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

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Sponsor: Astellas Pharma Global Development (APGD)

Medical Affairs, Americas

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Northbrook, IL 60062

STATISTICAL ANALYSIS PLAN

Version 2.00, 09 December 2019

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I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
ABMR	Antibody –Mediated Rejection
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
API	Astellas Pharma Inc
ASCM	Analysis Set Classification Meeting
AST	Aspartate Transaminase
ATC	Anatomical Therapeutic Chemical
BAS	Biopsy Analysis Set
BID	Twice Daily
BMI	Body Mass Index
BPAR	Biopsy-Proven Acute Rejection
CI	Confidence Intervals
CIT	Cold Ischemia Time
C1q	Complement Component 1, Q Subcomponent
CRF	Case Report Form
cPRA	Calculated Panel Reactivity Antibody
CSR	Clinical Study Report
DCD	Donation after Circulatory Death
DSA	Donor Specific Antibody
ECD	Extended Criteria Donor
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
ESRD	End of Stage Renal Disease
FAS	Full Analysis Set
GD	Global Development
HLA	Human Leukocyte Antigen
IA	Immune Activation
ICH	International Conference on Harmonization
IFTA	Interstitial Fibrosis and Tubular Atrophy
IgG3	Immunoglobulin G
IPV	Intrapatient Variability
IRT	Interactive Response Technology
IVIG	Intravenous Immunoglobulin
KDPI	Kidney Donor Profile Index
MDRD-4	Modification of Diet in Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
mFAS	Modified Full Analysis Set
MFI	Mean Fluorescence Intensity
MMF	Mycophenolate Mofetil
PD	Pharmacodynamic
PD1-x	Protocol Deviation 1-x
PDAS	Pharmacodynamic Analysis Set
PK	Pharmacokinetic
PKAS	Pharmacokinetics Analysis Set
PPS	Per-Protocol Analysis Set

Abbreviations	Description of abbreviations
PT	Preferred Term
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SoC	Standard of Care
SOC	System Organ Class
TBL	Total Bilirubin
TCMR	T-cell Mediated Rejection
TEAE	Treatment Emergent Adverse Event
TG	Transplant Glomerulopathy
TGI	Transplant Genomics Inc.
TLF	Tables, Listings and Figures
ULN	Upper Limit of Normal
WHO-DD	World Health Organization Drug Dictionary
WIT	Warm Ischemia Time
XM	Cross-Matching

List of Key Terms

Terms	Definition of terms
Endpoint	A variable that pertains to the trial objectives
Variable	Any quantity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

1 INTRODUCTION

This Statistical Analysis Plan (SAP) contains a more technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes detailed procedures for executing the statistical analysis of the primary and secondary endpoints and other data.

This statistical analysis is coordinated by the responsible biostatistician of Global Development (GD), Astellas Pharma Inc. (API). Any changes from the analyses planned in the SAP will be justified in the Clinical Study Report (CSR).

Prior to database hard 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 hard lock.

