation, and pharmacokinetic modeling suggests that a formulation that could allow periodic subcutaneous dosing is feasible. We hypothesize that use of such a formulation could reduce or eliminate many of the problem of bioavailability, toxicity, and adherence.

1.3. In vitro and Preclinical Experience

We have formulated sustained release injectable suspension of tacrolimus. We have measured the *in vitro* release and confirmed our ability to modify the release based on particle and coating parameters. We have developed an *in vitro / in vivo* correlation that will aid us in optimizing our formulation. We have demonstrated sustained release and preliminary safety in the rat.

Pharmacokinetic Modeling shows the feasibility of the approach

Pharmacokinetic modeling was carried out using USC*PACK version 11.8: a commercially available microcomputer software program (Figure 1).[32]

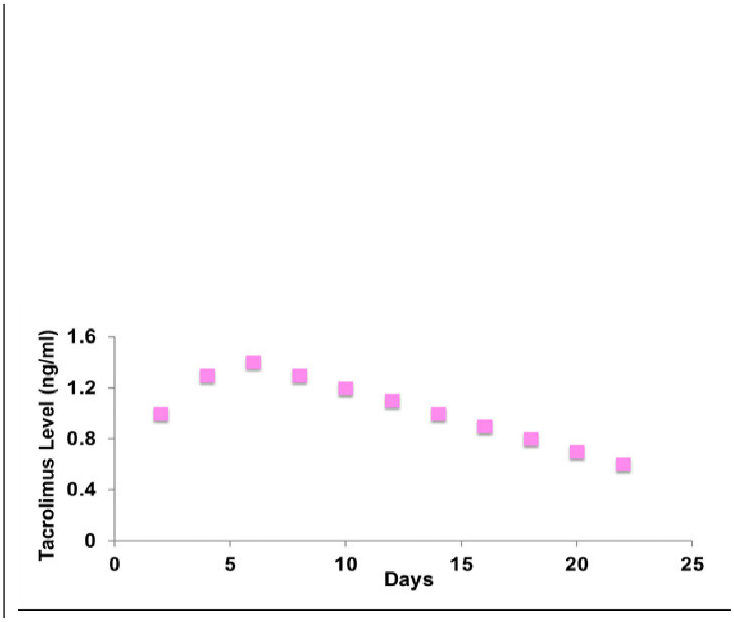


Figure 1: Pharmacokinetic Modeling of Sustained-Release Tacrolimus

The pharmacokinetics of tacrolimus can vary substantially depending on the population studied.[36] Assumptions used in this model for tacrolimus in humans were taken from Moller et al in healthy human subjects[37]: Volume of distribution: 1.2 L/kg; Elimination half-life: 44 hours; Delivery half-life: 10 days (K_{abs} 0.069).[38] As may be seen, dosing at 3 weekly intervals could maintain levels between 3 and 7ng/ml with a dose of 0.5 mg/kg.[39]

Safety

There was one unscheduled death of a tacrolimus injected rat in this study. All animals appeared normal: they showed no failure to eat or drink, failure to groom, or weight loss. There was no clinical evidence of inflammation at the injection sites.

Microscopic pathology

Microscopic findings were recorded manually on individual animal necropsy records. Microscopic findings were graded on a scale of 1-4 (minimal < mild < moderate < marked), according to the intensity and extent the change. Formalin-fixed injection site skin from three male SD rats (Group 1, #1, #3 and #6) was submitted for histopathologic evaluation following a study. No abnormalities were identified in individual animal necropsy records. No abnormalities were identified in any of the skin specimens.

1.4. Clinical Studies

Tacrolimus is an FDA-approved immunosuppressive calcineurin inhibitor indicated for the prophylaxis of organ rejection in patients receiving allogeneic liver, kidney, or heart transplants. Tacrolimus as an ointment has been approved for atopic dermatitis. Clinical data and postmarketing safety experience have demonstrated that systemic and topical tacrolimus is generally safe and effective. Additional information on clinical studies that support the safety and efficacy of tacrolimus are provided in the package inserts (PROGRAF® [tacrolimus] capsules, USP, PROGRAF® [tacrolimus] injection [for intravenous use])[40] and PROTOPIC [tacrolimus] ointment[41]).

2. Study Hypotheses/Objectives

Hypotheses

This is a pilot study designed to gather information regarding the safety and the pharmacokinetic profile of a new SR formulation of tacrolimus. This data will inform the clinical development program for SR tacrolimus, with the ultimate goal of offering an improved treatment option for prophylaxis of organ (kidney, liver or heart) transplant rejection. Our proposed sustained release dose levels of 0.1mg/kg tacrolimus chosen for this 30-day first-in-human study are approximately 30x less than the lowest marketed dose levels. As such, these doses are expected to be low enough to not cause adverse effects, yet high enough to provide data that will aid in future dose level adjustments for safety and efficacy determinations. Thus, we hypothesize that our SR tacrolimus formulation will be safe.

2.1. Primary Objectives

The primary objectives of this study are:

1. To evaluate the safety of a single dose of 1-month SR subcutaneous injectable tacrolimus in healthy subjects.
2. To evaluate the pharmacokinetic profile after a single dose of SR injectable tacrolimus for up to 60 days post-dose.

2.2. Secondary Objective(s)

Not applicable.

3. Study Design

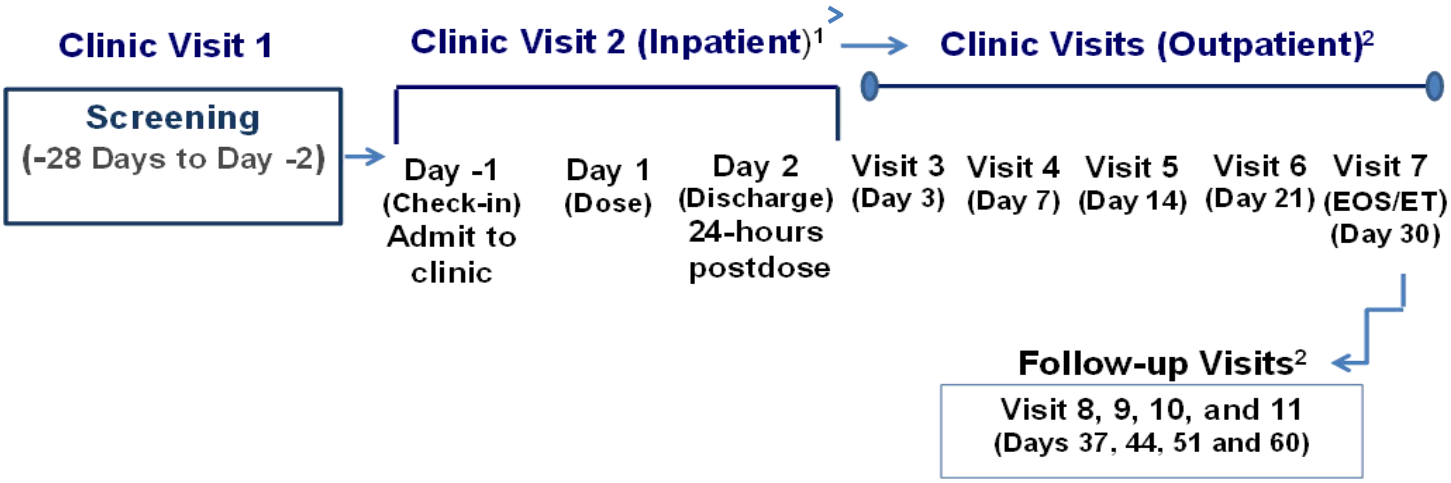
3.1. Description of Study Design

This is a single-center, nonrandomized, open-label, first-in-human study designed to assess the safety and pharmacokinetic (PK) profile of sustained-release (SR) tacrolimus, which will be administered as a single dose of 0.1 mg/kg by subcutaneous (SC) injection in 6 healthy subjects. Following a screening period of up to 30 days, eligible subjects will be admitted to the clinical research unit (CRU) on Day -1 (Check-in). On Day 1 (Dose), subjects will receive a single injection of SR tacrolimus. Subjects will remain at the CRU for at least 24 hours postdose for collection of serial blood samples for PK analysis and safety monitoring until discharge on Day 2. Subjects will return to the clinic for PK and safety evaluations (Outpatient Visits 3 through 7) for up to 30 days postdose, with an additional 30 day Follow-up period.

If a participant is unable or not willing to continue the study for 30 days, a new participant will be recruited to achieve a sample size of 6 pharmacokinetically evaluable subjects.

Neither a control group nor randomization is required for this type of study. The study schematic is shown in Figure 2. The study flow diagram is shown in Figure 3.

