Astagraf XL® to Understand the Impact of Immunosuppression on De Novo DSA Development and Chronic Immune Activation in Kidney Transplantation

ISN/Protocol IDTX-MA-3004

ClinicalTrials.gov Identifier: NCT02723591

Date of Protocol v2.1: 25 May 2018

Sponsor: Astellas Pharma Global Development, Inc. (APGD)

Medical Affairs, Americas

1 Astellas Way

Northbrook, IL 60062

Astagraf XL® to Understand the Impact of Immunosuppression on De Novo DSA Development and Chronic Immune Activation in Kidney Transplantation

™

Protocol for Phase 4 Study of Astagraf XL®

(tacrolimus extended-release capsules)

ISN/Protocol IDTX-MA-3004

Version 2.1

Incorporating Non-Substantial Amendment 2

May 25, 2018

IND 64148

Sponsor:

Astellas Pharma Global Development, Inc. (APGD)

Medical Affairs, Americas

1 Astellas Way

Northbrook, IL 60062

Investigator: Investigator information is on file at Astellas.

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I. SIGNATURES

1. SPONSOR'S SIGNATURE

Astagraf XL® to Understand the Impact of Immunosuppression on De Novo DSA Development and Chronic Immune Activation in Kidney Transplantation

ISN/Protocol IDTX-MA-3004 /

Version 2.1 / Incorporating Non-Substantial Amendment 2 / dated May 25, 2018

Required signatures (e.g., Protocol authors, Sponsor's reviewers and contributors, etc.) are located in **Section 14. Signatures**; e-signatures (when applicable) are located at the end of this document.

2. INVESTIGATOR'S SIGNATURE

Astagraf XL® to Understand the Impact of Immunosuppression on De Novo DSA Development and Chronic Immune Activation in Kidney Transplantation

ISN/Protocol IDTX-MA-3004 /

Version 2.1 / Incorporating Non-Substantial Amendment 2 / dated May 25, 2018

I have read all pages of this clinical study protocol for which Astellas is the Sponsor. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with ICH GCP guidelines and applicable local regulations. I will also ensure that sub-investigator(s) and other relevant members of my staff have access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

Principal Investigator:

Signature: .....

<Insert name and qualifications of the Investigator> Date (DD Mmm YYYY)

Printed Name: .....

Address: .....

.....

II. CONTACT DETAILS OF KEY SPONSOR'S PERSONNEL

24h-Contact for Serious Adverse Events (SAEs) See Section 5.5.5	PPD **, Transplant – Immunology** **Astellas Pharma Global Development, Inc.** **Medical Affairs, Americas** **1 Astellas Way** **Northbrook, IL 60062 USA** PPD **Please fax or email the SAE Worksheet to:** **Astellas Pharma Global Development, Inc.** **Global Pharmacovigilance** **North America telefax numbers: 888-396-3750** **(alternate 847-317-1241)** **Email: safety-us@astellas.com**
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III. LIST OF ABBREVIATIONS AND DEFINITION OF KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
ABMR	Antibody-mediated Rejection
ABO	Blood Group System (A, B, AB, and O)
ADNR	Acute Dysfunction/No Rejection
AE	Adverse event
Ag	Antigen
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase (GPT)
Anti-HBs	Hepatitis B surface antibody
APGD	Astellas Pharma Global Development, Inc.
AST	Aspartate Aminotransferase (GOT)
ATP	Adenosine 5'-Triphosphate
AUST	Astellas US Technology
BKV	BK Virus
BPAR	Biopsy-Proven Acute Rejection
C1q	Complement Component 1, Q Subcomponent
C4d	Complement Split Product, 4d
CAN	Chronic Allograft Nephropathy
CD52	Cambridge Pathology Antigen 1
CG	Chronic Glomerulopathy
CI	Confidence Intervals
CIT	Cold Ischemia Time
CLIA	Clinical Laboratory Improvement Amendments
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitors
cPRA	Calculated Panel Reactivity Antibody
CR	Chronic Rejection
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
CV	Coefficient of Variation
CYP3A	Cytochrome P450 3A System
DCD	Donation after Circulatory Death
DD	Deceased Donor
DeKAF	Deterioration of Kidney Allograft Trial
DILI	Drug-induced Liver Injury
DLDA	Diagonal Linear Discriminant Analysis
DSA	Donor-Specific Antibody
EBV	Epstein–Barr Virus
ECD	Extended Criteria Donor
ECG	Electrocardiogram

Abbreviations	Description of abbreviations
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
FAS	Full Analysis Set
GCP	Good Clinical Practice
GEE	General Estimating Equations
GMP	Good Manufacturing Practices
H&E	Hematoxylin and Eosin
HBc	Hepatitis B Virus Core
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HgA1c	Hemoglobin A1c
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HLA-DP	Human Leukocyte Antigen, DP locus
HLA-DQ	Human Leukocyte Antigen, Class II, DQ locus
IA	Immune Activation
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IFTA	Interstitial Fibrosis and Tubular Atrophy
IgG_3	Immunoglobulin G
IgM	Immunoglobulin M
IL-2	Interleukin-2
IND	Investigational New Drug
INR	International Normalized Ratio
IPV	Intrapatient Variability
IQ	Immune Quiescence
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISN	International Study Number
IVIG	Intravenous Immunoglobulin
KDPI	Kidney Donor Profile Index
KM	Kaplan-Meier
LA-CRF	Liver Abnormality Case Report Form
LD	Living Donor
LFT	Liver Function Tests
m^2	Meter squared

Abbreviations	Description of abbreviations
MA-A	Medical Affairs, Americas
MDRD-4	Modification of Diet in Renal Disease (4 variable)
MFC	Multiparameter flow cytometry
MFI	Mean Fluorescence Intensity
MHC	Major Histocompatibility Complex
MMF	Mycophenolate Mofetil
mFAS	Modified Full Analysis Set
mTOR	Mammalian target of rapamycin
NASH	Non-alcoholic steatohepatitis
NCBI	National Center for Biotechnology Information
NGT	Nasogastric Tube
NIH	National Institutes of Health
NTI	Narrow Therapeutic Index
OPO	Organ Procurement Organization
OPTN	Organ Procurement and Transplantation Network
PAS	Periodic Acid Schiff
PBS	Phosphate Buffered Saline
PCA	Principle Component Analysis
PD	Pharmacodynamics
PHI	Personal Health Information
PI	Package Insert
PK	Pharmacokinetics
PKAS	Pharmacokinetic Analysis Set
PPS	Per Protocol Set
PRCA	Pure Red Cell Aplasia
PRES	Posterior Reversible Encephalopathy Syndrome
PTC	Peritubular Capillaries
RMA	Robust Multi-array Average
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SDV	Source Document Verification
SGOT	Serum Glutamic Oxaloacetic Transaminase
SOC	Standard of Care
SOP	Standard Operating Procedure
SPA	Solid Phase Assay
SPGT	Serum Glutamic Pyruvic Transaminase
SRTR	Scientific Registry of Transplant Recipients
SUSAR	Suspected Unexpected Serious Adverse Reactions
TacSD	Standard Deviation of Tacrolimus
TB	Tuberculosis
TBL	Total Bilirubin

Abbreviations	Description of abbreviations
T-cell	Thymus Lymphocytes
TCMR	T-cell Mediated Rejection
TDM	Therapeutic Drug Monitoring
TG	Transplant Glomerulopathy
TGI	Transplant Genomics Inc.
TLFs	Tables, Listings, and Figures
TMF	Trial Master File
TX	Transplant eXcellent
ULN	Upper Limit of Normal
UNOS	United Network of Organ Sharing
USP	United States Pharmacopeia
WIT	Warm Ischemia Time
XM	Crossmatching

Definition of Key Study Terms

Terms	Definition of terms
Baseline	Observed values/findings which are regarded as the observed starting point for comparison.
Enroll	To register or enter into a clinical trial. NOTE: Once a subject has been enrolled, the clinical trial protocol applies to the subject.
Intervention	The drug, therapy or process under investigation in a clinical study that is believed to have an effect on outcomes of interest in a study (e.g., health-related quality of life, efficacy, safety, pharmacoeconomics).
Investigational period	Period of time where major interests of protocol objectives are observed, and where the test drug or comparative drug (sometimes without randomization) is usually given to a subject, and continues until the last assessment after completing administration of the test drug or comparative drug.
Screening period	Period of time before entering the investigational period, usually from the time of starting a subject signing consent until just before the test drug or comparative drug (sometimes without randomization) is given to a subject.
Randomization	The process of assigning trial subjects to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screening	A process of active consideration of potential subjects for enrollment in a trial.
Screen failure	Potential subject who did not meet one or more criteria required for participation in a trial.
Study period	Period of time from the first site initiation date to the last site completing the study.
Variable	Any quantity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

IV. SYNOPSIS

Date and Version # of Protocol Synopsis:	2.0, October 6, 2017
Sponsor: Astellas Medical Affairs, Americas	**Protocol Number:** IDTX-MA-3004
Name of Study Drug: Tacrolimus extended-release (Astagraf XL®)	**Phase of Development: 4**
Title of Study: ASTOUND Astagraf XL® to Understand the Impact of Immunosuppression on De Novo DSA Development and Chronic Immune Activation in Kidney Transplantation	
Planned Study Period: From 1Q FY2016 to 3Q FY2019	

Study Objective(s):

Primary Objective:

- Compare the incidence of a two-part composite endpoint consisting of de novo donor specific antibody (DSA) formation or a designation of "immune activation (IA)" on peripheral blood molecular profiling in patients maintained on twice daily, immediate-release tacrolimus versus those maintained on Astagraf XL® in the first two years post-transplant.

Secondary Objectives:

- To assess the risk factors for each of the following outcomes: DSA formation;, IA; transplant glomerulopathy (TG); acute and chronic forms of antibody-mediated rejection (ABMR); C1q-binding DSA; HLA-DQ DSA; DSA IgG_3 isotype; requirement for and type of antibody reduction required; various threshold levels of estimated glomerular filtration rate (eGFR) (less than 30, 40, and 50 mL/min/1.73 m^2); and the four components of the traditional composite endpoint, consisting of graft loss, mortality, biopsy-proven acute rejection (BPAR), and loss to follow-up; and additionally, the persistence of DSA and IA.
- Assess and compare, between treatment groups, the association/correlation of the appearance of DSA with the development of IA on molecular profiling.
- Compare, between treatment cohorts, the incidence and hazards of each of the following, occurring over the course of one year, and, where appropriate, at 24 months post-transplantation: DSA, HLA-DQ DSA, C1q-binding DSA, DSA IgG_3 isotype, IA, TG, select BANFF histology grades (acute and chronic forms of ABMR, acute and chronic active TCMR, borderline changes, interstitial fibrosis and tubular atrophy [IFTA]), graft loss, mortality, and BPAR.
- Compare between cohorts the incidence of various thresholds of eGFR (less than 30, 40, 50 mL/min/1.73 m^2, and a five-point decline).
- Compare the distribution of ordinal categories of antibody strength (weak, moderate, strong) between treatment groups across the study time course as well as at each time point.
- Compare raw mean fluorescence intensity (MFI) scores between treatment groups at each time point and across the duration of the study.
- Examine and compare histopathology between the treatment cohorts in biopsies obtained for cause and for maintenance during the course of clinical care.
- Examine and compare outcomes as a function of tacrolimus manufacturer and the number of switches between immediate-release tacrolimus products.

- Summarize adverse events (AEs) between study groups.
- Compare the change in eGFR over the study duration beginning from 30 days post-transplant between groups, and in patients who develop DSA or IA.
- Compare the persistence of the development of IA on molecular profiling between the two cohorts across the study duration in those patients who develop IA on molecular profiling.

Exploratory Objectives:

- Compare the frequency of those patients experiencing either component of a two-part composite endpoint consisting of DSA formation and the presence of TG (cg > 0) on centrally-interpreted, institutional protocol (maintenance) biopsy and / or biopsy obtained for cause during the first year post-transplant and/or up to the conclusion of the study.
- To assess and compare, between treatment groups, the association/correlation of incidence and strength of DSA, and the incidences of HLA-DQ DSA, C1q-binding DSA, and DSA IgG_3 isotype with each other, and individually, with each of the following: IA, TG, ABMR (both chronic forms and acute), Banff histology, various thresholds of eGFR (less than 30, 40, and 50 mL/min/1.73 m^2), eGFR change over time, TCMR, graft loss, and mortality.
- Examine the proportion of patients with the IA designation who have normal and abnormal creatinine.
- In patients with an IA designation and abnormal creatinine, examine biopsies (if available) for concordance.
- To assess the association/correlation of the histopathology with DSA, C1q-binding DSA, DSA IgG_3 isotype, molecular phenotyping, graft loss, and measures of renal function in each cohort in patients for whom one year institutionally-derived, protocol (maintenance) biopsies are available.
- To assess and compare, between treatment groups, the association/correlation of the coefficient of variation (CV) and standard deviation (SD) of tacrolimus trough concentrations with each of the following: the composite endpoint of DSA and IA, DSA formation and IA individually, TG, ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, histopathology, renal dysfunction, and the components of the traditional composite endpoint.
- To compare dose changes and intrapatient variability (IPV) between groups as a function of tacrolimus trough level after the first 6 weeks following transplantation in patients on stable doses of immediate-release tacrolimus and Astagraf XL.
- To assess and compare, between treatment groups, the association/correlation of the appearance of DSA with each of the following: TG, chronic forms of ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, IA, histopathology, graft loss, various thresholds of eGFR (< 30, 40, and 50 mL/min/1.73 m^2), acute ABMR, TCMR, and mortality in patients who develop IA on molecular profiling in each cohort.
- To compare the MFI shift to a lower category (and magnitude of shift) between groups (including C1q MFI shift), in those patients who develop DSA during the course of the study.
- To examine percent MFI reduction within each subgroup over time in each treatment arm (including C1q MFI reduction) in those patients who develop DSA during the course of the study.
- To map expression results to known immunological pathways implicated in immune-mediated, kidney transplant tissue injury.

- To determine the longitudinal consequences of DSA formation and immune activation, perform de-identified, long-term follow-up through participating transplant centers in select study participants who provide the appropriate informed consent by future linking to the Scientific Registry of Transplant Recipients (SRTR) after completion of ASTOUND.
- To examine the economic burden of treating patients with molecular evidence of immune activation and DSA formation using Medicare claims data or commercial payer cost data in patients required to transfer from Medicare to private insurance.
- To compare outcomes in like patients between cohorts managed with maintenance protocol biopsy vs. biopsy for cause with respect to the composite endpoint, the components thereof, complications, and resource utilization.

Planned Total Number of Study Centers and Location(s):
Approximately 25-30 centers
US

Study Population:
Living or deceased donor kidney transplant recipients 16 to 70 years of age.

Number of Subjects to be Enrolled / Randomized:
550 patients to be enrolled.

Study Design Overview:
This is an exploratory, two year, prospective, randomized, multi-center, open-label trial examining long-term kidney transplant outcomes through the use of an adaptive design and a two-part, composite surrogate endpoint. Specifically, it is designed to compare the effects of twice daily, immediate-release tacrolimus and once daily Astagraf XL on DSA formation and the development of a peripheral blood molecular profile indicating the presence of immune activation (IA) in de novo kidney transplant recipients during the first two years following transplantation. For the purposes of this study, IA will be defined as a positive molecular signature using the Trugraf v2.0 molecular assay (Transplant Genomics, Inc., Pleasanton, CA) in all patients.

Patients will be screened prior to surgery and randomized 1:1 to receive immediate-release tacrolimus, administered twice daily, or Astagraf XL, as a component of a standard immunosuppression maintenance regimen also consisting of corticosteroids (complete steroid avoidance not allowed – steroid taper and withdrawal permitted if part of institutional protocol) and mycophenolate mofetil (MMF) (or Myfortic® equivalent). Investigators are encouraged to start subjects on the randomized study treatment (immediate release tacrolimus or Astagraf XL) within 48 hours of transplantation (pre-transplant administration of study treatment is not allowed). However, if medically indicated per the treating physician's discretion, initiation of study treatment may be delayed for up to seven days post-transplant.

IF medically indicated, subjects who are unable to tolerate oral administration of their assigned study drug may be administered tacrolimus via an alternative route (for Astagraf XL treatment arm: intravenous [IV] tacrolimus; for immediate release tacrolimus: IV tacrolimus or nasogastric tube [NGT]) for up to seven consecutive days. Dosing via alternative routes should be discontinued as soon as the subject can tolerate oral administration, and is not allowed for more than seven consecutive days. Please see Section 5.1.1 and 4.1 for further details regarding non-oral administration of tacrolimus.

Given the variability of incidence rates for DSA reported in the literature, a stopping rule has been incorporated herein to mitigate the risk of continuing the study under incorrect prior assumptions relating to the true incidence rate of DSA formation. To apply the rule, the incidence rate of the primary endpoint in the control group will be examined once 50% of control patients have completed one year of therapy. Early termination of the study, or extension to a second year, will be predicated on this assessment (see Formal Stopping Rules below).

Induction therapy is required for all participants, and will be administered (dosing, type of agent) per center protocol. Intravenous corticosteroids will be administered prior to revascularization as part of the initial induction regimen, with dose and duration of therapy also determined by the participating transplant center's standard protocol. Thereafter, patients will be followed for up to 2 years during the open-label study period. Immediate-release tacrolimus will be obtained from pharmacies per the local standard of care. After discharge from the hospital, the patients randomized to immediate-release tacrolimus will receive study-provided vouchers and will be permitted to receive any immediate-release tacrolimus product available to them through normal dispensing mechanisms. Astagraf XL will be supplied by Astellas and dispensed by the participating institution.

Initial pre-transplant cross-matching (XM) will be performed per local protocol. Initial XM serum will be collected and stored centrally to facilitate the later assessment of pre-formed antibody on an as needed basis. Thereafter, antibody screening and molecular phenotyping will be performed by a central lab at one month, 3 months, 6 months, 9 months, 12 months, and 24 months. Antibody status, including HLA-DSA isotype and C1q-binding DSA status, will also be recorded when measured locally during the course of clinical care.

For central laboratory analysis, initial FlowPRA® (One Lambda, Canoga Park, CA) screening will be used to test for the presence of anti-HLA antibodies. Once identified, single antigen testing will be used to identify DSA specificity with an MFI greater than or approaching 1000 used to determine antibody positivity. In borderline cases, a three- member adjudication board of independent, qualified HLA Lab Directors will determine positivity based on relevant clinical data. In samples that meet threshold criteria for DSA positivity, additional classification will be performed to determine the degree of antibody strength. By performing a single dilution (1:16) of DSA-containing samples, the alloimmune response can be further elucidated [Tambur et al, 2015]. Samples in which MFI disappears (becomes < 1000 MFI) will be regarded as "weak." Samples increasing to > 10,000 MFI upon dilution will be regarded as having significant prozone and will be regarded as "strong." Samples which may increase or decrease, but nevertheless remain between 1000 and 10,000 MFI will be regarded as "moderate." In certain cases, samples with evidence of fluorescence that are nevertheless below MFI criteria for DSA positivity will also be subject to dilution. Samples increasing to above threshold criteria following dilution will be regarded as positive and assumed to be under the influence of a prozone effect that impairs antibody detection. For statistical purposes, the DSA with the highest MFI level obtained during the course of the study will be used for statistical comparisons between groups. However, the strongest antibody observed at each HLA-locus will be reported separately and tracked for patients who are DSA positive [Tambur et al, 2015]. In all cases, positive DSA and/or molecular testing results will be communicated to the patient's treating physician when available (DSA) or at the end of study (molecular testing). Testing will be performed in batches. De-identified typing reports (donor and recipient) will be requested from the participating centers to inform the central laboratory testing. De-identified results of recent local pre-transplant DSA testing (if performed per SOC) will also be requested to facilitate interpretation of central DSA testing results.

Follow-up visits will be performed quarterly during the first year of follow-up to collect standard clinical data. For follow-up beyond the first year, urinary protein and tacrolimus trough concentrations obtained closest to targeted date of visit, per SOC, should be recorded. The two closest serum creatinine levels to the study visit, obtained at least one month apart, if performed per SOC, should also be recorded. Additionally, all local kidney biopsy results, AEs, and concomitant medications pertaining to AEs will be recorded. At the terminal study visit at 24 months, the most recently obtained lab results as well as any episodes of rejection, need for antibody reduction, graft loss, and deaths occurring beyond the first year will be detailed in the eCRF. Final testing for DSA and molecular profiling will also be performed at the 24 month visit.

Pathology results [hematoxylin and eosin (H&E), light microscopy, and immunofluorescence] will be recorded in the eCRF when local kidney biopsies are performed and interpreted during the course of clinical care. De-identified copies of all kidney pathology reports must be submitted to Astellas. For purposes of data recording, substantiation by local biopsy will be required for cases of suspected rejection. For all participants who undergo at least one kidney biopsy during the first year post-transplant (either for cause or per institutional protocol), the kidney biopsy performed closest to the 12 month visit (but no later than 14 months post-transplant) will be submitted for central pathological grading. All biopsies that are submitted for central pathology review will be evaluated and scored using the most recent version of the 2007 Update to the Banff '97 Classification by a central pathologist blinded to clinical results and patient identifiers [Solez et al, 2008].

Inclusion/Exclusion Criteria:

Inclusion:

Subject is eligible for the study if all of the following apply.

1. Kidney transplant patient ≥ 16 years and ≤ 70 years old.
2. Institutional Review Board (IRB)-/Independent Ethics Committee (IEC)-approved written Informed Consent and privacy language as per national regulations (e.g., HIPAA Authorization for US sites) must be obtained from the subject or legally authorized representative prior to any study-related procedures (including withdrawal of prohibited medication, if applicable). For subjects < 18 years old, informed consent must be obtained from the subject's parent(s) or legal guardian(s); subject Assent (where appropriate) must be given.
3. Recipient of a de novo kidney from a living or deceased donor. Note: Recipient of an *en bloc* deceased donor kidney transplant from a pediatric donor ≥5 years of age AND weighing greater than 20 kg is allowed.
4. If deceased donor, a Kidney Donor Profile Index (KDPI) ≤ 85 [donation after circulatory death (DCD) and what was previously known as extended criteria donor (ECD) organ recipients *are* eligible for enrollment provided KDPI ≤ 85].
5. Removed
6. At least one antigen mismatch at major MHC (class I or class II).
7. Willingness to comply with study protocol.
8. Subject agrees not to participate in another investigational drug study while on treatment.

9. Female subject must be either:
    a. Of non-child-bearing potential,
        i. Post-menopausal (defined as at least 1 year without any menses) prior to screening, or
        ii. Documented surgically sterile or status post-hysterectomy
    b. Or, if of childbearing potential,
        i. Agree not to try to become pregnant during the study and for 90 days after the final study drug administration
        ii. And have a negative serum or urine pregnancy test within 7 days prior to transplant procedure
        iii. And, if heterosexually active, agree to consistently use two forms of highly effective birth control (at least one of which must be a barrier method) which includes consistent and correct usage of established oral contraception, established intrauterine device or intrauterine system, or barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository or vasectomy in the male partner, starting at screening and throughout the study period and for 90 days after the final study drug administration.
10. Male subject and their female spouse/partners who are of childbearing potential must be using highly effective contraception consisting of two forms of birth control (one of which must be a barrier method) starting at screening and continuing throughout the study period and for 90 days after the final study drug administration (acceptable forms of birth control are listed in Inclusion Criterion 9).
11. Male subject must not donate sperm starting at screening throughout the study period and for 90 days after the final study drug administration.
12. Female subject must agree not to breastfeed starting at screening and throughout the study period, and for 90 days after the final study drug administration.
13. Female subject must not donate ova starting at screening and throughout the study period, and for 90 days after the final study drug administration.
14. Will be receiving induction immunotherapy (either T-cell depleting agent, anti-CD52 monoclonal antibody, or IL-2 co-stimulation blocker), with dose and frequency of the chosen induction agent determined by local standard of care. Steroid-only induction therapy does not satisfy this criterion.

Waivers to the inclusion criteria will **NOT** be allowed.

Previous kidney transplants will be permitted.

Patients who are receiving a secondary transplant and who previously received Astagraf XL or who are currently on Astagraf XL as a component of maintenance immunosuppression and re-listed for transplant will be eligible to enroll in ASTOUND and will be randomized at the time of transplant to either cohort.

Exclusion:

Subject will be excluded from participation if any of the following apply.

1. Patient is known to have a positive test for latent tuberculosis (TB) and has not previously received adequate anti-microbial therapy or would require TB prophylaxis after transplant.
2. Uncontrolled concomitant infection or any unstable medical condition that could interfere with study objectives.
3. Significant liver disease, defined as having, during the past 28 days, consistently elevated AST (SGOT) and/or ALT (SPGT) levels greater than 3 times the upper value of the normal range of the investigational site.
4. Patient currently taking or maintained on another form of extended-release tacrolimus following his/her transplant procedure.
5. Patient who will be maintained on a non-tacrolimus-based maintenance immunosuppressive regimen following his/her transplant procedure.
6. Patient currently taking, having taken within 30 days, or who will be maintained on an mTOR inhibitor following his/her transplant procedure.
7. Use of an investigational study drug in the 30 days prior to the transplant procedure.
8. Contraindication or hypersensitivity to drugs or any of their components that constitute the immunosuppression regimen.
9. 6 Ag match or zero mismatch at major MHC (class I or class II).
10. Receipt of an ABO-incompatible organ. *Note: A2 donor to O recipient or A2 donor to B recipient is considered ABO-compatible and not excluded by this criterion.*
11. Removed
12. Removed
13. The presence of current *or historic*, pre-formed anti-HLA DSA against the current donor (evidence of pre-formed, *non-donor* HLA is not exclusionary) as defined by a subject meeting any of the following criteria*:
    a. Positive virtual crossmatch,
    b. Positive T- or B-cell crossmatch by NIH antiglobulin lymphocytotoxicity method,**
    c. Positive T- or B-cell flow cytometry crossmatch defined by the MFC criteria used by the center's HLA lab for their local proficiency testing,**
    d. An MFI greater than or approaching 1000 using flow cytometry/Luminex-based, specific anti-HLA antibody testing.

    * Patients *are* eligible to enroll with a negative virtual crossmatch if used in lieu of a physical crossmatch, if, in the opinion of the patient's attending physician, use of such is required to obviate the accrual of excessive ischemia time. However, continued participation is predicated on the performance of the physical crossmatch within 48 hours of transplant. If the physical crossmatch is positive, the subject will be discontinued.

    ** If *b* or *c* above are positive secondary to a suspected positive auto-crossmatch, that is not exclusionary as long as *a* and *d* above are not met.
14. Receipt of desensitization, antibody-removal, anti-B-cell, or anti-plasma cell therapy in the 90 days preceding the transplant procedure.

15. Planned initiation (prior to transplant) of desensitization, antibody-removal, anti-B-cell, or anti-plasma cell therapy within 7 days of the transplant procedure.
16. Donor or recipient with known hepatitis C infection (HCV antibody positive), HIV infection (HIV antibody positive), acute hepatitis B infection (HBsAg positive, anti-HBc positive, IgM anti-HBc positive, anti-HBs negative) chronic hepatitis B infection (HBsAg positive, anti-HBc positive, IgM anti-HBc negative, anti-HBs negative), or equivocal hepatitis B status (HBsAg negative, anti-HBc positive, anti-HBs negative). Patients (donor or recipient) who have normal liver function tests (LFT) and who are either hepatitis C positive with a negative viral load or have natural or vaccine-acquired immunity from hepatitis B are not excluded by this criterion.
17. Primary focal segmental glomerulosclerosis.
18. Subject has a current malignancy or history of malignancy (within the past 5 years), except non-metastatic basal or squamous cell carcinoma of the skin or carcinoma-in-situ of the cervix that has been successfully treated.
19. Recipient of multi-organ or dual kidney transplants (inclusive of current transplant and any prior non-renal transplants). *Note: Patients with prior kidney transplants are eligible*.
20. Recipient of an *en bloc*, pediatric deceased donor kidney from a donor less than 5 years of age OR weighing less than 20 kg.
21. Prior graft loss secondary to CMV or BK nephropathy.
22. Prior history of invasive organ disease in the presence of CMV or BKV or clinically significant CMV viremia.
23. History of clinically significant (per investigator's discretion) BK viruria
24. Any condition which, in the investigator's opinion, makes the subject unsuitable for study participation.
25. Planned complete steroid avoidance (Steroid initiation and subsequent taper / withdrawal will be allowed and will be under the purview of the treating physician.)
26. Planned receipt of post-transplant prophylactic HCV treatment.