2 FLOW CHART AND VISIT SCHEDULE

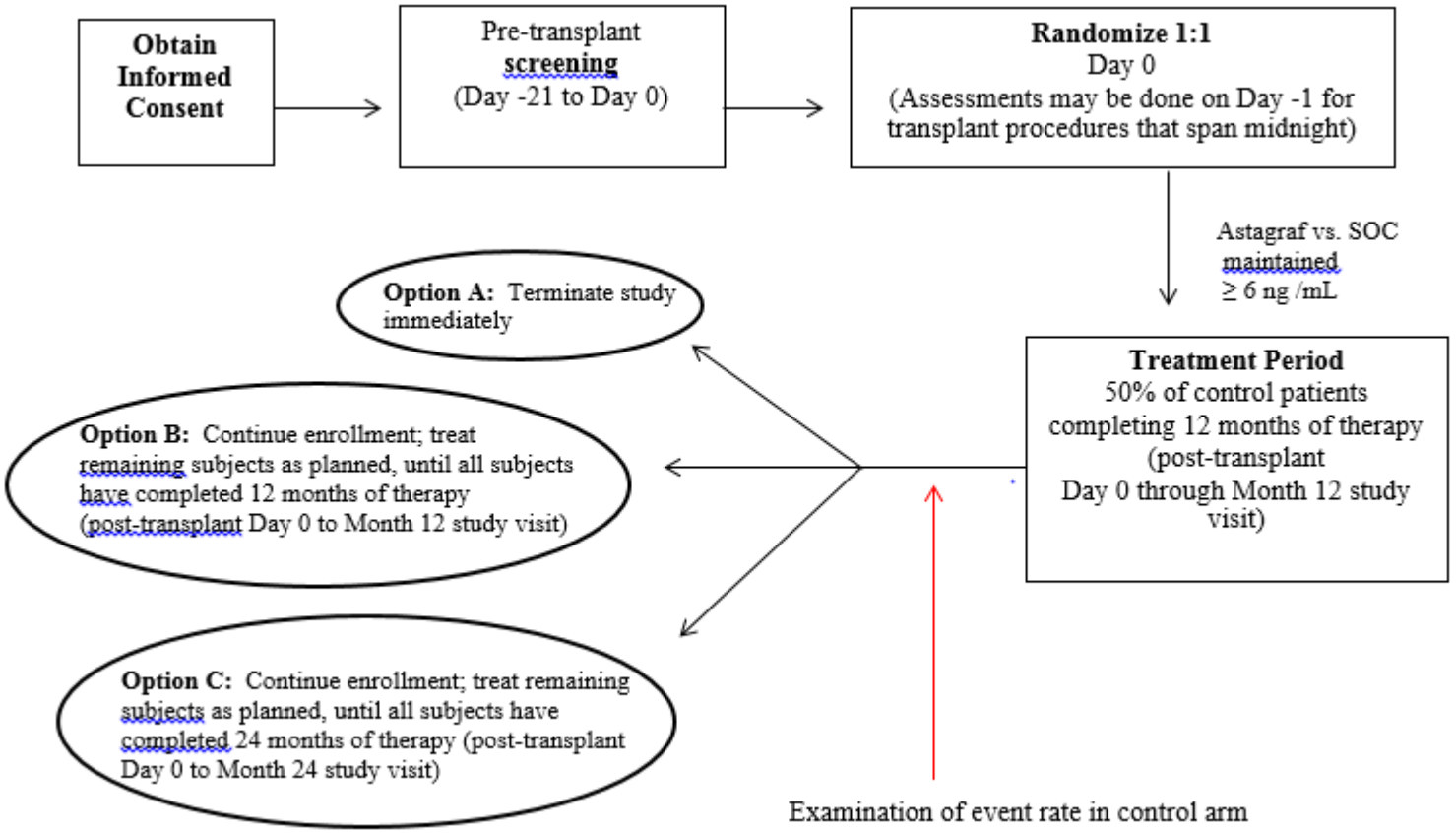

Table 1 Schedule of Assessments

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

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.

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.

3 STUDY OBJECTIVE(S) AND DESIGN

3.1 Study Objective(s)

3.1.1 Primary Objective

The primary objective is to 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 subjects maintained on twice daily, immediate-release tacrolimus versus those maintained on Astagraf XL in the first year post-transplant.

3.1.2 Secondary Objectives

The secondary objectives of the study, as stated in the protocol, have been further clarified and adjusted to more closely address the research questions and in consideration of data availability. They are to:

- Assess the risk factors for each of the following outcomes: Interstitial Fibrosis and Tubular Atrophy (IFTA), IFTA and Inflammation, biopsy-proven acute rejection (BPAR), the four-part traditional composite endpoint, early mycophenolate mofetil (MMF) interruption/discontinuation, and estimated glomerular filtration rate (eGFR) status based on three thresholds 30, 40, and 50 at one year.
- Assess and compare, between treatment groups, the association/correlation of the appearance of DSA with the development of IA on molecular profiling at one year.
- Assess and compare, between treatment groups, the incidence of each of the following, occurring over the course of one year : De novo DSA, C1q-binding DSA, DSA IgG_3 isotype, IA, transplant glomerulopathy (TG), select BANFF histology grades (acute and chronic forms of antibody-mediated rejection (ABMR), acute and chronic active T-cell mediated rejection (TCMR), borderline changes, and IFTA), graft loss, mortality, and BPAR.
- Compare, between treatment groups, the incidence of various thresholds of eGFR (less than 30, 40, 50 mL/min/1.73 m^2) at Day 365 visit and a five-point decline from Day 30 to Day 365).
- Summarize the distribution of ordinal categories of antibody strength (weak, moderate, strong) between treatment groups across the 1-year study time course.
- Summarize maximum Mean Fluorescence Intensity (MFI) scores by treatment group across the duration of the study.
- Examine histopathology in cases where biopsies were taken for maintenance (up to fourteen months) between treatment groups. In cases where biopsies were performed for cause, they will be examined separately.
- Summarize tacrolimus manufacturer and the number of switches between immediate-release tacrolimus products by the occurrence of study composite endpoint.
- Summarize tacrolimus manufacturer and the number of switches by the status of renal dysfunction.
- Summarize adverse events (AEs) between treatment groups.