Figure 2: Overview of Study Design

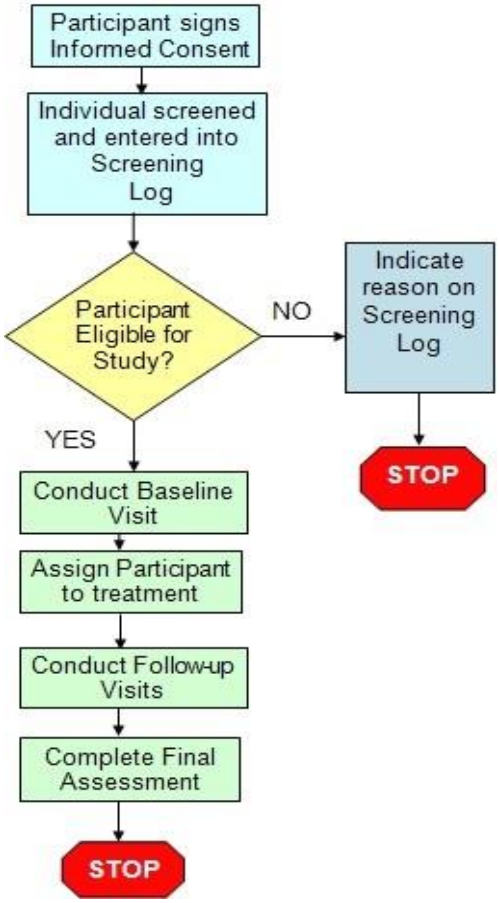


Abbreviations: EOS = End of Study; ET = Early Termination

1. **Blood samples for determination of plasma concentrations of SR tacrolimus will be collected predose, and 1, 3, 6, 12 hours (Day 1) and 24-hours postdose (Day 2).**
2. **A single blood sample for pharmacokinetic analysis will be collected at each Outpatient clinic visit (Visits 3 through 7) and each Follow-up clinic visit up to Day 60 (Visit 11), when it is expected that plasma concentrations of SR tacrolimus will not be detectable.**
3. **Safety will be monitored throughout the study.**

Number of subjects = 6
Dose: 0.1 mg/kg

Figure 3: Study Flow Diagram



3.2. Primary Endpoints/Outcomes

1) Safety endpoints for this study include the incidence, severity, and relatedness of adverse events (inclusive of injection site reactions), and changes from baseline (prior to dosing) in the following:

- Vital signs (blood pressure, pulse rate, respiratory rate and oral body temperature);
- Clinical laboratory evaluations (chemistry panel, hematology panel, liver function);
- Physical examinations (general appearance, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological skin, thyroid/neck, lymph nodes and neurological/psychiatric);
- Electrocardiograms (RR, PR, QT using Fridericia's formula, QT interval corrected using Fridericia's formula, QRS duration and ventricular heart rate).

2) Pharmacokinetic (PK) endpoints include calculation of the following PK parameters:

Parametersa	Description
AUC_{0-t}	Area under the concentration time curve from time zero to the time of last quantifiable concentration (C_{last}), calculated using the log-linear trapezoid rule.
$AUC_{0-\infty}$	Area under the concentration-time curve from the time of dosing (0 h), extrapolated to infinity. $AUC_{0-\infty}$ will be calculated as $AUC_{0-t} + C_{last}/\lambda_z$, where C_{last} is the last quantifiable whole blood concentration, and will be calculated using the log-linear trapezoidal method.
% AUC Extrap	Percent of $AUC_{0-\infty}$ due to extrapolation from t_{last} to infinity.
C_{max}	Maximum observed concentration.
t_{max}	Time to maximum observed concentration
$t_{1/2}$	Apparent terminal elimination half-life, calculated as ln(2) / λ_z, where λ_z = terminal rate constant, computed by log-linear regression of the terminal log-linear segment of the concentration versus time curve.
C_L/F	Apparent clearance, calculated either as Dose / $AUC_{0-\infty}$.
Vz/F	$C_L/F)/\lambda_z$

[a] PK parameters of tacrolimus (and potential metabolites) will be calculated following single dose and rich concentration-time profiles collected at the following timepoints Hour 0 (prior to dosing) on Day 1, and at 1, 3, 6, 12 and 24 hours postdose; Days 3, 7, 10, 14, 21, 30, 37, 44, 51 and 60.

3.3. Secondary Endpoint(s)/Outcome(s)
Not applicable

3.4. Exploratory Endpoint(s)/Outcome(s)
Not applicable

3.5. Stratification, Randomization, and Blinding/Masking

3.5.1. Procedure for Unblinding/Unmasking
This is an open-label study.

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

This is a first-in-human study. Only healthy subjects will be allowed to participate. Attempts will be made to enroll equal numbers of males and females. Since there is no data regarding any potential effect of SR injectable tacrolimus on pregnancy, women of childbearing potential may participate upon agreement to use appropriate contraception (see Section 4.2).

4.2. Inclusion Criteria

Subjects who meet the following criteria may be included in the study:

1. Subjects must be able to understand and provide informed consent. A signed informed consent form must be provided before any study assessments are done;
2. Males or females, between 18 and 45 years of age, inclusive;
3. Body mass index must be within the range 18.5 to 32.0 kg/m2, inclusive;
4. Must be in good health, as determined by no clinically significant findings from medical history, vital signs, and 12-lead electrocardiogram (ECG), inclusive of documented absence of QT prolongation;
5. Clinical laboratory evaluations (including clinical chemistry panel [fasted at least 10 hours], complete blood count [CBC], and urinalysis [UA]) must be within the reference range for the test laboratory, unless deemed not clinically significant by the Investigator;
6. Must be negative for autoimmune disorders in the past 3 months and at Screening - participant's medical history will be used for this evaluation;
7. Must not use any immunosuppressant calcineurin inhibitor product other than SR Injectable tacrolimus throughout the dosing period and until after the final visit;
8. Must agree to blood draws throughout the course of the study and venous access sufficient to allow for blood sampling as per the protocol;
9. Must be negative for selected drugs of abuse at Screening and at Check-in (Day -1);
10. Must have a negative hepatitis panel (including hepatitis B surface antigen [HBsAg] and hepatitis C virus antibody [HCV]) and negative human immunodeficiency virus [HIV] antibody screens;
11. Females will be nonpregnant, nonlactating, and either postmenopausal, defined as amenorrhea for at least 1 year and follicle-stimulating hormone levels of 40 mIU/mL or higher; surgically sterile (eg, tubal ligation, hysterectomy, oophorectomy) for at least 90 days prior to Screening; or agree to use, from the time of signing the informed consent or 10 days prior to Check-in on Day -1 of the Inpatient Period until 30 days after Study Discharge, one of the following forms of contraception: nonhormonal intrauterine device (IUD) with spermicide; female condom with spermicide; contraceptive sponge with spermicide; diaphragm with spermicide; cervical cap with spermicide; male sexual partner who agrees to use a male condom with spermicide; or sterile sexual partner; alternatively, women must agree to maintain abstinence (must agree to use a double barrier method if they become sexually active during the study. For all females of childbearing potential, the pregnancy test result must be negative at Screening and Check-in on Day -1 of the Inpatient Period (see Appendix 1). Women must also agree not to douche throughout the dosing period and until after the final visit;
12. Males will either be sterile or agree to use, from Check-in on Day -1 of the Inpatient Period until 90 days following Study Discharge, one of the following approved methods of contraception: male condom with spermicide; sterile sexual partner; or use by female sexual partner of an IUD with spermicide; a female condom with spermicide; a contraceptive sponge with spermicide; an intravaginal system (eg, NuvaRing®); a diaphragm with spermicide; a cervical cap with spermicide; or oral, implantable, transdermal, or injectable contraceptives. Subjects will refrain from sperm donation from Check-in on Day -1 of the Inpatient Period until 90 days following Study Discharge.