Waivers to the exclusion criteria will NOT be allowed.

Investigational Product(s):

Tacrolimus, extended-release, oral (Astagraf XL); 0.5 mg, 1 mg, 5 mg capsules

Dose(s):

Tacrolimus, extended-release (Astagraf XL); administered once daily at initial weight-based dose of 0.15 mg/kg. Dosing and monitoring thereafter predicated on clinical judgment to effect a minimum whole blood tacrolimus concentration of at least 6 ng/mL. A prolonged significant reduction of tacrolimus or withdrawal of tacrolimus will result in study discontinuation (see discontinuation criteria).

Mode of Administration:

Tacrolimus, extended-release (Astagraf XL); oral

IF medically indicated, subjects randomized to the Astagraf XL treatment arm who are unable to tolerate oral administration may be administered intravenous (IV) tacrolimus. In such cases, the patient must resume conventional oral Astagraf XL as soon as possible. Administration of IV tacrolimus may not exceed a period of seven consecutive days. **It is important to note that Astagraf XL cannot be given intravenously. Astagraf XL should not be given via NGT due to its extended release characteristics.** IV tacrolimus will not be provided by the Sponsor.

Comparative Drug(s):
Tacrolimus, immediate-release, oral; 0.5 mg, 1 mg, 5 mg capsules

Dose(s):
Tacrolimus, immediate-release; administered twice daily per clinical judgment of supervising physician (dosing and monitoring in accordance with center protocol) to effect a minimum whole blood tacrolimus concentration of at least 6 ng/mL. A prolonged significant reduction of tacrolimus or withdrawal of tacrolimus will result in study discontinuation (see discontinuation criteria).

Mode of Administration:
Tacrolimus, immediate-release; oral

IF medically indicated, subjects randomized to the immediate-release tacrolimus treatment arm who are unable to tolerate oral administration may be administered tacrolimus via NGT or IV, for no more than seven days. In such cases, the patient must resume conventional oral immediate release tacrolimus as soon as possible. Administration of immediate release tacrolimus via NGT or IV may not exceed a period of seven days. Additionally, a subject may not receive more than seven consecutive days of combined NGT and IV administration in cases where a subject is switched from one therapy to another. IV tacrolimus will not be provided by the Sponsor.

Drug(s) for Screening:
Not applicable.

Advanced Measures to Treat Rejections:
The need for advanced therapy to treat rejection will be determined by the supervising physician and predicated on the emergence of steroid-refractory antibody-mediated rejection. Typical rescue drugs employed under these conditions include, but are not limited to anti-thymocyte globulin, intravenous immunoglobulin (IVIG), rituximab, bortezomib, and eculizumab.

Concomitant Medication Restrictions or Requirements:
All patients will receive induction immunotherapy (either T-cell depleting agent, anti-CD52 monoclonal antibody, or IL-2 co-stimulation blocker – steroid-only induction therapy is not allowed). The dose and frequency of the chosen induction agent will be determined by the patient's treating physician and administered in conjunction with the participating transplant center's de novo kidney immunosuppression protocol. Anti-CMV, fungal, bacterial, and pneumocystis prophylaxis will be determined per local standard of care.

Non-tacrolimus based immunosuppressive regimens (i.e. cyclosporine, everolimus, sirolimus, belatacept), as well as all forms of extended-release tacrolimus other than Astagraf XL, are prohibited during the course of the study. As a clarifying statement, all patients must be maintained on MMF or its equivalent during the course of the study. Due to the influence the following medications have on tacrolimus blood concentrations, antiviral medications used in HCV and HIV treatment, as well as isoniazid, Rifampin, ethambutol, and pyrazinamide are also prohibited.

Duration of Treatment:
Study enrollees will receive study medication (single daily dose of Astagraf XL or standard of care twice daily, immediate-release tacrolimus) for up to two years following their transplant procedure. In cases where delayed renal function is suspected following transplantation, therapy initiation with either tacrolimus agent can be delayed for up to 7 days.

Formal Stopping Rules:

A decision to terminate the study early or extend to a second year will be predicated on the event rate in the control arm once 50% of control patients have completed one year of therapy. At that time, the estimated proportion will be used to calculate the Beta-binomial posterior Bayesian probability that the primary endpoint's incidence rate will, indeed, be at least 20%.

If this probability is ≥ 70%, the following rules apply:

1) The study will continue to enroll to completion.
2) The study duration will be such that patients will complete at least one year of study visits.
    a) Patients who have not yet completed one year of study (at the time of the interim analysis) will complete the study at the 12m visit.
    b) Patients who have already completed the 12m visit (at the time of the interim analysis) will complete the study at their next scheduled study visit.
3) Data analysis will be performed once all patients complete 12 months of study.

If this probability is < 70% and ≥ 30%:

1) The study will continue to enroll to completion.
2) The study duration will be 24 months; patients will complete the study at the 24 month visit.
3) Data analysis will be performed once all patients complete 24 months of therapy.

If this probability is < 30%, the study will be terminated at that time (50% of patients completing 12 months of therapy).

1) The study will be terminated upon completion of the interim analysis (done when 50% of patients have completed 12 months of therapy).
2) Patients will be discontinued from the study at their next scheduled study visit.

Discontinuation Criteria from Study for Individual Subjects:

- Subject develops allograft loss (as defined by subject death, retransplantation, transplant nephrectomy, or return to dialysis of ≥ 6 consecutive weeks duration);
- Subject develops unacceptable toxicity or is withdrawn at the discretion of the patient's supervising physician;
- Subject is lost to follow-up despite reasonable efforts by the investigator to locate the subject;
- Subject withdraws consent for further treatment or expires during the course of the study;
- Subject begins taking any prohibited medications;
- Subject undergoes a second organ transplant;
- Conversion to a non-tacrolimus-based maintenance regimen in either study arm if required to manage toxicities;
- Removal from an MMF-containing immunosuppressive regimen (or its equivalent);
- Failure to achieve a tacrolimus whole blood concentration ≥ 6 ng/mL for 4 consecutive weeks during the first 6 weeks following transplantation;
- Permanent withdrawal of tacrolimus;

- Interruption or prolonged significant reduction of tacrolimus (as an example, a 50% reduction or dose at a level of less than 3.5ng/mL) for periods exceeding 4 weeks;
- Conversion *by the investigator* to the therapy of the other study treatment arm (i.e., subject randomized to Astagraf XL is converted to immediate release tacrolimus OR subject randomized to immediate release tacrolimus is converted to Astagraf XL. Cases of inadvertent and temporary conversion [i.e., dispensing error] do not meet this discontinuation criterion);
- Subject is not initiated on Astagraf XL or immediate-release tacrolimus within 7 days of transplant;
- Gross non-compliance with protocol: The medical monitor or investigator may request permanent study discontinuation in the event of a major protocol deviation such as administration of prohibited concomitant medication, lack of cooperation, or noncompliance.

Endpoints for Evaluation:
Primary:

The primary endpoint is the combined incidence of either DSA or IA on peripheral blood molecular profiling at one year or at the conclusion of the study. For this purpose, DSA will be considered as a categorical (binary) variable with positivity determined at a threshold criteria approaching MFI = 1000 at any time during the study. For reporting purposes, IA will be considered either present or absent using the Trugraf™ v2.0 molecular assay. For the purposes of the study, a negative designation (Trugraf TX Normal) will be referred to as Immune Quiescence (IQ). Due to operating characteristics of the assay, a positive designation will be considered evidence of Immune Activation (IA) in all patients.

The modified Full Analysis Set (mFAS) will be used for the primary endpoint analysis.

Secondary:

The assessment and comparison of the incidence of DSA between treatment groups will rely on the binary variable of DSA positivity at a threshold criteria approaching an MFI of 1000. Comparisons of the cohorts with respect to the various molecular designations as well as correlating the results with DSA will rely on the categorical (binary) variable of positivity using the Trugraf™ v2.0 molecular assay.

Comparisons between treatment cohorts regarding incidence will rely upon assessments for each of the following results at any point in the study:

- DSA
- IA)
- TG
- Acute and chronic forms of ABMR
- C1q-binding DSA
- HLA-DQ DSA
- DSA IgG_3 isotype
- Required antibody reduction

- eGFR at various thresholds (less than 30, 40, and 50 mL/min/1.73 m^2) and whether a five-point decline in eGFR occurs
- Graft loss (defined as subject death, retransplantation, transplant nephrectomy, or a return to dialysis for at least a 6 week duration)
- Death
- BPAR (inclusive of ABMR and TCMR)
- Loss to follow-up

Time-to first occurrence will be assessed for each of the following: DSA, HLA-DQ DSA, C1q-binding DSA, DSA IgG3 isotype, IA, TG, select BANFF histology grades (acute and chronic forms of ABMR, acute and chronic active TCMR, borderline changes, and IFTA), mortality, and local BPAR.

The frequency of the type of antibody reduction employed will be assessed.

Patient MFI and eGFR values will be assessed over time.

Assessments of histopathology in biopsies will be made using the following endpoints:

- Incidence of ci, ct, and ptc scores greater than one
- Biopsy scores for g, t, v, i, cg, ct, ci, cv, ah, ptc, and mm

The safety endpoint for AEs will be the incidence of each type of AE based on system organ class and using the preferred term from the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA).

Persistence of DSA and IA will be additional endpoints for comparisons in patients who develop IA and DSA on molecular profiling.

Exploratory:

- The incidence of a second two-part composite endpoint encompassing the incidence of acquiring DSA or positive evidence of TG on a centrally-interpreted biopsy during the first year post-transplant or by the conclusion of the study
- Expression results, mapped to known immunological pathways implicated in immune-mediated, kidney transplant tissue injury.
- With appropriate informed consent, long-term graft and patient survival in study participants by future linking to the Scientific Registry of Transplant Recipients (SRTR) following completion of ASTOUND.
- The economic burden of treating patients with molecular evidence of immune activation and DSA formation may be assessed using Medicare claims data (or commercial payer cost data in patients required to transfer from Medicare to private insurance).
- Time to first DSA or IA positive test results
- Outcomes in like patients between cohorts managed with maintenance protocol biopsy vs. biopsy for cause with respect to the composite endpoint, the components thereof, complications, and resource utilization.

Statistical Methods:

Sample Size Justification:

The sample size for this study was determined based upon a comparison of the rate of the two-part composite endpoint in the Astagraf XL group compared to the immediate-release tacrolimus group. Assuming a rate of 20% would be observed for the immediate-release tacrolimus group compared to a rate of 10% for the Astagraf XL group, 220 subjects per group are needed to achieve an 80% power to detect a difference between Astagraf XL and immediate-release tacrolimus with a two-sided alpha level of 0.05. The study will enroll 275 subjects/group (550 total) to allow for 20% dropout.

Efficacy:

The analysis of efficacy will be conducted using the mFAS.

The primary efficacy endpoint is the incidence of any one of the following in the first two years post-transplant:

- DSA formation
- IA

The primary endpoint will be analyzed at a single time point per Section 7.4.1

The incidence of the primary endpoint will be summarized by treatment group with frequencies and percentages. Differences between treatment groups will be analyzed using logistic regression and controlled using pre-defined covariates.

For subjects with missing or incomplete endpoint data who did not permanently switch therapy or were discontinued from the study prior to DSA/IA event (who are lost to follow up, or those who expire for any reason), negative DSA/IA values will be imputed unless there is an otherwise positive result.

The hypothesis for comparison is as follows:

- H0: The probability of incidence for the primary endpoint is the same between patients receiving Astagraf XL and patients receiving immediate-release tacrolimus.
- H1: The probability of incidence for the primary endpoint is not the same between patients receiving Astagraf XL and immediate-release tacrolimus.

Hypothesis testing will be performed using a two-sided test with a 0.05 significance level. The 95% confidence interval for the odds ratio of the primary endpoint between Astagraf XL and twice daily tacrolimus will be included.

Analyses of treatment group differences for secondary endpoints will be evaluated by time and overall. The scope of these analyses will include the following: DSA formation, IA, TG, acute and chronic forms of ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, the requirement for antibody reduction therapy, chronic graft dysfunction, graft loss, local BPAR, mortality, loss to follow-up, ordinal antibody strength, MFI score, histopathology assessments, and tacrolimus trough levels.

Pharmacokinetics:
Not applicable.

Pharmacodynamics:
Not applicable.

Safety:
Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The number and percentage of AEs, SAEs, AEs leading to discontinuation, and AEs related to study drug will be summarized by system organ class, preferred term and treatment group. The number and percentage of AEs by severity will also be summarized. All AEs will be listed.

Interim Analyses:
Not applicable.

V. FLOW CHART AND SCHEDULE OF ASSESSMENTS

Flow Chart

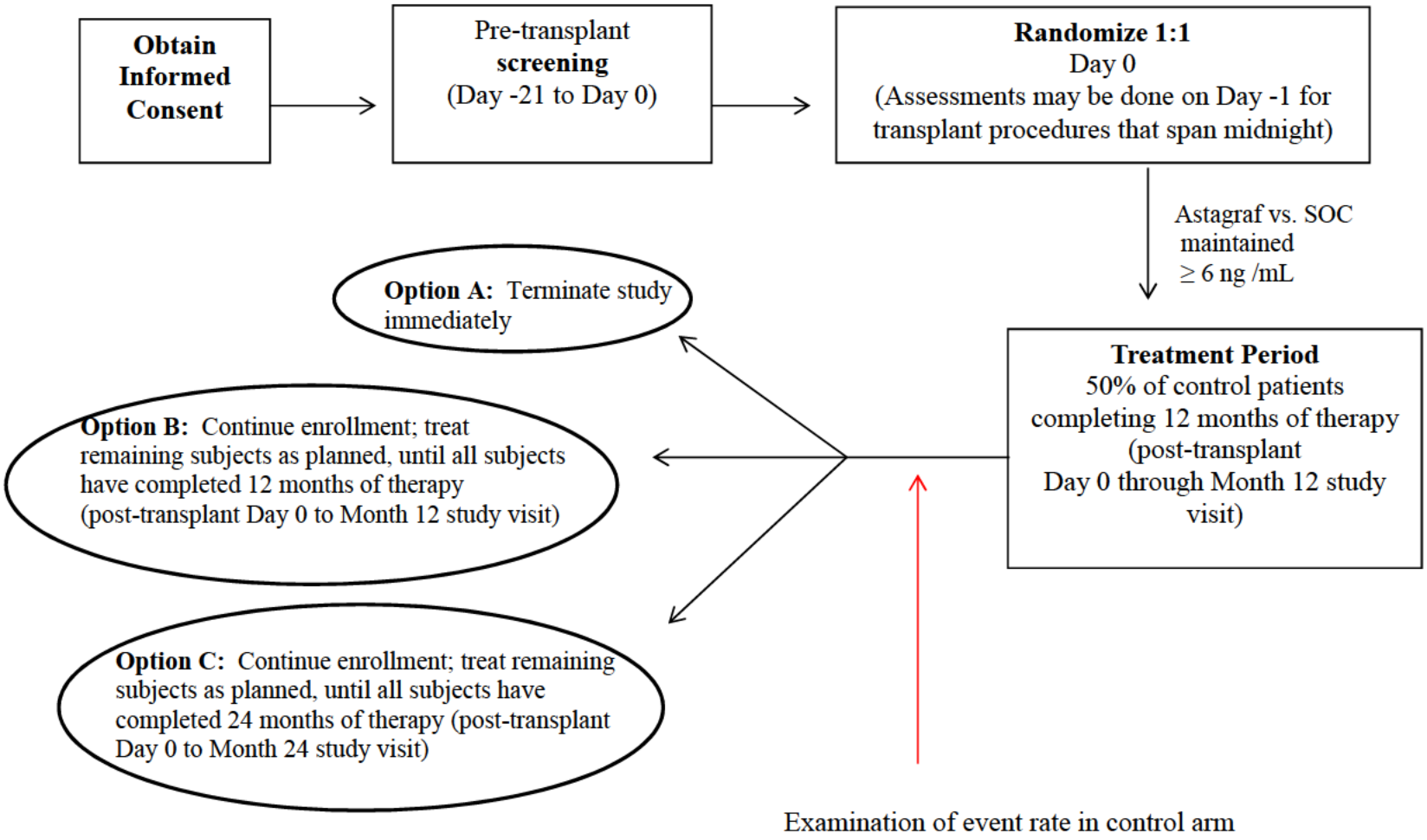


Table 1 Schedule of Assessments

Study Day	Screening (-21 to 0)[1]	0[1]	30	90	180	270	365	455[2]	545[2]	635[2]	730[3]	Unscheduled Visit[4]
Week	-3 to 0	0	4	12	24	36	52	64	76	88	104	n/a
Window (days)			± 21	± 21	± 21	±21	± 21	± 21	± 21	± 21	± 21	n/a
Screening: Inclusion/Exclusion Criteria	X											
Informed Consent/Assent	X											
Demographics & Medical History	X	X[5]										
Height, Pre-op Weight[6]	X											
Concomitant Medications[7]	X	X	X	X	X	X	X	X[8]	X[8]	X[8]	X[8]	X
Physical Exam	X											
Transplant Information and Donor Information/HLA typing[9]		X										
Randomization[10]		X										
Astagraf XL Arm: Study Drug Dispensing[11]		X	X	X	X	X	X	X	X	X	X	
BID Tacrolimus Arm: Study Drug Dispensing[12]		X →	→	→	→	→	→	→	→	→	X	
Clinical Labs Recording		X[13]	X[13]	X[13]	X[13]	X[13]	X[13]	X[14]	X[14]	X[14]	X[14]	
Kidney Biopsy Results[15]		X →	→	→	→	→	→	→	→	→	X	
Outcomes Review[16]			X	X	X	X	X				X	X
Adverse Events Recording[17]		X →	→	→	→	→	→	→	→	→	X	
Blood Samples for DSA/anti- HLA/IgG_3 isotyping, C1q-binding DSA[18]		X[19]	X	X	X	X	X				X	
Blood Samples for Molecular Diagnostics[18]			X	X	X	X	X				X	
Blood Sample for Potential Future Analysis (optional)[18, 20]			X	X	X	X	X				X	
BK Viremia Review[21]		X →	→	→	→	→	→	→	→	→	X	

Footnotes appear on next page

1. Per convention, Day 0 of transplant is the date of transplant completion (revascularization). Assessments may be done on day -1 for transplant procedures that span midnight. Screening and day 0 may occur on the same day. In all cases, blood obtained for baseline antibody testing must be obtained prior to surgery. For subjects that have a separate screening visit prior to day of transplant, inclusion/exclusion criteria will be reassessed prior to transplant to ensure continued eligibility.
2. Visits in 2nd year of follow-up to include quarterly drug dispensing visits for Astagraf XL cohort at which time AEs will be recorded in the eCRF. For patients receiving immediate-release tacrolimus in the control arm, AEs will be assessed by quarterly phone interview in the 2nd year and recorded in the eCRF.
3. Study visit at end of 2nd year to include measurement of DSA and molecular markers in both control and experimental groups along with the following clinical history: serum creatinine; urinary protein; interval biopsy results; and interval history of infection, BPAR, graft loss, or mortality.
4. Unscheduled visits may be performed at any time during the study whenever necessary to assess for or follow-up on adverse events, or if deemed necessary by the investigator.
5. For subjects whose screening visit occurred prior to day 0, any updates to medical history should be recorded at day 0 visit.
6. Height will only be obtained at the screening visit and dry weight will only be obtained at screening and pre-operatively on day 0.
7. Includes, but not limited to, concomitant immunosuppressant medication, tacrolimus dose/formulation(s)/manufacturer(s), recording of all anti-rejection therapy, and P_{450} inducers/inhibitors.
8. For the 2nd year of follow-up, only concomitant medications administered for a corresponding AE should be recorded in the eCRF.
9. Transplant information: type of transplant (living related, living non-related, DD, and whether organ was obtained in the setting of DCD), number of prior transplants and reason for prior graft loss (if applicable), total cold and warm ischemia time in hours and minutes, donor KDPI, ABO blood typing, HLA typing of donor and recipient, degree of HLA mismatch between donor and recipient, most recent panel reactive antibody testing (most recent cPRA level), and pre-transplant MFI status. Donor viral serology information (HBV, HCV, CMV and EBV), age, sex, height, weight, donor cause of death, ethnicity, ex vivo perfusion parameters, ABO typing, and results of any pre-implantation biopsies of the donor kidney.
10. Occurs at the pre-op visit, prior to transplant, if living donor (within 7 days). Randomization to occur upon hospital admission for deceased donors or for living donor recipients whose Screening and Day 0 visit occur on the same day.
11. Astagraf XL will be supplied by Astellas and dispensed by the participating institution. At the final study visit, subjects will return unused Astagraf XL. Additional Astagraf XL will not be provided to subjects at the final study visit.
12. After discharge from the hospital, patients randomized to receive twice daily immediate-release tacrolimus will receive study-provided vouchers and will be permitted to receive any immediate-release tacrolimus product available to them through normal dispensing mechanisms. At the final study visit, subjects will return unused vouchers; following the final study visit, vouchers previously provided to the subjects will be deactivated to prevent additional use. Additional vouchers will not be provided to subjects at the final study visit.
13. Recording of hematology, serum chemistries, urinalysis (including urine protein), tacrolimus trough concentrations, HgA1c, and whole blood tacrolimus concentrations obtained per SOC. See Section 5.4.3 for the components of the routine clinical labs that will be recorded. For all labs except whole blood tacrolimus concentrations and creatinine, only those labs obtained closest to targeted date of visit need to be recorded. For the Day 0 visit, pre-transplant labs should be recorded. For whole blood tacrolimus concentrations, at the Day 0 and Day 30 visits, only the concentration obtained closest to the targeted date of visit needs to be recorded. For the 90, 180, 270, and 365 day visits, all available outpatient tacrolimus concentration assessments done (per SOC) and available in the centralized medical records since the previous study visit should be recorded. For creatinine assessments, the assessment done closest to the targeted date of visit (obtained per SOC) as well as an additional assessment done at least one month prior to that assessment (if obtained per SOC) will be recorded on the eCRF.

Footnotes continued on next page

14. For visits following day 365, only serum creatinine levels, urinary protein, and tacrolimus trough concentrations obtained as part of SOC should be recorded. For all labs except creatinine, only labs obtained closest to targeted date of visit need to be recorded. For creatinine assessments, the assessment done closest to the targeted date of visit (obtained per SOC) as well as an additional assessment done at least one month prior to that assessment (if obtained per SOC) will be recorded on the eCRF.
15. All kidney biopsy results obtained during the study should be recorded in the eCRF. For all participants who undergo at least one kidney biopsy during the first year post-transplant (either for cause or per institutional protocol), the kidney biopsy performed closest to the 12 month visit (but no later than 14 months post-transplant) will be submitted for central pathological grading. Additionally, de-identified copies of all kidney pathology reports must be submitted to Astellas.
16. Inclusive of patient survival status, cause of death, rejection episodes, new diagnoses, and pathology review.
17. Adverse event collection will begin once informed consent/assent has been signed and continue throughout the subject's participation in the study. All new SAEs occurring up to 7 days after the end of study visit must be reported. *Note: The transplant procedure that occurs on Day 0 is not considered an AE or an SAE.*
18. Assessed via central labs.
19. On Day 0, sample drawn prior to transplant procedure and stored to confirm pre-op DSA (will be analyzed only as needed). Sample may be drawn on Day -1 for morning transplant procedures. De-identified copies of local typing reports (for donor and recipient typing) and recent local pre-transplant DSA testing results (if performed) will also be submitted to the central lab.
20. Patients will have the option of having an additional sample collected at each phlebotomy session for storage and analysis in future trials of transplant outcomes and biomarker discovery.
21. If a subject develops clinically significant BK viremia (as assessed per SOC) during study participation, the peak viremia level obtained per SOC will be retrospectively recorded in the eCRF at the time of subject's study discontinuation or completion.

1 INTRODUCTION

1.1 Background

Despite lower rates of acute rejection and short-term improvements in patient and graft survival, the rate of late allograft loss following kidney transplantation has remained unchanged for more than 30 years [Lamb et al, 2011; Kaneku and Terasaki, 2006]. During this time, chronic allograft nephropathy (CAN) became recognized as the major cause of late kidney allograft failure [Loupy et al, 2009; Seron & Moreso, 2007; Myers et al, 1984] and numerous attempts were made to correlate the progressive fibrosis and vascular injury seen on biopsy with the use of calcineurin inhibitors (CNIs) [Nankivell et al, 2003]. However, over the past three decades, the link between chronic nephrotoxicity and the use of CNIs has proven largely elusive. For example, in reports examining independent risk factors for chronic histological change, low CNI levels were often cited [Shihab et al, 2008; Ekberg, Grinyo, et al, 2007; Naesens et al, 2007; Nankivell et al, 2004; Seron et al, 2002]. Similarly, when investigators have attempted to minimize, avoid, or withdraw the use of tacrolimus during the course of a randomized clinical trial, they have routinely been confronted with inferior outcomes in the reduced tacrolimus/tacrolimus avoidance arms [de Sandes-Frietas et al, 2015; Hricik et al, 2015; Flechner et al, 2011; Weir et al, 2011; Durrbach et al, 2010; Vincenti et al, 2010; Lebranchu et al, 2009; Dean et al, 2008; Ekberg, Tedesco-Silva, et al, 2007; Srinvias et al, 2007; Ciancio et al, 2006; Larson et al, 2006; Medez et al, 2005; Meier-Kreische et al, 2005; Halloran, 2000; Andoh et al, 1996].

Late allograft injury as evidenced by moderate-to-severe arteriolar hyalinosis (a traditional hallmark of CNI nephrotoxicity) is found to a similar extent in patients never exposed to CNIs at 5 years [Stegall et al, 2011]. Likewise, when 1317 cases of allograft loss were re-examined by El-Zoghby and colleagues, alloimmunity was found to be the leading cause of kidney graft failure, prompting a reclassification of all biopsies with an original diagnosis of idiopathic interstitial fibrosis or CNI toxicity [2009]. There have also been several recent studies in which biopsies were performed in the setting of new-onset graft dysfunction. In the Deterioration of Kidney Allograft (DeKAF) Trial, the risk of graft failure was unrelated to immunosuppression and actually intensified in patients presenting with DSA. In fact, the severity of clinical injury strongly correlated with the magnitude of antibody response [Gaston et al, 2010]. In another large study, the Edmonton group demonstrated that the majority of kidney failures were not attributable to CNI use, but rather, rejection, non-adherence, and the presence of alloantibody. In this study and others, the incidence of allograft dysfunction hastened by the presence of antibody increased steadily over time, becoming more noticeable with increasing time from transplant [Wiebe et al, 2015; Sellares et al, 2012].

Indeed, the link between subclinical immune-mediated graft injury and progressive worsening of graft function is now firmly established. However, given that the appearance of chronic renal allograft injury may also take years to manifest, any randomized trial involving new forms of therapy and utilizing this parameter as an endpoint may still be difficult to conduct from a practical standpoint [Stegall et al, 2015]. For this reason, the identification of

an appropriate surrogate endpoint becomes an important consideration. In the context of long-term investigations, a surrogate offers a theoretical advantage as a study endpoint in lieu of a parameter such as graft loss; the primary benefit being to decrease the interval between therapeutic intervention and the ability to determine efficacy [Stegall et al, 2015]. Unfortunately, many prospective biomarkers are relatively non-specific, poorly-correlated, or may be causally influenced by a disease process without, itself, influencing the desired clinical outcome [Stegall et al, 2015; Fleming, 2005]. Therefore, in order to establish that the effect of a particular intervention on a clinical efficacy endpoint is reliably predicted by the effect of that intervention on a surrogate, Prentice identified two conditions that, if satisfactorily validated, would be sufficient to authenticate any proposed biomarker as an appropriate surrogate. First and foremost, the biological marker must be correlated with the clinical endpoint – in this case, graft failure. Secondly, it must fully capture the net effect of an intervention (immunosuppression) on the clinical efficacy endpoint [Prentice, 1989].

