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STUDY PROTOCOL

# A Prospective, Pilot Trial to Compare the Efficacy of Tacrolimus Extended-Release (Envarsus XR) to Tacrolimus Immediate-Release on Suppression of Donor-Specific Antibodies in HLA Sensitized Kidney Transplant Recipients

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## 1. Background and Rationale

Extended-release tacrolimus (Envarsus XR) received FDA approval in July, 2015 for the prevention of allograft rejection in kidney transplantation on the basis of two separate phase 3 trials of de novo and stable kidney transplant recipients that demonstrated non-inferiority to immediate-release tacrolimus for the composite outcome of death, graft failure, biopsy-proven acute rejection, or loss to follow-up within 12 months (1,2).

Both phase 3 trials involved mostly low immunologic risk recipients with follow-up to one year. It has been previously shown that the incidence of de novo donor-specific antibodies (DSA) in the first year after kidney transplant in low-immunologic patients is low, developing in only 2%-11% of unsensitized de novo kidney transplant recipients (3-6). Donor-specific antibodies (DSA) are the primary mediator of antibody-mediated rejection and their development after transplant is a major risk factor for late allograft failure (7). It is now believed that antibody-mediated rejection is the most common cause of late allograft failure (8,9). However, neither of the two phase 3 trials were able to adequately assess the effect of Envarsus XR on the development of donor specific antibodies and therefore, the efficacy of Envarsus XR in higher immunologic risk recipients is not known. Therefore, a comparative study of extended- and immediate-release tacrolimus in highly-sensitized recipients is warranted.

### 1.1 Key differences between Envarsus XR and immediate-release tacrolimus

In contrast to the immediate-release formulation of tacrolimus, which is intended for twice-daily administration, Envarsus XR is an extended-release formulation designed for once-daily administration. Envarsus XR is manufactured using the MeltDose® delivery technology, which is based upon the principle that a smaller particle size yields a greater surface area and facilitates more rapid dissolution and greater absorption in the gastrointestinal tract. MeltDose® technology involves heating the active ingredient (tacrolimus) to create a "MELT" solution, which is then linked to a carrier before incorporation into a tablet. In doing so, Envarsus XR is more readily absorbed upon ingestion and is associated with more consistent tacrolimus concentrations.

### 1.2 Pharmacokinetics of Envarsus XR compared to immediate-release tacrolimus

A phase 2, open-label, multicenter study of sixty adult stable kidney transplant recipients who were converted from immediate-release tacrolimus (Prograf) to Envarsus XR was performed to compare the pharmacokinetics of Prograf compared to Envarsus XR (10). In this study, a 30%

lower total daily dose of Envarsus XR was associated with a similar $AUC_{24}$ as Prograf. Envarsus XR was associated with a lower peak ($C_{max}$), ranging from 71.6% to 73.9% of the $C_{max}$ associated with Prograf (figure 1). The percent fluctuation (peak-to-trough change in drug concentration around the average concentration) and percent swing (peak-to-trough change in drug concentration relative to the minimum concentration) were significantly lower for Envarsus XR compared to Prograf (p=0.0001 and p=0.0004, respectively; figure 1). Thus, Envarsus XR is associated with more stable whole blood concentrations compared to Prograf.