4.3. Exclusion Criteria

Subjects will be excluded from the study if they meet the following criteria:

1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol;
2. Prisoners or patients who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (eg, infectious disease) illness must not be enrolled into this trial;

3. Females of childbearing potential, or who are breast-feeding, or who have a positive urine or serum pregnancy test result prior to receiving study drug;
4. Presence of an uncontrolled, unstable clinically significant medical condition that in the opinion of the Investigator may increase the risk to the subject or may interfere with the interpretation of safety and PK evaluations, or could impair the subject's ability to complete the trial, or could impair the decisional capacity of the subject;
5. Presence of clinically significant vital signs or a physical examination finding that, in the opinion of the Investigator, could increase the risk to the subject or may potentially interfere with the ability to evaluate safety and tolerability in the trial;
6. History of tacrolimus use, or hypersensitivity and/or adverse reaction to calcineurin inhibitor drugs;
7. History of toxic shock syndrome;
8. Currently receiving chemotherapy or immunosuppressive agents;
9. Use of investigative drugs within 30 days or 5 half-lives of study participation;
10. Currently using sirolimus;
11. Currently using live vaccines;
12. Currently on concomitant substrates and/or inhibitors of CYP3A4;
13. Requires the use of any concomitant medication, except for treatment of an adverse event (AE) during the study;
14. Any abnormality on clinical laboratory tests, or ECG finding that is considered to be clinically significant by the Investigator.
15. Known or suspected (nonfebrile) seizure disorder;
16. Use of any other depot medications within the last three months.
17. Unwilling to commit to avoid eating grapefruit or drinking grapefruit juice during the first 30 days of this exploratory study.
18. Grade ≥ 1 finding as described in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

4.4. Selection of Clinical Sites/Labs

This single site study will be conducted at Worldwide Clinical Trials Early Phase Services, LLC. The site is equipped with an onsite laboratory that will perform all clinical laboratory assessments.

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product or Intervention as cited in Investigator Brochure or Package Insert

This is a single dose study and the dose level selected is not expected to cause any adverse events (AEs). Effects such as inflammation at the site of delivery will be closely monitored.

Some side effects associated with tacrolimus are lymphomas and other malignancies (particularly of the skin), bacterial, viral, fungal and protozoal infections, including opportunistic infections, polyoma virus infections, CMV viremia and CMV disease, acute or chronic nephrotoxicity, neurotoxicity, hyperkalemia, hypertension, anaphylactic reactions, QT prolongation, myocardial hypertrophy, pure red cell aplasia, gastrointestinal perforation and anaphylactic reactions. Side effects reported with the intake of much higher doses of tacrolimus include baldness, anemia, loss of appetite, diarrhea, high concentrations of potassium in the blood, high blood pressure, nausea, vomiting, tingling sensation in the extremities, itching, tremor, fever, headache, rash, high blood sugar concentrations, and abdominal pain. Subjects will be asked to not use other drugs during the course of this study.

There is a theoretical concern of “drug dumping” i.e. complete delivery of the investigational product’s drug load (0.1 mg/kg) at the time of dose administration. Current dosage guidelines for marketed oral formulations of tacrolimus (Prograf®, Astagraf XL® and Envarsus®) are 0.075 mg/kg/day to 0.2 mg/kg/day, hence, the dosage resulting from possible dose dumping of the investigational drug is similar to the dosage delivered through commercial tacrolimus products. However, if the bioavailability of the investigational product is found to be higher than the currently marketed oral formulations because of its injectable nature, then the dose dumping will result in higher levels of whole blood tacrolimus concentrations. The bioavailability of the investigational product will be studied in this trial.

5.2. Risks of Investigational Product or Intervention cited in Medical Literature
Tacrolimus has been marketed since 1994. No new risks have been identified in the literature with the use of tacrolimus.

5.3. Risks of Other Protocol Specified Medications
No concomitant medications are allowed during the study.

5.4. Risks of Study Procedures
There are minimal risks associated with blood draws for clinical laboratory analysis and PK analysis. There are also risks associated with the subcutaneous administration of a therapeutic agent. These risks are as follows:

- **Venipuncture**: The risks of drawing blood include temporary discomfort from the needle stick, bruising, bleeding, and, rarely, fainting or infection.
- **Subcutaneous injections**: Injections to the skin may be less convenient than some other forms of treatment, such as oral medications. In addition, injections may cause momentary discomfort and other local symptoms, such as bleeding, bruising, and, rarely, infection.

At each post-dose clinic visit, subjects will be monitored for AEs, inclusive of injection site reactions.

5.5. Potential Benefits
There is a large database for conventional delivery of tacrolimus in animals. The safety of the injectable platform – Plexis® -has been studied in animals administered tacrolimus, as well as with other APIs. The risk-benefit ratio strongly favors the conduct of this study. While there are no direct benefits to the subject, clinical laboratory results will impart knowledge of kidney and liver function, ECGs will provide information regarding cardiac status, and physical examination may detect other diseases. The results of the research may contribute to scientific knowledge and may benefit patients in the future. There are theoretical risks associated with the investigational product, which have been described in Section 5.1.

6. Investigational Agent /Device/Intervention
6.1. Investigational Agents/Devices/Interventions
Tacrolimus is an FDA-approved immunosuppressive calcineurin inhibitor.

6.1.1. Investigational Agent
Sustained-release tacrolimus, single dose, 0.1 mg/kg, SC injectable

Drug Substance:	tacrolimus, an immunosuppressive calcineurin inhibitor
Pharmacological Class:	Immunosuppressive calcineurin inhibitor agent.
Drug Product:	Sustained release injectable tacrolimus: particles of drug substance coated with layers of polyvinyl alcohol.
Total Drug Dose Levels:	Sustained-release injectable tacrolimus, releasing at 0.1 mg/kg
Route of Administration:	Injectable sustained release.
Duration of Therapy	30 days

6.1.1.1. Formulation, Packaging, and Labeling
Each injectable vial will be manufactured by Auritec Pharmaceuticals, Inc. (Pasadena, CA). Each vial to be used during the study will be enclosed in a pouch (3.5 x 5.25 inches; Crosstex International, Santa Fe Springs, CA). Each individually packaged SR injectable tacrolimus monohydrate vial will be labeled with the following information:

Protocol Number: Lot Number: Drug: Sustained release (0.1 mg/kg) injectable tacrolimus IND Sponsor and Principal Investigator: Caution: New Drug- Limited by Federal Law to Investigational Use Only

6.1.1.2. Dosage, Preparation, and Administration

The dose of SR tacrolimus is 0.1 mg/kg. It will be administered as a single SC injection. This formulation has demonstrated pseudo-zero order release *in vitro* and *in vivo* (rats), suggesting that the formulation could be developed as weekly or monthly injectable, which could help improve treatment adherence and compliance in transplant patients, who are currently prescribed daily pills.

The tacrolimus injectables will be stored at room temperature in the clinical research center, that employsfull-time state-licensed pharmacists familiar with first-in-human clinical studies. Although some responsibilities may be delegated, all activities related to study conduct are the responsibility of the Principal Investigator. The Pharmacy employees will adhere to site SOPs, GCP, USP 797, DEA and FDA Guidance. Pharmacy staff will ensure that the study drug is properly stored, prepared, and dispensed according to federal, state, and local laws, calculate appropriate study drug dosage, maintain study drug inventory records, and assist in the preparation of intravenous, intramuscular, and subcutaneous injections, when required. The Research Pharmacist will maintain an accurate inventory and accountability record of study products received and subsequently dispensed. After the study is completed, all unused study product will be returned to Auritec Pharmaceuticals or their designated recipient.

The drug product will be subjected to gamma sterilization and then be put on an accelerated stability (40°C and 75% relative humidity) program and analyzed for appearance, purity, and dissolution at time 0, 1 and 6 months using HPLC and/or ^{1}H-NMR spectroscopy to verify the drug product is stable throughout the period of use.

6.2. Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the Investigator will maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed. The Investigator (or designee) will maintain an accurate record of the receipt of the test materials as shipped by the Auritec (IND Sponsor), including the date received. One copy of this receipt will be returned to Auritec when the contents of the test material shipment have been verified. In addition, an accurate drug disposition record will be kept, specifying the amount dispensed to each subject and the date of dispensation. This drug accountability record will be available for inspection at any time. At the completion of the study, the original drug accountability record will be available for review by Auritec upon request.

Records for receipt, storage, use, and disposition will be maintained by the study site. All records regarding the disposition of the investigational product will be available for inspection. At the completion of the study, all unused drug supplies will be returned to Auritec (or designee) or disposed of by the study site, per Auritec's (or designee's) written instructions.

6.3. Assessment of Participant Compliance with Investigational Agent
This is a single dose study. The dose will be administered when the subject is confined to the site (inpatient period). The subject's actual time of study drug administration will be recorded in the source documents and transcribed into the Case Report Form (CRF).

6.4. Toxicity Prevention and Management
This is a single dose study. The proposed dose for this exploratory study is 0.1mg/kg tacrolimus. In the event that there is an initial burst of tacrolimus (ie, 100% drug release on Day 1), subjects will be exposed to a maximum dose of 0.1 mg/kg/day, which is the prescribed dosage of the current marketed products, Prograf® and Astagraf XL®. As such, this dose is expected to be low enough to not cause adverse effects yet high enough to provide data that will aid in future dose level adjustments for safety and efficacy determinations.