- Summarize the absolute and percent changes in eGFR over the study duration beginning from 30 days post-transplant between groups, and in subjects who develop DSA or IA.
- Compare the persistence of the development of IA on molecular profiling between two treatment groups across the study duration in those subjects who develop IA on molecular profiling.

3.1.3 Exploratory Objectives

Exploratory objectives are:

- Compare the frequency of those subjects experiencing either component of a two-part composite endpoint consisting of DSA formation and the presence of TG (cg > 0) on centrally-interpreted institutionally-derived protocol (maintenance) biopsy and /or biopsy obtained for cause during the first year post-transplant.
- Examine the proportion with IA designation in subject groups at various thresholds of eGFR (<30, 40, and 50 mL/min/1.73 m^2), and those with threshold of serum creatinine (> 1.2 mg/dL) at one year.
- 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, IFTA, renal dysfunction, various thresholds of eGFR (< 30, 40, and 50 mL/min/1.73 m^2), and the four components of the traditional composite endpoint.
- Summarize intrapatient variability (IPV) by treatment group in subjects with at least three non-missing tacrolimus trough level assessments after the first six weeks following transplantation and on or before Day 365.

3.2 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 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 Transplant Genomics (TGI) Trugraf™ v.2.0 molecular assay (Transplant Genomics, Inc., Pleasanton, CA).

This study will enroll 550 living or deceased donor kidney transplant recipients, 16 to 70 years of age. Subjects 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 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.

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 4). To apply the rule, the incidence rate of the primary endpoint in the control group will be examined once 50% of control subjects 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 7.9).

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, subjects will be followed for up to two years during the open-label study period.

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, three months, six months, nine 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. For borderline cases, a three member adjudication panel 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. 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 of DSA positivity between groups. However, the strongest antibody observed at each HLA-locus will be reported separately and tracked for subjects who are DSA positive. In all cases, positive DSA and/or molecular testing results will be

communicated to the subject'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 standard of care [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. Up to two serum creatinine levels 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. If the study progresses to two years, 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 electronic Case Report Form (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 appended to 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 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 subject identifiers.

3.3 Randomization

Randomization will be carried out using permuted blocks. 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:

- Planned use of alemtuzumab (Campath) (yes/no)
- Kidney donor profile index (KDPI) [three 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.

Subjects enrolled will be randomized using one central list 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. Each subject

will be assigned a randomization number that is five digits long. The first two digits are chosen to be fixed for each strata; and the last three digits correspond to the sequential subject number (001 to 550) for the study subjects in each strata.

4 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.

5 ANALYSIS SETS

In accordance with International Conference on Harmonization (ICH) recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

Detailed criteria for analysis sets will be laid out in Analysis Dataset Specifications and the allocation of subjects to analysis sets will be finalized prior to database hard lock.

5.1 Full Analysis Set (FAS)

The full analysis set (FAS) will consist of all subjects who are randomized and receive at least one dose of study drug (Astagraf XL or immediate-release tacrolimus). Subjects will be included in the FAS based on randomized study drug assignment. This population will be used for selected demographic and baseline summaries and for sensitivity analysis of primary efficacy endpoint.

5.2 Modified Full Analysis Set (mFAS)

The modified full analysis set (mFAS) consists of all subjects who are randomized and received 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, and subjects will be included based on randomized study drug assignment.

The selection of subjects for the mFAS will be confirmed in the Analysis Set Classification Meeting (ASCM) / Data Review Meeting.

The mFAS will be used for summaries and primary analyses of efficacy data, as well as selected demographic and baseline characteristics.

5.3 Per Protocol Set (PPS)

The Per-Protocol Set (PPS) includes all subjects of the mFAS who do not experience any protocol deviation criteria defined in Section 7.2.2.

Final judgments on exclusion of subjects from the PPS will be made at the ASCM and data review meeting, held prior to database lock.

The PPS will be used for secondary analyses of efficacy data.

5.4 Safety Analysis Set (SAF)

The Safety Analysis Set (SAF) consists of all subjects who enrolled into the study and took at least one dose of study medication.

The SAF will be used for summaries of all safety, tolerability and medication compliance related variables, and subjects will be included based on actual study medication received.

5.5 Biopsy Analysis Set (BAS)

The Biopsy Analysis Dataset (BAS) consists of all mFAS subjects who had at least one post-transplant central pathology assessment.