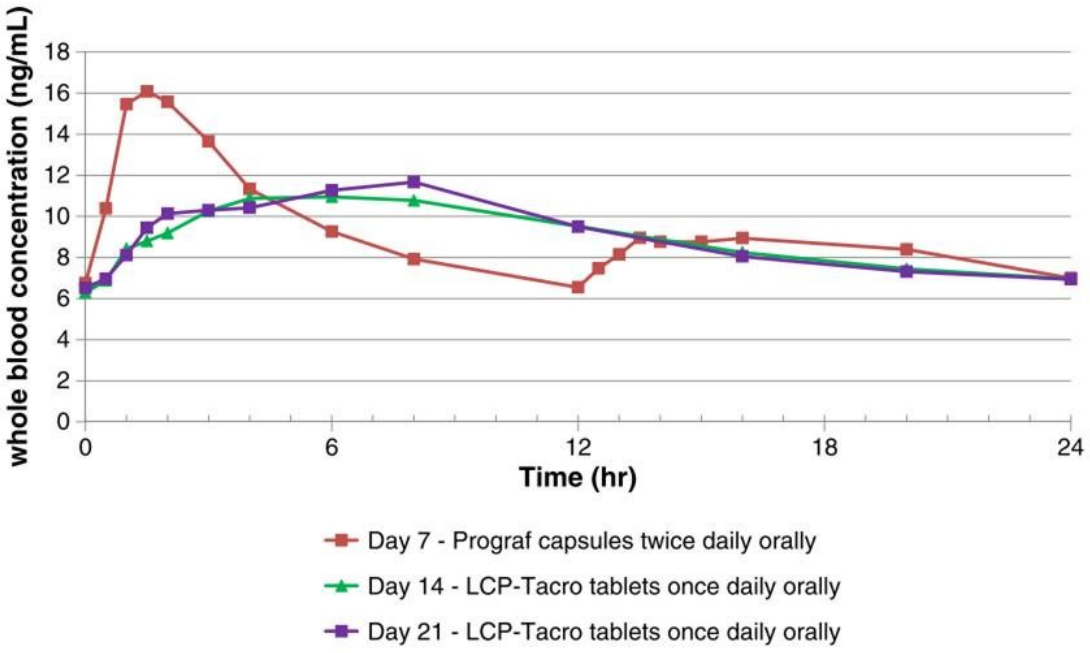


**Figure 1. Mean whole-blood tacrolimus concentration associated with immediate-release tacrolimus (Prograf) and extended-release tacrolimus (Envarsus XR). Figure reproduced from Gaber, et al. Transplantation. 2013 Jul 27, 96(2): 191-7.**

### 1.3 Two-year results of a double-blind, randomized controlled trial of extended- and immediate release tacrolimus in de novo kidney transplant recipients.

A phase 3 randomized, double-blind, double-dummy multicentre trial was performed to assess the efficacy and safety of Envarsus XR compared to immediate-release tacrolimus (13). In this study, 543 de novo kidney transplant recipients were randomized to receive Envarsus XR (n=268) or immediate-release tacrolimus (n=275) in conjunction with mycophenolate mofetil or mycophenolic acid, and corticosteroids. The primary composite endpoint was composed of death, graft failure, biopsy-proved acute rejection, and loss to follow-up within 24 months. Using a pre-

specified 10% non-inferiority margin, Envarsus XR was non-inferior to immediate-release tacrolimus for the primary endpoint (at 24 months: Envarsus XR: 23.1%; immediate-release tacrolimus 27.3%; treatment difference -4.14%, 95% CI: -11.38%-3.17%). There were no observed differences in each component of the primary endpoint between Envarsus XR and immediate-release tacrolimus [death, p=0.8); graft failure (p=0.5), biopsy-proven acute rejection (p=0.8), loss to follow-up (p=0.4)]. Furthermore, the mean number of adverse events per patient was similar between the two drugs (Envarsus XR: 14.3%; immediate-release tacrolimus: 14.4%) as were the mean number of serious adverse events per patient (Envarsus XR: 1.8%; immediate-release tacrolimus: 1.9%).

### 1.4 One-year results among stable kidney transplant recipients converted from immediate-release tacrolimus to Envarsus XR

A phase III, prospective, randomized, open-label study was performed among 324 patients (162 in each arm) who were considered stable. A stable patient was defined as one who was between 3 months and 5 years post-transplant, on a stable tacrolimus dose, with an estimated glomerular filtration rate ≥30 ml/min, and without an episode of acute rejection requiring antibody therapy within 3 months of enrollment. Patients were randomized to remain on immediate-release tacrolimus or convert to Envarsus XR. The primary endpoint was a composite outcome of death, graft failure, biopsy-proven acute rejection, or loss to follow-up within 12 months. There were only 4 events in each group, corresponding to a primary efficacy failure rate of 2.5% (95% CI: -4.2%-4.2%) which fell within the pre-specified non-inferiority margin. There was no difference in each component of the composite outcome – death, graft failure, or biopsy-proven acute rejection. Furthermore, there was no difference in adverse events between the two groups.

