Trial Title: Extension protocol for subjects previously enrolled in the "Use of donor derived-cell free DNA (AlloSure) and gene expression profiling (AlloMap Kidney) to facilitate Belatacept monotherapy in kidney transplant patients"

Chief Investigator: Dr. David Wojciechowski

Study Center: UT Southwestern Medical Center

Funding: Internal/Patient Gift Fund

Study Design: Prospective single-center, single arm extension study

Objectives: 1. To determine if donor derived-cell free DNA (AlloSure) and gene expression monitoring (TruGraf) can be utilized to facilitate Belatacept monotherapy in patients who were not weaned to Belatacept monotherapy in the initial protocol.

2. To determine if Belatacept monotherapy is safe and effective as immunosuppression in kidney transplant recipients who were weaned to Belatacept monotherapy in the initial protocol out to 24 months.

Primary Endpoints: Incidence of biopsy-proven acute rejection at 24 months after starting immunosuppression wean and percentage of subjects successfully weaned to belatacept monotherapy

Secondary Endpoints: Kidney graft survival, patient survival, change in eGFR, development of proteinuria, and appearance of de-novo donor specific antibodies at 12 months after study enrollment

Sample Size: maximum of 25 subjects

Protocol Version: 1.0

Maximum Follow-Up Duration: 12 months

Inclusion Criteria:

1. Completion of the parent study (STU-2020-1339)
2. Able to provide informed consent
3. Absence of donor specific antibodies
4. Stable renal function (eGFR>40mL/min for 3 months prior to enrollment)

Exclusion Criteria:

1. Female participant who is pregnant, lactating or planning pregnancy during the course of the trial
2. Significant hepatic impairment
3. Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the trial, or may influence the result of the trial, or the participant's ability to participate in the trial
4. Proteinuria > 0.5 g/g creatinine on spot urine sample within 3 months of enrollment

Purpose: The purpose of the study is to provide immunosuppression weaning and/or monitoring for an additional 12-months to evaluate the safety and efficacy of belatacept monotherapy in patients previously enrolled in the clnical trial: "Use of donor derived-cell free DNA (AlloSure) and gene expression profiling (AlloMap Kidney) to facilitate Belatacept monotherapy in kidney transplant patients."

Background:

Patients who have undergone a kidney transplantation are traditionally treated with a multi-drug maintenance immunosuppression regimen that generally includes mycophenolate mofetil, a calcineurin inhibitor, with or without maintenance steroids. While this regimen has been traditionally effective, it is also fraught with adverse

effects including renal toxicity, cardiovascular effects, hypertension, hyperglycemia, and more[1–3]. These side effects become critical as chronic renal allograft dysfunction and cardiovascular disease are the leading causes of graft loss and death in kidney transplant recipients, respectively[4,5]. Belatacept was developed with the goal of reducing the adverse effects of the multi-drug regimens, while maintaining adequate immunosuppression.

Belatacept inhibits T-cell activation by binding co-stimulatory ligands CD80 and CD86. These ligands would otherwise lead to activation of T-cells by binding to the CD28 co-stimulatory receptor on T-cells [6]. As an immunosuppressive agent in kidney transplant recipients, Belatacept is associated with better renal function compared with cyclosporine after 12 months and up to 7 years of therapy, with similar rates of serious adverse events[6–10]. Even though a significantly higher rate of acute allograft rejection at 12 months was found in the Belatacept group, this group had a lower rate of death and graft loss compared with the group treated with Cyclosporine at 12, 36, 60, and 84 months (a 43% reduction at 84 months)[9]. Similar results were found in a sample of extended criteria donation patients[11,12] and a Cochrane meta-analysis review where no statistical difference in rates of acute rejection were found[13]. In addition, patients treated with Belatacept had lower blood pressure, better lipid profiles, and lower rates of new-onset diabetes compared with those treated with calcineurin inhibitors[6,7,11,13]. These studies suggest that Belatacept could be a safe alternative to calcineurin inhibitors with improved long-term outcomes and reduced metabolic side effect profile.