6.5. Premature Discontinuation of Investigational Agent
The Investigator may remove a subject from the study if, in the Investigator's opinion, it is not in the best interest of the subject to continue the study. Subjects may be discontinued due to the following:

- Change in compliance with inclusion/exclusion criterion that is clinically relevant and affects subject safety;
- Occurrence of AEs;
- Intake of non-permitted concomitant medication that might affect subject safety or study assessments/objectives.
- Reasons of noncompliance such as failure to comply with study guidelines, refusal to use the injectable, or missing follow-up visits.

Notification of discontinuation will immediately be made to Auritec and the DAIT/NIAID Medical Monitor. In case of premature discontinuation of study participation, efforts will be made to perform all final study day assessments. The date the subject is withdrawn from the study and the reason for discontinuation will be recorded on the subject's CRF. All dropouts will be followed until resolution of all their AEs or until the unresolved AEs are judged by the Investigator to have stabilized.

7. Other Medications
7.1. Concomitant Medications
7.1.1. Protocol-mandated
Only a single dose of SR tacrolimus at a dose of 0.1 mg/kg will be administered during this study. There are no protocol-mandated concomitant medications.

7.1.2. Other permitted concomitant medications
The administration of any other concomitant medications during the study is prohibited without prior approval of the Investigator unless its use is deemed necessary in a medical emergency. Any medication taken by a subject during the course of the study and the reason for its use will be documented in the source documents and the CRF.

7.2. Prophylactic Medications
No prophylactic medications are allowed in this study.

7.3. Prohibited Medications
No prescription or over-the counter medications are allowed during this study. No depot medications are allowed within 3 months prior to study participation. No other investigational study drug is allowed during this study or within 5 half-lives or 30 days, whichever is longer, prior to Check-in to the inpatient unit (Day -1). The use of immunosuppressive drugs or chemotherapeutic agents will not be permitted during the course of the study. Subjects will be queried on the use of medications prior to enrollment and during the course of the study.
All concomitant prescription medications taken during study participation will be recorded on the subject's CRF. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications,

over-the-counter medications, and medications taken at the time of adverse events (all grades).

7.4. Rescue Medications

In the event of an injection site reaction, symptomatic eruptions can be treated with cold compresses, topical corticosteroids, oral antihistamines, or acetaminophen. If a subject experiences a hypersensitivity reaction after administration of SR tacrolimus, basic equipment and medication will be readily available in the clinical research unit.

8. Study Procedures

The schedule of study procedures is shown in Appendix 1.

8.1. Enrollment

The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures. Once the informed consent has been signed, screening procedures will be initiated to confirm eligibility.

8.2. Screening Visit

The purpose of the screening period is to confirm eligibility to participate in the study. The screening period is up to 28 (Day -28) days prior to Check-in (Day -1) to the inpatient unit. The following procedures, assessments, and laboratory measures will be conducted to determine participant eligibility:

- Signed informed consent.
- Medical history/demographics/height, weight, and body mass index.
- An abbreviated physical examination, which includes assessment of general appearance, skin, thorax/lungs, cardiovascular status, and abdomen.
- A 12-lead electrocardiogram (ECG). The ECG is to be recorded after the subject has been supine for at least 5 minutes. The ECG data will be obtained directly from the 12-lead ECG traces, including RR, PR, QT, and QT interval corrected using Fridericia's formula, QRS duration, and ventricular heart rate.
- Vital signs (blood pressure, pulse rate, respiratory rate and oral body temperature). Vital signs will be measured in the supine position after the subject has rested comfortably for at least 5 minutes.
- Clinical laboratory (chemistry, hematology, urinalysis)
- Alcohol and drug screen, Serology (HIV, HBsAg, HCV), TB skin test
- For females of childbearing potential, a serum qualitative pregnancy test (HCG). For postmenopausal women only, FSH.
- Adverse events and concomitant medications will be recorded.

8.3. Study Visits or Study Assessments

Enrollment/Baseline

Study participants must have signed study participation consent form to be considered enrolled into the trial and have all study screening complete to confirm eligibility requirements have been met before the study drug is administered. Evaluations prior to the first administration are the baseline for subsequent safety evaluations. Females of nonchildbearing potential must have negative serum pregnancy test results, if documentation of sterility has not been provided, and an FSH level greater than 40 IU/L verified prior to enrollment and dosing.

At Check in (Day -1) to the inpatient unit, the following procedures, assessments, and laboratory measures will be conducted:

- Confirmation of eligibility
- Interim medical history will be recorded
- Complete physical examination. A complete medical examination includes general appearance, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric assessments.
- Vital signs (blood pressure, pulse rate, respiratory rate and oral body temperature)
- Body weight
- Clinical laboratory (chemistry, hematology, LFTs, urinalysis) after 10-hour fast
- Serum qualitative pregnancy test (HCG)
- Alcohol and Drug Screen
- How Do You Feel Inquiry
- Adverse events and concomitant medications will be recorded.
- Blood collection for baseline measurement of drug levels.
- Blood collection for possible post-study genetic evaluation of potential cytochrome P450 genetic variations

Inpatient Period

Day 1: Day 1 is defined as the day of enrollment and administration of the study drug. Following a, complete physical examination, recording of predose vital signs, and collection of a predose blood sample for PK analysis, subjects will receive a single SC injection of SR tacrolimus. Following injection, subjects will be monitored for anaphylactic reactions for the first 30 minutes. Vital signs, , clinical laboratory tests (chemistry, hematology, LFTs, urinalysis and blood samples for PK analysis will be obtained at 1, 3, 6, and 12 hours postdose. Adverse events and concomitant medications will be recorded.

Day 2: Subjects will be discharged from the inpatient unit with instructions to return to the clinic the next day following the procedures, assessments, and laboratory measures listed below.

- Complete physical examination
- Vital signs (blood pressure, pulse rate, respiratory rate and oral body temperature).
- Clinical laboratory (chemistry, hematology, LFTs, urinalysis) after a 10-hour fast
- How Do You Feel Inquiry
- Blood samples for PK analysis
- 12-lead electrocardiogram (ECG). The ECG is to be recorded after the subject has been supine for at least 5 minutes
- Adverse events and concomitant medications will be recorded.

Outpatient Visits (Visit 3, 4, 5, 6, and 7 [End of Study (EOS)/Early Termination (ET)])

After discharge from the inpatient unit, subjects will return the next day (Visit 3, Day 3), again at 7 days postdose (Visit 4, Day 7), and weekly, thereafter (Visits 5, 6, and 7 [EOS/ET], Days 14, 21, and 30 [EOS/ET]). The following procedures, assessments, and laboratory measures will be conducted:

- Complete physical examination. (Visits 3, 4, 5, and 6).
- Abbreviated physical examination (Visit 7 [EOS/ET])
- Vital signs (blood pressure, pulse rate, respiratory rate and oral body temperature). Vital signs should be assessed at approximately the same time of day at postdose visits on days 3, 7, 14, 21, 30 at end of the study ie, Day 60). The date and time of each vital sign assessment will be recorded in the CRF.
- Clinical laboratory (chemistry, hematology, LFTs, urinalysis) at each visit after a 10-hour fast
- How Do You Feel Inquiry

- Blood samples for PK analysis should be collected at approximately the same time of day at each postdose visit. The date and time of each PK blood sample will be recorded in the CRF).
- Urine pregnancy test (visits 4, 5 and 6)
- Serum qualitative pregnancy test (HCG) (visit 7)
- 12-lead electrocardiogram (ECG). The ECG is to be recorded after the subject has been supine for at least 5 minutes (visit 7)
- Adverse events and concomitant medications will be recorded.

Follow-up Visits (8, 9, 10, and 11)

Subjects will return within one week after the 30-day study period, and weekly thereafter (Visits 8, 9, 10, and 11 [Final Visit], Days 37, 44, 51 and 60, respectively]). The following procedures, assessments, and laboratory measures will be conducted:

- Vital signs (blood pressure, pulse rate, respiratory rate and oral body temperature). Vital signs should be assessed at approximately the same time of day (ie, Day 60). The date and time of each vital sign assessment will be recorded in the CRF.
- Clinical laboratory (chemistry, hematology, LFTs, urinalysis) at each visit after a 10-hour fast
- How Do You Feel Inquiry
- Blood samples for PK analysis should be collected at approximately the same time of day at each postdose visit. The date and time of each PK blood sample will be recorded in the CRF).
- Urine Pregnancy Test (visits 8, 9, 10)
- Serum qualitative pregnancy test (HCG) (visit 11)
- Adverse events and concomitant medications will be recorded.