Histologic changes, such as transplant glomerulopathy (TG) or subclinical inflammation, are moderately good predictors of subsequent graft loss [Issa et al, 2008; Cosio et al, 2005]. Patients with graft fibrosis, especially with inflammatory cell infiltrates, have worse outcomes and return to dialysis and/or undergo repeat transplantation with greater frequency. Likewise, subclinical histologic abnormalities found only on protocol biopsies have been associated with the development of chronic injury and decreased renal function over time [Loupy et al, 2009; Moreso et al, 2006; Nankivell et al, 2004; Shishido et al, 2003; Nankivell et al, 2001; Kirk et al, 1999; Rush et al, 1994].

While some transplant centers routinely perform protocol biopsies as part of their standard of care (SOC), others do not, despite the fact that there is compelling evidence that subclinical acute rejection present in the graft leads to chronic rejection and early graft loss. For this reason, a search for molecular biomarkers which can predict these phenomenon has been undertaken in the allograft, the urine, and the blood [El Ters et al, 2013; Li et al, 2013; Loupy et al, 2009; Kee et al, 2006; Moreso et al, 2006; Nankivell et al, 2004; Shishido et al, 2003; Nankivell et al, 2001; Kirk et al, 1999], the latter two of which having been proposed as a minimally invasive way to diagnose and monitor subclinical rejection, to optimize and personalize immunosuppression decisions, and as a means to potentially reduce the incidence of chronic rejection, which, in turn, could lead to significantly improved graft survival and function over the long-term. In fact, the need to complement histopathological grading with an interpretation of accompanying molecular signals was recently identified at the 2013 Banff Conference on Allograft Pathology [Haas et al, 2014]. However, up to now, relatively few individual molecular transcripts have been shown to correlate with chronic injury. Interestingly, an approach examining global genomic gene expression profiling in the peripheral blood was recently described. Using multiple, three-way classifier tools to validate 200 of the highest value probesets, the groups at Northwestern and Scripps have identified a variety of molecular signatures in the peripheral blood. Mapping these gene signatures to what is concomitantly revealed in biopsy specimens has demonstrated a sensitivity ranging from 82% to 100%, a specificity of 76% to 95%, a positive predictive value of 76% to 95%, and a negative predictive value of 79% to 100% [Kurian et al, 2014].

If similar methodology can be used to predict subclinical acute rejection, before the appearance of kidney dysfunction from the cumulative effects of tissue injury, this, too, could satisfy the necessary conditions for use as a surrogate marker.

Given the wealth of accumulated evidence for fulfillment of the Prentice criteria, DSA also seems to be a relatively good surrogate marker to examine the effects of chronic antibody-mediated injury. For example, the disease process itself, but also the contribution of DSA, has been well-elucidated with regard to chronic, alloimmune injury [Einecke et al, 2009; Sis et al, 2007]. Further strengthening its role as a surrogate, DSA appears to act late in the biological injury pathway, and based on clinical insight and empirical evidence, is likely the predominant mechanism precipitating chronic, immune-mediated graft injury [Jordan & Vo, 2014; Loupy et al, 2012]. In this regard, studies examining the impact of DSA on transplant outcomes allude to a quantitative epidemiologic relationship as well [Marfo et al, 2014; Alachkar et al, 2012; Zachary & Reinsmoen, 2011; Lefaucheur et al, 2010]. Additionally, newer reports are also beginning to link antibody formation to non-adherence [Wiebe et al, 2015; Wiebe et al, 2012]. The "real world" evidence linking DSA to at least two primary clinical outcome measures [antibody-mediated rejection (ABMR) and graft survival] has been extensive as well as consistent. For example, DSA dramatically accelerates the progression of histological lesions [Hill et al, 2011], is independently associated with TG [Issa et al, 2008; Gloor et al, 2007], and is a strong predictor of both ABMR and graft failure [Guidicelli et al, 2015; Wiebe et al, 2015; DeVos et al, 2014; Kim et al, 2014; Banasik et al, 2013; Everly et al, 2013; Freitas et al, 2013; DeVos et al, 2012; Ginevri et al, 2012; Sellares et al, 2012; Wiebe et al, 2012; Willicombe et al, 2012; Cooper et al, 2011; Ntokou et al, 2011; Gaston et al, 2010; El-Zoghby et al, 2009; Hidalgo et al, 2009; Lachmann et al, 2009; Mao et al, 2007; Cardarelli et al, 2005; Campos et al, 2006; Zhang et al, 2005]. It is also highly pragmatic that the measurement of DSA is relatively non-invasive and unlike biopsy, can be performed sequentially in the same patient.

Recent publications describe that the major causes for de novo development of DSA relate primarily to medication non-adherence and immunosuppression minimization or elimination protocols [Matas & Gaston, 2015; Nickerson & Rush, 2015; Jordan & Vo, 2014; Issa et al, 2013]. Therefore, strategies which improve adherence and optimize exposure to immunosuppression should be evaluated to reduce the consequences of alloimmune-mediated injury. Astagraf XL® is an oral, extended-release formulation of tacrolimus developed to enable a once daily dosing regimen with a similar safety and efficacy profile as Prograf®. Available in the European Union as Advagraf® since 2007, its decreased intrapatient variability (IPV) [Stifft et al, 2014; Kurnatowska et al, 2011; Wu et al, 2011] and flatter kinetics [van Hooff et al, 2012; Alloway et al, 2011] should ensure a more consistent immunosuppression exposure over the long-term and the once daily dosing has been described as improving medication adherence [Sabbatini et al, 2014; Doesch et al, 2013; Eberlin et al, 2013; Kuypers et al, 2013]. Given the negative impact of IPV on kidney transplant outcomes [Sapir-Pichhadze et al, 2014; Pollock-Barziv et al, 2010] and the association of non-adherence with DSA production [Wiebe et al, 2015; Wiebe et al, 2012],

the ability to target both with a single agent may be a highly relevant mechanism to improve long-term outcomes.

To this end, Astellas proposes a prospective, randomized, controlled trial comparing two immunosuppressant agents, specifically powered and designed to detect a difference in surrogates of chronic immune-mediated graft loss: DSA and real-time molecular profiling from individual patient, peripheral blood samples. Both components will be combined as part of a two-part composite endpoint designed to test the hypothesis that the consistency of immunosuppression with Astagraf XL decreases the development of DSA and reduces the molecular signature of immune activation (IA). Accordingly, this study will perform tests of correlation between DSA and peripheral blood molecular profiling as well as correlate each with information gained from SOC or institutional-derived protocol biopsies. In the course of this study, patients in the control arm will receive immediate-release formulations of tacrolimus in accordance with local SOC dispensing practices, thus preserving the real-world conditions in which the patient could receive any manufacturer's approved product. In this manner, it is hoped that this research will improve the understanding of chronic alloimmune injury and provide greater insight into how tacrolimus exposure impacts DSA production.

1.2 Clinical Data

1.2.1 Clinical Summary

Pharmacokinetics of Tacrolimus (Prograf) in Humans

Following oral administration, tacrolimus is generally rapidly absorbed (t_{max} of approximately 1.5 hours). The mean oral bioavailability is low, approximately 20% to 25%, with a large amount of variation between individuals. The drug binds strongly to erythrocytes resulting in the distribution ratio of whole blood/plasma concentrations of tacrolimus of approximately 20:1. In plasma, the drug is highly bound to plasma proteins (> 98.8%), mainly to serum albumin and alpha-1-acid glycoprotein. Tacrolimus is also extensively distributed in the body as indicated by a large steady state volume of distribution.

Systemically available tacrolimus is cleared by hepatic metabolism, specifically, from isoforms of the CYP3A system, a fact that contributes to a significant first pass effect. Pre-systemic metabolism by the gastrointestinal CYP3A and the ATP-binding cassette transporter protein (ABC-transporter), p-glycoprotein, has also been demonstrated and is considered responsible for the limited oral bioavailability of tacrolimus.

Tacrolimus is primarily eliminated in the bile; urinary excretion amounts to < 2% of the dose and < 1% as unchanged drug.

As tacrolimus is metabolized via CYP3A system enzymes, concomitant use of substances known to inhibit or induce CYP3A system enzymes may influence the metabolism of tacrolimus and thereby increase or decrease blood levels. Tacrolimus, itself, is an inhibitor of CYP3A4; thus, concomitant use of tacrolimus with other drugs known to be metabolized by CYP3A4-dependent pathways may, in turn, affect the metabolism of such drugs (e.g., cortisone, testosterone). Tacrolimus is also extensively bound to plasma proteins; therefore,

interactions with other drugs known to have high affinity for plasma proteins should be considered (e.g., NSAIDs, oral anticoagulants or oral antidiabetics).

Pharmacokinetics of Tacrolimus (Astagraf XL/Advagraf) in Humans

Biopharmaceutics in Healthy Volunteers

Absorption is variable and the mean oral bioavailability of tacrolimus (investigated with the Prograf formulation) is in the range of 20% to 25% (individual range in adult patients of 6% to 43%). The oral bioavailability of tacrolimus for Advagraf or Prograf was approximately 35% lower in the evening relative to morning administration. Bioavailability was also reduced when Advagraf or Prograf capsules were administered with, or up to, 1.5 hours after consumption of food. It is therefore recommended that Advagraf or Astagraf XL capsules be taken consistently in the morning either with or without food.

Pharmacokinetics in De Novo Transplant Patients

Two phase 2 pharmacokinetic studies in de novo liver and de novo kidney transplant patients have been performed in order to compare the pharmacokinetics of tacrolimus for the Advagraf and Prograf formulations. The pharmacokinetic data from the study in de novo kidney transplant recipients revealed that the systemic exposure to tacrolimus on day 1 was lower for Advagraf than for Prograf despite similar doses. By day 4, systemic exposure as measured by trough levels was similar for both formulations.

To obtain more data on the pharmacokinetics of tacrolimus for Advagraf during the early post-transplant period, a phase 3 study protocol, in which the first dose of tacrolimus was given preoperatively, included a pharmacokinetic substudy which was conducted in selected centers. This pharmacokinetic substudy aimed to obtain information on the pharmacokinetics of tacrolimus for Prograf and Advagraf during the first 2 weeks after transplantation in de novo kidney transplant patients. The data for all patients who completed the study as planned and who provided 4 complete blood concentration-time profiles (day 1, day 3, day 7, and day 14) without major protocol deviations or factors which could influence the pharmacokinetic evaluations showed that the systemic exposure to tacrolimus (ln[AUC_{24}]) on day 1 was approximately 16% lower for Advagraf than for Prograf, although the mean total daily dose (mg/kg) was approximately the same. On days 3, 7 and 14, the ln(AUC_{24}) for Advagraf was 5%, 22% and 22% higher than that for Prograf, with the 90% Confidence Intervals (CIs) for ln(AUC_{24}) being outside the equivalence range of 80% to 125%.

However, the mean total daily doses (mg/kg) of Advagraf were approximately the same as the corresponding mean Prograf doses at the times of the day 1, day 3, and day 14 profiles. These data suggest that systemic exposure is similar by day 3.

Across all studies where stable kidney, liver, and heart transplant recipients were converted from twice daily Prograf to once daily Advagraf on a 1:1 (mg:mg) total daily dose basis, the geometric mean ratios of systemic exposure of tacrolimus (Advagraf:Prograf) ranged from 88.8% to 100.9%. The 90% CIs across these studies were all contained within the equivalence limits of 80% to 125%.

There was a high correlation between AUC_{24} and trough levels in de novo kidney transplant recipients (correlation coefficients of 0.83 and 0.87 in Studies FG-506E-12-01 and FG-506E-12-03, respectively) and in stable kidney, liver, and heart transplant recipients converted from Prograf to Advagraf (correlation coefficients of 0.86 to 0.934 in kidney transplant recipients, 0.88 to 0.90 in liver transplant recipients and 0.94 in heart transplant recipients). The slope of the line of best fit was also similar for both Advagraf and Prograf, indicating that for therapeutic drug monitoring (TDM), the same target trough level range is applicable for both formulations.

1.2.2 Clinical Studies of Efficacy and Safety

Tacrolimus as Prograf has been proven to be effective in adult and pediatric liver, kidney, and heart transplant recipients. Several controlled clinical trials have confirmed that the efficacy of Prograf in this population is therapeutically equivalent, in terms of preventing acute rejection, to a cyclosporine-based regimen.

The efficacy of Advagraf in adult de novo kidney transplant recipients was evaluated in 3 phase 3/3b clinical studies that provided comparative efficacy for 1 year (Studies 02-0-158 and FG-506E-12-03) and 6 months (Study PMR-EC-1210). The 6-week data from phase 2 pharmacokinetic Study FG-506E-12-01 provides additional support of efficacy. Efficacy in adult de novo liver transplant recipients was evaluated in phase 3 Study FG-506E-11-03 for patients treated for at least 1 year.

Data from the studies in adult de novo kidney transplant recipients indicate that Advagraf is non-inferior to Prograf in both the short and longer-term prevention of kidney allograft rejection and in extending allograft survival. Efficacy was maintained in terms of patient and graft survival and prevention of biopsy-proven acute rejection (BPAR). This was seen at 3 years post-transplant in patients for the phase 3 de novo studies (Studies FG-506E-12-03 and FG-506E-11-03) from the Advagraf treatment group who were included in the long-term follow-up Study FG-506-14-02.

Overall Safety Profile

The safety profile of Prograf has been established worldwide in over 100,000 transplant patients treated since its introduction in 1993. Clinically important events include impairment of renal function, abnormal glucose metabolism, and neurological disorders. Many of the adverse drug reactions are reversible and/or responded to dose reduction. Transplant patients on immunosuppressive therapy are also known to be at increased risk of developing post-transplant lymphoproliferative disorders and other malignancies. This risk is considered to be related to the overall immunosuppressive burden.

The safety of Advagraf was evaluated in 4201 transplant patients in 16 phase 2/3 studies, of who 2801 received Advagraf.

The adverse events observed following administration of Advagraf in the three large phase 3/3b studies in de novo kidney transplant recipients (Study 02-0-158, Study FG-506E-12-03 and Study PMR-EC-1210 [OSAKA]), and in the two phase 2 pharmacokinetic studies in de novo kidney (Study FG-506E-12-01) transplant recipients were consistent with the

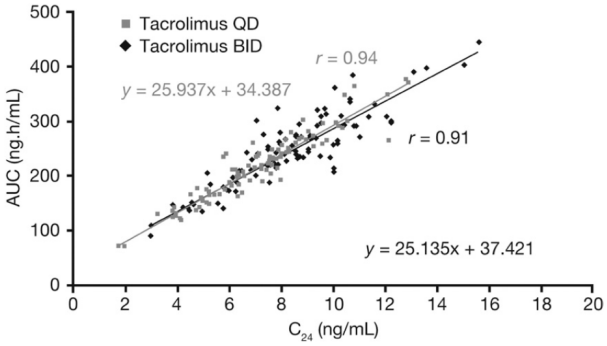
established safety profile of Prograf. No new adverse events that would suggest a clinical concern were identified, with Advagraf having a similar safety profile to that of Prograf, despite small differences in individual adverse events.

The safety profile of Advagraf and Prograf were also similar in stable kidney, liver, and heart transplant recipients converted from twice daily Prograf to once daily Advagraf.

1.2.3 Whole Blood Trough Concentrations Can Be Used to Target the Same Systemic Exposure for Both Prograf and Astagraf

Tacrolimus is an active substance with a narrow therapeutic index (NTI). Like other NTI drugs, tacrolimus requires regular monitoring to establish and maintain appropriate drug exposure. Immunosuppressive therapy with once daily tacrolimus is usually initiated at a dose between 0.1 mg/kg per day and 0.3 mg/kg per day in orally divided doses. Subsequent doses are adjusted on the basis of clinical signs aided by tacrolimus whole blood trough concentration monitoring. To ensure that appropriate systemic exposure is achieved, the prevailing practice is to conduct TDM based on the trough whole blood concentrations (C_{min}). Although tacrolimus systemic exposure (AUC) is the critical parameter for both safety and efficacy in transplant recipients [Kuypers et al, 2004; Undre et al, 1999], AUC monitoring is not feasible in clinical practice. For this reason, C_{min} (i.e., trough concentration) is used as a surrogate for overall exposure. Moreover, as shown in Figure 1, the relationship between C_{min} and AUC is the same for both Prograf and Astagraf XL. Therefore, for both formulations, the established trough concentration goals can be used to target the same systemic exposure level. It is not known whether this is the case for other formulations.

Figure 1 Correlation between AUC and C_{min} of Advagraf (tacrolimus QD) and Prograf (tacrolimus BID) in heart transplant recipients



Reproduced from Alloway et al, 2011.

1.2.4 Increased Potential for Adverse Clinical Outcomes with Too High or Too Low Tacrolimus Exposure

Several studies have shown that with increasing tacrolimus exposure, the decreased risk of acute rejection is offset by a potential for increased risk of toxicity and infection [(Cockfield et al, 2014; Kuypers et al, 2004) reviewed in (Staatz & Tett, 2004)]. Additional studies have

shown that modest, short-term changes in tacrolimus exposure may result in adverse clinical outcomes. These studies, explained in greater detail below, demonstrated:

- A significantly (26.9%) lower AUC in patients who experienced acute rejection at day 2/3 post-kidney transplant, compared with those who remained rejection-free (157 ng·h/mL vs. 215 ng·h/mL, P = 0.007) [Undre et al, 1999];
- Increased risk of BPAR in patients with AUC < 150 ng·h/mL at day 7 post-kidney transplant in comparison to those above this threshold [Kuypers et al, 2004];
- A 7.2% increased risk of acute rejection with every 1 ng/mL decrease in tacrolimus trough concentration within the first 6 months following kidney transplant; an additional 23% increased risk of acute rejection with every 1 ng/mL decrease in trough between 3 and 6 months [Israni et al, 2013];
- Significantly increased incidence of BPAR in de novo kidney transplant recipients receiving low-dose tacrolimus ER compared with those receiving standard-dose tacrolimus ER (actual tacrolimus exposure differed by 30% to 40%) [Cockfield et al, 2014];
- Significantly higher mean AUC in de novo kidney transplant recipients who had infection at day 7 and week 6, compared with those who remained infection-free (197.4 ± 70.5 ng·h/mL vs. 160.5 ± 47.9 ng·h/mL, P = 0.01 at day 7; 177.5 ± 28.3 ng·h/mL vs. 149.2 ± 31.3 ng·h/mL, P = 0.02 at week 6) [Kuypers et al, 2004].

Based on the AUCs associated with these adverse events, it appears that risk of acute rejection and infection increased outside of the range of 150–177 ng·h/mL.

Similar to the studies described above, the association between early acute rejection (within the first 6 months of transplantation) and decreased tacrolimus trough concentration was shown in a post hoc analysis of the prospective DeKAF study. DeKAF was a landmark study that has shaped our understanding of post-transplantation immunosuppression and long-term kidney transplant function. The authors of this analysis reported that for every 1 ng/mL decrease in tacrolimus trough concentration, a 7.2% increased risk of acute rejection within the first 6 months could be expected post-transplant (P = 0.03). Between months 3 and 6, when centers typically reduce tacrolimus exposure to limit long-term side effects, there was an additional 23% increased risk of acute rejection with each 1 ng/mL decrease in tacrolimus trough level (P = 0.008) [Israni et al, 2013]. The latter result is especially concerning, as stable patients are typically monitored no more than once in 3 months.

Together, these studies show that small deviations in tacrolimus exposure places patients at increased risk of acute rejection and opportunistic infection, highlighting the fine balance between tacrolimus under- and overexposure.

1.2.5 Association Between the Potential for Adverse Clinical Outcomes and Increased Tacrolimus Exposure Variability

High IPV in tacrolimus concentration over time may put the patient at risk of over- or under-immunosuppression. Tacrolimus IPV has been assessed in several studies enrolling stable transplant patients, in which the coefficient of variation (CV) of tacrolimus trough

concentration is consistently calculated to be approximately 15%. The following section describes study results suggesting that increased IPV is associated with adverse long-term (≥ 6 month post-transplant) clinical outcomes.

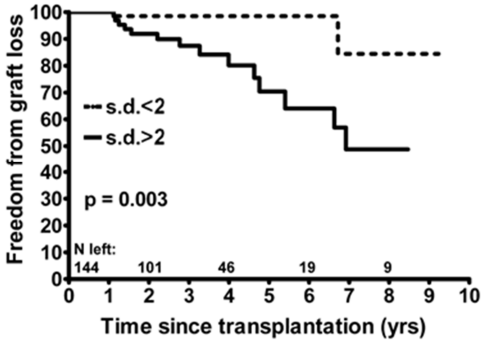
Study Details

Borra et al. reported that high tacrolimus IPV was associated with significantly higher risk of graft failure at ≥ 1 year post-transplant, compared with those with low IPV (P = 0.003). IPV was calculated from a retrospective analysis of 6–12 month post-transplant trough measurements in 297 kidney transplant recipients. The median tacrolimus IPV in this study (14.9%) was used as the cut-off for high vs. low variability. Notably, mean ± SD tacrolimus concentration was similar between patients who experienced or did not experience graft failure (6.9 ± 2.5 ng/mL vs. 7.4 ± 2.9 ng/mL, P = NS). However, multivariate analysis showed that IPV was indeed an independent predictor of graft failure [2010].

Another retrospective analysis in kidney transplant recipients demonstrated a similar association between high variability and poor graft function. In a retrospective analysis of 200 kidney transplant patients, Makowski et al. reported a significant association between high tacrolimus IPV, defined here as CV > 40%, and increased development of delayed graft function and diminished renal function [2013].

Pollock-Barziv et al. reported that increased standard deviation of tacrolimus (TacSD) was an independent predictor of acute rejection at ≥ 6 months post-transplant. In this retrospective analysis of 144 adolescent heart, kidney, liver, or lung transplant patients, TacSD was calculated from trough level measurements since transplant. In recipients with TacSD < 2 at least 6 months post-transplant, graft survival was significantly better than in those with TacSD > 2 [Figure 2]. Moreover, each 1-point increase in TacSD > 2 of tacrolimus trough was associated with a 58% increase in risk of graft loss (P = 0.003) [2010].

Figure 2 Variability in tacrolimus trough levels associated with increased risk of graft loss in adolescents



Reproduced from Pollock-Barziv et al, 2010

1.3 Summary of Key Safety Information for Study Drugs

As with other potent immunosuppressive agents, patients receiving tacrolimus are at an increased risk for infections (viral, bacterial, fungal, and protozoal). Likewise, the course of pre-existing infections may be aggravated. Overall, infections have been reported frequently in patients being treated with tacrolimus. Both generalized and localized infections can occur. Patients receiving immunosuppressive agents are also at an increased risk of developing malignancies, particularly of the skin. Benign as well as malignant neoplasms, including Epstein-Barr virus (EBV)-associated lymphoproliferative disorders, have been reported in association with tacrolimus treatment.

Tacrolimus can result in renal function impairment in post-transplant patients due to its vasoconstrictive effect on renal vasculature. Acute renal impairment, that is usually reversible, can result in high serum creatinine, hyperkalemia, decreased secretion of urea, and hyperuricemia.

Additional adverse reactions reported in patients receiving tacrolimus include allergic and anaphylactoid reactions, ventricular hypertrophy or hypertrophy of the septum (reported as cardiomyopathies; most cases have been reversible), encephalopathies (such as posterior reversible encephalopathy syndrome [PRES], and pure red cell aplasia [PRCA]). In the case of PRCA, all patients reported risk factors such as parvovirus B19 infection, underlying disease, or concomitant medications associated with PRCA. Gastrointestinal perforation has also been reported in patients treated with tacrolimus, although all cases were considered a complication of transplant surgery or accompanied by infection, diverticulum, or malignant neoplasm. Tacrolimus may prolong the QT interval and may cause Torsade de Pointes.

For additional information regarding possible adverse drug reactions related to tacrolimus or Astagraf XL, please refer to the Package Inserts (PIs) for Astagraf XL or the various immediate-release tacrolimus products.

1.4 Risk-Benefit Assessment

The research proposed herein compares two FDA-approved medications indicated for the prophylaxis of acute rejection in kidney transplantation: 1) tacrolimus, extended-release (Astagraf XL) and 2) tacrolimus, immediate-release. Tacrolimus (in the twice daily formulation of Prograf) has been extensively studied and used in clinical practice for many years. The safety profile of tacrolimus has been well-established. The use of tacrolimus in the current study is consistent with the recommendations described in the approved package inserts, and as such, is not expected to shift the known risk-benefit profile in the transplant population under study. Clinical studies have confirmed that Astagraf XL is as efficacious as Prograf, with a similar safety profile [Trunecka et al, 2008; Fischer et al, 2007; Alloway, 2005; Undre, 2005].

2 STUDY OBJECTIVE(S), DESIGN, AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective

The primary objective is to compare the incidence of a two-part composite endpoint consisting of de novo DSA formation or a designation of immune activation (IA) on peripheral blood molecular profiling in patients maintained on twice daily, immediate-release tacrolimus versus those maintained on Astagraf XL in the first two years post-transplant.

2.1.2 Secondary Objectives

The secondary objectives of the study are to:

- To assess the risk factors for each of the following outcomes: DSA formation; IA; transplant glomerulopathy (TG); acute and chronic forms of antibody-mediated rejection (ABMR); C1q-binding DSA; HLA-DQ DSA; DSA IgG_3 isotype; requirement for and type of antibody reduction required; various threshold levels of estimated glomerular filtration rate (eGFR) (less than 30, 40, and 50 mL/min/1.73 m^2); and the four components of the traditional composite endpoint, consisting of graft loss, mortality, biopsy-proven acute rejection (BPAR), and loss to follow-up; and additionally, the persistence of DSA and IA.
- Assess and compare, between treatment groups, the association/correlation of the appearance of DSA with the development of IA on molecular profiling.
- Compare, between treatment cohorts, the incidence and hazards of each of the following, occurring over the course of one year and, where appropriate, at 24 months post-transplantation: DSA, HLA-DQ DSA, C1q-binding DSA, DSA IgG_3 isotype, IA, TG, select BANFF histology grades (acute and chronic forms of ABMR, acute and chronic active TCMR, borderline changes, and IFTA), graft loss, mortality, and BPAR.
- Compare between cohorts the incidence of various thresholds of eGFR (less than 30, 40, 50 mL/min/1.73 m^2, and a five-point decline).
- Compare the distribution of ordinal categories of antibody strength (weak, moderate, strong) between treatment groups across the study time course as well as at each time point.
- Compare raw MFI scores between treatment groups at each time point and across the duration of the study.
- Examine and compare histopathology between the treatment cohorts in biopsies obtained for cause and for maintenance during the course of clinical care.
- Examine and compare outcomes as a function of tacrolimus manufacturer and the number of switches between immediate-release tacrolimus products.
- Summarize AEs between study groups.
- Compare the change in eGFR over the study duration beginning from 30 days post-transplant between groups, and in patients who develop DSA or IA.

- Compare the persistence of the development of IA on molecular profiling between the two cohorts across the study duration in those patients who develop IA on molecular profiling.

2.1.3 Exploratory Objectives

Exploratory objectives are:

- Compare the frequency of those patients experiencing either component of a two-part composite endpoint consisting of DSA formation and the presence of TG (cg > 0) on centrally-interpreted institutional protocol (maintenance) biopsy and / or biopsy obtained for cause during the first year post-transplant and/or up to the conclusion of the study.
- To assess and compare, between treatment groups, the association/correlation of incidence and strength of DSA, and the incidences of HLA-DQ DSA, C1q-binding DSA, and DSA IgG_3 isotype with each other, and individually, with each of the following: IA, TG, ABMR (both chronic forms and acute), Banff histology, various thresholds of eGFR (less than 30, 40, and 50 mL/min/1.73 m^2), eGFR change over time, TCMR, graft loss, and mortality.
- Examine the proportion of patients with the IA designation who have normal and abnormal creatinine.
- In patients with an IA designation and abnormal creatinine, examine biopsies (if available) for concordance.
- To assess the association/correlation of the histopathology with DSA, C1q-binding DSA, DSA IgG_3 isotype, molecular phenotyping, graft loss, and measures of renal function in each cohort in patients for whom one year institutionally-derived, protocol (maintenance) biopsies are available.
- To assess and compare, between treatment groups, the association/correlation of the CV and SD of tacrolimus trough concentrations with each of the following: the composite endpoint of DSA and IA, DSA formation and IA individually, TG, ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, histopathology, renal dysfunction, and the components of the traditional composite endpoint.
- To compare dose changes and IPV between groups as a function of tacrolimus trough level after the first 6 weeks following transplantation in patients on stable doses of immediate-release tacrolimus and Astagraf XL.
- To assess and compare, between treatment groups, the association/correlation of the appearance of DSA with each of the following: TG, chronic forms of ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, IA, histopathology, graft loss, various thresholds of eGFR (< 30, 40, and 50 mL/min/1.73 m^2), acute ABMR, TCMR, and mortality in patients who develop IA on molecular profiling in each cohort.
- To compare the MFI shift to a lower category (and magnitude of shift) between groups (including C1q MFI shift), in those patients who develop DSA during the course of the study.