Cell Free DNA (cfDNA) is a biomarker that measures the circulating DNA products that are released after cells have been damaged[14]. Across multiple specialties of medicine, it has been used as a non-invasive method of monitoring adverse events including monitoring and screening of cancer[15–17], acute kidney injury development in cardiac surgery[18], prognostication in trauma and sepsis[19,20], and in pre-natal testing for fetal chromosomal abnormalities[21,22]**.** Specifically within transplantation, donor derived cfDNA (dd-cfDNA) released from allograft cells in multiple solid organs can be used to monitor graft rejection[14]**.** In kidney transplant recipients, multiple studies have shown that elevated levels of dd-cfDNA correspond to episodes of acute rejection and may identify rejection better than creatinine levels, donor specific antibodies, or other traditional measurements of kidney function[23–28]. Similar to cfDNA, gene expression testing has been explored as a biomarker for detection of acute graft rejection. TruGraf is a blood-based biomarker test that measures differential expression of a collection of genes shown to correlate with surveillance biopsy results. TruGraf has shown to confirm immune quiescence with a high negative predictive value (98%) without the need to perform a surveillance kidney biopsy[39].

At our institution, the current post-transplant maintenance regimen includes a de novo Belatacept option. Our de novo Belacept regiment includes rATG induction with maintenance Belatacept, Everolimus, with or without steroids. We also employ a Belatacept conversion strategy from tacrolimus in the setting of prolonged delayed graft function. Belatacept does not impart the same cardiovascular complications associated with traditional immunosuppression and is associated with a decreased risk of DSA formation. Kirk et al has shown in living donor recipients Belatacept monotherapy or with Sirolimus can prevent acute rejection without the need for calcineurin inhibitors (CNI) or steroids[30]. Other studies, including a randomized multi-center trial, have shown that Belatacept-based regimens can be used safely and effectively without the need for CNIs or steroids[31,32]. There are no current recommendations on the type of patients or the timing of transition to Belatacept monotherapy in order to reduce long-term side effects of maintenance immunosuppression. We hypothesize that patients who reach immune quiescence, as judged by normal levels of dd-cfDNA and traditional markers of rejection, can be safely transitioned to Belatacept monotherapy without increasing the incidence of acute graft rejection.

Current Practice:

Currently at UT Southwestern, post-transplant de-novo Belatacept regiment follows the FDA recommended dosing. In patients with prolonged delayed graft function (DGF) Tacrolimus is the primary immunosuppression and levels are targeted at 8-10 ng/mL. During this time patients are not eligible for early corticosteroid withdrawal until DGF resolves. A biopsy will be done on post-operative day 14 on all patients with prolonged or on-going DGF. If there is no demonstration of rejection and are EBV seropositive negative, they may be converted to Belatacept. Patients <6 months post-transplant are weaned off of their calcineurin inhibitor

(tacrolimus) to 50% of baseline at the 1st dose of Belatacept, 25% of baseline at the second dose, and discontinued at the third dose. A routine kidney biopsy is performed prior to conversion of therapy[33].

Post-transplant patients are normally seen in clinic every 4 weeks for the first 6 months, then at 9, 12, 19, and 24 months and annually thereafter. Blood work is drawn weekly for the first 3 months, every 2 weeks from 3-6 months, and monthly thereafter until 3 years[34].

For serial monitoring of immunosuppression blood is drawn at 4 week intervals for evaluation of blood counts, a metabolic panel, and immunosuppression medication trough levels. DSA is measured at 6 months and 12 months post-transplant for patients with DSA pre-transplant or cPRA>80%. Urinalysis with protein/creatinine ratio is performed 4 weeks post-transplant and then quarterly. Immunosuppression is adjusted as necessary, based on the serum levels and type of medication they are on. If there is an elevation in creatinine, a drop in eGFR, or other clinical suspicion of graft rejection (fever, pain overlying the graft) then a renal biopsy is performed to assess for graft rejection. If graft rejection is present they will be treated with pulse steroids and/or thymoglobulin based on the Banff criteria. Specific treatment details are listed in the referenced policy document.[35]

All UT Southwestern clinical guidelines are found in the health system portal under hospital policies & procedures.