The last follow-up visit will be held on Visit 11 (Day 60) after enrollment. At this time, if a subject has continued symptoms, they may be given a phone call to discuss resolution of symptoms and asked to return again in one week for an exam for resolution of findings. Subjects having an allergic reaction or a serious adverse event deemed related to a study drug will be asked to return for a clinical examination and follow-up until the event has resolved.

Early Termination Visit

Subjects who withdraw or discontinue the study will be asked to return for a final clinic visit. The procedures and assessments will be the same as those included in Visit 7 (Day 30).

8.4. Unscheduled Visits

If disease activity increases or other concerns arise between regularly scheduled visits, participants should be instructed to contact study personnel and may be asked to return to the study site for an “unscheduled” visit. The reason for the visit will determine the procedures and assessments included.

8.5. Visit Windows

Study visits should take place within ± 2 days for each scheduled visit. Visit and procedure windows are also indicated on the Schedule of Events (Appendix 1).

8.6. Additional Visits

If the subjects experience certain side effects or have a serious finding during an exam at a follow-up visit, they will be asked to return to the clinic. Subjects will be asked to come to the clinic until the symptoms go away. During these visits, subjects will be asked questions about their symptoms. Research blood will be collected to measure tacrolimus levels. Blood samples may be collected for safety analysis.

9. Mechanistic Assays

Research blood will be collected for PK analysis and post-study CYP450 genetic evaluation as specified in the schedule of events. All samples will be stored at Worldwide Clinical Trials in San Antonio, TX for the duration of the study.

10. Biospecimen Storage

Blood samples collected during the study for clinical laboratory will be destroyed or discarded in compliance with state or local biological waste disposal requirements. If subject blood samples will be used for future research, this protocol (and other appropriate study documents, e.g., the informed consent and the statistical analysis plan) will be amended and submitted to the appropriate regulatory authorities, ethics committees, and IRBs for approval. Permission from the subject will be secured before additional evaluations are performed.

11. Criteria for Participant and Study Completion and Premature Study Termination

11.1. Participant Completion

A subject who attends all visits and follows all procedures in accordance with the protocol, from Screening through the last study Follow-up visit (Visit 11), will have completed the study.

11.2. Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is “lost to follow-up” (ie, no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.
5. Individual safety stopping rules

11.3. Participant Replacement

Data will be obtained from all enrolled subjects, including those that discontinue the study. Subjects will not be replaced. If needed, we will recruit up to 8 subjects to obtain six completers, while also reporting all data on all subjects who enter the study. The goal will be to have 6 fully evaluable subjects, who have completed all safety and PK assessments per protocol.

11.4. Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant will be asked to complete a final visit and/or final assessments. At a minimum, the following information must be collected when a subject discontinues:

- The reason the subject discontinued.
- The date of the last dose of study drug
- The date of the last assessment and/or contact. A follow-up contact (telephone or visit) will be arranged as appropriate.
- Adverse events, including SAEs
- Compliance with the study drug administration as specified in this protocol

Final assessments: unless there is a withdrawal of consent or death, every effort should be made to ensure that all procedures and evaluations scheduled for the final visit are performed.

11.5. Study Stopping Rules

The study may be prematurely terminated for the following reasons:

The entire study may be discontinued at the discretion of the Investigator, Auritec, or DAIT/NIAID based on the occurrence of the following:

- Adverse events unknown to date with respect to their nature, severity, and duration;
- Increased frequency and/or severity and/or duration of known AEs;
- Medical or ethical reasons affecting the continued performance of the study;
- Difficulties in the recruitment of subjects;
- Cancellation of drug development.

In order to ensure six completers, up to 8 subjects may be enrolled.

12. Safety Monitoring and Reporting

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, *Reporting of Serious Adverse Events and Adverse Events*) to Auritec and DAIT/NIAID. Appropriate notifications will also be made to the site principal investigator, Institutional Review Board (IRB), and health authorities.

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice*, 21CFR Parts 312 and 320, and applies the standards set forth in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials:

https://www.fda.gov/downloads/BiologicsBloodVaccines/ucm091977 .

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with, but not limited to:

- Study therapy regimen: Sustained release (0.1 mg/kg) injectable tacrolimus
- Study mandated procedures: Research Blood Draw

12.2.1.1 Suspected Adverse Reaction (SAR)

Any AE for which there is a reasonable possibility that the investigational drug or study procedure caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug (21 CFR 312.32(a)).

12.2.2 Unexpected Adverse Event

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator Brochure or package insert or is not listed at the specificity, severity or rate of occurrence that has been observed; or, is not consistent with the risk information described in the general investigational plan or elsewhere in the IND.

"Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the Investigator Brochure or package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR 312.32[a]).

12.2.3 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, Auritec *or DAIT/NIAID*, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator, Auritec or DAIT/NIAID*,* its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

Pregnancy will not be reported as an AE or SAE. However, if at any time the pregnancy falls under the scope and definition of an SAE it will then be reported as such.

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. This Grading Scale has been reviewed by the Site Principal Investigator and has been deemed appropriate for the subject population to be studied in this protocol.

Adverse events will be graded on a scale from 1 to 4 according to the following standards in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials manual:

Grade 1 = mild adverse event.
Grade 2 = moderate adverse event.
Grade 3 = severe adverse event.
Grade 4 = potentially life-threatening adverse event.

Events of all grades will be recorded on the appropriate AE/SAE *CRF* for this study.

12.3.2 Attribution Definitions

The relationship, or attribution, of an AE to the study drug, regimen, or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE/SAE CRF. Final determination of attribution for safety reporting will be determined by Auritec and DAIT/NIAID. The relationship of an AE to study drug or procedure will be determined using the descriptors and definitions provided in Table 12.3.2.

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other study procedure)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

Adverse events will be collected from the time of enrollment until a subject completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the subject.
- Interviewing the subject [e.g., using a checklist, structured questioning, etc.].
- Receiving an unsolicited complaint from the subject.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 12.3, *Grading and Attribution of Adverse Events.*

12.4.3 Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously (Section 12.2, *Definitions)* on the appropriate AE/SAE CRF regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to Auritec

Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.
The site investigator will report all serious adverse events (see Section 12.2.3, *Serious Adverse Event)*, regardless of relationship or expectedness within 24 hours of discovering the event.

For serious adverse events, all requested information on the AE/SAE CRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE CRF will be updated and submitted. The site will send a written confirmation or summary of the AE to Auritec within 3 working days of the original notification.

12.5.2 Reporting Serious Adverse Events to DAIT/NIAID

The DAIT/NIAID Medical Monitor will be notified within 24 hours of when a FDA-reportable SAE is first recognized or reported.

1) All SAEs will be reported within 24 hours of awareness using the AE/SAE CRF. Notification of these events will be sent to Auritec and DAIT upon completion of the CRF.
2) Any unanticipated study problem that does not fit the definition of an adverse event, but which may, in the opinion of the affiliate Site Principal Investigator, involve risk to the participant, affect others in the research study, or significantly impact the integrity of research data will be reported to Auritec and DAIT within 24 hours of awareness.
3) SAEs will be entered into the NIAID/DAIT CRIS system.

12.5.3 Reporting to Health Authority

After an AE requiring 24-hour reporting (per Section 12.5.1, *Reporting of Serious Adverse Events to Sponsor*) is submitted by the site investigator and assessed by Auritec and DAIT/NIAID, there are two options for the Auritec to report the AE to the appropriate health authorities:

12.5.3.1 Annual Reporting

Auritec will include in the annual study report to health authorities all AEs classified as:

- Serious, expected, suspected adverse reactions (see Section 12.2.1.1, *Suspected Adverse Reaction,* and Section 12.2.2, *Unexpected Adverse Event*).
- Serious and not a suspected adverse reaction (see Section 12.2.2, *Suspected Adverse Reaction*).
- Pregnancies.

Note that all adverse events (not just those requiring 24-hour reporting) will be reported in the Annual Report.

12.5.3.2 Expedited Safety Reporting

This option, with 2 possible categories, applies if the AE is classified as one of the following:
Category 1: **Serious and unexpected suspected adverse reaction** [**SUSAR]** (see Section 12.2.1.1, *Suspected Adverse Reaction* and Section 12.2, *Unexpected Adverse Event* and 21 CFR 312.32(c)(1)i).

Auritec shall report any suspected adverse reaction that is both serious and unexpected. Auritec shall report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);
2. One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
3. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Category 2: **Any findings from studies that suggests a significant human risk**

Auritec shall report any findings from other epidemiological studies, analyses of adverse events within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (eg, mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure or package insert or other aspects of the overall conduct of the study.