The Biopsy Analysis Set will be used for summaries of all secondary and exploratory efficacy analyses that need to use central pathology findings.

5.6 Pharmacokinetics Analysis Set (PKAS)

Not applicable

5.7 Pharmacodynamic Analysis Set (PDAS)

Not applicable

6 ANALYSIS VARIABLES

6.1 Efficacy Endpoints

Following the decision from the interim analysis (see Section 7.9), subjects will complete at least one year of study visits, and data analysis will be performed once all subjects complete 12 months of study. Accordingly, efficacy analyses will base on data reported within the one year period.

6.1.1 Primary Efficacy Endpoint(s)

The primary endpoint is a composite endpoint of either the presence of DSA or IA (as assessed by a central lab) on peripheral blood molecular profiling by one year post-transplant.

6.1.2 Secondary Efficacy Endpoints

Comparisons between treatment groups regarding incidence will rely upon assessments for the following results:

- De novo DSA formation over the course of 1 year
    - De novo DSA (positive / negative / indeterminate) up to Day 365
    - Class I and Class II DSA will be assessed by a central HLA lab at 1, 3, 6, 9, and 12 months. Overall, Class I and Class II DSA occurrence (positive / negative) of

the period from transplant to Month 12 will be assessed based on assessments at each visit.

- Endpoints for the subset of DSA positive subjects:
  - initial MFI values and antibody strength (mild, moderate, strong) based on MFI
  - post-transplant peak MFI and antibody strength
  - DSA persistent (yes/no) will be assessed by sponsor based on DSA lab data
  - C1q-binding DSA (positive/negative)
  - DSA IgG3 isotype (positive/negative)

For de novo DSA positive subjects, their initial MFI values and antibody strength, peak MFI and antibody strength, and DSA persistence (yes/no) will be assessed by sponsor based on DSA lab data.

- IA
  - Immune Activation (IA) from Day 1 to Day 365 visit
  - Immune Activation (IA) during the period from Day 30 to Day 365 visit

IA will be regarded as persistent if IA is detected for positivity for any two measurements on or before Day 365 visit.

- TG during the first year

  The presence of TG will be reported where TG is defined as cg > 0 on centrally-interpreted institutional protocol biopsy or biopsy obtained for cause during the first year post-transplant with +2 months visit window.

- Microcirculatory Inflammantion during the first year

  The presence of microcirculatory inflammation will be reported where microcirculatory inflammantion is defined as g + ptc >=2 on centrally-interpreted institutional protocol biopsy or biopsy obtained for cause during the first year post-transplant, with +2 months visit window.

- IFTA and Inflammantion during the first year

  The presence of IFTA and inflammation will be reported where IFTA and inflammation is defined as IFTA positive and inflammation positive (i >0) on centrally-interpreted institutional protocol biopsy or biopsy obtained for cause during the first year post-transplant, with +2 months visit window.

- Thresholds for eGFR
  - Values less than 30, 40, and 50 mL/min/1.73 m^2

These threshold endpoints will be calculated for months 1, 3, 6, 9, and 12using the Modification of Diet in Renal Disease (four variable-MDRD) criteria. Assessments are positive or negative.

- Five-point decline in eGFR

  The decline in eGFR will be calculated with respect to the eGFR value at Day 30 visit.

- Traditional transplantation composite endpoint

  Incidence will be assessed at month 12 for the following:

  - Graft loss (defined as retransplantation, transplant nephrectomy, or a return to dialysis for at least a six week duration, or subject death)
  - Death
  - BPAR – positivity will be determined by local biopsy, central pathology, or reported adverse events.
  - Composite occurrence of either graft loss, death, BPAR, and loss-to-follow-up

- Histopathology grading within 1 year

  Central histopathology grading over 1 year will be diagnosed according to the BANFF diagnostic categories and subcategories that include the following:

  - Normal
  - Antibody mediated changes
    - C4d deposition without morphologic evidence of active rejection
    - Acute ABMR (grades: I, II, and III)
    - Chronic active ABMR
    - ABMR: either Acute ABMR or Chronic Active ABMR
  - Borderline changes
  - TCMR
    - Acute TCMR (grades: IA, IB, IIA, IIB, and III)
    - Chronic active TCMR
  - IFTA (grades: I, II, and III)
  - Other: all categorical findings will be summarized.
  - In addition to the categories above, specimens will be given individual scores for the following: glomerulitis (g), tubulitis (t), intimal arteritis (v), mononuclear cell interstitial inflammation (i), early allograft Glomerulopathy (cg), tubular atrophy (ct), interstitial fibrosis (ci), vascular fibrous intimal thickening (cv), arteriolar hyaline thickening (ah), peritubular capillaries (ptc) and mesangial matrix (mm).