Study Design:

This is a one year extension of the prospective, single arm, pilot study entitled "Use of donor derived-cell free DNA (AlloSure) and gene expression profiling (AlloMap Kidney) to facilitate Belatacept monotherapy in kidney transplant patients." Patients in the initial protocol who underwent successful weaning of immunosuppression to Belatacept monotherapy will undergo an additional 12-months of monitoring according to Table 1. Patients who were not successfully weaned to Belatacept monotherapy will undergo potential immunosuppression weaning throughout an additional 12-month monitoring period according to Table 1.

Research Methods:

Patient Selection:

Inclusion/exclusion criteria will be evaluated through medical record review to pre-identify eligible patients from the parent study. These subjects will include those currently enrolled and those who have already completed study.

Patient Withdrawal:

Subjects will be withdrawn from the study if:

- They withdraw consent or decline to continue
- Any reason deemed necessary by the investigator and in the interest of the subject

Procedures:

Eligible patients attend monthly infusion visits as part of their routine care. All research procedures will be scheduled to take place at this regularly scheduled appointment, with additional visits occurring only as needed to minimize any additional burden on the subject. Allosure and TruGraf will be collected alongside patient's standard transplant labs. These blood samples will be correlated with any renal biopsies performed to evaluate the secondary objective.

Eligible patients will be enrolled into the study at their final visit for the parent study. This visit will also function as the Month 0 timepoint for this extension protocol. For eligible patients who are agreeable to study and have already completed the parent study, informed consent will be obtained at their next regularly scheduled infusion appointment. The timeframe between all monthly interval visits will include a +/- 7 day window period.

Urine protein and DSA will be checked at quarterly timepoints, while BMP and CBC will be drawn monthly per local standard of care. Allosure samples will be collected at monthly intervals to monitor for any signs of rejection. To monitor changes in the immunosuppressant side effect profile during the wean, a lipid panel will be evaluated at enrollment (Month 0) and at study completion (Month 12) for patients on mTOR inhibitors (i.e. Sirolimus or Everolimus). Additionally, a hemoglobin A1C will be drawn for patients with a history of diabetes at the same timepoints.

A pre-enrollment surveillance kidney biopsy may be performed as per institutional standard of care but is not required for enrollment. If patients have stable kidney function and are deemed immune quiescent at Month 0, then they will begin their immunosuppression wean as described in Table 1.

If at any point during a wean an episode of biopsy proven acute rejection occurs, the patient will return to the standard immunosuppression regimen and will no longer be a candidate for weaning. The full schedule of all sample retrievals is shown below in Tables 1.

Kidney Biopsies:

Kidney biopsies will be performed under these circumstances per instutional standard of care.

- Unexplained consistent decrease in eGFR >40% above baseline value
- New persistent proteinuria >1.0g/g
- Any clinical suspicion of graft rejection including, but not limited to, fever, pain at graft site, etc.
- TruGraf result of 'Not-TX'
- Allosure dd-cfDNA level of >1%
- Development of new de-novo DSA
- At the discretion of the principal investigator for those patients who do not meet the above criteria, but have a high clinical suspicion of acute rejection

If the patient is deemed to be immune quiescent then his/her immunosuppression will be tapered down in a stepwise fashion. We evaluate immune quiescence based on the following factors, but it is ultimately determined by PI discretion.

- Renal function is stable, defined as eGFR no more than 20% lower compared to value at inclusion
- AlloSure dd-cfDNA level of < 1%[24]
- No change in AlloSure level >61% above baseline that coincides with an absolute AlloSure level of >0.5%[24,36]
- No appearance of any de novo DSA during study
- No proteinuria > 0.5 g/g creatinine on spot urine sample
- No new occurrence of biopsy-proven acute rejection (based on Banff 2017 Criteria[37])

If any of these are present and the PI deems the subject to not be immune quiescent, then the patient's immunosuppression weaning will be paused and they will be classified as treatment failure. They will continue with the surveillance protocol as described, however will no longer continue any study intervention (immunosuppressant wean).