## 2. Primary objective

The primary objective is to assess whether the incidence of biopsy-proven acute rejection within the first 12 months of transplant is comparable between highly sensitized patients maintained on Envarsus XR and immediate-release tacrolimus.

### 2.1 Secondary Objectives

Secondary objectives of the study are to assess markers of immunosuppression efficacy between Envarsus XR-treated and immediate-release tacrolimus-treated highly sensitized recipients. Markers to be assessed include the presence of de novo and pre-existing donor-specific HLA antibodies, estimated glomerular filtration rate (eGFR; CKD-Epi equation), and the level of donor-derived cell-free DNA (Allosure®), a biomarker with high sensitivity/specificity for rejection.

## 3. Inclusion criteria

1. Recipient of a deceased or living donor kidney allograft

2. Patients must have undergone desensitization therapy with or without plasma exchange prior to transplant or be administered desensitization therapy peri-operatively post-transplant

3. Age 18 and over

4. Able to understand and provide informed consent

5. At transplant, patient must have an acceptable crossmatch (as defined by a T- or B-FCMX ≤ 225 MCS) from a non-HLA identical donor. A negative crossmatch is defined as a T pronase FCMX <70 MCS or a T- FCMX <50 MCS and a B pronase FCMX <130 MCS or a B-FCMX <100 MCS.

## 3.1 Exclusion criteria

1. Recipients of a dual simultaneous kidney/liver, kidney/heart, kidney/lung, or kidney/pancreas transplant

2. History of hypersensitivity to any of the study drug or to drugs of similar chemical classes

3. Patients with a clinically significant systemic infection within 30 days prior to transplant

4. Patients who have any history of a surgical or medical condition that may affect absorption of drug, such as severe diarrhea, active peptic ulcer disease, or uncontrolled diabetes mellitus, which in the opinion of the investigator at the time of enrollment, might significantly alter the absorption, distribution, metabolism and/or excretion of study

medication.

5. Women of childbearing potential who are either pregnant, lactating, planning to become pregnant during this trial, or with a positive serum or urine pregnancy test. Women of childbearing potential must be willing to agree to contraceptive practices.

6. Patients who are PCR positive for hepatitis B, hepatitis C, or HIV.

## 4. Study design and methods

This is a single center pilot study employing an open-label, randomized controlled trial design of twenty subjects assigned to receive either Envarsus XR or immediate-release tacrolimus as part of their maintenance immunosuppression regimen (10 subjects will be randomized to each arm). All patients aged 18 and older and requiring desensitization prior to or at kidney transplantation may be included in the study. Subjects will take part in the study until they are one-year post-transplant. All subjects will require informed consent.

At the time of screening, subjects will receive a physical exam and undergo lab testing as detailed below in the evaluation schedule. Alemtuzumab (Campath 1H, Lemtrada) or thymoglobulin will be administered to all subjects for induction immunosuppression immediately post-transplant. Maintenance immunosuppression will consist of Envarsus XR or immediate-release tacrolimus, mycophenolate mofetil (Cellcept) or mycophenolic acid (Myfortic), and prednisone. The starting dose of Envarsus XR will be 4 mg PO daily, immediate release tacrolimus will be started at 0.1mg/kg/day PO BID; Envarsus XR and immediate release tacrolimus will be titrated based on drug levels as per usual standard practice. Depending on the time of the kidney transplant, study drug or immediate release tacrolimus will be given the same day as transplant or next day. Study drug initiation may be delayed to post-operative day #2 per PI discretion. Patients will receive antimicrobial prophylaxis as per Cedars-Sinai Medical Center (CSMC) protocol (valganciclovir x 6 months, TMP/SMX x 9-12 months, and fluconazole x 1 month). Lab tests and physical exams for safety will take place according to the evaluation schedule below. Tacrolimus trough level, complete metabolic panel, complete blood count with differential, donor specific antibodies, and urinalysis with culture will be assessed according to the evaluation schedule below. Subjects will complete the study at one year post-transplant. Consent may be withdrawn by the study participant at any time. The investigator may also withdraw the study participant at any time if there are any safety concerns.