- To examine percent MFI reduction within each subgroup over time in each treatment arm (including C1q MFI reduction) in those patients who develop DSA during the course of the study.
- To map expression results to known immunological pathways implicated in immune-mediated, kidney transplant tissue injury.
- To determine the longitudinal consequences of DSA formation and immune activation, perform de-identified, long-term follow-up through participating transplant centers in select study participants who provide the appropriate informed consent by future linking to the Scientific Registry of Transplant Recipients (SRTR) after completion of ASTOUND.
- To examine the economic burden of treating patients with molecular evidence of immune activation and DSA formation using Medicare claims data or commercial payer cost data in patients required to transfer from Medicare to private insurance.
- To compare outcomes in like patients between cohorts managed with maintenance protocol biopsy vs. biopsy for cause with respect to the composite endpoint, the components thereof, complications, and resource utilization.

2.2 Study Design and Dose Rationale

2.2.1 Study Design

This is an exploratory, two year, prospective, randomized, multi-center, open-label trial examining long-term kidney transplant outcomes through the use of an adaptive design and a two-part, composite surrogate endpoint. Specifically, it is designed to compare the effects of twice daily, immediate-release tacrolimus and once daily Astagraf XL on DSA formation and the development of a peripheral blood molecular profile indicating the presence of immune activation (IA) in *de novo* kidney transplant recipients during the first two years following transplantation. For the purposes of this study, IA will be defined as a positive molecular signature using the Trugraf™ v.2.0 molecular assay (Transplant Genomics, Inc., Pleasanton, CA) in all patients.

Patients will be screened prior to surgery and randomized 1:1 to receive immediate-release tacrolimus, administered twice daily, or Astagraf XL, as a component of a standard immunosuppression *maintenance* regimen also consisting of corticosteroids (complete steroid avoidance not allowed – steroid taper and withdrawal permitted if part of institutional protocol) and mycophenolate mofetil (MMF) (or Myfortic® equivalent). Investigators are encouraged to start subjects on the randomized study treatment (immediate release tacrolimus or Astagraf XL) within 48 hours of transplantation (pre-transplant administration of study treatment is not allowed). However, if medically indicated, per the treating physician's discretion, initiation of study treatment may be delayed for up to seven days post-transplant.

IF medically indicated, subjects who are unable to tolerate oral administration of their assigned study drug may be administered tacrolimus via an alternative route (for Astagraf XL treatment arm: intravenous [IV] tacrolimus; for immediate release tacrolimus: IV tacrolimus

or nasogastric tube [NGT]) for up to seven consecutive days. Dosing via alternative routes should be discontinued as soon as the subject can tolerate standard oral administration, and is not allowed for more than seven consecutive days. Please see Section 5.1.1 and 4.1 for further details regarding non-oral administration of tacrolimus.

Given the variability of incidence rates for DSA reported in the literature, a stopping rule has been incorporated herein to mitigate the risk of continuing the study under incorrect prior assumptions relating to the true incidence rate of DSA formation (Section 7.1). To apply the rule, the incidence rate of the primary endpoint in the control group will be examined once 50% of control patients have completed one year of therapy. Early termination of the study, or extension to a second year, will be predicated on this assessment (see Section 7.8.1).

Induction therapy is required for all participants, and will be administered (dosing, type of agent) per center protocol. Intravenous corticosteroids will be administered prior to revascularization as part of the initial induction regimen, with dose and duration of therapy also determined by the participating transplant center's standard protocol. Thereafter, patients will be followed for up to 2 years during the open-label study period. Immediate-release tacrolimus will be obtained from pharmacies per the local standard of care. After discharge from the hospital, the patients randomized to immediate-release tacrolimus will receive study-provided vouchers and will be permitted to receive any immediate-release tacrolimus product available to them through normal dispensing mechanisms. Astagraf XL will be supplied by Astellas and dispensed by the participating institution.

Initial pre-transplant cross-matching (XM) will be performed per local protocol. Initial XM serum will be collected and stored centrally to facilitate the later assessment of pre-formed antibody on an as needed basis. Thereafter, antibody screening and molecular phenotyping will be performed by a central lab at one month, 3 months, 6 months, 9 months, 12 months, and 24 months. Antibody status, including HLA-DSA isotype and C1q-binding DSA status, will also be recorded when measured locally during the course of clinical care.

For central laboratory analysis, initial FlowPRA® (One Lambda, Canoga Park, CA) screening will be used to test for the presence of anti-HLA antibodies. Once identified, single antigen testing will be used to identify DSA specificity with an MFI greater than or approaching 1000 used to determine antibody positivity. In borderline cases, a three-member adjudication board of independent, qualified HLA Lab Directors will determine positivity based on relevant clinical data. In samples that meet threshold criteria for DSA positivity, additional classification will be performed to determine the degree of antibody strength. By performing a single dilution (1:16) of DSA-containing samples, the alloimmune response can be further elucidated [Tambur et al, 2015]. Samples in which MFI disappears (becomes < 1000 MFI) will be regarded as "weak." Samples increasing to > 10,000 MFI upon dilution will be regarded as having significant prozone and will be regarded as "strong." Samples which may increase or decrease, but nevertheless remain between 1000 and 10,000 MFI will be regarded as "moderate." In certain cases, samples with evidence of fluorescence that are nevertheless below MFI criteria for DSA positivity will also be subject to dilution. Samples increasing to above threshold criteria following dilution will be regarded as positive and

assumed to be under the influence of a prozone effect that impairs antibody detection. For statistical purposes, the DSA with the highest MFI level obtained during the course of the study will be used for statistical comparisons between groups. However, the strongest antibody observed at each HLA-locus will be reported separately and tracked for patients who are DSA positive [Tambur et al, 2015]. In all cases, positive DSA and/or molecular testing results will be communicated to the patient's treating physician when available (DSA) or at the end of study (molecular testing). Testing will be performed in batches. De-identified typing reports (donor and recipient) will be requested from the participating centers to inform the central laboratory testing. De-identified results of recent local pre-transplant DSA testing (if performed per SOC) will also be requested to facilitate interpretation of central DSA testing results.

Follow-up visits will be performed quarterly during the first year of follow-up to collect standard clinical data. For follow-up beyond the first year, urinary protein, and tacrolimus trough concentrations obtained closest to targeted date of visit, per SOC, should be recorded. The two closest serum creatinine levels to the study visit, obtained at least one month apart, if performed per SOC, should also be recorded. Additionally, all local kidney biopsy results, AEs, and concomitant medications pertaining to AEs will be recorded. At the terminal study visit at 24 months, the most recently obtained lab results as well as any episodes of rejection, need for antibody reduction, graft loss, and deaths occurring beyond the first year will be detailed in the eCRF. Final testing for DSA and molecular profiling will also be performed at the 24 month visit.

Pathology results [hematoxylin and eosin (H&E), light microscopy, and immunofluorescence] will be recorded in the eCRF when local kidney biopsies are performed and interpreted during the course of clinical care. De-identified copies of all kidney pathology reports must be submitted to Astellas. For purposes of data recording, substantiation by local biopsy will be required for cases of suspected rejection. For all participants who undergo at least one kidney biopsy during the first year post-transplant (either for cause or per institutional protocol), the kidney biopsy performed closest to the 12 month visit (but no later than 14 months post-transplant) will be submitted for central pathological grading. All biopsies that are submitted for central pathology review will be evaluated and scored using the most recent version of the 2007 Update to the Banff '97 Classification by a central pathologist blinded to clinical results and patient identifiers [Solez et al, 2008].

2.2.2 Dose Rationale

In line with recommendations from the US PI, the initial dose of Astagraf XL should be weight-based in order to avoid the possibility of under-immunosuppression and ranged between 0.1 mg/kg/day and 0.2 mg/kg/day in the two pivotal trials considered by the FDA for Astagraf XL approval [Silva et al, 2007; Kramer et al, 2010]. **For the purposes of the ASTOUND study, patients randomized to the Astagraf XL treatment arm will be administered an initial, weight-based dose of 0.15 mg/kg/day**. Dry weight, or estimated dry weight, if dry weight is not available, should be used to calculate the initial dose. Subsequent dosing should be based on clinical assessments of rejection and tolerability in

each individual patient and should be aided by whole blood trough concentration monitoring. To assist in dose titration of Astagraf XL, it is important to note that the relationship between C_{min} and AUC has been validated for both Prograf and Astagraf XL, allowing practitioners to rely on their clinical experience with Prograf to appropriately titrate Astagraf XL. For the purpose of this study, immediate-release tacrolimus will be dosed in accordance with local center protocol.

2.3 Endpoints

2.3.1 Primary Endpoints

The primary endpoint is the combined incidence of either DSA or IA on peripheral blood molecular profiling at one year or at the conclusion of the study. For this purpose, DSA will be considered as a categorical (binary) variable with positivity determined at a threshold criteria approaching MFI = 1000 at any time during the study. For reporting purposes, IA will be considered either present or absent using the Trugraf™ v2.0 molecular assay. For the purposes of the study, a negative designation (Trugraf TX Normal) will be referred to as Immune Quiescence (IQ). Due to operating characteristics of the assay, a positive designation will be considered evidence of Immune Activation (IA) in all patients.

The mFAS will be used for the primary endpoint analysis.

2.3.2 Secondary Endpoints

The assessment and comparison of the incidence of DSA between treatment groups will rely on the binary variable of DSA positivity at a threshold criteria approaching an MFI of 1000. Comparisons of the cohorts with respect to the various molecular designations as well as correlating the results with DSA will rely on the categorical (binary) variable of positivity using the Trugraf™ v2.0 molecular assay. Comparisons between treatment cohorts regarding incidence will rely upon assessments for each of the following results at any point in the study:

- DSA
- IA
- TG
- Acute and chronic forms of ABMR
- C1q-binding DSA
- HLA-DQ DSA
- DSA IgG_3 isotype
- Required antibody reduction
- eGFR at various thresholds (less than 30, 40, and 50 mL/min/1.73 m^2, and whether a five-point decline in eGFR occurs
- Graft loss (defined as subject death, retransplantation, transplant nephrectomy, or a return to dialysis for at least a 6 week duration)
- Death
- BPAR (inclusive of ABMR and TCMR)
- Loss to follow-up

Time-to first occurrence will be assessed for each of the following: DSA, HLA-DQ DSA, C1q-binding DSA, DSA IgG_3 isotype, IA, TG, select BANFF histology grades (acute and chronic forms of ABMR, acute and chronic active TCMR, borderline changes, and IFTA), mortality, and local BPAR.

The frequency of the type of antibody reduction employed will be assessed.

Patient MFI and eGFR values will be assessed over time.

Assessments of histopathology in biopsies will be made using the following endpoints:

- Incidence of ci, ct, and ptc scores greater than one
- Biopsy scores for g, t, v, i, cg, ct, ci, cv, ah, ptc, and mm

The safety endpoint for AEs will be the incidence of each type of AE based on system organ class and using the preferred term from the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA).

Persistence of DSA and IA will be the additional endpoints for comparisons in patients who develop IA and DSA on molecular profiling.

2.3.3 Exploratory Endpoints

Additional exploratory endpoints include the following:

- A second two-part composite endpoint encompassing the incidence of acquiring DSA or positive evidence of TG on biopsy during the first year post-transplant or by the conclusion of the study.
- Expression results, mapped to known immunological pathways implicated in immune-mediated, kidney transplant tissue injury.
- With appropriate informed consent, long-term graft and patient survival in de-identified study participants by future linking to the Scientific Registry of Transplant Recipients (SRTR) following completion of ASTOUND.
- The economic burden of treating patients with molecular evidence of immune activation and DSA formation using Medicare claims data (or commercial payer cost data in patients required to transfer from Medicare to private insurance).
- Outcomes in like patients between cohorts managed with maintenance protocol biopsy vs. biopsy for cause with respect to the composite endpoint, the components thereof, complications, and resource utilization.

3 STUDY POPULATION

3.1 Selection of Study Population

This study will enroll 550 living or deceased donor kidney transplant recipients, 16 to 70 years of age. Patients will be screened and randomized 1:1 prior to surgery to receive, twice daily, immediate-release tacrolimus or Astagraf XL as a component of a standard immunosuppression maintenance regimen also consisting of corticosteroids (complete steroid avoidance not allowed – steroid taper and withdrawal permitted if given per institutional protocol) and MMF (or Myfortic equivalent) (see Section 4.5 for randomization details). After discharge from the hospital, patients randomized to receive twice-daily immediate-release tacrolimus will receive study-provided vouchers and will be permitted to receive any immediate-release tacrolimus product available to them through normal dispensing mechanisms. Patients randomized to receive Astagraf XL will receive this treatment for the duration of the study and will not be permitted to use other extended-release tacrolimus formulations.

Upon request from the investigator, the Sponsor's medical monitor will consider requests for patient re-screenings on a case-by-case basis.

3.2 Inclusion Criteria

Subject is eligible for the study if all of the following apply.

1. Kidney transplant patient ≥ 16 years and ≤ 70 years old.
2. Institutional Review Board (IRB)-/Independent Ethics Committee (IEC)-approved written Informed Consent and privacy language as per national regulations (e.g., HIPAA Authorization for US sites) must be obtained from the subject or legally authorized representative prior to any study-related procedures (including withdrawal of prohibited medication, if applicable). For subjects < 18 years old, informed consent must be obtained from the subject's parent(s) or legal guardian(s); subject Assent (where appropriate) must be given.
3. Recipient of a de novo kidney from a living or deceased donor. Note: Recipient of an *en bloc* deceased donor kidney transplant from a pediatric donor ≥5 years of age AND weighing greater than 20 kg is allowed.
4. If deceased donor, a Kidney Donor Profile Index (KDPI) ≤ 85 [donation after circulatory death (DCD) and what was previously known as extended criteria donor (ECD) organ recipients *are* eligible for enrollment provided KDPI ≤ 85]
5. Removed
6. At least one antigen mismatch at major MHC (class I or class II).
7. Willingness to comply with study protocol.
8. Subject agrees not to participate in another investigational drug study while on treatment.

9. Female subject must be either:
    a. Of non-child-bearing potential
        i. Post-menopausal (defined as at least 1 year without any menses) prior to screening, or
        ii. Documented surgically sterile or status post-hysterectomy
    b. Or, if of childbearing potential,
        i. Agree not to try to become pregnant during the study and for 90 days after the final study drug administration
        ii. And have a negative serum or urine pregnancy test within 7 days prior to transplant procedure
        iii. And, if heterosexually active, agree to consistently use two forms of highly effective birth control (at least one of which must be a barrier method) which includes consistent and correct usage of established oral contraception, established intrauterine device or intrauterine system, or barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository or vasectomy in the male partner, starting at screening and throughout the study period and for 90 days after the final study drug administration.
10. Male subject and their female spouse/partners who are of childbearing potential must be using highly effective contraception consisting of two forms of birth control (one of which must be a barrier method) starting at screening and continuing throughout the study period and for 90 days after the final study drug administration (acceptable forms of birth control are listed in Inclusion Criterion 9).
11. Male subject must not donate sperm starting at screening throughout the study period and for 90 days after the final study drug administration.
12. Female subject must agree not to breastfeed starting at screening and throughout the study period, and for 90 days after the final study drug administration.
13. Female subject must not donate ova starting at screening and throughout the study period, and for 90 days after the final study drug administration.
14. Will be receiving induction immunotherapy (either T-cell depleting agent, anti-CD52 monoclonal antibody, or IL-2 co-stimulation blocker), with dose and frequency of the chosen induction agent determined by local standard of care. Steroid-only induction does not satisfy this criterion.

Waivers to the inclusion criteria will **NOT** be allowed.

Previous kidney transplants will be permitted.

Patients who are receiving a secondary transplant and who previously received Astagraf XL or who are currently on Astagraf XL as a component of maintenance immunosuppression and re-listed for transplant will be eligible to enroll in ASTOUND and will be randomized at the time of transplant to either cohort.

3.3 Exclusion Criteria

Subject will be excluded from participation if any of the following apply.

1. Patient is known to have a positive test for latent tuberculosis (TB) and has not previously received adequate anti-microbial therapy or would require TB prophylaxis after transplant.
2. Uncontrolled concomitant infection or any unstable medical condition that could interfere with study objectives.
3. Significant liver disease, defined as having, during the past 28 days, consistently elevated AST (SGOT) and/or ALT (SPGT) levels greater than 3 times the upper value of the normal range of the investigational site.
4. Patient currently taking or maintained on another form of extended-release tacrolimus following his/her transplant procedure.
5. Patient who will be maintained on a non-tacrolimus-based maintenance immunosuppressive regimen following his/her transplant procedure.
6. Patient currently taking, having taken within 30 days, or who will be maintained on an mTOR inhibitor following his/her transplant procedure.
7. Use of an investigational study drug in the 30 days prior to the transplant procedure.
8. Contraindication or hypersensitivity to drugs or any of their components that constitute the immunosuppression regimen.
9. 6 Ag match or zero mismatch at major MHC (class I or class II).
10. Receipt of an ABO-incompatible organ. *Note: A2 donor to O recipient or A2 donor to B recipient is considered ABO-compatible and not excluded by this criterion.*
11. Removed
12. Removed
13. The presence of current *or historic*, pre-formed anti-HLA DSA against the current donor (evidence of pre-formed, *non-donor* HLA is not exclusionary) as defined by a subject meeting any of the following criteria*:
    a. Positive virtual crossmatch,
    b. Positive T- or B-cell crossmatch by NIH antiglobulin lymphocytotoxicity method,**
    c. Positive T- or B-cell flow cytometry crossmatch defined by the MFC criteria used by the center's HLA lab for their local proficiency testing,**
    d. An MFI greater than or approaching 1000 using flow cytometry/Luminex-based, specific anti-HLA antibody testing.

    *Patients are eligible to enroll with a negative virtual crossmatch if used *in lieu* of a physical crossmatch, if, in the opinion of the patient's attending physician, use of such is required to obviate the accrual of excessive ischemia time. However, continued

participation is predicated on the performance of the physical crossmatch within 48 hours of transplant. If the physical crossmatch is positive, the subject will be discontinued.
** If *b* or *c* above are positive secondary to a suspected positive auto-crossmatch, that is not exclusionary as long as *a* and *b* above are not met.

14. Receipt of desensitization, antibody-removal, anti-B-cell, or anti-plasma cell therapy in the 90 days preceding the transplant procedure.
15. Planned initiation (prior to transplant) of desensitization, antibody-removal, anti-B-cell, or anti-plasma cell therapy within 7 days of the transplant procedure.
16. Donor or recipient with known hepatitis C infection (HCV antibody positive), HIV infection (HIV antibody positive), acute hepatitis B infection (HBsAg positive, anti-HBc positive, IgM anti-HBc positive, anti-HBs negative) chronic hepatitis B infection (HBsAg positive, anti-HBC positive, IgM anti-HBC negative, anti-HBs negative), or equivocal hepatitis B status (HBsAg negative, anti-HBc positive, anti-HBs negative). Patients (donor or recipient) who have normal liver function tests (LFT) and who are either hepatitis C positive with a negative viral load or have natural or vaccine-acquired immunity from hepatitis B are not excluded by this criterion.
17. Primary focal segmental glomerulosclerosis.
18. Subject has a current malignancy or history of malignancy (within the past 5 years), except non-metastatic basal or squamous cell carcinoma of the skin or carcinoma-in-situ of the cervix that has been successfully treated.
19. Recipient of multi-organ or dual kidney transplants (inclusive of current transplant and any prior non-renal transplants). *Note: Patients with prior kidney transplants are eligible*.
20. Recipient of an *en bloc*, pediatric deceased donor kidney from a donor <u>less than 5 years of age OR weighing less than 20 kg.</u>
21. Prior graft loss secondary to CMV or BK nephropathy.
22. Prior history of invasive organ disease in the presence of CMV or BKV or clinically significant CMV viremia.
23. History of clinically significant (per investigator's discretion) BK viruria.
24. Any condition which, in the investigator's opinion, makes the subject unsuitable for study participation.
25. Planned complete steroid avoidance. (Steroid initiation and subsequent taper / withdrawal will allowed and will be under the purview of the treating physician.)
26. Planned receipt of post-transplant, prophylactic HCV treatment.

Waivers to the exclusion criteria will **NOT** be allowed.

4 TREATMENT(S)

4.1 Identification of Investigational Product(s)

4.1.1 Test Drug(s)

Astellas will provide the following for patients randomized to the Astagraf XL treatment arm for the study duration of up to 24 months:

Astagraf XL 0.5 mg

Oblong capsule with a light yellow cap and orange body. Capsule is branded with red "647" on capsule body and "0.5 mg" on capsule cap. The capsule is supplied in 30-count short, square bottles with brown caps.

Astagraf XL 1 mg

Oblong capsule with a white cap and orange body. Capsule is branded with red "677" on capsule body and "1 mg" on capsule cap. The capsule is supplied in 30-count short, square bottles with blue caps.

Astagraf XL 5 mg

Oblong capsule with a grayish-red cap and orange body. Capsule is branded with red "687" on capsule body and "5 mg" on capsule cap. The capsule is supplied in 30-count short, square bottles with orange caps.

IF medically indicated, subjects randomized to the Astagraf XL treatment arm who are unable to tolerate oral administration of Astagraf XL, may be administered tacrolimus via IV solution (**Prograf** solution for intravenous use), administered as a 24-hour continuous infusion. Administration of IV tacrolimus should be discontinued as soon as the subject can tolerate oral administration, and may not exceed a period of seven consecutive days. **Astagraf XL cannot be given intravenously. Astagraf XL should not be given via NGT due to its extended release characteristics**. IV tacrolimus will not be provided by the Sponsor.

4.1.2 Comparative Drug(s)

Tacrolimus, immediate-release, oral; 0.5 mg, 1 mg, 5 mg capsules to be obtained by the transplanting center during a participant's inpatient stay, and by the patients themselves, thereafter, at retail pharmacies via a study-provided voucher for the study duration of up to 24 months.

IF medically indicated, subjects randomized to the immediate release tacrolimus treatment arm who are unable to tolerate oral administration may be administered tacrolimus via either IV solution, (**Prograf** solution for intravenous use) administered as a 24-hour continuous infusion, or via NGT for no more than seven consecutive days. Dosing via these routes should be discontinued as soon as the subject can tolerate oral administration, and may not exceed a period of seven consecutive days. Additionally, a subject may not receive more than

seven days of combined NGT and IV administration in cases where a subject is switched from one therapy to another. Intravenous tacrolimus will not be provided by the Sponsor.

4.2 Packaging and Labeling

Astagraf XL will be prepared, packaged, and labeled under the responsibility of qualified staff at AUST in accordance with APGD-AUST Standard Operating Procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, ICH GCP guidelines, and applicable local laws/regulations.

Each bottle will bear a label conforming to regulatory guidelines, Good Manufacturing Practice and local laws and regulations which identifies the contents as investigational drug.

Tacrolimus, immediate-release will be obtained by the transplanting center during a participant's inpatient stay and by the patients, themselves, thereafter, at retail pharmacies via a study-provided voucher.

Astagraf XL should be stored at 25°C (77°F); excursions permitted to 15°C-30°C (59°F to 86°F) [see United States Pharmacopeia (USP) Controlled Room Temperature].

Tacrolimus, immediate-release should be stored according to the product label.

4.3 Study Drug Handling

Current ICH GCP Guidelines require the investigator to ensure that study drug deliveries from the Sponsor are received by the investigator/or designee and

- that such deliveries are recorded,
- that study drug is handled and stored according to labeled storage conditions,
- that study drug with appropriate expiry/retest and is only dispensed to study subjects in accordance with the protocol, and
- that any unused study drug is returned to the Sponsor.

Drug inventory and accountability records for the study drugs will be kept by the investigator/or designee. Study drug accountability throughout the study must be documented and reconciled. The following guidelines are therefore pertinent:

- The investigator agrees not to supply study drugs/vouchers to any persons except the eligible subjects in this study in accordance with the protocol.
- The investigator or designee will keep the study drugs in a pharmacy or other locked and secure storage facility under controlled storage conditions, accessible only to those authorized by the investigator to dispense these test drugs.
- A study drug inventory will be maintained by the investigator or designee. The inventory will include details of material received and a clear record of when they were dispensed and to which subject.
- At the conclusion or termination of this study, the investigator or designee agrees to conduct a final drug supply inventory and to record the results of this inventory on the Drug Accountability Record. It must be possible to reconcile delivery records with those of used and/or returned medication. Any discrepancies must be accounted for and

documented. Appropriate forms of deliveries and returns must be signed by the site staff delegated this responsibility.

- The site must return study drug to the Sponsor or designee at the end of the study (or, with prior Sponsor approval, after interim reconciliation by the monitor) or upon expiration.

It is prohibited to remotely dispense study Astagraf XL (i.e., by mail or courier) to subjects unless there are exceptional circumstances and prior approval from the Sponsor is received in writing.

4.4 Blinding

This section is not applicable as this is an open-label study.

4.5 Assignment and Allocation

Randomization will be performed via Interactive Response Technology (IRT). Prior to the initiation of the study treatment, the site staff will contact the IRT in order to determine the randomly assigned treatment. Specific procedures for randomization through the IRT are contained in the study procedures manual.

- The randomization allocation will consist of a 1:1 ratio for twice daily, immediate-release tacrolimus or Astagraf XL. The randomization will be stratified according to the following three factors: Use of alemtuzumab (Campath) (yes/no)
- KDPI [3 levels: N/A (living donors) vs. ≤ 50 vs. > 50]
- HLA Class II mismatch (yes/no)

Additionally, enrollment of Campath users will be capped to not exceed 20% of the study population.

5 TREATMENTS AND EVALUATION

5.1 Dosing and Administration of Study Drug(s) and Other Medication(s)

5.1.1 Dose/Dose Regimen and Administration Period

Study participants will receive either Astagraf XL (single daily dose) or SOC, twice daily, immediate-release tacrolimus for up to 730 consecutive days (2 years) following their transplant procedure. Investigators are encouraged to start subjects on the randomized study treatment (immediate release tacrolimus or Astagraf XL) within 48 hours of transplantation. If medically indicated, therapy initiation with either tacrolimus agent can be delayed for up to 7 days. Administration of the randomized study treatment prior to the transplant is not allowed for the purposes of the current study.

When possible, patients should receive their daily dose of Astagraf XL using the fewest number of pills possible (i.e., a patient on 15 mg of Astagraf XL daily should ideally receive three 5 mg tablets). **Because of its extended-release pharmacokinetics, it is critical that Astagraf XL be initiated at a dose of 0.15/mg/kg/day as a single daily dose. Failure to**

dose patients in this manner will result in a protocol violation. Dry weight (or estimated dry weight, if dry weight is not available) should be used for the initial dosing. There is no dosing adjustment for subjects with gastric sleeves. After the initial dose, Astagraf XL dosing should be titrated according to the clinical judgment of the attending physician and appropriate TDM of tacrolimus trough concentrations, typically occurring 48 hours after a dose change. This dosing should account for the fact that when identical trough levels are targeted, systemic exposure to tacrolimus is the same for both Prograf and Astagraf XL.

Patients receiving immediate-release tacrolimus should be initiated on therapy per center protocol.