A full schedule of tests and medication tapering is shown below in Table 1:

Table 1.

Month (+/- 7 days)	0	1	2	3	4	5	6	7	8	9	10	11	12
Physical Exam**	x			x			x			x			x
Allosure	x	x	x	x	x	x	x	x	x	x	x	x	x

TruGraf	x			x			x			x			x
DSA	x			x			x			x			x
Urine protein	x			x			x			x			X
BMP/CBC	x	x	x	x	x	x	x	x	x	x	x	x	X
Lipid panel/HgA1C*	x												X
Quality of Life Measurement	x												x
EMR Clinical Events	x	x	x	x	x	x	x	x	x	x	x	x	X
Medication taper***	1			2			3			4			

*Lipid panel only evaluated for patients on mTOR inhibitors and HgA1C only evaluated for patients with history of diabetes

**If subject is classified as treatment failure, physical exams will no longer be done quarterly. Next physical exam will be at EOS (Month 12).

***Medication taper 1 will be completed pending negative TruGraf and DSA results from Month 0

Immunosuppression Taper:

Patients currently on prednisone will begin their Month 0 taper at step #1, but those not on prednisone will begin their month 0 taper at step #2. Each step will occur in three month intervals (i.e months 0, 3, 6, and 9, if necessary)

For patients on a baseline immunosuppression regimen that includes Everolimus, the following taper will occur:

1: Weaning will begin with termination of prednisone (standard dosing is only 5mg).

2: The trough goal for Everolimus will be reduced from 4-8 (standard) to 3-6

3: Further reduction of Everolimus trough goal to 2-4

4: Everolimus will be stopped

For patients on a baseline immunosuppression regimen that includes mycophenolate (MMF or MPA), the following taper will occur:

1: Weaning will begin with termination of prednisone

2: The total daily dose of mycophenolate will be reduced by 25%

3: The total daily dose of mycophenolate will be reduced by another 25%

4: Mycophenolate will be stopped

Acute Rejection Treatment:

If acute allograft rejection is diagnosed on a kidney biopsy by the Banff criteria[37] then the patient will be treated according to our institutional standard care. This includespulse dose of steroids and/or thymoglobulin depending on the Banff criteria as listed in the UT Southwestern rejection treatment guidelines[35].

Patients who meet criteria for a kidney biopsy based on laboratory criteria (eGFR, proteinuria, etc) and have a borderline result by Banff criteria will be considered a treatment failure. These patients are then treated with a pulse dose of steroids as per institutional guidelines. However, patients who meet criteria for a kidney biopsy on clinical suspicion or investigator discretion alone, without abnormal laboratory markers, will not be classified as a treatment failure if there is a borderline result; these patients may continue in the protocol. Finally, patients

who meet criteria for a kidney biopsy based on laboratory findings but have a negative biopsy result will not be classified as treatment failure and may also continue through the protocol.

If treatment of rejection is necessary then, as stated above, the patient will be classified as a treatment failure and be converted back to combination immunosuppression therapy. Patients classified as treatment failure will continue with the surveillance protocol as described, however they will no longer continue with any study intervention (immunosuppressant wean).

Quality of Life Measurement:

Quality of life measurements will be assessed through the PROMIS global health and medication management surveys. This survey was chosen for it ease of administration and its validation across multiple populations of chronic medical disease[38]. The goal will be to understand the impact of decreased number of medications and potentially improved side effect profile of Belatacept monotherapy on the quality of life. The PROMIS global health and medication management surveys include 18 questions that evaluate the patient's perception of their overall health, functional status, and ability to maintain compliance with their medication regimen. Surveys will be administered at month 0 and at month 12 to evaluate changes through the timeframe of the study. Surveys will be given to all enrolled patients whether or not they were able to successfully complete the wean to Belatacept monotherapy.

Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form before any trial specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the trial. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site.

*can be substituted parent/guardian or legally authorised representative, as appropriate, make sure that the term is consistent throughout the document.