Auritec shall notify the FDA, DAIT/NIAID and all participating investigators of expedited Safety Reports within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

12.5.4 Reporting of Adverse Events to IRBs/IECs

The investigator shall report AEs, including expedited reports, in a timely fashion to the IRB in accordance with applicable regulations and guidelines. The IRB will be notified in writing (eg, facsimile) within 24 hours (1 working day) of when an FDA-reportable SAE is first recognized or reported. In addition, a copy of the written confirmation or summary of the SAE, as submitted to Auritec, will also be submitted to the IRB within 3 working days of when the FDA-reportable SAE is first recognized or reported. The IRB Serious and Unexpected Adverse Experience Submission Form will be completed and submitted with the copy of the written confirmation or summary of the SAE.

12.6 Pregnancy Reporting

The investigator/Auritec shall be informed immediately of any pregnancy in a study subject or a partner of a study subject. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The investigator shall report all pregnancies to Auritec using the Pregnancy *CRF*. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy *CRF* shall be updated when details about the outcome are available. When possible, similar information shall be obtained for a pregnancy occurring in a partner of a study subject.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and

medically indicated abortion will be considered SAE. Such pregnancy events shall be reported using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as Auritec when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an AE.

12.8 Review of Safety Information

12.8.1 DAIT/NIAID Medical Monitor Review

- The DAIT/NIAID Medical Monitor shall receive a copy of the annual study report to health authorities from Auritec (See Sections 12.5.3.1, *Annual Reporting*).

- The DAIT/NIAID Medical Monitor shall review and make decisions on the disposition of SAEs (See Sections 12.5.1, *Reporting of Serious Adverse Events to Sponsor*).

- The DAIT/NIAID Medical Monitor shall be informed of any participant who experiences a large burst of tacrolimus release detected at any point during the study.

- The DAIT/NIAID Medical Monitor shall be informed of any premature study drug discontinuation.

12.9 DSMB Review

The DAIT/NIAID Data and Safety Monitoring Board (DSMB) shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include a listing of all reported SAEs.

The DAIT/NIAID DSMB will be informed of any unanticipated problems involving risks to subjects or others in a timely manner

13. Statistical Considerations and Analytical Plan

The Statistical Analysis Plan (Appendix 2) provides details on Data Source, Analysis Objectives, Analysis sets/populations/subgroups, endpoints, covariates, handling of missing values, statistical procedures, measures to adjust for multiplicity/confounders/heterogeneity, sensitivity analysis, rationale for any deviation from pre-specified analysis plan and programming plans.

14. Identification and Access to Source Data

14.1. Source Data

Source documents and source data are considered to be the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial.

14.2. Access to Source Data

The site investigator and site staff will make all source data available to the DAIT/NIAID and Auritec as well as to the FDA. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15. Protocol Deviations

15.1. Protocol Deviation Definitions

Protocol Deviation – The investigator and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. If necessary, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a deviation from the IRB-approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

15.2. Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by Auritec. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

16. Ethical Considerations and Compliance with Good Clinical Practice

16.1. Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the IRB. Any amendments to the protocol or to the consent materials will also be approved by the IRB before they are implemented.

16.2. Informed Consent Process

Written informed consent for the study will be obtained from all subjects before protocol specific procedures are carried out. The ICF will be approved (along with the protocol) by the IRB and will be acceptable to Auritec and DAIT/NIAID.

The Investigator (or designee) will explain the nature of the study and the action of the test product. The subjects will be informed that participation is voluntary and that they can withdraw from the study at any time. In accordance with 21 CFR 50, the informed consent process shall be documented by the use of a written ICF approved by the IRB and signed by the subject prior to protocol-specific procedures being performed.

A copy of the signed Informed Consent Document will be given to the subject; the original signed document will be maintained with the subject's records.

16.3. Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to Auritec or their representatives.

17. Publication Policy

Any publication of the results, either in part or in total (eg, articles in journals or newspapers, oral presentations, abstracts) by the Investigator or his/her representative(s), shall require prior notification and review, within a reasonable time frame, by Auritec, and cannot be made in violation of the Auritec's confidentiality restrictions or to the detriment of the Auritec's intellectual property rights.

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Appendix 1: Schedule of Procedures and Evaluations

	Screening	Inpatient			Outpatient					Follow Up			
Study Visit Window					+/- 2 days					+/- 2 days			
Study Day	-28 to -2	-1	1	2	3	7	14	21	30	37	44	51	60
Visit Number	1	2			3	4	5	6	7	8	9	10	11
Informed Consent	X												
Eligibility	X	X											
Confine to the Clinical Site		X	X										
Discharge from Clinical Site				X									
Demographics	X												
Medical History	X	X[a]											
Height, Weight[b], and BMI	X	X											
Physical Examination[c]	X	X	X	X	X	X	X	X	X				
12-Lead ECG[d]	X			X					X				
Vital Signs[e]	X	X	X	X	X	X	X	X	X	X	X	X	X
Injection Site Reactions			X	X	X	X	X	X	X	X	X	X	X
HDYF? Inquiry[f]		X	X	X	X	X	X	X	X	X	X	X	X
Dose (SR tacrolimus SC injection)			X										
Blood sample for CYP450 genetic variability evaluation		X											
PK Blood Samples[g]		X	X	X	X	X	X	X	X	X	X	X	X
Clinical Laboratory Evaluations (chemistry, hematology, liver and kidney function, urinalysis)	X	X	X	X	X	X	X	X	X	X	X	X	X
TB Skin Test	X												
Serology (HIV, HBsAg, HCV)	X												
Urine Drug Screen	X	X											
Pregnancy Test[l,j]	X[i]	X[i]				X	X	X	X[i]	X	X	X	X[gi]
Adverse Events	← Monitor and Record Throughout Study →												
Concomitant Medication	← Monitor and Record Throughout Study →												

Abbreviations: BMI = body mass index; ECG = electrocardiogram; EOS = End of Study; ET = Early Termination; FSH = follicle-stimulating hormone; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HDYF? = How do you feel; HIV = human immunodeficiency virus; PK = pharmacokinetic; SC = subcutaneous; SR = sustained release; TB = tuberculosis.

a Interim medical history only.

b Weight only will be measured on Day -1

c An abbreviated physical examination will be performed at Screening and at the End of Study/Early Termination Visit: a full physical examination will be performed at all other visits. A full medical examination includes general appearance, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatricassessments. An abbreviated physical examination includes assessment of general appearance, skin, thorax/lungs, cardiovascular status, and abdomen.

d A 12-lead ECG will be obtained at Screening, Discharge (Day 2), and at the End of Study/Early Termination Visit. The ECG is to be recorded after the subject has been supine for at least 5 minutes. The ECG data will be obtained directly from the 12-lead ECG traces, including RR, PR, QT, and QT interval corrected using Fridericia's formula, QRS duration, and ventricular heart rate.

e Vital signs include blood pressure, pulse rate, respiratory rate and oral body temperature. Vital signs will be measured in the supine position after the subject has rested comfortably for at least 5 minutes. Vital signs will be obtained at Screening, predose at Day -1; and post-dose at 1, 3, 6, 12 hours (Day 1), and 24 hours (Day 2) postdose during the inpatient period, and at each outpatient and at end of the study (ie, Day 60).

f Subjects will be asked a nonleading HDYF question, such as "Have there been any changes in your health status since Screening/since you were last asked?" Subjects will also be encouraged to voluntarily report AEs occurring at any other time during the study. The "How Do You Feel" inquiry is to be asked during each day of the inpatient period and at all outpatient clinic visits.

g Pharmacokinetic (PK) blood samples for the determination of SR tacrolimus concentrations are to be collected predose and at approximately 1, 3, 6, 12 hours (Day 1), and 24 hours (Day 2) following dose administration. A single PK sample will be obtained at each postdose clinic visit.

h Clinical laboratory assessments will be performed in the fasted state (10 hours). Basic safety labs (ie, standard chemistry panel [CMP], UA and hematology panel) will be done at baseline (Day -1) and at least 1 time on the day of dosing, at 24-hours postdose, and at each outpatient visit to the clinic.

ii Women of childbearing potential will have a serum qualitative pregnancy test (human chorionic gonadotropin) on designated visits. A urine pregnancy test will be performed at other timepoints.

j Follicle stimulating hormone levels will be measured at screening only to determine eligibility (postmenopausal women only). In the absence of documentation of sterility, a serum qualitative pregnancy test will also be performed (human chorionic gonadotropin) at screen and Day -1.

Additional Visits - If the subjects experience certain side effects or have a serious finding during an exam at a follow-up visit, they will be asked to return to the clinic. Subjects will be asked to come to the clinic until the symptoms go away. During these visits, subjects will be asked questions about their symptoms. Research blood will be collected to measure tacrolimus levels. Blood samples may be collected for safety analysis.