For the purposes of this study, patients receiving Astagraf XL or immediate-release tacrolimus should maintain a minimal tacrolimus trough concentration of 6 ng/mL at all times during the study. The failure to do so for four out of 6 weeks (continuous) during the first 6 weeks after transplantation will result in a subject level discontinuation. Patients requiring a reduction in tacrolimus levels in either cohort for treatment of infection, malignant disease, or other complication for four out of the first 6 weeks after transplantation will also result in a subject level discontinuation. Patients whose tacrolimus is withdrawn or who have a prolonged significant reduction of tacrolimus levels will be discontinued from the study (see Section 6.1).

Patients meeting inclusion criteria at the time of randomization and not initiated on Astagraf XL or immediate-release tacrolimus within 7 days of transplant will be discontinued.

IF medically indicated, subjects in either treatment arm who are unable to tolerate oral administration of their study drug may be administered tacrolimus via an alternative route. Subjects randomized to the Astagraf XL treatment arm may be administered IV tacrolimus. **Note, however, that Astagraf XL cannot be given intravenously**. Likewise, Astagraf XL should not be given via NGT due to its extended release characteristics. Subjects randomized to the immediate release tacrolimus arm may be administered tacrolimus via IV or NGT. Dosing via alternative routes should be discontinued as soon as the subject can tolerate oral administration, and may not be done for more than seven consecutive days. Additionally, for subjects in the immediate release tacrolimus arm, a subject may not receive more than seven consecutive days of combined NGT and IV administration in cases where a subject is switched from one therapy to another.

Intravenous tacrolimus will not be provided by the Sponsor.

Instructions for NGT Administration of Immediate Release Tacrolimus

NGT administration of immediate release tacrolimus is only allowed for subjects randomized to the immediate release tacrolimus treatment arm. If a decision is made to administer immediate release tacrolimus via NGT, the capsules may be opened and the contents mixed with 50 ml of water to form a suspension. The suspension should be drawn into a syringe and administered via the NGT. The container should then be refilled with another 50 ml of water, which should be drawn into the same syringe to flush the NGT tube and the container. After administration, the NGT may taken off suction in line with hospital practice. Clinical studies

have recommended removing the NGT from suction for 45 to 60 minutes after medication administration [Marubashi et al, 2012; PMR-EC-1106].

Appropriate safety precautions should be taken when preparing a suspension of immediate release tacrolimus.

Healthcare professionals should bear in mind that tacrolimus in any form is not compatible with PVC (polyvinylchloride). Tubing, syringes and other equipment used to prepare a suspension of immediate release tacrolimus capsule contents should not contain PVC.

5.1.2 Increase or Reduction in Dose of the Study Drug(s)

After initial dosing, subsequent dosing in patients receiving either Astagraf XL or immediate-release tacrolimus should be predicated on clinical experience, patient tolerability, and monitoring of whole blood tacrolimus drug concentrations, the measurement of which, along with target tacrolimus levels, should be guided by individual center protocol and currently approved US Package Insert. For the ASTOUND study, the lower limit of acceptable tacrolimus trough concentrations in both the Astagraf XL and immediate-release tacrolimus treatment arms is 6 ng/mL for the duration of the study. If a prolonged period of reduced immunosuppression will be required to treat infection, sepsis, delayed wound healing, or other circumstances, patients should be removed from the study as per the discontinuation criteria (Section 6.1).

5.1.3 Previous and Concomitant Treatment (Medication and Non-Medication Therapy)

Previous medication (all medications taken up to 30 days prior to transplant) and concomitant medication will be entered on the eCRF. All patients will receive induction immunotherapy (either T-cell depleting agent, IL-2 co-stimulation blocker, or anti-CD52 monoclonal antibody – steroid only induction therapy is not allowed). The dose and frequency of the chosen induction agent will be determined by the patient's treating physician and administered in conjunction with the participating transplant center's de novo kidney immunosuppression protocol. Anti-CMV, fungal, bacterial, and pneumocystis prophylaxis will be determined per local standard of care. As a participant in ASTOUND, in addition to tacrolimus, study subjects will need to concomitantly receive MMF (or Myfortic equivalent) and, if given per institutional protocol, corticosteroids (dose and duration of therapy of both medications to be determined according to clinical judgment of the patient's attending physician) as their maintenance immunosuppressive regimen.

5.1.4 Treatment Compliance

Study subjects should be counseled on the need to exhibit 100% compliance with their medication regimen in a manner that reflects a center's own standard of care. As this study is designed to assess the real-world impact of either once-daily Astagraf XL or a twice daily tacrolimus regimen, investigators or their designee should not change how they counsel patients on compliance based on their patient's randomization status or participation in ASTOUND.

Astagraf XL will be monitored by the accounting of unused medication returned by the subject at quarterly visits. Patients receiving immediate-release tacrolimus will be monitored by a third party vendor (via voucher system). Manufacturer, dose, number of pills dispensed, and the date of prescription refills will be captured using a voucher system with a built-in reporting mechanism.

5.1.5 Restrictions During the Study

The following are prohibited medications during ASTOUND (see Appendix 12.1):

1) Extended-release formulations of tacrolimus, other than Astagraf XL
2) Non-tacrolimus based immunosuppressive regimens (i.e., cyclosporine, everolimus, sirolimus, belatacept)
3) Antiviral medications used in HCV treatment
4) Antiviral medications used in HIV treatment
5) Isoniazid
6) Rifampin
7) Ethambutol
8) Pyrazinamide

5.2 Demographics and Baseline Characteristics

5.2.1 Demographics

The following demographic information will be collected during screening: date of birth, sex, race, and ethnicity.

5.2.2 Medical History

Site personnel will collect and record information regarding the subject's medical history (if available) at screening and any updated information will be recorded on study day 0. Medical history will include: subject etiology of renal failure, viral serology (e.g., HBV, HCV, CMV, EBV), duration and severity of renal disease, and diabetes history (as applicable) at enrollment.

5.2.3 Transplant Information

The following transplant information will be obtained at the Day 0 visit: type of transplant (living related, living non-related, DD, and whether organ was obtained in the setting of DCD), number of prior transplants and reason for prior graft loss (if applicable), total cold and warm ischemia time in hours and minutes, KDPI, ABO blood typing, HLA typing of donor and recipient (obtained via de-identified copies of the donor and recipient HLA typing reports, submitted to the Central HLA laboratory), degree of HLA mismatch between donor and recipient, and most recent panel reactive antibody testing (most recent cPRA level). Donor viral serology information (HBV, HCV, CMV and EBV), if available, will be collected and recorded. Additional donor information will include age, sex, height, weight,

donor cause of death, ethnicity of the donor, ex vivo perfusion parameters, ABO typing, and results of any pre-implantation biopsies of the donor kidney. De-identified copies of recent local pre-transplant DSA testing results (if performed) will b also be obtained.

5.2.4 Height and Body Weight

Height and dry weight will be recorded at screening. Dry weight will also be collected pre-operatively on study day 0.

5.2.5 Diagnosis of the Target Disease, Severity, and Duration of Disease

Medical history, including etiology of renal failure, will be collected during screening.

5.3 Efficacy Assessment

5.3.1 Efficacy

5.3.2 Donor Specific Antibody

Class I and Class II DSA will be assessed by a central HLA lab at 1, 3, 6, 9, 12, and 24 months in all patients, and as needed at baseline to confirm local testing results. Otherwise, initial DSA screening prior to transplant will be performed locally as per standard of care with results reported on the eCRF. For the purpose of assessing the impact of the proposed intervention (Astagraf XL vs. twice daily, immediate-release tacrolimus), detection of DSA at any time during the course of the study will be treated as a positive result. For the purposes of the study, DSA positivity will be defined at an MFI threshold approaching 1000. An independent adjudication board will take into account relevant clinical information to qualify samples in borderline cases. In cases where DSA is quantifiable, but nevertheless at an MFI < 1000, patient serum will be diluted and re-measured to mitigate the possible impact of the prozone phenomenon [Tambur et al, 2015]. Samples with new MFI levels above the target threshold will subsequently be regarded as positive.

In each case of quantifiable DSA or C1q-binding DSA at or above the MFI threshold, patient sera may be diluted 1:16 and re-assessed [Tambur et al, 2015]. MFI readings remaining above 10,000 following dilution or those increasing to over 10,000 following dilution (thus indicating prozone activity) will be regarded as “strong.” Samples with MFI values from 1000 to 10,000 will be designated “moderate.” Serum samples that demonstrate disappearance of MFI or reduction to < 1000 following dilution will be regarded as “weak.”

All borderline MFI threshold results will be reviewed by the Adjudication Board (see Section 10.2) in conjunction with relevant clinical information to confirm that the results have been categorized correctly. The Adjudication Board will also 1) review and adjudicate all cases antibody formation (per central HLA lab assessment) post-transplantation and 2) determine the need for ancillary testing of pre-transplant sera in equivocal cases in which such testing could indicate the presence of pre-transplant DSA.

5.3.3 Antibody Persistence

DSA and C1q-binding DSA will be regarded as persistent under the following conditions: 1) DSA is detected and remains above the threshold for positivity (MFI = 1000) for two consecutive or non-consecutive measurements, or 2) the new appearance of a DSA at the threshold for positivity when preceded by a DSA of a different specificity that has subsequently become non-detectable.

5.3.4 C1q-binding DSA

C1q-binding DSA will be assessed in all patients who are DSA positive. Results will be reported as positive or negative. MFI values and DSA strength will also be recorded.

5.3.5 IgG_3 Isotyping

IgG_3 isotyping will be performed in patients who are DSA positive. Results will be reported as positive or negative.

5.3.6 Requirement for Antibody Removal

The number of plasmapheresis sessions will be tracked in patients who develop DSA. The need for IV corticosteroids, IVIG (grams), thymoglobulin (mg), eculizumab, and bortezomib will be recorded per patient.

5.3.7 Histopathology

For all participants who undergo at least one kidney biopsy during the first year post-transplant (either for cause or per institutional protocol), the kidney biopsy performed closest to the 12 month visit (but no later than 14 months post-transplant) will be submitted forcentral histopathology grading. During the central histopathology grading, published criteria will be used to diagnose the following: 1) antibody mediated changes, 2) acute cellular and ABMR, 3) borderline changes (suspicious acute TCMR), and 4) IFTA. The diagnosis of acute and chronic forms of ABMR will be based on C4d positivity with prior or current evidence of DSA during the study or evidence of chronic tissue injury, such as glomerular double contours, and/or peritubular capillary basement membrane multilayering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries, also in-line with published criteria [Solez et al, 2008]. Biopsies will be regarded as suspicious for ABMR if C4d is not demonstrated in the biopsy, but morphologic evidence of tissue injury is nonetheless present, coincident with, or following DSA development [Solez et al, 2008].

Specimens will be similarly scored for g, t, v, i, cg, ct, ci, cv, ah, ptc, and mm in the manner previously described [Racusen et al, 1999]. Specimens will be regarded as positive for a particular feature if they receive a score > 0 for that particular characteristic. TG will be defined, for the purposes of this study, as cg > 0.

For specimens that are read locally, the above information will be extracted from the official biopsy report for inclusion in the eCRF.

5.3.8 Molecular Endpoints

Validated molecular signatures will be used to classify patients dichotomously at each blood sampling (i.e., 1, 3, 6, 9, 12, and 24 months following transplantation) as "IA" or Immune Quiescence (IQ). It is assumed that other causes for the IA signature other than tacrolimus exposure will be equally and randomly distributed between the 2 groups. The frequency of IA and IQ signatures will be compared between cohorts receiving Astagraf XL and immediate-release tacrolimus. The use of clinical and biopsy-proven samples from over 500 patients at 7 clinical centers in the United States and one from Brazil has enabled creation of locked Transplant Genomics Inc. (TGI) classifiers based on core data sets for each possible molecular phenotype. Thus, each new patient in the ASTOUND study will be treated as an unknown and run against each of the core data sets for classification using a TGI automated workflow based on multiple R Bioconductor tools and enabling scripts.

For the purposes of the study, assessment and comparison of the incidence of the molecular endpoint will rely on a binary molecular designation in accordance with the TruGraf v2.0 molecular assay. A negative designation (Trugraf TX Normal) will be referred to as Immune Quiescence (IQ). Due to operating characteristics of the assay, a positive designation will be considered evidence of Immune Activation (IA) in all patients.

The above-mentioned classifier has already been "locked" during the discovery process to avoid over-training a classifier on new data. As a dichotomous variable, 'IQ' will represent any patient sample classified as not positive. In turn, 'IA' will represent any patient sample not classified as 'IQ'. Biopsies, when available, will be used to inform the molecular diagnosis when alternative pathology is recognized (i.e. recurrent disease).

5.3.9 Estimated Glomerular Filtration Rate

Estimated glomerular filtration rate (eGFR) will be calculated at day 0 and months 1, 3, 6, 9, 12, and 24 (where available) using the Modification of Diet in Renal Disease (4 variable – MDRD) criteria.

5.3.10 Patient Survival

Patient survival is any subject that is known to be alive at the study conclusion.

5.3.11 Graft Survival

Graft survival is defined as any subject that does not fit the following definition of graft loss: subject death, retransplantation, transplant nephrectomy, or return to dialysis for a period of $\geq$ 6 weeks by study end.

5.3.12 Acute Rejection

For study purposes, diagnoses of rejection require biopsy confirmation. Both acute TCMR and ABMR (acute and chronic) will be assessed and graded according to published criteria [Solez et al, 2008].

5.4 Safety Assessment

5.4.1 Vital Signs

Vital signs will not be collected for this study.

5.4.2 Adverse Events

Adverse event collection will begin once informed consent/assent has been signed and continue throughout the subject's participation in the study. The kidney transplant surgery at Day 0 is not considered an AE or SAE. See **Section 5.5 Adverse Events and Other Safety Aspects** for information regarding adverse event collection and data handling.

5.4.2.1 Adverse Events of Possible Hepatic Origin

See **Appendix 12.2 Liver Safety Monitoring and Assessment** for detailed information on liver abnormalities, monitoring and assessment, if the AE for a subject enrolled in a study and receiving study drug is accompanied by increases in liver function testing (LFT, e.g.: AST, ALT, bilirubin, etc.) or is suspected to be due to hepatic dysfunction.

Subjects with AEs of hepatic origin accompanied by LFT abnormalities should be carefully monitored.

5.4.3 Laboratory and Pathological Assessments

A subset of the routine laboratory assessments obtained per SOC, including creatinine, hemoglobin, hematocrit, platelet count, white blood cell count, serum sodium, potassium, blood urea nitrogen, glucose, urinalysis (including urinary protein), tacrolimus trough concentrations, and HgA1c will be recorded on the eCRF. Of these, excepting creatinine and tacrolimus trough concentrations, only the assessments done closest to the targeted dates of visits will be recorded on the eCRF.

For creatinine assessments, the measurement done closest to the targeted visit date (obtained per SOC) as well as an additional assessment done at least one month prior to that assessment (if obtained per SOC) will be recorded on the eCRF.

For tacrolimus trough concentrations, only the assessments (as per SOC) done closest to the targeted visit dates will be recorded for the visits occurring at baseline, Day 30, Day 455, Day 545, Day 635, and day 730. For the visits occurring at Day 90, Day 180, Day 270, and Day 365, all available outpatient tacrolimus concentration assessments done (per SOC) and available in the centralized medical records since the previous study visit should be recorded.

All kidney transplant biopsy results obtained during the study will also be recorded on the eCRF.

For subjects who develop clinically significant BK viremia (as assessed per standard of care) during study participation, the peak viremia level obtained per standard of care will be retrospectively recorded in the eCRF at the time of the subject's study discontinuation or completion.

Additionally, blood will be collected at the baseline visit, and the study visits at months 1, 3, 6, 9, 12, and 24 for the following central laboratory assessments:

- DSA and determination of MFI and antibody strength, IgG_3 isotyping, and C1q-binding DSA capability
- Molecular diagnostics (not collected at baseline)
- Long term storage for potential future analyses (optional; not collected at baseline)

Please refer to Sections 5.7 and 5.8 for more information regarding sample collection.

5.4.4 Physical Examination

A complete physical exam consisting of an examination of general appearance, eyes, nose, throat, neck (including thyroid), lymph nodes, chest lungs, cardiovascular, abdominal, skin, extremities, musculoskeletal, and neurological system including mental status will be conducted at screening. Any abnormal findings must be assessed and documented as not clinically significant if a subject is to be enrolled in the study. The investigator or qualified designee will conduct the exam, determine findings, and assess any abnormalities as to clinical significance.

5.5 Adverse Events and Other Safety Aspects

5.5.1 Definition of Adverse Events (AEs)

An AE is defined as any untoward medical occurrence in a subject administered a study drug or who has undergone a study procedure which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An abnormality identified during a medical test (e.g., laboratory parameter, vital sign, ECG data, physical exam) should be defined as an AE only if the abnormality meets one of the following criteria:

- Induces clinical signs or symptoms
- Requires active intervention
- Requires interruption or discontinuation of study medication
- The abnormality or investigational value is clinically significant in the opinion of the investigator.

5.5.2 Definition of Serious Adverse Events (SAEs)

An adverse event is considered “serious” if, in the view of either the investigator or Sponsor, it results in any of the following outcomes:

- Results in death
- Is life threatening (an adverse event is considered “life-threatening” if, in the view of either the investigator or Sponsor, its occurrence places the subject at immediate risk of

death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death)
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly, or birth defect
- Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious)
- Other medically important events

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These events, including those that may result in disability/incapacity, should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Special situations on the medicinal products administered to the subject as part of the study (e.g., study drug, comparator, background therapy) that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Overdose of the medicinal product(s)
- Suspected abuse/misuse of the medicinal product(s)
- Drug exposure during pregnancy or via breast milk
- Medication error involving the medicinal product(s) (with or without subject/patient exposure to the Sponsor medicinal product, e.g., name confusion)

All of the special situations noted above should be recorded on the eCRF. Any special situations that also meets the criteria for an SAE should be recorded on the AE page of the eCRF and marked ‘serious’ and the SAE worksheet.

The Sponsor has a list of events that they classify as “always serious” events. If an adverse event is reported that is considered to be an event per this classification as “always serious”, additional information on the event may be requested.

5.5.3 Criteria for Causal Relationship to the Study Drug

Adverse events that fall under either "Possible" or "Probable" should be defined as "adverse events whose relationship to the study drugs could not be ruled out".

Causal relationship to the study drug	Criteria for causal relationship
Not Related	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and/or in which other drugs, chemicals, or underlying disease provide plausible explanations.

Causal relationship to the study drug	Criteria for causal relationship
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Probable	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on re- administration (rechallenge) or withdrawal (dechallenge).

5.5.4 Criteria for Defining the Severity of an Adverse Event

The investigator will use the following definitions to rate the severity of each adverse event

- Mild: No disruption of normal daily activities (Asymptomatic, or mild symptoms, clinical or diagnostic observations noted; intervention not indicated.)
- Moderate: Affect normal daily activities (Local or noninvasive intervention indicated.)
- Severe: Inability to perform daily activities (Medically significant but not immediately life threatening, hospitalization or prolonged hospitalization.)

5.5.5 Reporting of Serious Adverse Events (SAEs)

In the case of a serious adverse event (SAE), the investigator must contact the Sponsor by telephone or fax immediately (within 24 hours of awareness).

The investigator should complete and submit an SAE Worksheet containing all information that is required by the Regulatory Authorities to the Sponsor by fax immediately (within 24 hours of awareness). If the faxing of an SAE Worksheet is not possible or is not possible within 24 hours, the local drug safety contact should be informed by phone.

For contact details, see Section II Contact Details of Key Sponsor's Personnel. Please fax the SAE Worksheet to:

Astellas Global Pharmacovigilance	**Email: Safety-US@astellas.com** **North America telefax numbers: 888-396-3750 (alternate 847-317-1241)**

If there are any questions, or if clarification is needed regarding the SAE, please contact the Sponsor's Medical Monitor/Expert or his/her designee (see Section II Contact Details of Key Sponsor's Personnel).

Follow-up information for the event should be sent promptly (within 7 days of the initial notification.

Full details of the SAE should be recorded on the medical records and on the eCRF.

The following minimum information is required:

- ISN/Study number,
- Subject number, sex, and age,
- The date of report,
- A description of the SAE (event, seriousness of the event), and
- Causal relationship to the study drug.

The Sponsor or Sponsor's designee will submit expedited safety reports (i.e., IND Safety Reports) to the regulatory agencies (i.e., FDA) as necessary, and will inform the investigators of such regulatory reports. Investigators must submit safety reports as required by their Institutional Review Board (IRB)/Independent Ethics Committee (IEC) within timelines set by regional regulations (i.e., EU, (e)CTD, FDA). Documentation of the submission to and receipt by the IRB/IEC of expedited safety reports should be retained by the site.

The Sponsor will notify all investigators responsible for ongoing clinical studies with the study drug of all SAEs which require submission per local requirements IRB/IEC/ head of the study site.

The investigators should provide written documentation of IRB/IEC notification for each report to the Sponsor.

You may contact the Sponsor's Medical Monitor/Expert for any other problem related to the safety, welfare, or rights of the subject.

All AEs occurring after informed consent/assent is signed should be collected on the eCRF. The transplant operation on Day 0 is not considered an AE or SAE.

All new SAEs occurring up to 7 days after the end of study visit must be reported. SAEs starting after the subject's end of study visit but within 7 days shall be recorded via the SAE form only. After the 7 day period, Adverse Drug Reactions (non-serious and serious AEs related to systemic tacrolimus) will be captured through spontaneous reporting (please see Packet Insert for reporting information).

5.5.6 Follow-up of Adverse Events

All AEs occurring during the study are to be followed up until resolved or judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized. If during AE follow-up, the adverse event progresses to an ″SAE″, or if a subject experiences a new SAE, the investigator must immediately report the information to the Sponsor. Please refer to **Appendix 12.2 Liver Safety Monitoring and Assessment** for detailed instructions on Drug Induced Liver Injury (DILI).

5.5.7 Monitoring of Common Serious Adverse Events

Common serious adverse events are SAEs commonly anticipated to occur in the study population independent of drug exposure. SAEs classified as "common" are provided in **Appendix 12.3 Common Serious Adverse Events** for your reference. The list does NOT change your reporting obligations or prevent the need to report an AE meeting the definition

of an SAE as detailed above. The purpose of this list is to alert you that some events reported as SAEs may not require expedited reporting to the FDA based on the classification of "common serious adverse events" as specified in **Appendix 12.3 Common Serious Adverse Events**. The Sponsor will monitor these events throughout the course of the study for any change in frequency. Any changes to this list will be communicated to the participating investigational sites and regulatory authorities as appropriate. Investigators must report individual occurrences of these events as stated in **Section 5.5.5 Reporting of Serious Adverse Events.**

5.5.8 Procedure in Case of Pregnancy

If a female subject or partner of a male subject becomes pregnant during the study dosing period or within 30 days from the discontinuation of dosing, the investigator should report the information to the Sponsor as if it is an SAE. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result and neonatal data etc., should be included in this information.

The investigator will follow the medical status of the mother, as well as the fetus, as if the pregnancy is an SAE and will report the outcome to the Sponsor.

When the outcome of the pregnancy falls under the criteria for SAEs [spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly (including anomaly in a miscarried fetus)], the investigator should respond in accordance with the report procedure for SAEs. Additional information regarding the outcome of a pregnancy (which is categorized as an SAE) is mentioned below.

- ″Spontaneous abortion″ includes miscarriage, abortion, and missed abortion
- Death of an infant within 1 month after birth should be reported as an SAE regardless of its relationship with the study drug
- If an infant dies more than 1 month after the birth, it should be reported if a relationship between the death and intrauterine exposure to the study drug is judged as ″possible″ by the investigator
- In the case of a delivery of a living newborn, the "normality" of the infant is evaluated at the birth
- Unless a congenital anomaly are identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination

If during the conduct of a clinical trial, a male subject makes his partner pregnant, the subject should report the pregnancy to the investigator. The investigator will report the pregnancy to the Sponsor as an SAE.

5.5.9 Emergency Procedures and Management of Overdose

Experience with overdosage of tacrolimus is limited. Several cases of accidental overdosage have been reported; symptoms have included tremor, headache, nausea and vomiting, infections, urticaria, lethargy, increased blood urea nitrogen and elevated serum creatinine concentrations, and an increase in alanine aminotransferase levels. No specific antidote to systemic tacrolimus therapy is available. If overdosage occurs, general supportive measures

and symptomatic treatment should be conducted. Based on its high molecular weight, poor aqueous solubility, and extensive erythrocyte and plasma protein binding, it is anticipated that systemic tacrolimus will not be dialyzable. In isolated patients with very high plasma levels, hemofiltration or diafiltration have been effective in reducing toxic tacrolimus concentrations. In cases of oral intoxication, gastric lavage and/or the use of adsorbents (such as activated charcoal) may be helpful, if used shortly after intake.

5.5.10 Supply of New Information Affecting the Conduct of the Study

When new information becomes available necessary for conducting the clinical study properly, the Sponsor will inform all investigators involved in the clinical study as well as the regulatory authorities. Investigators should inform the IRB/IEC of such information when needed.

5.6 Test Drug Concentration

Not applicable.

5.7 Other Measurements, Assessments or Methods

5.7.1 Sample for the Analysis of Validated Gene Expression Profiling in the Peripheral Blood

Peripheral blood for the analysis of validated gene expression profiling will be collected at the study visits occurring 1, 3, 6, 9, 12, and 24 months following transplantation. Validated gene expression profiling analyses will be completed by Transplant Genomics (TGI). Whole blood will be collected at each of the above mentioned visits and shipped for central analysis, in accordance with the central laboratory manual.

Affymetrix Hu133PM Peg Arrays will be used in conjunction with a fully automated GeneAtlas instrument, which interrogates > 28,000 well-annotated genes along with Expressed Sequence Tags and other sequences representing over 47,000 analytes, such as long, non-coding RNAs from RefSeq and Ensembl. TGI automated bioinformatics workflows will be used for analysis of array-based RNA expression profiling in addition to multiple other tools including quantile frozen robust multi-array average (RMA) normalization, batch detection and correction, outlier detection by Principle Component Analysis (PCA), signal filtering by $\log_2$ histograms, differential expression by ANOVA methods with multiple correction testing, and multi-class prediction using DLDA validated by an independent classification tool, Vector Machines. Mapping to functional biological pathways will also be done using multiple tools including Gene Ontology, Ingenuity's Pathway Analysis™ (Ingenuity Systems, Redwood, CA), TGI's proprietary tool called ImmuneMap, WikiPathways, GSEA Broad, Panther, and National Center for Biotechnology Information (NCBI) databases such as DAVID. Visualization of networks and hub genes will be done with Cytoscape. Reports on functional pathway mapping will be prepared and provided to Astellas by TGI.

5.7.2 Blood Sample for DSA, C1q-binding DSA Analysis, and IgG_3 DSA Isotyping

Blood samples will be collected and shipped, in accordance with the central laboratory manual, for analysis at a central lab at baseline (prior to transplant; this baseline sample will be stored centrally for analysis as-needed), and at the study visits occurring 1, 3, 6, 9, 12 and 24 months post-transplant. These samples will be screened for anti-HLA class-I and class-II antibodies using Flow PRA. For samples testing positive, the Luminex solid phase assay (SPA) will be used to determine antibody specificity, using LabScreen® Single Antigen Detection Tests and the C1q Screen® (both One Lambda, Inc.) following the manufacturer's recommendations to obtain neat MFI values for DSA. C1q MFI will also be determined for those samples testing positive for DSA. Results from the centrally-performed Single Antigen Detection Tests on recipient serum will be compared to the donor HLA typing report furnished by the participating center to ascertain the presence of donor-specific anti-HLA antibody. De-identified local typing reports will be used to confirm the degree of HLA mismatch between donor and recipient. De-identified local recent pre-transplant DSA testing results (if performed) will be used to inform interpretation of the central DSA results.