### 4.1 Primary endpoint

The primary endpoint is the incidence of biopsy-proven acute rejection (cell-mediated and/or antibody-mediated) over twelve months among patients maintained on Envarsus XR and immediate-release tacrolimus.

### 4.2 Secondary endpoints

Secondary endpoints include:

a) the proportion of patients in each arm who develop de novo donor specific antibodies (dnDSA) at one year after transplant;

b) the time to development of de novo DSA;

c) mean change in MFI of pre-existing DSA at transplant over one year among patients on Envarsus XR versus immediate-release tacrolimus;

d) mean percentage of donor-derived cell-free DNA (Allosure®) at six months and one year among patients on Envarsus XR versus immediate-release tacrolimus;

e) estimated GFR (CKD-EPI equation) over one year after transplant among patients on Envarsus XR versus immediate-release tacrolimus;

f) graft survival over one year among patients on Envarsus XR versus immediate-release tacrolimus;

g) patient survival over one year among patients on Envarsus XR versus immediate-release tacrolimus.

### 4.3 Statistical analysis

The Kaplan-Meier product limit method will be performed to assess freedom from biopsy-proven acute rejection (cell-mediated and/or antibody-mediated), graft loss, and death among patients maintained on Envarsus XR and immediate-release tacrolimus over twelve months. Statistical comparison will be made with the log-rank test.

The proportion of patients in each arm who develop de novo donor specific antibodies (dnDSA) at one year after transplant will be compared using Fisher's exact test. The Kaplan-Meier product limit method will be used to calculate time to development of de novo DSA, using the log-rank test for statistical comparison between patients receiving Envarsus XR and immediate-release tacrolimus.

The mean change in MFI of pre-existing DSA at transplant at baseline and at 12 months among patients on Envarsus XR versus immediate-release tacrolimus will be compared using the t-test.

The mean percentage of donor-derived cell-free DNA (Allosure®) at six months and one year among patients on Envarsus XR versus immediate-release tacrolimus will be compared using the t-test.

Estimated GFR (CKD-EPI equation) over one year after transplant among patients on Envarsus XR versus immediate-release tacrolimus will be assessed with a mixed linear effects model.

## 5. Monitoring for AE/SAEs

Adverse events (AE) and serious adverse events (SAE) will be monitored with careful attention to calcineurin inhibitor side effects, nephrotoxicity, or infectious complications. Side effects associated with Envarsus XR have not been reported to occur more frequently than with immediate-release tacrolimus (1,2). Common side effects associated with tacrolimus include tremor, paresthesia, hypertension, new-onset diabetes after transplant, and acute kidney injury (14).

## 6. Project enrollment

As patients receive treatment for desensitization, the study team will approach patients who meet Inclusion/ Exclusion criteria to evaluate patients' interest in study participation. The screening visit will occur once patients are considered for kidney transplant offer.

Twenty highly sensitized patients will be enrolled for this study. We anticipate enrolling approximately two to three patients a month and estimate that all patients will be enrolled within 10-12 months. Once eligible patients are identified and consented, they will be randomized 1:1 by the Kidney Transplant research pharmacist to study drug, Envarsus XR (N=10) or immediate release tacrolimus (N=10).
The study is designed to follow each enrolled patient for 12 months. Therefore, it is anticipated that the total time required for study completion will be approximately two years.