Appendix 2: Statistical Analysis Plan

1. Introduction

The Lead Project Biostatistician, Dr. Martin Lee will serve as the primary contact for all issues related to the analysis of the data. Dr. Lee will perform any necessary SAS-related work to coordinate the programming, validation, and documentation of statistical programs for use in creating analysis datasets, tables, listing, and figures for the study.

The aim of this study is an exploratory assessment of the safety and pharmacokinetic behavior of a sustained release (SR) injectable formulation of tacrolimus. As such, the statistical approach is largely descriptive and preliminary in nature. The analysis will provide both a qualitative visual representation of the behavior of the whole blood concentration as well as providing a tentative predictive model to estimate both mean and variance over time for use in the development of further studies.

1.1. Background

This first-in-man (FIM) study will entail injecting a new Plexis® polymer-based sustained-release formulation of tacrolimus, which is not FDA approved. Volunteers in this study will receive a single injection releasing the drug, tacrolimus at a dose (0.1 mg per kg) designed to have no therapeutic effect and at which we expect no side effects. Tacrolimus is currently approved as oral formulations; however, the investigational injectable product is not FDA-approved.

At the completion of this study we will be in a much improved position to rationally select a sustained release injectable formulation for further development. The formulation chosen will then be manufactured under appropriate cGMP and a further IND will be applied for to carry out a first-in-human clinical trial following an orthodox product development program.

1.2. Study design

We will carry out this study at a fully cGCP certified Contract Research Organization. Our SR injectable tacrolimus formulation is designed to release the drug over at least 30 days. Safety examinations to include AE monitoring will be performed on Day 1 at 1, 3, 6 and 12 hours after injection. Adverse event (AE) monitoring will then be conducted on Days 3, 7, 14, 21, 30, 37, 44, 51 and 60.

1.3. Schedule of activities table

Monitoring Activity	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8, 9, 10 11**
	Day -28 to -2	**Day -1 to 2**	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 30**	**Days 37, 44, 51, 60**
	Screening	**Inject SR tacrolimus**						
Informed Consent	X							
Blood for Drug Concentration		X	X	X	X	X	X	X
Record AEs		X	X	X	X	X	X	X
Clinical Exams	X	X	X	X	X	X	X	X

*On Day 1, patients will be monitored and blood samples will be collected at 1, 3, 6, 12 and 24 hours post-injection

2. Data Source

2.1. Description of the data sets to be analyzed.

This is a single-center, nonrandomized, unblinded first-in-human study to assess the safety and drug concentration levels of sustained injectable delivery of tacrolimus in eight healthy volunteers for up to 30

days. Eight healthy subjects are to be enrolled in this study. Each subject will be injected with the injectable sustained release tacrolimus formulation on the first day of enrollment. The anticipated sustained dose to be administered subcutaneously from an injection is approximately 0.1 mg/kg of tacrolimus over a 30-day study period. Safety examinations to include AE monitoring will be performed on Day 1 at 1, 3, 6, 12 and 24 hours after injection.
Data will be obtained from the 8 subjects over a period of 30 days. Blood samples will be collected pre-dpse and at 1, 3, 6, 12 and 24 hours post-injection on Day 1 from each subject. After this, one blood sample will be collected from each subject on the following visits: Days 3, 7, 14, 21 and 30, 37, 44, 51 and 60. Cumulatively, 15 samples (including blood collection prior to injection) will be taken from each of the six subjects for a total of 90 samples.
Other covariates, such as age, gender, race/ethnicity, etc., will be collected as a matter of course, but are ancillary to the primary objectives, and will likely not be employed in the analysis or model unless a major effect is observed.

2.2. Primary Endpoints/Outcomes

1) Safety endpoints for this study include the incidence, severity, and relatedness of adverse events (inclusive of injection site reactions), and changes from baseline (prior to dosing) in vital signs, clinical laboratory evaluations, physical examinations, and electrocardiograms.
2) Pharmacokinetic (PK) endpoints include calculation of the following PK parameters: maximum observed tacrolimus whole blood concentration (Cmax), time to maximum observed tacrolimus whole blood concentration (tmax), area under the concentration-time curve (AUC) and elimination constant (kel).

3. Analysis Objectives

3.1. Overall scientific objectives of the analyses

1) The first primary objective of this first-in-human study is to evaluate the safety of 1-month SR injectable containing the approved drug - tacrolimus in healthy volunteers.
2) Another primary objective of this first-in-human study is to evaluate the pharmacokinetics of the drugs by measuring blood concentrations of the drug formulation in healthy volunteers.

3.2. Key unanswered questions that these analyses are designed to address

This research is designed to study a new formulation that is not approved by the FDA, to see how much tacrolimus is delivered to the body when released from a SR injectable formulation see if it is safe. The results of the research may contribute to scientific knowledge and may benefit patients in the future.
The short-term general investigational plan is to evaluate sustained release tacrolimus in healthy volunteers for up to 30 days in an open-label, first-in-human study to determine **safety** and **drug concentrations in blood**. Additional exploratory studies will be considered and planned based in part on the results obtained in this study. The long-term investigational plan is to evaluate the safety and efficacy of sustained release tacrolimus for the prophylaxis of organ rejection in patients receiving allogeneic liver, kidney, or heart transplants.

3.3. Additional detail to formulate the objectives in statistical terms.

In making an initial determination of the safety of this formulation, it is necessary to observe both the incidence and time of onset for any adverse events to allow for appropriate planning and design of additional studies.

4. Analysis Sets/ Populations/Subgroups

4.1. Brief definition of analysis sets/populations to be used

Eight healthy participants in the age group 18-45 years of age. All subjects who receive any amount of the investigational product will be included in the safety population. A pharmacokinetic population will be defined as all subjects in the safety population who provide sufficient tacrolimus levels to enable a PK model to be fitted (in the opinion of the pharmacokineticist).

4.2. Criteria for inclusion/exclusion for the population

Please see protocol sections 4.2 and 4.3.

4.3. Subgroups/subsets

Not applicable

5. Endpoints and Covariates

The primary outcome variables will be the various pharmacokinetic parameters described in more detail in section 7.2.
Safety as an outcome will be assessed through the recording of any adverse events, together with type of event and time of event as covariates.
Various other common covariates, such as age, gender, race/ethnicity, etc., will be examined but will likely not be feasible to include in any modeling due to the small sample size.

6. Handling of Missing Values and Other Data Conventions

No missing data will be imputed. Missing blood tacrolimus data will be ignored in the calculation of PK parameters. This is the standard approach in the analysis of pharmacokinetic data.

7. Statistical Methodology

7.1 Statistical Procedures

The sample size choice was based on the preliminary nature of the study and the goals of an initial analysis of pharmacokinetics of the sustained-release version of injectable tacrolimus as well as safety in a cohort study of healthy volunteers.
Qualitative examination of the behavior of the blood concentration will be performed via a profile plot, which allows for the visual inspection of the data for all subjects simultaneously. Specific pharmacokinetic analyses are described below.

7.2 Pharmacokinetic Analysis

PK samples for the PK period are planned for all subjects (irrespective of their CYP-enzyme genotype since the injectable formulation of tacrolimus will not be metabolized by this enzyme). Blood samples will be collected before the injection (baseline) and 1, 3, 6, 12 and 24 hours post-injection on Day 1 from each subject. After this, one blood sample will be collected from each subject on days 3, 7, 14, 21 and 30, 37, 44, 51 and 60. Actual sampling times post start of the injection will be recorded and used for calculation. The following PK parameters will be calculated by a non-compartmental injection model, using WinNonlin® (Version 5.2 or higher) based on the blood levels of tacrolimus:

The PK parameters will be calculated using the unadjusted and the baseline adjusted concentrations and actual sampling times post start of injection, for each subject and each PK period for which sufficient data are available to allow such calculations:

- Cmax (ng/mL): Observed maximum tacrolimus whole blood concentration;
- Cmin (ng/mL): Observed minimum tacrolimus whole blood concentration;
- tmax (h): Time to reach Cmax;
- λz: Elimination rate constant (kel);
- AUC0-t (h*ng/mL): Area under the tacrolimus whole blood concentration time curve from time point zero to the last quantifiable time point;
- AUC0-inf (h*ng/mL): Area under the tacrolimus whole blood concentration time curve from time point

zero to infinite time;
- t1/2 (h): Terminal phase half life
- MRT (h): Mean residence time;
- CL (L/h*kg): Total clearance from the body, calculated as dose/AUC0-inf; where AUC0-inf can be reliably calculated;
- Vss (L/kg): Volume of distribution at steady state, calculated as CL*MRT.