Samples positive for DSA per study protocol will be tested a second time after diluting the serum 1:16 with phosphate buffered saline (PBS) to provide a semi-quantitative measure of antibody strength. Samples in which MFI disappears (becomes < 1000 MFI) will be regarded as "weak." Samples increasing to > 10,000 MFI upon dilution will be regarded as having significant prozone and will be regarded as "strong." Samples which may increase or decrease, but nevertheless remain between 1000 and 10,000 MFI will be regarded as "moderate." Samples with evidence of fluorescence that are nevertheless below MFI criteria for DSA positivity will also be subject to dilution. Samples increasing to above threshold criteria following dilution will regarded as positive and assumed to be under the influence of a prozone effect that impairs antibody detection. For statistical purposes, the antibody with the highest MFI level obtained during the course of the study will be used for statistical comparisons between groups. However, the strongest antibody observed at each HLA-locus will be reported separately and tracked for patients who are DSA positive.

C1q-binding DSA, MFI, and DSA IgG_3 isotyping will be performed in accordance with established protocol [Lefaucheur et al, 2015; Honger et al, 2011].

5.7.3 Storage of Donor Tissue for DNA Typing

In order to determine the presence of HLA-DSA with a high degree of accuracy, it is expected that in some rare situations, additional typing will be required using donor DNA due to the fact that frequently, only partial donor typing is available through the United Network of Organ Sharing (UNOS). This will be especially important in situations where antibodies might arise to HLA-DP, a locus that is infrequently typed in donors. Other examples may be situations where a donor is not typed at the level of resolution necessary to discern when a recipient has antibodies to some alleles of an antigen group and not others. Thus, to accommodate such contingencies, organ procurement organizations (OPOs) and HLA labs are required to maintain donor/recipient material by their respective regulatory bodies. In most cases, the relationships maintained by transplant centers with their local

OPO or HLA lab affords them access to this material if it is needed for subsequent, additional HLA typing. If this situation should arise in the course of ASTOUND, the participating transplant center will be asked to request such material from their OPO or HLA lab for additional donor HLA typing, the results of which will also be communicated to a patient's participating transplant physician. If additional typing is sought for recipients of living donor organs, donors will be approached and separately consented, as needed.

5.7.4 Banking of Blood for Future Studies

Patients participating in ASTOUND will be provided, through the informed consent/assent process, the option of having an additional sample collected at each phlebotomy session for storage and analysis in future trials of transplant outcomes and biomarker discovery. In the event additional investigations are contemplated, patients will be re-consented for subsequent use of their biological material. Samples will be stored for a maximum of 5 years and then destroyed thereafter. Samples will be stored at -80°C at a facility designated by the Sponsor.

5.8 Total Amount of Blood

Prior to transplant, participants will have one 10 mL tube of blood collected.

At each post-transplant study-designated phlebotomy session, participants will have one 10 mL tube and two or three 2.5 mL tubes of blood. The third 2.5 mL tube of blood will only be collected from patients consenting to additional blood storage. Seven study-specific phlebotomy sessions have been incorporated into ASTOUND and timed to what is likely to coincide with a participant's regularly scheduled therapeutic drug monitoring (TDM). The total amount of additional blood for research purposes to be collected from an individual study participant over the course of two years is expected to be between 100 and 115 mL.

6 DISCONTINUATION

6.1 Discontinuation of Individual Subject(s)

A discontinuation is a subject who enrolled in the study and for whom study treatment is permanently discontinued prematurely for any reason.

The subject is free to withdraw from the study treatment and/or study for any reason and at any time without giving reason for doing so and without penalty or prejudice. The investigator is also free to terminate a subject's involvement in the study at any time if the subject's clinical condition warrants it.

If a subject is discontinued from the study with an ongoing AE or an unresolved laboratory result that is significantly outside of the reference range, the investigator will attempt to provide follow-up until the condition stabilizes or no longer is clinically significant. However, other than pertinent information regarding the AE (i.e., follow-up laboratory values, AE information updates, etc.), no additional clinical information will be collected following the discontinuation.

Discontinuation Criteria from Study for Individual Subjects:

- Subject develops allograft loss (as defined by subject death, retransplantation, transplant nephrectomy, or return to dialysis of ≥ 6 consecutive weeks duration);
- Subject develops unacceptable toxicity or is withdrawn at the discretion of the patient's supervising physician;
- Subject is lost to follow-up despite reasonable efforts by the investigator to locate the subject;
- Subject withdraws consent for further treatment or expires during the course of the study;
- Subject begins taking any prohibited medications (see Section 5.1.5);
- Subject undergoes a second organ transplant;
- Conversion to a non-tacrolimus-based maintenance regimen in either study arm if required to manage toxicities;
- Removal from an MMF-containing immusosuppressive regimen (or its equivalent);
- Failure to achieve a tacrolimus whole blood concentration ≥ 6 ng/mL for 4 consecutive weeks during the first 6 weeks following transplantation;
- Permanent withdrawal of tacrolimus;
- Interruption or prolonged significant reduction of tacrolimus (as an example, a 50% reduction or dose at a level of less than 3.5ng/mL) for periods exceeding 4 weeks;
- Conversion *by the investigator* to the therapy of the other study treatment arm (i.e., subject randomized to Astagraf XL is converted to immediate release tacrolimus OR subject randomized to immediate release tacrolimus is converted to Astagraf XL. Cases of inadvertent and temporary conversion [i.e., dispensing error] do not meet this discontinuation criterion);
- Subject is not initiated on Astagraf XL or immediate-release tacrolimus within 7 days of transplant;
- Gross non-compliance with protocol: The medical monitor or investigator may request permanent study discontinuation in the event of a major protocol deviation such as administration of prohibited concomitant medication, lack of cooperation, or noncompliance.

6.2 Discontinuation of the Site

If an investigator intends to discontinue participation in the study, the investigator must immediately inform the Sponsor.

6.3 Discontinuation of the Study

The Sponsor may terminate this study prematurely, either in its entirety or at any study site, for reasonable cause provided that written notice is submitted in advance of the intended termination. Advance notice is not required if the study is stopped due to safety concerns. If the Sponsor terminates the study for safety reasons, the Sponsor will immediately notify the investigator and subsequently provide written instructions for study termination.

7 STATISTICAL METHODOLOGY

The statistical analysis will be coordinated by the responsible Astellas Medical Affairs, Americas (MA-A) biostatistician. A Statistical Analysis Plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be finalized before the database soft lock at the latest. Any changes from the analyses planned in the SAP will be justified in the Clinical Study Report (CSR).

Prior to Database Lock, a Final Review of Data and TLFs Meeting will be held to allow a review of the clinical trial data and to verify the data that will be used for analysis set classification. If required, consequences for the statistical analysis will be discussed and documented. A meeting to determine analysis set classifications may also be held prior to database lock.

In general, all data will be summarized by treatment group with descriptive statistics (number of subjects, mean, standard deviation, minimum, median, maximum, and interquartile range) for continuous endpoints, and frequency and percentage for categorical endpoints.

7.1 Sample Size

The sample size for this study was determined based upon a comparison of the rate of the two-part composite endpoint in the Astagraf XL group compared to the immediate-release tacrolimus group. Assuming a rate of 20% would be observed for the immediate-release tacrolimus group compared to a rate of 10% for the Astagraf XL group, 220 subjects per group are needed to achieve an 80% power to detect a difference between Astagraf XL and immediate-release tacrolimus with a two-sided alpha level of 0.05. The study will enroll 275 subjects/group (550 total) to allow for 20% dropout.

7.2 Analysis Set

Detailed criteria for analysis sets will be laid out in Classification Specifications and the allocation of subjects to analysis sets will be determined prior to database hard-lock.

7.2.1 Full Analysis Set (FAS)

The full analysis set will consist of all subjects who are randomized and receive at least one dose of study drug (Astagraf XL or immediate-release tacrolimus). Subjects with pre-transplant cross-match samples that later demonstrate pre-formed antibody (undetected at the time of transplant), as assessed by the adjudication board, will be included in the FAS but not in the modified FAS (see next section).

7.2.2 Modified Full Analysis Set (mFAS)

The modified full analysis set will consist of all subjects who are randomized and receive at least one dose of study drug (Astagraf XL or immediate-release tacrolimus), and whose pre-transplant cross-match antibody samples do not demonstrate pre-formed DSA for the duration of the study. This will be the primary analysis set for efficacy assessments.

7.2.3 Per Protocol Set (PPS)

The per protocol set will consist of the subset of the FAS who do not experience any major protocol deviations. Final adjudication and assessment of the classification for any protocol deviations will be conducted prior to database hard-lock. Further criteria may be defined in the SAP.

7.2.4 Safety Analysis Set (SAF)

For the statistical summary and analysis of safety data, the safety analysis set (SAF) will be used. The SAF consists of all subjects who enrolled into the study and took at least one dose of study medication.

7.2.5 Pharmacokinetic Analysis Set (PKAS)

Not applicable.

7.3 Demographics and Other Baseline Characteristics

Demographics and other baseline characteristics will be summarized by treatment group for the SAF. Descriptive statistics will be included for continuous endpoints, and frequency and percentage for categorical endpoints.

7.4 Analysis of Efficacy

The analysis of Efficacy will be conducted using the mFAS.

7.4.1 Analysis of Primary Endpoint

7.4.1.1 Primary Analysis

The primary efficacy endpoint is the combined incidence of either DSA or IA on molecular blood profiling at one year or at the conclusion of the study.

The primary endpoint will be analyzed at a single time point, corresponding to end of study and with respect to the formal stopping rule (see Section 7.8.1). The primary analysis of this endpoint will be based on the mFAS. The incidence of the primary endpoint will be summarized by treatment group with frequencies and percentages. Differences between treatment groups will be analyzed using logistic regression with the following covariates: treatment group (Astagraf XL group versus the immediate-release group), the pre-defined randomization stratification factors, recipient age, race, and gender; type of induction therapy, delayed graft function, and pre-transplant cPRA.

For subjects with missing or incomplete endpoint data who did not permanently switch therapy or were discontinued from the study prior to a DSA/IA event, who are lost to follow up, or those who expire for any reason), negative DSA/ IA values will be imputed unless there is an otherwise positive result.

The hypothesis for comparison is as follows:

H0: The probability of incidence for the primary endpoint is the same between patients receiving Astagraf XL and patients receiving immediate-release tacrolimus.

H1: The probability of incidence for the primary endpoint is not the same between patients receiving Astagraf XL and those receiving immediate-release tacrolimus.

Hypothesis testing will be performed using a two-sided test with a 0.05 significance level. The 95% confidence interval for the odds ratio of the primary endpoint between Astagraf XL and twice daily tacrolimus will be included.

7.4.1.2 Sensitivity Analysis

A sensitivity analysis using the FAS will be conducted on the primary endpoint. The same logistic regression analysis described in Section 7.4.1.1 will be used.

Additionally, a sensitivity analysis for mFAS patients will be performed exclusively using assessments with a basis in normal creatinine function.

7.4.1.3 Secondary Analysis

A secondary analysis using the PPS will be conducted on the primary endpoint data. The same logistic regression analysis described in Section 7.4.1.1 will be used.

7.4.1.4 Subgroup Analyses of the Primary Endpoint

Subgroup analyses of the primary endpoint, using the mFAS, will be conducted by patient age, sex, and race as well as donor race.

7.4.2 Analysis of Secondary Endpoints

Risk factor analyses will be conducted for all 15 binary assessments. In addition to the treatment effect, the risk factors assessed will include: manufacturer and type of immediate-release tacrolimus; medication compliance, prior transplantation recipient, IPV of tacrolimus whole blood concentrations; absolute tacrolimus whole blood concentration dose and duration of MMF (or Myfortic equivalent); amount and duration of post-transplant corticosteroid use, type and amount of induction therapy used; age, sex, race, cold ischemia time (CIT), warm ischemia time (WIT), KDPI, DCD status, LD vs. DD status, recipient pre-transplant dialysis status > 3 months (yes/no), donor race, pre-transplant donor kidney *ex vivo* perfusion parameters, degree of HLA mismatch, and pre-transplant cPRA; and the presence of post-transplant bacterial and viral infection (including BK and CMV). Additional risk factors may be included per the SAP.

Binary endpoints will be summarized with frequencies and percentages by treatment group and time point. The incidence of events will be analyzed for treatment group differences at each time point, in addition to overall. Analysis of treatment group differences will be implemented with generalized estimating equations (GEEs) using random effect(s), as necessary, to account for the temporal structure of data collection. This analysis applies to each of the following incidence-based endpoints:

1. DSA formation
2. IA
3. TG

4. Acute and chronic forms of ABMR
5. C1q-binding DSA
6. HLA-DQ DSA
7. DSA IgG_3 isotype
8. Requirement for antibody reduction therapy
9. eGFR at various thresholds (less than 30, 40, 50 mL/min/1.73 m^2 at one year or later; and a five-point decline)
10. Graft loss (defined as subject death, retransplantation, transplant nephrectomy, or a return to dialysis for at least a 6 week duration)
11. Mortality
12. BPAR (inclusive of ABMR and TCMR)
13. The four-part traditional composite endpoint: graft loss, local BPAR, mortality, and lost to follow-up (note – lost to follow-up will be summarized descriptively only)

GEE models used for analysis of treatment group differences will use the same covariates detailed in Section 7.4.1.1 with the exception of biopsy-based endpoints (i.e., TG and ABMR). In addition to the set of covariates from Section 7.4.1.1, biopsy-based endpoints will also include the covariates of CIT, WIT, KDPI, DCD status, and the three ex vivo perfusion parameters recorded pre-transplant. Similar to the primary endpoint, additional changes/details for the set of covariates will be included in the SAP.

For study purposes, lost to follow-up is defined as a subject failing to complete their final study visit no more than 30 days prior to the scheduled visit, and without a prior incidence of local BPAR, graft loss, or death. The incidence of lost to follow-up will be summarized with frequencies and percentage by treatment group.

The assessments of time to first occurrence for DSA, HLA-DQ DSA, C1q-binding DSA, DSA IgG_3 isotype, IA, TG, select BANFF histology grades (acute and chronic forms of ABMR, acute and chronic active TCMR, borderline changes, and IFTA), graft loss, mortality, local BPAR, and the four-part traditional composite endpoint will be summarized from the date of transplant. For patients who did not experience the endpoint event and/or were lost to follow-up or died prior to reporting the event, they will be censored at the date of their last known assessment/follow-up/death. Summaries will include estimable quartiles of time-to-first event with 95% confidence interval, Kaplan-Meier (KM) figures, and hazard ratios with 95% confidence interval. In the event that the median times are not estimable, the proportion of subjects with no DSA formation at the one and two year time points (as applicable) will be summarized by treatment group. Treatment group differences will be assessed with Cox regression models that account for the same covariates as used in the analogous GEE models.

The type of antibody reduction therapy will be summarized at each time point using frequencies and percentages. Analysis of treatment group differences will be conducted

using multinomial logistic regression and GEEs with the same covariates used in the primary endpoint's model and random effects(s), as necessary.

Ordinal assessments of antibody strength (weak, moderate, and strong) will be summarized at each time point using frequencies and percentages. Analysis of treatment group differences will be done by time point and overall using a proportional odds model and GEEs with the same covariates used in the primary endpoint's model and random effect(s), as necessary, to account for the temporal structure of data collection.

The continuous endpoint of MFI score will be summarized with descriptive statistics by time point. Due to the expected non-normality of errors, an analysis of treatment group differences will be implemented with a semi-parametric, mixed repeated measures model on rank scores using a random effect of subject and the same covariates included in the analysis of Section 7.4.1.1. Least square mean (LS Mean) estimates will be provided at each time point and treatment group differences will be assessed at each time point and overall.

Histopathology assessments will be summarized with both descriptive statistics and frequencies and percentages. Raw biopsy scores, including g, t, v, i, cg, ct, ci, cv, ah, ptc, and mm will be summarized as ordinal outcomes. Analysis of treatment group differences will be performed using the same proportional odds model and GEEs implemented for the analysis of antibody strength. The incidence of binary outcomes including ci, ct, and ptc scores greater than 1 will be summarized with frequencies and percentages. Analysis of treatment group differences will be done consistent with the models using GEEs for the other binary endpoints. For biopsy-based analyses, additional covariates will be added to the existing set in order to account for CIT, WIT, KDPI, DCD status, and the three ex vivo perfusion parameters recorded pre-transplant.

Assessments of association between DSA and IA will be analyzed overall and by treatment group with logistic regression models using DSA formation as the response and IA as predictor.

For patients who develop IA or DSA, change in eGFR from the time of diagnosis will be summarized with descriptive statistics by treatment group and by time point. An analysis of treatment group differences will also be made using the same mixed, repeated measures model that is fit for the raw MFI scores.

The binary endpoints (bulleted early in this section) and the persistence of DSA will be analyzed under the subgroups of patients who developed DSA and patients who developed IA. Treatment group differences by time point and overall will be assessed with the same GEE models and covariates detailed in this section.

7.4.3 Analysis of Exploratory Endpoints

Analysis will be performed on the secondary composite endpoint consisting of DSA formation and the presence of TG (cg > 0). The same analysis from Section 7.4.1.1 will be conducted for treatment group differences. The analysis will be performed using the mFAS population.

The associations between each of HLA-DQ DSA incidence, ordinal DSA strength, DSA formation, C1q-binding DSA, DSA IgG_3 isotype will be analyzed with respect to each other and with IA, TG, acute and chronic ABMR, histopathology, eGFR at various thresholds (less than 30, 40, and 50 mL/min/1.73 m^2), ABMR (acute and chronic), TCMR, graft loss, and mortality. Binary GEE models and proportional odds models will be fit for incidence and ordinal strength of HLA-DQ DSA, respectively. Associations will be analyzed between treatment groups and overall as well as across time points. Analogous methods will be used to assess the association between DSA and each component of the traditional composite endpoint.

The incidence of patients with normal and abnormal creatinine assessments and an IA designation will be summarized with descriptive statistics. Concordance between the IA and biopsy results will be summarized with descriptive statistics for each of the normal and abnormal creatinine assessments. Analysis of concordance between the IA and biopsy results will be conducted using Cohen's Kappa for each of the abnormal and normal creatinine assessments.

Percent MFI reduction will be summarized with descriptive statistics by time point. Analysis of treatment group differences will be done using the same semi-parametric, mixed, repeated measures model implemented for raw MFI scores. MFI shift will be summarized with frequencies and percentages and analyzed for treatment group differences using the same proportional odds model used to analyze DSA antibody strength.

Mapping of expression results to known immunological pathways implicated in immune-mediated, kidney transplant injury will be performed using multiple tools including Gene Ontology, Ingenuity's Pathway Analysis™ (Ingenuity Systems, Redwood, CA), TGI's proprietary tool called ImmuneMap, WikiPathways, GSEA Broad, Panther, and NCBI databases such as DAVID. Visualization of networks and hub genes will be done with Cytoscape. Reports on functional pathway mapping will be prepared and provided to Astellas by TGI.

Additional sensitivity and secondary analyses for secondary and exploratory endpoints may be conducted using the PPS or FAS.

7.5 Analysis of Safety

7.5.1 Adverse Events

Adverse events will be coded using the MedDRA. The number and percentage of AEs, SAEs, AEs leading to discontinuation, and AEs related to study drug will be summarized by system organ class, preferred term and treatment group. The number and percentage of AEs by severity will also be summarized. All AEs will be listed.

7.5.2 Laboratory Assessments

For quantitative laboratory measurements obtained during standard of care, descriptive statistics will be used to summarize results and change from baseline by treatment group and time point. Shifts relative to normal ranges from baseline to each time point during the

treatment period with regard to lab tests will also be tabulated. Laboratory data will be displayed in listings.

7.5.3 Histopathology

Data collected based on light microscopy, electron microscopy, and immunofluorescent images will be summarized by treatment group, pathologic designation, and time point stratified by local and central readings.

7.6 Analysis of Tacrolimus Dose and Trough Concentrations

Descriptive statistics (e.g., n, mean, standard deviation, minimum, median, maximum) as well as interquartile range, coefficient of variation, and geometric mean will be provided for tacrolimus dose and whole blood concentrations of tacrolimus for both study cohorts.

Tacrolimus concentrations will be summarized with descriptive statistics. Assumptions and methods for estimation and time point windowing will be detailed in the SAP as appropriate.

Tacrolimus trough by-patient CV and SD estimates will be assessed for association across time and overall with each of the following: the primary endpoint, the secondary composite endpoint of DSA and IA, DSA formation and IA individually, TG, chronic ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, histopathology, renal dysfunction, and the components of the traditional composite endpoint. Analysis details will be included in the SAP.

IPV of dose-adjusted tacrolimus trough levels after the 6 weeks following transplantation will be assessed for treatment group differences. The SAP will detail the analysis methods and time points.

The association between whole blood concentrations and the following endpoints will be assessed: DSA presence, DSA strength, C1q-binding DSA, IgG_3, IA,eGFR, histopathology, and conventional measures of transplant outcomes.

Additionally, the effect of switching product manufacturers during the study will be assessed with tacrolimus concentration. Changes will be assessed between treatment groups and for patients taking bid tacrolimus with the bid tacrolimus group analyzed for differences between those who did and did not switch manufacturers during the study.

7.7 Protocol Deviations and Other Analyses

Protocol deviations as defined in Section 8.1.6 will be summarized for all randomized subjects by treatment group and total as well as by site. A data listing will be provided by site and subject. The protocol deviation criteria will be uniquely identified in the summary table and listing. The unique identifiers will be as follows:

PD1 - Entered into the study even though they did not satisfy entry criteria,

PD2 - Developed withdrawal criteria during the study and was not withdrawn,

PD3 - Received wrong treatment or incorrect dose,

PD4 - Received excluded concomitant treatment.

7.8 Interim Analysis (and Early Discontinuation of the Clinical Study)

No formal interim analysis is planned.

7.8.1 Formal Stopping Rule

Given the variability of incidence rates for DSA reported in the literature, a stopping rule has been incorporated into ASTOUND to mitigate the risk of continuing the study under incorrect prior assumptions (Section 7.1). This stopping rule will be based on the incidence of the primary endpoint (combined incidence of either DSA or IA).

Patients will remain on treatment for 12 months or potentially longer (up to a maximum of 24 months). Nevertheless, in order to minimize the risk of conducting a study that could be, in the end, underpowered, a decision tree [Figure 3] will be actuated once 50% of control patients have completed one year of therapy. If, based on the enclosed algorithm, the primary endpoint's event rate in the control arm at that time is high enough for efficacy to be assessed at one year for the totality of subjects entering the study, the trial will continue enrolling and those subjects who, up until that point have completed <12 months of therapy, will be permitted to remain on such until study completion (1 year). However, it is acknowledged that in deploying a potential stopping rule once 50% of patients have completed one year of therapy, that a certain percentage of patients may have progressed beyond one year of therapy by the time the decision algorithm is applied. If this situation should occur, only the one year data for these patients will be included in the efficacy assessment, the exception being if the decision algorithm dictates that the study should continue for an additional year, in which case, the efficacy assessment will be made at 24 months for all patients.

For the decision algorithm, the estimated proportion will be used to calculate the Beta-binomial posterior Bayesian probability that the primary endpoint's incidence rate will, indeed, be at least 20% (see Appendix 12.4 for assumption details).

As mentioned above, a decision to terminate the study early or extend to a second year will be predicated on the event rate in the control arm once 50% of control patients have completed one year of therapy. At that time, the estimated proportion will be used to calculate the Beta-binomial posterior Bayesian probability that the primary endpoint's incidence rate will, indeed, be at least 20%.

If this probability is ≥ 70%, the following rules apply:

1) The study will continue to enroll to completion.
2) The study duration will be such that patients will complete at least one year of study visits.
    a) Patients who have not yet completed one year of study (at the time of the interim analysis) will complete the study at the 12m visit.
    b) Patients who have already completed the 12m visit (at the time of the interim analysis) will complete the study at their next scheduled study visit.
3) Data analysis will be performed once all patients complete 12 months of study.

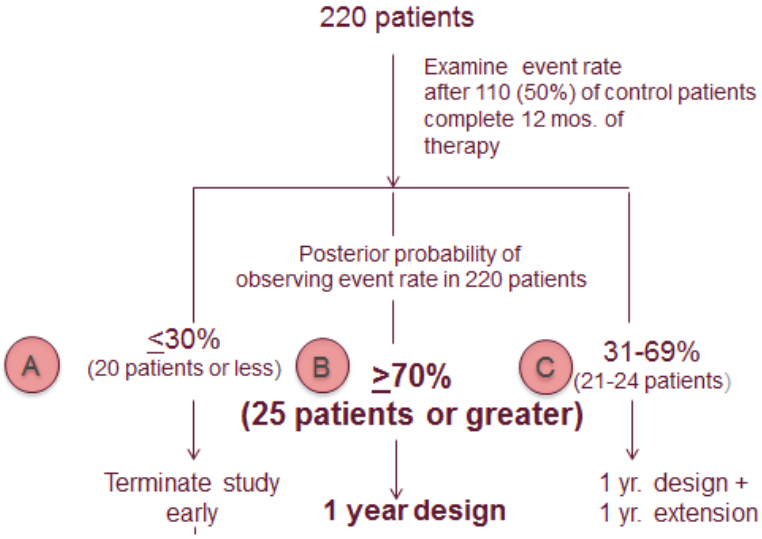
If this probability is < 70% and ≥ 30%:

1) The study will continue to enroll to completion.
2) The study duration will be 24 months; patients will complete the study at the 24 month visit.
3) Data analysis will be performed once all patients complete 24 months of therapy.

If this probability is < 30%, the study will be terminated at that time (50% of patients completing 12 months of therapy).

1) The study will be terminated upon completion of the interim analysis (done when 50% of patients have completed 12 months of therapy).
2) Patients will be discontinued from the study at their next scheduled study visit.

Figure 3 Application of Stopping Rule



7.9 Handling of Missing Data, Outliers, Visit Windows, and Other Information

In the primary efficacy analysis, other than imputation specifically stated in Section 7.4.1.1 there is no planned imputation of missing values.

See the SAP for details of the definitions for windows to be used for analyses by visit.

8 OPERATIONAL AND ADMINISTRATIVE CONSIDERATIONS

8.1 Procedure for Clinical Study Quality Control

8.1.1 Data Collection

The investigator or site designee will enter data collected using an Electronic Data Capture (EDC) system. In the interest of collecting data in the most efficient manner, the investigator or site designee should record data (including local laboratory values, as applicable) in the electronic case report form (eCRF) within 5 days after the subject visit.

The investigator or site designee is responsible to ensure that all data in the eCRFs and queries are accurate and complete and that all entries are verifiable with source documents. These documents should be appropriately maintained by the site.

The monitor should verify the data in the eCRFs with source documents according to the study monitoring plan.

Laboratory testing performed during the course of routine clinical care, including HLA typing and pre-transplant cross-match results, will be performed according to local standards. De-identified copies of HLA typing reports (donor and recipient) as well as recent pre-transplant local DSA testing results (if performed per SOC) will be sent to the central lab.

For the antibody and molecular assessments conducted by the central laboratory (performed at baseline, months 1, 3, 6, 9, 12, and 24), results will be compiled and the data electronically transferred to Astellas or its designee for inclusion in the clinical study database. The results of these central assessments for each participant will be shared with the corresponding Investigator. The results of the antibody assessments will be provided, as available (testing performed in batches), on an on-going basis, throughout the study. The results of the molecular assessments will not be shared with the corresponding Investigator until the end of the study.

For all participants who undergo at least one kidney biopsy during the first year post-transplant (either for cause or per institutional protocol), the kidney biopsy performed closest to the 12 month visit (but no later than 14 months post-transplant) will be submitted for central pathology review for pathological grading. It is preferred that centers submit whole slide digital imaging using a standard protocol on at least Hematoxlyin and Eosin (H&E), Periodic Acid Schiff (PAS), Trichrome-stained slides, silver slides, immunoperoxidase C4d, and control immunoperoxidase slides for C4d (as available). If an institution does not do whole slide imaging, then the paraffin section slides must also be submitted for central review along with the available digital images. Absent the availability of digital pathology transmission capabilities, centers will submit representative H&E, PAS, Trichrome-stained, silver, immunoperoxidase C4d, and control immunoperoxidase C4d slides (as available) for central pathology review. The results for each participant with a central pathology review will be shared with the corresponding Investigator at the end of study, upon Investigator/site request.

For screen failures, the demographic data, reason for failing, informed consent/assent, inclusion and exclusion criteria, and AEs will be collected in the eCRF.