Baseline Assessments

No additional assessments outside standard clinical testing is needed as part of this trial. Access to clinical test results through the patient EMR will be made through the consent process.

Subsequent Visits

Participants will attend outpatient visits/follow-up visits as part of their standard care, these will include appointments where they will have blood tests taken as part of post-transplant surveillance.

No additional study visits are anticipated.

Sample Handling

Whole blood will be taken in a number of vacutainers for clinical assessment, as part of clinical care. These samples will be analyzed for normal post-transplant surveillance markers as part of normal care, such as BMP, CBC, and DSA. Additional samples for dd-cfDNA (AlloSure) and TruGraf will be taken for the study during this standard phlebotomy procedure. Specifically, whole blood will be collected in 2 DNA streck tubes.

Protocol Deviations

The investigator and site staff will conduct the study in accordance to the protocol and any change, divergence, or departure from the study design constititues a protocol deviation. As a result of any deviation, corrective action will be developed by the site and implemented properly.

Out of window monthly study visits due to alterations in the subject's standard of care belatcept infusion schedule is acceptable and not considered a protocol deviation. Missed or out of window local sample collection is not considered a protocol deviation due to its adherence to site's standard of care monitoring.

The principal investigator has the responsibility to identify, document and report the protocol deviations to the appropriate institutional research board (IRB). Protocol deviations may also be identified during study monitoring visits or during other forms of study contact review.

Adverse Events

For this study, the following categories of adverse events (AEs) will be tracked and recorded.

•Adverse events, as defined by any untoward medical occurrence, unintended disease or injury, or untoward clinical signs, related to study subjects' kidney function/graft

•Serious Adverse Events, as defined by any event that results in death, is life-threatening, requires inpatient hospitalization, or prolongs an existing hospitalization

All adverse events will be monitored and tracked by the study team and graded by the Principal Investigator. These events will be reported via UTSW's HRPP Reporting Guidelines.

Definition of End of Trial

The end of trial is the date of the last visit of the last participant.

Description of Statistical Methods

The primary analysis performed will be descriptive data of the entire cohort. A secondary subgroup analysis will be performed comparing those with acute rejection during the study against those without acute rejection. The primary outcome will be the incidence of acute allograft rejection and the secondary outcomes will be change in eGFR from enrolment to the end of the study, incidence of proteinuria, incidence of appearance of donor specific antibodies,and recipient and graft survival at 12 months after the start of immunosuppression wean. Continuous data, i.e. change in eGFR, will be compared using either the Student's t-test or Wilcoxon rank-sum as appropriate, and categorical data, i.e. any incidence comparison, will be compared using either Fisher's exact test or Chi-squared analysis, as appropriate. For survival analyses, Kaplan Meier analysis will be done to create survival curves.

The Number of Participants

The study is an observational experimental study, with a projected enrollment of 25 participants.

The Level of Statistical Significance

Results with a p value <0.05 will be deemed significant.

DATA MANAGEMENT

Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in a locked cabinet in the Department of Surgery research office.

Access to Data

Direct access will be granted to authorised representatives from the host institution and regulatory authorities to permit trial-related monitoring, audits and inspections.

Data Recording and Record Keeping

All trial data will be entered on to a secure electronic data collection system. The participants will be identified by a unique trial specific number and/or code in any database.

QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

ETHICAL AND REGULATORY CONSIDERATIONS

Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate institutional research board (IRB) / Research Ethics Committee (REC), for approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all trial documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

Expenses and Benefits

No patients will be reimbursed for travel expenses for any visits additional to normal care, as all testing intervals are anticipated to occur with normal clinical care visits.

FINANCE AND INSURANCE

Insurance

Hospitals are legally liable for the negligent acts and omissions of their employees. If you are harmed whilst taking part in a clinical trial as a result of negligence on the part of a member of the trial team this liability cover would apply.

Non-negligent harm is not covered by the hospital indemnity scheme. Therefore, the hospital cannot agree in advance to pay compensation in these circumstances.

PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

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