Half-life will be calculated as ln (2) / λz, where ln (2) is the natural logarithm of 2.
λz will be estimated by linear regression after log transformation of the concentrations:

- Only those data points that are judged to describe the terminal log linear decline will be used in the regression.
- λz will then be estimated using the highest adjusted R squared value, with R squared being the coefficient of determination from the regression (the PK scientist will review all profiles and PK parameters. The decision will be made based on his or her scientific judgment).
- A minimum number of three data points in the terminal phase will be used in calculating λz with the line of regression starting at any post-Cmax data point and including Clast, tlast.
- An appropriate number of decimal places will be used for λz to enable the reported value of t½ to be calculated.
- For subjects who do not have a λz estimation, t1/2, Vss, AUC0-inf and MRT will not be estimated.

The λz lower and λz upper times will be reported.
Area under the curve (AUC0-t) to the last measured blood level will be calculated from the observed data using the linear trapezoidal method with linear/log interpolation.
AUC0-inf will be calculated as AUC0-t + Ct/λz where Ct is the last quantifiable concentration in the profile;
Peak concentration (Cmax) will be the observed maximum value during the collection period. The time to peak concentration (tmax) will be the time at which Cmax was observed (or first observed, if the peak value occurs more than once).
Post injection concentrations will be baseline corrected as follows:
Adjusted post injection value = measured post injection value-pre injection value.
For calculation of clearance CL and incremental recovery, the dose of tacrolimus received as entered in the CRF will be used. These doses will be sumarized and listed. Unadjusted and baseline tacrolimus blood concentrations will be summarized by planned sampling time point by means of descriptive statistics, including the geometric mean and the coefficient of variation (CV%, calculated as 100 * SD / mean). For AUC0 t and Cmax, the 90% and 95% CI for the mean and the 90% and 95% CI for the geometric mean will also be computed. The CI for the geometric mean will be calculated assuming a log normal distribution. Four decimal places will be provided for all CIs.

7.2 Safety Analyses

7.2.1 Adverse events

All AEs experienced will be recorded during the study. Details to be collected include AE diagnosis, date and time of onset and resolution, whether the event is ongoing, whether the event is serious, frequency, severity, outcome status, action taken and relationship to study drug.
An overall summary table of AEs, including patients experiencing at least one AE, at least one SAE, related to study drug, discontinued due to AE, and death, will be presented.
AEs will be summarized by system organ class and preferred term, showing the number and percentage of patients experiencing a given event. In addition, AEs will be summarized by system organ class, preferred term and worst severity, and also by system organ class, preferred term and relationship to study drug. A patient will be counted only once for each preferred term when multiple AEs are coded to the same preferred term. If a patient experiences multiple AEs coded to the same preferred term, the maximum toxicity grade will be used for the summary.

7.2.2 Serious adverse events
Similar to AE reporting above, SAEs will be summarized by system organ class and preferred term, showing the number and percentage of patients experiencing a given event. In addition, SAEs will be summarized by system organ class, preferred term and relationship to study drug.

7.2.3 Deaths
Patients who die during study participation will be listed. Death will be determined from the AE CRF page (where outcome is death).

7.2.4 Discontinuation due to AE
Patients who discontinue the study because of an AE will be listed. Patient discontinuation will be determined from the evaluation (where reason for study termination is adverse event) and the specific adverse event will be determined from the AE CRF page (where action taken is discontinuation of study drug).

7.2.5 Interruption in study drug **injection**
Patients who have interruption in study drug injection will be listed. Listing will include start time of interruption, stop time of interruption and reason of interruption. Duration of interruption of study drug injection will be summarized.

7.2.6 Safety laboratory tests
Specimens for laboratory analysis at screen and predose baseline visit will be obtained to ascertain if the subject meets eligibility criteria and to provide a baseline to assess for possible AEs post drug administration. Complete blood count (CBC) panel, whole blood chemistry panel, urinalysis, and urine pregnancy test at baseline (as applicable) will be performed.
Laboratory specimens will be obtained for testing to assess safety, including CBC panel, whole blood chemistry panel, and urinalysis panel at all study visits.
Descriptive statistics will be provided for each test and for change-from-baseline values. Laboratory test results along with their normal ranges will be listed. Pregnancy test results will be listed.
Clinically significant changes from baseline in laboratory test findings and abnormalities will be reported as AEs.

7.2.7 Physical examination and vital signs
Vital signs will be collected to include body temperature, blood pressure, respiratory rate, and heart rate. Descriptive statistics will be provided for vital signs and physical examination. Predose vital signs (Visit 2 baseline predose) will be used as baseline to derive change from baseline of vital signs. Descriptive statistics will be provided for change from baseline vital signs at each available assessment time points. Shift tables on physical examination between normal and abnormal will be provided. Listings of vital signs and physical examination data will also be listed.

7.3 Measures to Adjust for Multiplicity, Confounders, Heterogeneity, Etc.
Not applicable for this study.

8. Sensitivity Analyses
Not applicable for this study.

9. Rationale for any Deviation from Pre-Specified Analysis Plan Performed by Auritec
Any change from the planned analysis as per final SAP will be documented in the study report along with the rationale.

10. Programming Plans
Data will be analyzed using WinNonlin® (Version 5.2 or higher) based on the blood levels of tacrolimus
A profile plot will be constructed using the PROC SGPLOT procedure in SAS 9.4. A smoothing algorithm such as penalized B-splines or LOESS will be chosen as appropriate, depending on the behavior of the data.

A summary table delineating common demographic characteristics such as age, gender, etc. will be produced.

11. Regulatory Compliance and Quality Assurance

11.1 Record keeping

The Investigator is responsible for maintaining all records pertaining to the clinical trial and for ensuring complete and accurate documentation.

The Investigator is responsible for maintaining a subject identification log. This confidential subject identification code provides the link between named subject source records in the subject file and anonymous CRF data provided to the sponsor.
The Sponsor requires that the Investigator retain records (all regulatory documents such as the protocol, study approval letters, all CRFs, drug dispensing and accountability logs, all original subject consent forms and all correspondence pertaining to the conduct of the study) for a period of no less than 5 years from the date of final regulatory approval or as per local regulations, whichever is longer. If the study is discontinued, or if no application/license is to be filed or if the application/license is not approved for such indication, records should be retained for 5 years after the investigation is discontinued or as per local regulations, whichever is longer.

11.2 Quality control and quality assurance

The Clinical Research Associates will monitor the data collected throughout the study thus providing Quality Control (QC) of the study. Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically at the contract research organization (Worldwide Clinical Trials) by qualified Quality Assurance (QA) auditors. Clinical monitoring will be performed by ASKE CRA services.
The investigator must make himself or herself available for CRAs during their visits and ensure that CRAs have direct access to all documents that they require, including direct access to the subjects' files. The investigator agrees to cooperate with the CRAs to make certain that any problems detected in the course of monitoring visits are resolved. The investigator will permit direct access to the source data and documents to the appropriate regulatory authorities to verify the accuracy of this data.
The present study will be conducted in accordance with ICH GCP. The clinical team will systematically control the essential documents generated during this trial. The trial will be monitored by the clinical team and will be subject to internal audits by Quality Assurance. All clinical study monitoring visits and audits by QA will be followed by internal reports and corrective actions, if needed. Follow-up letters will be forwarded to sites after all visits and any findings should be addressed by the investigator. The follow-up letters should be filed with the study correspondence and other essential documentation.

11.3 Changes in the conduct of the study

Any changes of the protocol (substantial amendments and non-substantial amendments) will be integrated into an updated study protocol, with a listing of all changes and reasoning for them. A protocol amendment must be submitted for DAIT/NIAD and IRB approval and consideration to the applicable regulatory agencies.
Minor procedural changes will be implemented by Study Notes to File, with supporting documentation at each site, if appropriate.
A non-substantial amendment of a study protocol includes minor correction or clarification that have no significant impact on the way the clinical study is to be conducted and no effect on subject safety (i.e., administrative changes like change of telephone number(s), logistical changes, etc.).

11.4 Confidentiality

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational product(s) used in this study.

The Investigator will ensure that the subjects' anonymity will be maintained. The privacy rules of the US HIPAA will be followed to obtain authorization for most uses and disclosures of Protected Health Information. On CRFs or other documents submitted to the Sponsor or its designee, subjects will not be identified by their names, but by an identification code, consisting of the combination of subject's initials and study number. Documents not for submission to Sponsor or its designee (e.g., the site confidential subject enrollment log and original subjects' consent forms) will be maintained by the Investigator in strict confidence. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of study subjects confidential.

The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator to allow direct access to his or her original medical records for study-related monitoring, audit, IRB, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The subject has the right to request through the investigator access to his/her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps should be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.