## 7. Permitted drug dose adjustments and interruptions

Dose adjustments and interruptions are permitted in order to keep the patient on study drug, per standard practice. The study drug may be temporarily interrupted if patient develops short term intolerance or is unable to take oral medication. Administration of alternative immunosuppression or alternative route per SOC is acceptable. Patients should be returned to study drug as soon as possible.

## 8. Schedule of events

IRB No: Pro00054474
Protocol: Envarsus XR Study

**FLOWCHART OF PROCEDURES**

| Procedures | Screening Visit | Tx Day 0 | Day 2 ± 1 day | Day 4 ± 2 day | Day 7± 3 day | Day 14 - 3 day /+5 days | Day 30± 7 day | Month 2± 14 day | Month 3± 14 day | Month 6 ± 14 day | Month 9 ± 14 day | Month 12 ± 14 day |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Informed Consent[6] | R | | | | | | | | | | | |
| Inclusion/Exclusion[6] | R | | | | | | | | | | | |
| Randomization | R | | | | | | | | | | | |
| Physical Exam | S | S | S | S | S | S | S | S | S | S | S | S |
| Chest X-ray[1] | S | | | | | | | | | | | |
| EKG[1] | S | | | | | | | | | | | |
| Alemtuzumab or thymoglobulin Administration | | S | | | | | | | | | | |
| CBC with diff | S | S | S | S | S | S | S | S | S | S | S | S |
| Complete Metabolic Panel | S | S | S | S | S | S | S | S | S | S | S | S |
| Urinalysis | | | | S | S | S | S | S | S | S | S | S |
| Tacrolimus trough level[2] | | | S | S | S | S | S | S | S | S | S | S |
| Viral testing: Polyomavirus BK | | | | | | | S | | S | S | S | S |
| Viral testing: Cytomegalovirus | | | | | | | S | | S | S | S | S |
| Serum Pregnancy Test[3] | S | | | | | | | | | | | |
| Donor Specific Antibody[4] | S | | | | | | S | | S | S | S | S |
| Donor -derived cell-free DNA (Allosure) | | | | | | | S | | S | S | S | S |
| Adverse event monitoring | R | R | R | R | R | R | R | R | R | R | R | R |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Valganciclovir | S | S | S | S | S | S | S | S | S | S | | |
| TMP/SMX (Bactrim) | S | S | S | S | S | S | S | S | S | S | S* | S* |
| Fluconazole | S | S | S | S | S | S | S | | | | | |
| Envarsus XR [5] | | R[2] | R | R | R | R | R | R | R | R | R | R |
| Immediate-Release Tacrolimus[5] | | S[2] | S | S | S | S | S | S | S | S | S | S |
| Mycophenolate mofetil (cellcept) or mycophenolate acid (myfortic) | | S | S | S | S | S | S | S | S | S | S | S |
| Prednisone | | S | S | S | S | S | S | S | S | S | S | S |

**LEGEND**
**R** = Research item/procedure done only for research purposes and covered by the study
**S** = Standard of care item/procedure that is part of regular care and billed to the patient/insurance

**Footnotes:**

*TMP/SMX (Bactrim) may be discontinued as per standard of care at months 9, 10, 11 or 12

1: Can be done within 6 months of screening date

2: Depending on the time of the kidney transplant, Envarsus XR and Immediate-Release Tacrolimus will be given either same day as transplant or the next day or delayed to post-operative day #2 per PI discretion

3: If subject is of childbearing age. (Not required if subject has a histroy of hysterectomy).

4: DSA will be doneat time of transplant and quarterly for the first year. For deceased donor, sample will be drawn pre-op, prior to IVIG. Majority of pts would have received desensitization within the past 6-9 months prior to transplant. For living donors, sample will be drawn prior to IVIG2 but pt would already have received IVIG1 and ritux 5 weeks prior to sample drawn

5: Subject will be taking either Envarsus XR or Immediate-Release Tacrolimus; not both. Envarsus XR will be provided by study staff.

6: Consent & Inclusion/Exclusion can be obtained prior to screening visit. Inclusion/Exclusion will be reviewed at time of screening to confirm the patient remains a study candidate.

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