The investigator or designee must record all protocol-required data in the provided electronic Case Report Forms (eCRF). In the interest of collecting data in the most efficient manner, the investigator or site designee should record data (including laboratory values, if applicable) onto the eCRF as soon as possible after the subject visit. ECRFs and any supporting documents should be available for retrieval by the Sponsor/delegated CRO at any given time. The monitor should verify the data in the eCRFs with source documents to confirm that there are no inconsistencies between them.

If any inconsistency is detected on the collected eCRFs, the monitor or data manager should query the investigator/sub-investigator. The investigator/sub-investigator should provide an answer to the query and provide the resolved query to the Sponsor.

The monitor should verify the revised data of the eCRFs with source documents and confirm that there are no inconsistencies between them, and also check that appropriate records on the correction/addition of data are maintained.

8.1.2 Specification of Source Documents

Source data must be available at the site to document the existence of the study subjects and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the subject.

The following information should be included in the source medical records:

- Demographic data (age, sex, race, ethnicity, height and body weight)
- cPRA
- Cross-match results
- Separate donor source data including donor demographics, KDPI of donor kidney and donor HLA typing.
- Inclusion and exclusion criteria details
- Participation in study and original signed and dated informed consent/assent forms
- Visit dates
- Medical history and physical examination details
- Key efficacy and safety data, if applicable (as specified in the protocol)
- Adverse events (including causality) and concomitant medication
- Results of relevant examinations (e.g., ECG charts, X-ray films etc.)
- Laboratory printouts (if applicable)
- Dispensing and return of study drug details
- Reason for premature discontinuation (if applicable)
- Randomization number (if applicable)

8.1.3 Clinical Study Monitoring

Astellas or Astellas' delegated CRO is responsible for monitoring the clinical study to ensure that subjects' human rights, safety, and well-being are protected, that the study is properly conducted in adherence to the current protocol and GCP, and that study data reported by the

investigator/sub-investigator are accurate and complete and that they are verifiable with study-related records such as source documents. Astellas or Astellas' delegated CRO is responsible for assigning a study monitor(s) to this study to ensure proper study oversight. Monitoring will occur in accordance with planned monitoring procedures.

8.1.4 Direct Access to Source Data/Documents

The investigator and the study site must accept monitoring and auditing by Astellas or its delegated CRO as well as inspections from the IRB/IEC and relevant regulatory authorities. In these instances, they must provide all study-related records, such as source documents (refer to Section 8.1.2 Specification of Source Documents) when they are requested by Astellas monitors and auditors, the IRB/IEC, or regulatory authorities. The confidentiality of the subject's identities shall be well protected consistent with local and national regulations when the source documents are subject to direct access.

8.1.5 Data Management

Data Management will be in accordance with the standard operating procedures (SOPs) of Astellas or Astellas' designated CRO. Data analysis will be performed in adherence to Astellas data standards. All study specific processes and definitions will be documented by Data Management. ECRF completion will be described in the eCRF instructions. Coding of medical terms and medications will be performed using MedDRA and World Health Organization (WHO) Drug Dictionary respectively.

8.1.6 Protocol Deviations

A protocol deviation is generally an unplanned excursion from the protocol that is not implemented or intended as a systematic change. The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol and for protecting the rights, safety, and welfare of subjects. The investigator should not implement any deviation from, or changes to, the protocol, unless it is necessary to eliminate an immediate hazard to trial subjects.

A protocol waiver is a documented prospective approval of a request from an investigator to deviate from the protocol. Protocol waivers are strictly prohibited.

For the purposes of this protocol, deviations requiring notification to Sponsor are defined as any subject who:

- Entered into the study even though they did not satisfy entry criteria.
- Developed withdrawal criteria during the study and not withdrawn
- Received wrong treatment or incorrect dose.
- Received excluded concomitant treatment.

When a deviation from the protocol is identified for an individual subject, the investigator or designee must ensure Astellas is notified. Astellas will follow-up with the investigator, as applicable, to assess the deviation and the possible impact to the safety and/or efficacy of the subject and to determine subject continuation in the study.

If a deviation impacts the safety of a subject, the investigator must contact the Sponsor immediately.

The investigator will also assure that deviations meeting IRB/IEC and applicable regulatory authorities' criteria are documented and communicated appropriately. All documentation and communications to the IRB/IEC and applicable regulatory authorities will be provided to the Sponsor and maintained within the Trial Master File (TMF).

NOTE: Other deviations outside of the categories defined above that are required to be reported by the IRB/IEC in accordance with local requirements will be reported, as applicable.

8.1.7 End of Trial in All Participating Countries

The end of trial in all participating countries is defined as the Last Subject's Last Visit.

8.2 Ethics and Protection of Subject Confidentiality

8.2.1 Institutional Review Board (IRB) / Independent Ethics Committee (IEC) / Competent Authorities (CA)

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator's Brochure, the informed consent, all other forms of subject information related to the study (e.g., advertisements used to recruit subjects), and any other necessary documents be reviewed by an IEC/IRB. The IEC/IRB will review the ethical, scientific and medical appropriateness of the study before it is conducted. IEC/IRB approval of the protocol, informed consent/assent, and subject information and/or advertising, as relevant, will be obtained prior to the authorization of drug shipment to a study site.

Any substantial amendments to the protocol will require IEC/IRB approval prior to implementation of the changes made to the study design at the site. The investigator will be required to submit, maintain, and archive study essential documents according to ICH GCP.

Any serious adverse events that meet reporting criteria, as dictated by local regulations, will be reported to both responsible Ethics Committees and Regulatory Agencies, as required. During the conduct of the study, the investigator should promptly provide written reports (e.g., ICH Expedited Reports, and any additional reports required by local regulations) to the IEC/IRB of any changes that affect the conduct of the study and/or increase the risk to subjects. Written documentation of the submission to the IEC/IRB should also be provided to Sponsor.

If required by local regulations, the investigator shall make accurate and adequate written progress reports to the IEC/IRB at appropriate intervals, not exceeding one year. The investigator shall make an accurate and adequate final report to the IRB/IEC within 90 days after the close-out visit for APGD-sponsored studies, or for APEB/APEL-sponsored studies within one year after last subject out (LSO) or termination of the study.

8.2.2 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, ICH guidelines, applicable regulations and guidelines governing clinical study conduct and the ethical principles that have their origin in the Declaration of Helsinki.

8.2.3 Informed Consent of Subjects

8.2.3.1 Subject Information and Consent/Assent

The investigator or his/her representative will explain the nature of the study to the subject or his/her parents/guardian (if applicable) or legal representative, and answer all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the informed consent (and assent, if applicable) statement will be reviewed and signed and dated by the subject or his/her parents/guardian or legal representative, the person who administered the informed consent, and any other signatories according to local requirements. A copy of the signed informed consent form (and assent, if applicable) will be given to the subject and the original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that informed consent (and assent, if applicable) was obtained prior to any study-related procedures and that the subject received a signed copy.

The signed consent forms (and assent, if applicable) will be retained by the investigator and made available (for review only) to the study monitor, regulatory authorities and other applicable individuals upon request.

8.2.3.2 Supply of New and Important Information Influencing the Subject's Consent and Revision of the Written Information

1. The investigator or his/her representative will immediately inform the subject orally whenever new information becomes available that may be relevant to the subject's consent or may influence the subject's willingness to continue to participate in the study (e.g., report of serious drug adverse drug reaction). The communication must be documented in the subject's medical records and must document whether the subject is willing to remain in the study or not.
2. The investigator must update their ICF and submit it for approval to the IRB/IEC. The investigator or his/her representative must obtain written informed consent (and assent, if applicable) from the subject on all updated ICFs throughout their participation in the study. The investigator or his/her designee must re-consent subjects with the updated ICF even if relevant information was provided orally. The investigator or his/her representative who obtained the written informed consent and the subject should sign and date the informed consent (and assent, if applicable) form. A copy of the signed informed consent (and assent, if applicable) form will be given to the subject and the original will be placed in the subject's medical record. An entry must be made in the subject's records documenting the re-consent process.

8.2.4 Subject Confidentiality

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Such medical information may be given only after approval of the subject to the subject's physician or to other appropriate medical personnel responsible for the subject's well-being.

For the purpose of fulfilling exploratory objectives, ASTOUND has made allowances for the conduct of future studies by which the long-term outcomes of patients testing positive for DSA or IA, as well as the health care costs associated with those outcomes, are examined.

For those patients agreeing to contribute to the possible future study of health care costs, Astellas would receive de-identified Medicare claims or commercial payer costs data from the participating transplant centers. If this follow up study is conducted, it will be done in accordance with a separate, IRB-approved study protocol.

For those patients agreeing to contribute to the possible future study of long-term outcomes, ASTOUND will make use of each patient’s unique transplant identifier, maintained by the OPTN for recording of data within the SRTR and UNOS databases. This identifier is maintained by each individual transplant center and permits access to the aforementioned databases for required reporting purposes. ASTOUND will not ask for or maintain a record of this identifier. In the event future long-term follow-up studies are warranted, individual transplant centers and investigators who participated in ASTOUND will be asked, subject to separate IRB approval, to query the respective databases in the context of those studies, with reporting of de-identified results (graft survival, patient survival, and immune-mediated phenomenon) to a central study location. Astellas shall not disclose any confidential information on subjects obtained during the performance of their duties in the clinical study without justifiable reasons.

Astellas affirms the subject's right to protection against invasion of privacy. Only a subject identification number (different and separate than that maintained by the OPTN) and/or initials will identify subject data retrieved by Astellas. However, Astellas requires the investigator to permit Astellas, its representative(s), the IRB/IEC and when necessary, and representatives of the regulatory health authorities to review and/or to copy any medical records relevant to the study.

Astellas will ensure that the use and disclosure of protected health information (PHI) obtained during a research study complies with the federal and/or regional legislation related to the privacy and protection of personal information (i.e., HIPAA).

8.3 Administrative Matters

8.3.1 Arrangement for Use of Information and Publication of the Clinical Study

Information concerning the study drug, patent applications, processes, unpublished scientific data, the Investigator's Brochure, and other pertinent information is confidential and remains the property of the Sponsor. Details should be disclosed only to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose

of the study only. It is understood by the investigator that the Sponsor will use the information obtained during the clinical study in connection with the development of the drug and therefore may disclose it as required to other clinical investigators or to regulatory agencies. In order to allow for the use of the information derived from this clinical study, the investigator understands that he/she has an obligation to provide the Sponsor with all data obtained during the study.

Publication of the study results is discussed in the Clinical Study Agreement.

8.3.2 Documents and Records Related to the Clinical Study

The investigator will archive all study data (e.g., Subject Identification Code List, source data, eCRFs, and Investigator's File) and relevant correspondence. These documents are to be kept on file for the appropriate term determined by local regulation (for US sites, two years after approval of the NDA or discontinuation of the IND). The Sponsor will notify the site/investigator if the NDA/MAA/J-NDA is approved or if the IND/IMPD/CHIKEN TODOKE is discontinued. The investigator agrees to obtain the Astellas' agreement prior to disposal, moving, or transferring of any study-related records. Astellas will archive and retain all documents pertaining to the study according to local regulations.

Data generated by the methods described in the protocol will be recorded in the subjects' medical records and/or study progress notes. All data will be entered on the eCRFs supplied for each subject.

8.3.3 Protocol Amendment and/or Revision

Any changes to the study that arise after approval of the protocol must be documented as protocol amendments/substantial amendments and/or /non-substantial amendments. Depending on the nature of the amendment, either IRB/IEC/CA approval or notification may be required. The changes will become effective only after the approval of Astellas, the investigator, the regulatory authority, and the IRB/IEC (if applicable).

Amendments to this protocol must be signed by Astellas and the investigator. Written verification of IRB/IEC approval will be obtained before any amendment is implemented which affects subject safety or the evaluation of safety, and/or efficacy. Modifications to the protocol that are administrative in nature do not require IRB/IEC approval, but will be submitted to the IRB/IEC for their information, if required by local regulations.

If there are changes to the Informed Consent/Assent, written verification of IRB/IEC approval must be forwarded to the Sponsor. An approved copy of the new Informed Consent/Assent must also be forwarded to Astellas.

8.3.4 Signatory Investigator for Clinical Study Report

ICH E3 guidelines recommend and EU Directive 2001/83/EC requires that a final study report which forms part of a marketing authorization application be signed by the representative for the Coordinating Investigator(s) or the Principal Investigator(s). The representative for the Coordinating Investigator (s) or the Principal Investigator(s) will have the responsibility to review the final study results to confirm to the best of his/her knowledge

it accurately describes the conduct and results of the study. The representative for Coordinating Investigator(s) or the Principal Investigator(s) will be selected from the participating investigators by Astellas prior to database lock.

9 QUALITY ASSURANCE

Astellas is implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted and data are generated, documented, recorded, and reported in compliance with the protocol, GCP, and applicable regulatory requirement(s).

Astellas or Astellas' designee may arrange to audit the clinical study at any or all investigational sites and facilities. The audit may include on-site review of regulatory documents, case report forms, and source documents. Direct access to these documents will be required by the auditors.

10 STUDY ORGANIZATION

10.1 Independent Data-Monitoring Committee (IDMC) | Data and Safety Monitoring Board (DSMB) | Monitoring Committee | Other Evaluation Committee(s)

Not applicable

10.2 Adjudication Board: MFI Adjudication Committee

An Adjudication Board will be formed to review all borderline MFI threshold results that occur during the study to confirm that the results have been categorized correctly. The Board will consist of three independent members of appropriate expertise who are not directly involved in the clinical study and who are blinded to the treatment allocation. Information related to the borderline threshold results will be sent to the committee for blinded assessment. A separate charter will describe which results will require adjudication and the specification of the result-related documents, listings, data flow, method(s) for data collection and data transfer.

The Adjudication Board will 1) review and adjudicate all cases of antibody formation (per central HLA lab assessment) post-transplantation and 2) determine the need for ancillary testing of pre-transplant sera in equivocal cases in which such testing could indicate the presence of pre-transplant DSA.

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Company Reports:

Astagraf XL US Package Insert. Northbrook, IL: Astellas Pharma US, Inc.; June 2015.

12 APPENDICES

12.1 List of Excluded Concomitant Medications

The following are prohibited medications during ASTOUND:

1) Extended-release formulations of tacrolimus, other than Astagraf XL
2) Non-tacrolimus based immunosuppressive regimens (i.e. cyclosporine, everolimus, sirolimus, belatacept)
3) Antiviral medications used in HCV treatment
4) Antiviral medications used in HIV treatment
5) Isoniazid
6) Rifampin
7) Ethambutol
8) Pyrazinamide

12.2 Liver Safety Monitoring and Assessment

Any subject enrolled in a clinical study with active drug therapy and reveals an increase of serum aminotransferases (AT) to > 3 × ULN, or bilirubin > 2 × ULN, should undergo detailed testing for liver enzymes (including at least ALT, AST, ALP, and TBL). Testing should be repeated within 48-72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central lab regarding moderate and severe liver abnormality to inform the investigator, study monitor and study team. Subjects should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN:

	ALT or AST		Total Bilirubin
Moderate	> 3× ULN	or	> 2× ULN
Severe*	> 3× ULN	and	> 2× ULN

In addition, the subject should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8× ULN
- ALT or AST > 5× ULN for more than 2 weeks
- ALT or AST > 3× ULN and INR > 1.5 (If INR testing is applicable/evaluated).
- ALT or AST > 3× ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%).

The investigator may determine that abnormal liver function results, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and laboratory tests. The site should complete the Liver Abnormality Case Report Form (LA-CRF) that has been developed globally and can be activated for any study> or an appropriate document. Subjects with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal LFTs should be repeated 2-3 times weekly then weekly or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as a Serious Adverse Event (SAE). The Sponsor should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to study drug are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new onset-diseases should be recorded as 'adverse events' on the AE page of the eCRF. Illnesses and conditions such as hypotensive events, and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Non-alcoholic steatohepatitis (NASH) is seen in obese hyperlipoproteinemic, and/or diabetic patients and may be associated with fluctuating aminotransferase levels. The investigator should ensure that the medical history form captures any illness that pre-dates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including non-prescription medication, complementary and alternative medications), alcohol use, recreational drug use, and special diets. Medications, including dose, should be entered on the concomitant medication page of the eCRF. Information on alcohol, other substance use, and diet should be entered on the LA-CRF or an appropriate document.
- Obtain a history of exposure to environmental chemical agents.
- Based on the subject's history, other testing may be appropriate including:
    - acute viral hepatitis (A,B, C, D, E or other infectious agents)
    - ultrasound or other imaging to assess biliary tract disease
    - other laboratory tests including INR, direct bilirubin
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

Study Discontinuation

In the absence of an explanation for increased LFT's, such as viral hepatitis, pre-existing or acute liver disease or exposure to other agents associated with liver injury, the subject may be discontinued from the study. The investigator may determine that it is not in the subject's best interest to continue study enrollment. Discontinuation of treatment should be considered if:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks
- ALT or AST > 3 × ULN and TBL > 2 × ULN or INR > 1.5) (If INR testing is applicable/evaluated)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%).

In addition, if close monitoring for a subject with moderate or severe hepatic laboratory tests is not possible, drug should be discontinued.

*Hy's Law Definition-Drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10–50% mortality (or transplant)." The two "requirements" for Hy's Law are: 1) Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in transaminase elevations higher

3 times the upper limit of normal ("2 x ULN elevations are too common in treated and untreated patients to be discriminating"). 2) Cases of increased bilirubin (at least 2 x ULN) with concurrent transaminase elevations at least 3x ULN and no evidence of intra- or extra-hepatic bilirubin obstruction (elevated alkaline phosphatase) or Gilbert's syndrome. [Temple R. Hy's law: predicting serious hepatotoxicity. *Pharmacoepidemiol Drug Saf* 2006 Apr;15(4):241-3.]

Reference

Guidance for Industry titled "Drug-Induced Liver Injury: Premarketing Clinical Evaluation" issued by FDA on July 2009.

12.3 Common Serious Adverse Events

The following list of adverse events are considered common for the renal transplant study population and single occurrences of the AEs if serious, unexpected and related will not be reported in an expedited manner to the FDA and investigators. Frequency of these events will be analyzed according to the protocol Section 7.5 and/or the Statistical Analysis Plan. If aggregate analysis of these events indicate that they occur more frequently with study drug an expedited report will be submitted to FDA as well as investigators.

1. Kidney transplant rejection
2. Kidney allograft loss
3. Increase in blood creatinine
4. Diabetes mellitus
5. Hyperglycemia
6. Increase in blood glucose

12.4 Interim Decision Rule Details

The Interim report stopping rules for this study are based on a Bayesian methodology of the *Beta-Binomial* posterior distribution.

The prior distribution used as the basis of the posterior is the *Beta(α=2, β=8)*. This prior was chosen due to its expectation (mean) of 0.20, and therefore remains consistent with the assumption that the twice daily tacrolimus DSA/IA composite endpoint incidence rate is 0.20 (Section 7.1). The variance of the distribution was also analyzed for robustness to the scaling of α and β, holding the expectation constant (mean of 0.20). Note that in this case, the variance of the *Beta* distribution is inversely related to the scaling of parameters. The decision rule cutoff values found in Section 7.8.1 remain robust to scaling of the parameters for anything from 1/1000 to unity (results not shown). Decreasing the variance beyond what is associated with a *Beta(α=2, β=8)* distribution was not considered in order to maintain the conservative nature of weighting prior information.

Let x be the number of occurrences of the DSA/IA composite primary endpoint, n be the number of evaluable subjects at the time of analysis, and p be the actual probability of the primary endpoint's occurrence in the population of twice daily tacrolimus use. Therefore, the posterior probability is based on the assumption that x given n and p is distributed as $Binomial(n, p)$. Therefore given the prior distribution of $p|\alpha, \beta \sim Beta(\alpha = 2, \beta = 8)$ and a binomial distribution for the frequency of the DSA/IA primary endpoint ($x|n, p \sim Binomial[n, p]$), then by Bayes' rule the conditional distribution of p follows as a *Beta-Binomial* posterior of $p|x, n, \alpha, \beta \sim Beta(2 + x, 8 + n - x)$.

The following table [Table 1] illustrates the possible posterior probabilities of $p \geq 0.2$ for the twice daily tacrolimus group given 50% of the planned subjects completed the 12 month follow-up visit ($n = 110$). In particular, the distribution in this case is $p|x, n, \alpha, \beta \sim Beta(2 + x, 118 - x)$.

Table 1 50% of Subjects Complete 12 Month Visit (*n*=110)

Incidence of DSA/IA	**Observed Proportion**	**Posterior Probabilities**
x	***p=x/110***	**P(p≥0.20|*x,n,alpha,beta*)**
10	0.091	0.001
11	0.1	0.003
12	0.109	0.006
13	0.118	0.013
14	0.127	0.024
15	0.136	0.042
16	0.146	0.07
17	0.155	0.11
18	0.164	0.162
19	0.173	0.228
20	0.182	0.305
21	0.191	0.391
22	0.2	0.482
23	0.209	0.572
24	0.218	0.659
25	0.227	0.737
26	0.236	0.804
27	0.246	0.859
28	0.255	0.902
29	0.264	0.934
30	0.273	0.958
31	0.282	0.974
32	0.291	0.984
33	0.3	0.991
34	0.309	0.995
35	0.318	0.997
36	0.327	0.999

12.5 Interim Stopping Rules Risk Analysis

The purpose of this appendix is to provide an assessment of favorable scientific outcomes that are possible under the conditions of early termination of the IDTX-MA-3004 study. This analysis explores how it is possible to have a favorable scientific outcome if the study would be terminated early due to the immediate-release tacrolimus arm having a DSA/IA incidence rate less than 0.20.

All analyses in this report are based on SAS version 9.3, a 2-sided test, significance level of 0.05, and power of at least 0.80. Additionally, the conservative distributional assumptions of Fisher's Exact Test are used.

The following figure and table illustrate the difference in statistical significance tests between having a sample of 220 subjects per treatment group (study data from either the one year or two year study design) versus 110 subjects (early termination study data). In this case, the difference between the boundaries of statistical significance for the two possible sample sizes illustrates the risk of the study having insufficient power to statistically detect a difference between the treatment arms due to early termination of the study.

For example, the first line of Table 1 represents a potential scenario where the immediate-release tacrolimus cohort has a 0.19 incidence of DSA/ IA. In this scenario, if the sample size per treatment group is N=220, statistical significance will be met with at least 80% power if the Astagraf XL cohort's incidence is 0.093 or less. However, if the size of each treatment group is N=110, statistical significance will be met with at least 80% power if Astagraf XL cohort's incidence of DSA/IA is less than or equal to 0.059. Therefore, in this example scenario, the range between 0.059 and 0.093 for DSA/IA incidence in the Astagraf XL cohort shows the risk of study failure due to early study termination – i.e. had the study not terminated early, the Astagraf XL cohort's DSA/IA incidence would be statistically distinguishable from the immediate-release tacrolimus cohort's incidence; however, with the early termination, there is not sufficient statistical power to detect a difference in DSA/IA incidence between the two cohorts.

The calculations in the following table and figure show the incidence boundaries for at least 80% power under incidence rates of DSA/IA in the immediate-release tacrolimus arm of 0.04 to 0.19. Values of Astagraf XL less than immediate-release tacrolimus values were investigated exclusively, as values of Astagraf XL being larger than immediate-release tacrolimus are not of scientific interest for this study.

Table 1 Statistically significant outcomes with at least 80% power.

	Astagraf XL			
	N = 220		N = 110	
BID Tac	Statistical Significance with ≥ 80% Power	Percent Decrease	Statistical Significance with ≥ 80% Power	Percent Decrease
0.19	≤ 0.093	51.05	≤ 0.059	68.95
0.18	≤ 0.085	52.78	≤ 0.053	70.56
0.17	≤ 0.078	54.12	≤ 0.047	72.35
0.16	≤ 0.071	55.63	≤ 0.041	74.38
0.15	≤ 0.064	57.33	≤ 0.035	76.67
0.14	≤ 0.057	59.29	≤ 0.030	78.57
0.13	≤ 0.050	61.54	≤ 0.025	80.77
0.12	≤ 0.043	64.17	≤ 0.020	83.33
0.11	≤ 0.037	66.36	≤ 0.015	86.36
0.10	≤ 0.031	69.00	≤ 0.011	89.00
0.09	≤ 0.025	72.22	≤ 0.007	92.22
0.08	≤ 0.019	76.25	≤ 0.003	96.25
0.07	≤ 0.014	80.00	NA	NA
0.06	≤ 0.009	85.00	NA	NA
0.05	≤ 0.005	90.00	NA	NA
0.04	≤ 0.001	97.5	NA	NA

Figure 1 Astagraf XL and immediate-release tacrolimus DSA/IA proportions that are statistically significant with 220 and 110 subjects per group and at least 80% power.

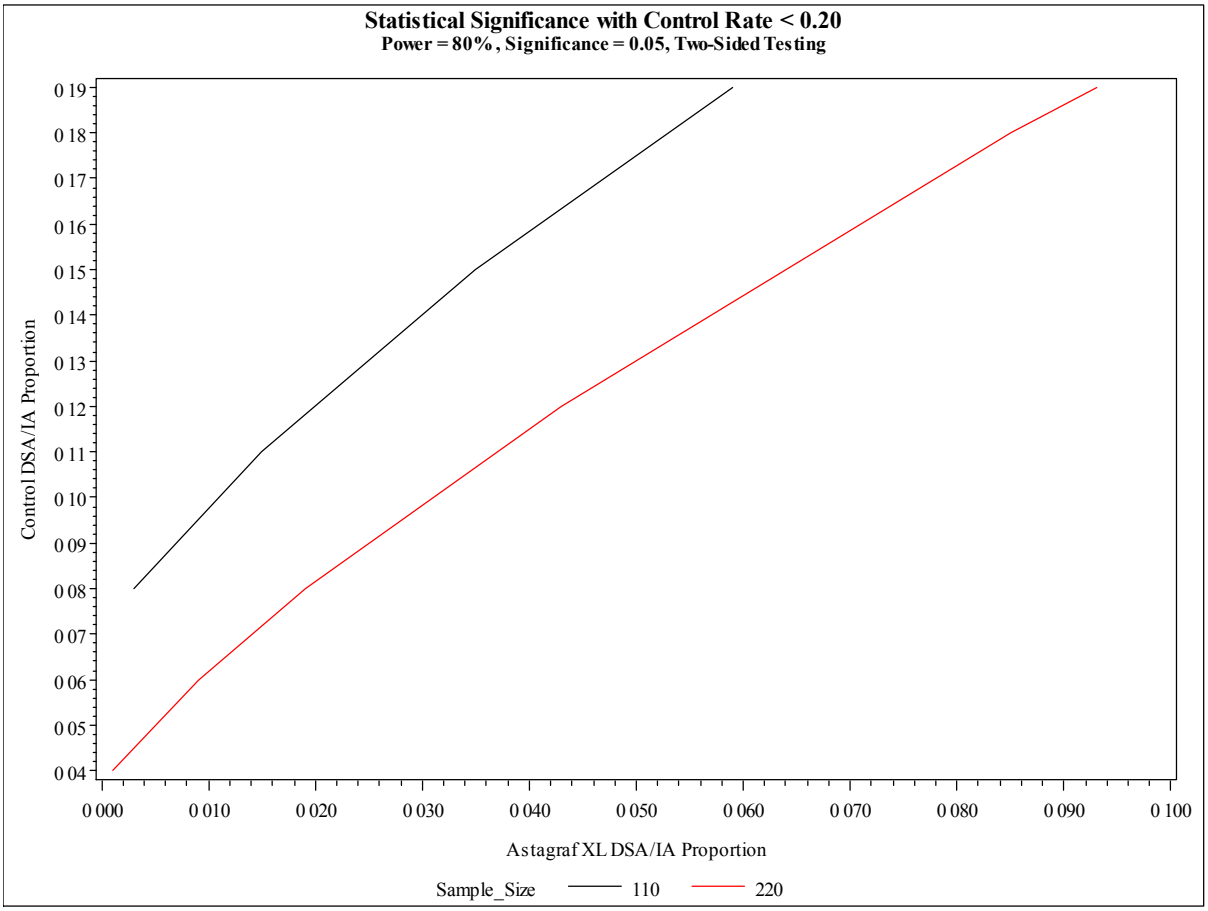

Figure 1 illustrates statistical significance with 80% power on the continuum for all points falling above each of the respective lines. For example, any points above the red line represent outcomes of statistical significance with at least 80% power for the full study sample of 220 subjects. The same applies to the black line representing N=110 as the early termination sample size. Therefore, the risk of early termination for the study is all points between each of the two lines in the figure.

This area of risk has been identified and mitigated with the current stopping rules in Section 7.8.1 of the study protocol.

13 ATTACHMENT 1: NON-SUBSTANTIAL AMENDMENT 2

ISN/Protocol IDTX-MA-3004
Non-Substantial Amendment 2
May 25, 2018

I. The purpose of this amendment is:

Non-Substantial Changes
1. Revision of molecular profiling categories
DESCRIPTION OF CHANGE
Sub-categories for the molecular profiling endpoints of cAR (clinical acute rejection) and subAR (subacute rejection) were removed throughout the protocol.
RATIONALE:
Since the approval of the previous protocol amendment, Transplant Genomics, Inc. (the vendor performing the molecular categorization analyses) have revised their molecular categorizations; the changes in the amendment, therefore, reflect the categorizations available in the most contemporary version of Transplant Genomics' TruGraf v2.0 test.
2. Addition of two exploratory objectives to compare the molecular profiling results with creatinine and (where available) biopsy results
DESCRIPTION OF CHANGE
Two exploratory objectives were added to assess creatinine results in subjects with the immune activation (IA) designation and to compare biopsy results for concordance, where available, in patients with an IA designation and abnormal creatinine.
RATIONALE:
Given that the TruGraf 2.0 test has only been formally validated in patients with normal renal function, additional exploratory objectives were included to improve the confidence of results in all study patients (which also includes patients with abnormal creatinines).
3. Clarification of the requirement that all subjects must be taking Mycophenolate Mofetil (MMF) or its equivalent while on study
DESCRIPTION OF CHANGE
Language was inserted into the concomitant medications section and discontinuation criteria to clarify that patients must be maintained on MMF or its equivalent throughout the study.
RATIONALE
Correction of previous oversight; MMF or its equivalent is given with tacrolimus per standard practice / product labeling requirements.
4. Addition of discontinuation criteria related to tacrolimus discontinuation or

interruption
DESCRIPTION OF CHANGE
Discontinuation criteria were added to include discontinuation for subjects who permanently withdraw from tacrolimus or who have a significant reduction or interruption of tacrolimus for a period exceeding four weeks.
RATIONALE:
In the previous amendment, the requirement for a minimum tacrolimus trough concentration was removed (due to variation in standard of care therapeutic drug monitoring levels at participating centers). This raised questions about whether subjects whose tacrolimus was intentionally reduced (e.g., temporarily, due to infection or other medical reasons) should be discontinued. These revised discontinuation criteria address those questions.
5. Clarification that Astagraf XL may not be remotely dispensed without prior written approval from the Sponsor
DESCRIPTION OF CHANGE
Language inserted to confirm that, due to study drug handling requirements, remotely dispensing Astagraf XL (for example, by mail or by courier) is prohibited without prior written approval from the Sponsor.
RATIONALE
Several participating centers have provided feedback that language related to study drug handling is not sufficiently clear in the protocol for them to discern whether Astagraf XL may be sent directly to subjects via courier. Language was added to prevent centers from couriering study Astagraf XL to study subjects without adequate quality assurance.
6. Minor edits to improve clarity of language
DESCRIPTION OF CHANGE
Grammatical, consistency, and other minor edits throughout the document.
RATIONALE:
The changes are intended to facilitate better understanding and implementation of the study protocol.

II Amendment Summary of Changes: Non-Substantial

1. Revision of molecular profiling categories	
III LIST OF ABBREVIATIONS AND DEFINITION OF KEY TERMS *Page 11, List of Abbreviations and Definitions of Key Terms*	
DELETED:	
~~cAR~~	~~Clinical Acute Rejection~~

1. Revision of molecular profiling categories	
Page 13, List of Abbreviations and Definitions of Key Terms	
DELETED:	
~~subAR~~	~~Subacute Rejection~~

1. Revision of molecular profiling categories
IV Synopsis, Study Objective(s) and 2.1 Study Objectives *Page 15; IV. Synopsis – Study Objective(s)* *Page 42; Section 2.1.2 Secondary Objectives*
WAS:
• To assess the risk factors for each of the following outcomes: DSA formation; IA (and separately for cAR [clinical acute rejection] and subAR [subacute rejection]); transplant glomerulopathy (TG); acute and chronic forms of antibody-mediated rejection (ABMR); C1q-binding DSA; HLA-DQ DSA; DSA IgG_3 isotype; requirement for and type of antibody reduction required; various threshold levels of estimated glomerular filtration rate (eGFR) (less than 30, 40, and 50 mL/min/1.73 m^2); and the four components of the traditional composite endpoint, consisting of graft loss, mortality, biopsy-proven acute rejection (BPAR), and loss to follow-up; and additionally, the persistence of DSA and IA.
IS AMENDED TO:
• To assess the risk factors for each of the following outcomes: DSA formation~~;~~**,** IA ~~(and separately for cAR [clinical acute rejection] and subAR [subacute rejection])~~; transplant glomerulopathy (TG); acute and chronic forms of antibody-mediated rejection (ABMR); C1q-binding DSA; HLA-DQ DSA; DSA IgG_3 isotype; requirement for and type of antibody reduction required; various threshold levels of estimated glomerular filtration rate (eGFR) (less than 30, 40, and 50 mL/min/1.73 m^2); and the four components of the traditional composite endpoint, consisting of graft loss, mortality, biopsy-proven acute rejection (BPAR), and loss to follow-up; and additionally, the persistence of DSA and IA.

1. Revision of molecular profiling categories
IV Synopsis, Study Objective(s) and 2.1 Study Objectives *Page 16; IV. Synopsis – Study Objective(s)* *Page 43; Section 2.1.2 Secondary Objectives*
WAS:
• Compare the persistence of the development of cAR and subAR on molecular profiling between the two cohorts across the study duration in those patients who develop IA on molecular profiling.
IS AMENDED TO:
• Compare the persistence of the development of ~~cAR and subAR~~ **IA** on molecular profiling between the two cohorts across the study duration in those patients who develop IA on molecular profiling.

1. Revision of molecular profiling categories
IV Synopsis, Study Objective(s) and 2.1 Study Objectives *Page 16; IV. Synopsis – Study Objective(s)* *Page 43; Section 2.1.3 Exploratory Objectives*
WAS:
• To assess and compare, between treatment groups, the association/correlation of incidence and strength of DSA, and the incidences of HLA-DQ DSA, C1q-binding DSA, and DSA IgG_3 isotype with each other, and individually, with each of the following: IA (and components thereof), TG, ABMR (both chronic forms and acute), Banff histology, various thresholds of eGFR (less than 30, 40, and 50 mL/min/1.73 m^2), eGFR change over time, TCMR, graft loss, and mortality.
IS AMENDED TO:
• To assess and compare, between treatment groups, the association/correlation of incidence and strength of DSA, and the incidences of HLA-DQ DSA, C1q-binding DSA, and DSA IgG_3 isotype with each other, and individually, with each of the following: IA ~~(and components thereof)~~, TG, ABMR (both chronic forms and acute), Banff histology, various thresholds of eGFR (less than 30, 40, and 50 mL/min/1.73 m^2), eGFR change over time, TCMR, graft loss, and mortality.

1. Revision of molecular profiling categories
IV Synopsis, Study Objective(s) and 2.1 Study Objectives *Page 16; IV. Synopsis – Study Objective(s)* *Page 43; Section 2.1.3 Exploratory Objectives*
ADDED:
• **Examine the proportion of patients with the IA designation who have normal and abnormal creatinine.** • **In patients with an IA designation and abnormal creatinine, examine biopsies (if available) for concordance.**

1. Revision of molecular profiling categories
IV Synopsis, Study Objective(s) and 2.1 Study Objectives *Page 16; IV. Synopsis – Study Objective(s)* *Page 43; Section 2.1.3 Exploratory Objectives*
WAS:
• To assess and compare, between treatment groups, the association/correlation of the appearance of DSA with each of the following: TG, chronic forms of ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, IA (and components thereof), histopathology, graft loss, various thresholds of eGFR (< 30, 40, and 50 mL/min/1.73 m^2), acute ABMR, TCMR, and mortality in patients who develop IA on molecular profiling in each cohort.
IS AMENDED TO:
• To assess and compare, between treatment groups, the association/correlation of the appearance of DSA with each of the following: TG, chronic forms of ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, IA**,** ~~(and components thereof)~~, histopathology, graft loss, various thresholds of eGFR (< 30, 40, and 50 mL/min/1.73 m^2), acute ABMR, TCMR, and mortality in patients who develop IA on molecular profiling in each cohort.

1. Revision of molecular profiling categories
IV Synopsis, Study Design Overview and 2.2 Study Design and Dose Rationale *Page 17; IV. Synopsis – Study Design Overview* *Page 44; Section 2.2.1 Study Design*
WAS:
For the purposes of this study, IA will be defined as a molecular signature indicating either clinical acute rejection (cAR) or subacute rejection (subAR).
IS AMENDED TO:
For the purposes of this study, IA will be defined as a **positive** molecular signature ~~indicating either clinical acute rejection (cAR) or subacute rejection (subAR)~~ **using the Trugraf v2.0 molecular assay (Transplant Genomics, Inc., Pleasanton, CA) in all patients.**

1. Revision of molecular profiling categories
IV Synopsis, Endpoints for Evaluation and 2.3 Endpoints *Page 24; IV. Synopsis – Endpoints for Evaluation (Primary)* *Page 47; Section 2.3.1 Primary Endpoints*
WAS:
For reporting purposes, IA will be considered either present or absent.
IS AMENDED TO:
For reporting purposes, IA will be considered either present or absent **using the Trugraf™ v2.0 molecular assay. For the purposes of the study, a negative designation (Trugraf**

TX Normal) will be referred to as Immune Quiescence (IQ). Due to operating characteristics of the assay, a positive designation will be considered evidence of Immune Activation (IA) in all patients.

1. Revision of molecular profiling categories

IV Synopsis, Endpoints for Evaluation and 2.3 Endpoints
Page 24; IV. Synopsis – Endpoints for Evaluation (Secondary)
Page 47; Section 2.3.2 Secondary Endpoints

WAS:

Comparisons of the cohorts with respect to the various molecular designations as well as correlating the results with DSA will rely on the categorical (binary) variable of positivity for the following molecular designations: Transplant Normal (TX), otherwise referred to as Immune Quiescence (IQ); and Clinical Acute Rejection [cAR, inclusive of Sub-acute Acute Rejection (subAR)], also referred to as Immune Activation (IA). A designation of subAR will be given to patients who exhibit cAR on peripheral blood molecular profiling in the setting of normal renal function as defined by a stable serum creatinine ranging from between 0.7 and 2.4 ng/mL on two separate measurements at least one month apart (stability further defined by no more than a 20% difference between the two individual measurements). Biopsies, when available, will be used to inform the molecular diagnosis when alternative pathology is recognized (i.e. recurrent disease).

Comparisons between treatment cohorts regarding incidence will rely upon assessments for each of the following results at any point in the study:

- DSA
- IA (inclusive of cAR and subAR)
- TG

IS AMENDED TO:

Comparisons of the cohorts with respect to the various molecular designations as well as correlating the results with DSA will rely on the categorical (binary) variable of positivity **using the Trugraf™ v2.0 molecular assay.** ~~for the following molecular designations: Transplant Normal (TX), otherwise referred to as Immune Quiescence (IQ); and Clinical Acute Rejection [cAR, inclusive of Sub-acute Acute Rejection (subAR)], also referred to as Immune Activation (IA). A designation of subAR will be given to patients who exhibit cAR on peripheral blood molecular profiling in the setting of normal renal function as defined by a stable serum creatinine ranging from between 0.7 and 2.4 ng/mL on two separate measurements at least one month apart (stability further defined by no more than a 20% difference between the two individual measurements). Biopsies, when available, will be used to inform the molecular diagnosis when alternative pathology is recognized (i.e. recurrent disease)~~.

Comparisons between treatment cohorts regarding incidence will rely upon assessments for each of the following results at any point in the study:

- DSA
- IA ~~(inclusive of cAR and subAR)~~

• TG

1. Revision of molecular profiling categories
IV Synopsis, Endpoints for Evaluation and 2.3 Endpoints *Page 25; IV. Synopsis – Endpoints for Evaluation (Secondary)* *Page 48; Section 2.3.2 Secondary Endpoints*
WAS:
Persistence of DSA and IA will be additional endpoints for comparisons in patients who develop IA and DSA on molecular profiling, with specific focus on subAR.
IS AMENDED TO:
Persistence of DSA and IA will be additional endpoints for comparisons in patients who develop IA and DSA on molecular profiling~~, with specific focus on subAR~~.

1. Revision of molecular profiling categories
IV Synopsis, Statistical Methods *Page 26; IV. Synopsis – Statistical Methods (Efficacy)*
WAS:
The primary efficacy endpoint is the incidence of any one of the following in the first two years post-transplant: • DSA formation • IA (cAR and subAR)
IS AMENDED TO:
The primary efficacy endpoint is the incidence of any one of the following in the first two years post-transplant: • DSA formation • IA ~~(cAR and subAR)~~

1. Revision of molecular profiling categories
IV Synopsis, Statistical Methods *Page 27; IV. Synopsis – Statistical Methods (Efficacy)*
WAS:
The scope of these analyses will include the following: DSA formation, IA (and components thereof), TG, acute and chronic forms of ABMR, C1q-binding DSA, HLA-DQ DSA, DSA IgG_3 isotype, the requirement for antibody reduction therapy, chronic graft dysfunction, graft loss, local BPAR, mortality, loss to follow-up, ordinal antibody strength, MFI score, histopathology assessments, and tacrolimus trough levels.
IS AMENDED TO:
The scope of these analyses will include the following: DSA formation, IA ~~(and components thereof)~~, TG, acute and chronic forms of ABMR, C1q-binding DSA, HLA-DQ DSA, DSA

IgG_3 isotype, the requirement for antibody reduction therapy, chronic graft dysfunction, graft loss, local BPAR, mortality, loss to follow-up, ordinal antibody strength, MFI score, histopathology assessments, and tacrolimus trough levels.

1. Revision of molecular profiling categories
1.1 Background *Page 33; Section 1.1 Background*
WAS:
Using multiple, three-way classifier tools to validate 200 of the highest value probesets, the groups at Northwestern and Scripps have identified molecular signatures in the peripheral blood for the following five clinical phenotypes: 1) clinical acute rejection (cAR), 2) subAR, 3) immune quiescence (Transplant eXcellent [TX]), 4) acute dysfunction/no rejection (ADNR), and 5) chronic rejection (CR)…
IS AMENDED TO:
Using multiple, three-way classifier tools to validate 200 of the highest value probesets, the groups at Northwestern and Scripps have identified **a variety of** molecular signatures in the peripheral blood**.** ~~for the following five clinical phenotypes: 1) clinical acute rejection (cAR), 2) subAR, 3) immune quiescence (Transplant eXcellent [TX]), 4) acute dysfunction/no rejection (ADNR), and 5) chronic rejection (CR).~~

1. Revision of molecular profiling categories
5.3 Efficacy Assessment *Page 60; Section 5.3.8 Molecular Endpoints*
WAS:
5.3.8 Molecular Endpoints (cAR, subAR, and TX)
IS AMENDED TO:
5.3.8 Molecular Endpoints ~~(cAR, subAR, and TX)~~

1. Revision of molecular profiling categories
5.3 Efficacy Assessment *Page 61; Section 5.3.8, Molecular Endpoints*
WAS:
Each patient will be classified into one of the three currently validated phenotypes (TX/cAR/ subAR, see above). Patients with the cAR designation in the setting of a normal creatinine (as defined by a value between 0.7 and 2.4 mg/dL and stable based on at least two reads over one month apart where stable is defined as no more than 20% difference between the creatinine levels) will be further given the sub-designation of subAR. Classification by phenotypes will be done with every scheduled blood test completed. A final phenotype will be created when patients complete the protocol at 1 year and / or at the conclusion of the study. The posterior probabilities for each new classification (essentially equivalent to CIs) will also be recorded. The above-mentioned classifiers have already been "locked" during

the discovery process to avoid over-training a classifier on new data. 'IA' signatures will be recorded. As a dichotomous variable, 'IA' will represent any patient sample classified as cAR or subAR by the Diagonal Linear Discriminant Analysis (DLDA) and Vector Machines classifiers. In turn, 'IQ' will represent any patient sample classified as TX.
IS AMENDED TO:
~~Each patient will be classified into one of the three currently validated phenotypes (TX/cAR/ subAR, see above). Patients with the cAR designation in the setting of a normal creatinine (as defined by a value between 0.7 and 2.4 mg/dL and stable based on at least two reads over one month apart where stable is defined as no more than 20% difference between the creatinine levels) will be further given the sub-designation of subAR. Classification by phenotypes will be done with every scheduled blood test completed. A final phenotype will be created when patients complete the protocol at 1 year and / or at the conclusion of the study. The posterior probabilities for each new classification (essentially equivalent to CIs) will also be recorded.~~ **For the purposes of the study, assessment and comparison of the incidence of the molecular endpoint will rely on a binary molecular designation in accordance with the TruGraf v2.0 molecular assay. A negative designation (Trugraf TX Normal) will be referred to as Immune Quiescence (IQ). Due to operating characteristics of the assay, a positive designation will be considered evidence of Immune Activation (IA) in all patients.** The above-mentioned classifier~~s have~~ **has** already been "locked" during the discovery process to avoid over-training a classifier on new data. ~~'IA' signatures will be recorded~~. As a dichotomous variable, ~~'IA'~~ **'IQ'** will represent any patient sample classified as ~~cAR or subAR~~ **not positive.** ~~by the Diagonal Linear Discriminant Analysis (DLDA) and Vector Machines classifiers.~~ In turn, ~~'IQ'~~ **'IA'** will represent any patient sample **not** classified as ~~TX~~ **'IQ'**.

1. Revision of molecular profiling categories
5.4 Safety Assessment *Page 63; Section 5.4.3 Laboratory and Pathological Assessments*
WAS:
● Molecular diagnostics (cAR, subAR, and TX; not collected at baseline)
IS AMENDED TO:
● Molecular diagnostics (~~cAR, subAR, and TX;~~ not collected at baseline)

1. Revision of molecular profiling categories
7.4 Analysis of Efficacy *Page 75; Section 7.4.2 Analysis of Secondary Endpoints*
WAS:
1. DSA formation

2. IA (and components thereof) 3. TG
IS AMENDED TO:
1. DSA formation 2. IA ~~(and components thereof)~~ 3. TG

1. Revision of molecular profiling categories
7.4 Analysis of Efficacy *Page 76; Section 7.4.2 Analysis of Secondary Endpoints*
WAS:
Assessments of association between DSA and IA (and its subcomponents) will be analyzed overall and by treatment group with logistic regression models using DSA formation as the response and IA as predictor.
IS AMENDED TO:
Assessments of association between DSA and IA ~~(and its subcomponents)~~ will be analyzed overall and by treatment group with logistic regression models using DSA formation as the response and IA as predictor.

1. Revision of molecular profiling categories
7.4 Analysis of Efficacy *Page 77; Section 7.4.3 Analysis of Exploratory Endpoints*
WAS:
The associations between each of HLA-DQ DSA incidence, ordinal DSA strength, DSA formation, C1q-binding DSA, DSA IgG_3 isotype will be analyzed with respect to each other and with IA, TG, acute and chronic ABMR, histopathology, cAR (and subAR), eGFR at various thresholds (less than 30, 40, and 50 mL/min/1.73 m^2), ABMR (acute and chronic), TCMR, graft loss, and mortality.
IS AMENDED TO:
The associations between each of HLA-DQ DSA incidence, ordinal DSA strength, DSA formation, C1q-binding DSA, DSA IgG_3 isotype will be analyzed with respect to each other and with IA, TG, acute and chronic ABMR, histopathology, ~~cAR (and subAR),~~ eGFR at various thresholds (less than 30, 40, and 50 mL/min/1.73 m^2), ABMR (acute and chronic), TCMR, graft loss, and mortality.

1. Revision of molecular profiling categories
7.6 Analysis of Tacrolimus Dose and Trough Concentrations

Page 78; Section 7.6 Analysis of Tacrolimus Dose and Trough Concentrations
WAS:
The association between whole blood concentrations and the following endpoints will be assessed: DSA presence, DSA strength, C1q-binding DSA, IgG3, IA (cAR / subAR), eGFR, histopathology, and conventional measures of transplant outcomes.
IS AMENDED TO:
The association between whole blood concentrations and the following endpoints will be assessed: DSA presence, DSA strength, C1q-binding DSA, IgG3, IA ~~(cAR / subAR)~~, eGFR, histopathology, and conventional measures of transplant outcomes.

2. Added two exploratory objectives to compare the molecular profiling results with creatinine and (where available) biopsy results.
IV Synopsis, Study Design Overview and 2.2 Study Design and Dose Rationale *Page 18; IV. Synopsis – Study Design Overview* *Page 46; Section 2.2.1 Study Design*
WAS:
Up to two serum creatinine levels obtained at least one month apart, if performed per SOC, should also be recorded.
IS AMENDED TO:
~~Up to~~ **The** two **closest** serum creatinine levels **to the study visit**, obtained at least one month apart, if performed per SOC, should also be recorded.

2. Added two exploratory objectives to compare the molecular profiling results with creatinine and (where available) biopsy results
7.4 Analysis of Efficacy *Page 74; Section 7.4.1.2 Sensitivity Analysis*
ADDED:
Additionally, a sensitivity analysis for mFAS patients will be performed exclusively using assessments with a basis in normal creatinine function.

2. Added two exploratory objectives to compare the molecular profiling results with creatinine and (where available) biopsy results
7.4 Analysis of Efficacy *Page 78; Section 7.4.2 Analysis of Secondary Endpoints*
ADDED:

The incidence of patients with normal and abnormal creatinine assessments and an IA designation will be summarized with descriptive statistics. Concordance between the IA and biopsy results will be summarized with descriptive statistics for each of the normal and abnormal creatinine assessments. Analysis of concordance between the IA and biopsy results will be conducted using Cohen's Kappa for each of the abnormal and normal creatinine assessments.

3. Clarification of the requirements for subjects to be taking MMF or its equivalent.
IV Synopsis, Concomitant Medication Restrictions or Requirements *Page 23; IV. Synopsis – Concomitant Medication Restrictions or Requirements*
WAS:
Non-tacrolimus based immunosuppressive regimens (i.e. cyclosporine, everolimus, sirolimus, belatacept), as well as all forms of extended-release tacrolimus other than Astagraf XL, are prohibited during the course of the study. Due to the influence these medications have on tacrolimus blood concentrations, antiviral medications used in HCV and HIV treatment, as well as isoniazid, Rifampin, ethambutol, and pyrazinamide are also prohibited.
IS AMENDED TO:
Non-tacrolimus based immunosuppressive regimens (i.e. cyclosporine, everolimus, sirolimus, belatacept), as well as all forms of extended-release tacrolimus other than Astagraf XL, are prohibited during the course of the study. **As a clarifying statement, all patients must be maintained on MMF or its equivalent during the course of the study.** Due to the influence ~~these~~ **the following** medications have on tacrolimus blood concentrations, antiviral medications used in HCV and HIV treatment, as well as isoniazid, Rifampin, ethambutol, and pyrazinamide are also prohibited.

3. Clarification of the requirements for subjects to be taking MMF or its equivalent.
IV Synopsis, Formal Stopping Rules and 6.1 Discontinuation of Individual Subject(s) *Page 24; IV. Synopsis – Discontinuation Criteria from Study for Individual Subjects* *Page 71; Section 6.1 Discontinuation of Individual Subject(s)*
ADDED:
• **Removal from an MMF-containing immunosuppressive regimen (or its equivalent);**

4. Addition of discontinuation criteria for subjects who permanently withdraw from tacrolimus or who have a prolonged significant reduction or interruption of tacrolimus for a period of greater than four weeks.
IV Synopsis, Investigational Product(s) & Comparative Drug(s) *Page 22; IV. Synopsis – Investigational Product(s)* *Page 22; IV. Synopsis – Comparative Drug(s)*
ADDED:
A prolonged significant reduction of tacrolimus or withdrawal of tacrolimus will result

in study discontinuation (see discontinuation criteria).

4. Addition of discontinuation criteria for subjects who permanently withdraw from tacrolimus or who have a prolonged significant reduction or interruption of tacrolimus for a period of greater than four weeks.

IV Synopsis, Formal Stopping Rules and 6.1 Discontinuation of Individual Subject(s)
Page 24; IV. Synopsis – Discontinuation Criteria from Study for Individual Subjects
Page 71; Section 6.1, Discontinuation of Individual Subject(s)

WAS:

- With regard to subjects receiving Astagraf XL, failure to achieve a tacrolimus whole blood concentration ≥ 6 ng/mL for 4 consecutive weeks during the first 6 weeks following transplantation;
- With regard to subjects receiving immediate-release tacrolimus, failure to achieve a tacrolimus whole blood concentration ≥ 6 ng/mL for 4 consecutive weeks during the first 6 weeks following transplantation;

IS AMENDED TO:

- **Removal from an MMF-containing immunosuppressive regimen (or its equivalent);**
- ~~With regard to subjects receiving Astagraf XL, failure to achieve a tacrolimus whole blood concentration ≥ 6 ng/mL for 4 consecutive weeks during the first 6 weeks following transplantation;~~
- ~~With regard to subjects receiving immediate release tacrolimus, f~~Failure to achieve a tacrolimus whole blood concentration ≥ 6 ng/mL for 4 consecutive weeks during the first 6 weeks following transplantation;
- **Permanent withdrawal of tacrolimus;**
- **Interruption or prolonged significant reduction of tacrolimus (as an example, a 50% reduction or dose at a level of less than 3.5ng/mL) for periods exceeding 4 weeks;**

4. Addition of discontinuation criteria for subjects who permanently withdraw from tacrolimus or who have a prolonged significant reduction or interruption of tacrolimus for a period of greater than four weeks.

5.1 Dosing and Administration of Study Drug(s) and Other Medication(s)
Page 56; Section 5.1.1 Dose/Dose Regimen and Administration Period

ADDED:

Patients whose tacrolimus is withdrawn or who have a prolonged significant reduction of tacrolimus levels will be discontinued from the study (see Section 6.1).

4. Addition of discontinuation criteria for subjects who permanently withdraw from tacrolimus or who have a prolonged significant reduction or interruption of tacrolimus for a period of greater than four weeks.

5.1 Dosing and Administration of Study Drug(s) and Other Medication(s)
Page 57; Section 5.1.2 Increase or Reduction in Dose of Study Drug(s)

ADDED:
If a prolonged period of reduced immunosuppression will be required to treat infection, sepsis, delayed wound healing, or other circumstances, patients should be removed from the study as per the discontinuation criteria (Section 6.1).

5. Confirmation that study Astagraf XL may not be remotely dispensed without prior written approval from the Sponsor.
4.3 Study Drug Handling *Page 55; Section 4.3 Study Drug Handling*
ADDED:
It is prohibited to remotely dispense study Astagraf XL (i.e. by mail or courier) to subjects unless there are exceptional circumstances and prior approval from the Sponsor is received in writing.

III Non-Substantial Amendment Rationale:

Rationale for Non-Substantial Designation
The changes incorporated into this amendment do not increase any potential harm or affect the safety of the subject. The revisions are meant to provide clarity for protocol procedures and allow for enrollment of appropriate subjects.

14 SPONSOR'S SIGNATURE

Astagraf XL® to Understand the Impact of Immunosuppression on De Novo DSA Development and Chronic Immune Activation in Kidney Transplantation

ISN/Protocol IDTX-MA-3004 /

Version 2.1 incorporating Non-Substantial Amendment 2 / dated May 25, 2018

1.1. PROTOCOL AUTHORS:

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(GPF 4.00)