6.1.3 Exploratory Efficacy Endpoints

Additional exploratory endpoints include the following:

- The validation of peripheral blood molecular profiling will employ the use of 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.

6.1.4 Other Efficacy Variables

Not applicable

6.2 Safety Variables

Safety will be assessed by evaluation of the following variables:

- Treatment-emergent adverse events (TEAEs; frequency, severity, seriousness, and relationship to study drug).

  TEAE is defined as an AE observed on or after the day of starting the administration of the test drug/comparative drug. If a subject experiences an event both during the pre-investigational period and during the investigational period, the event will be considered as TEAE only if it has worsened in severity (i.e., it is reported with a new start date). All AEs collected that begin within seven days after taking the last dose of study drug will also be counted as TEAE. Based on conservative rule, all AEs that could not be determined as started before transplant or after seven days of the last study dose will be assumed to be TEAEs.

  A drug-related TEAE is defined as any TEAE with at least a possible relationship to study treatment, as assessed by the investigator.

  For TEAE (and serious TEAE), the exposure-adjusted incidence rate (EAIR) will be calculated using following formula:

  EAIR (%) = 100 * n / sum of exposure

  In the formula, n is the number of subjects with an event (e.g. one SOC/PT category). Sum of exposure is the total exposure time of all subjects in the group. For subject with one event, exposure time = time from transplant to the event; for subject with multiple events, exposure time = time from transplant to the earliest event; for subject with no event, exposure time = time from transplant to end of study. Because of the large sample size of the study, time unit is set to Year.

- 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 Medical Dictionary for Regulatory Activities (MedDRA) version 20.1.
- Clinical laboratory variables (hematology, biochemistry including liver enzymes and total bilirubin, urinalysis, coagulation, and tacrolimus trough levels).

6.3 Pharmacokinetic Variables

Not applicable

6.4 Pharmacodynamic Variables

Not applicable

6.5 Other Variables

Baseline

Baseline renal function (serum creatinine, eGFR) will be defined as the value measured at the Day 30 visit. For all other data, the baseline value will be defined as the last observation collected prior to or at the time of reperfusion of the transplanted kidney.

Extended Criteria Donor (ECD)

The donor characteristics that define an ECD kidney include age > or = 60 years, or age 50-59 years plus two of the following: cerebrovascular accident as the cause of death, preexisting hypertension, or terminal serum creatinine greater than 1.5 mg/dl.

Study Days

For the purpose of analyzing time to event endpoints, the time-in-study for each subject will be measured relative to Day 0, the day of transplantation; defined as the date in which reperfusion to the transplanted organ is established. From this starting point, the day of reperfusion will be considered the first day in study for each subject for analysis and the study days will be calculated as:

Date of event - Date of reperfusion

Study Treatment Dose Increased, Decreased, Interrupted, or Treatment Arm Switched

During the whole study treatment period, a subject is considered as:

- Dose Increased – at least one treatment record has higher dose than that of at least one previous record
- Dose Decreased – at least one treatment record has lower dose than that of at least one previous record
- Dose Interrupted – at least one gap (i.e. non-treated days between two records) is >= 7 days
- Arm Switched – at least one treatment record with non-assigned treatment (i.e. not the randomized treatment group)

Time on Treatment (days)

For each subject, the length of time from first dose of study medication to date of discontinuation from study treatment will be calculated in days, using the following formula: (‘Date last dose of study drug’ - ‘Date first dose’) + 1

Actual Treatment Exposure (days)

For each subject, Actual Treatment Exposure (days) will be the sum of treatment days of all treatment records = Sum of all treatment records' ('Stop Date' – 'Start Date' + 1), where the treatment record must have Dose > 0.

Average Daily Dose (mg/day)

Average Daily Dose = Total dose (mg) of study drug / Actual Treatment Exposure (day).

Intrapatient Variability (IPV) of Tacrolimus Trough Level

If the subject had at least three non-missing tacrolimus trough concentration assessments between Week 6 (Day 42) and Day 365, the IPV will be estimated from CV%, geometric CV%, and SD:

CV% = (σ/μ) x 100, where σ is standard deviation and μ is mean of the tacrolimus trough levels

Geometric CV% = $\text{sqrt}(\exp(\sigma_{log}^2)-1)$ x 100, where σ_{log} is the standard deviation of log-transformed tacrolimus concentration value.

SD = standard deviation

Above three IPVs will also be calculated from dose-adjusted tacrolimus concentration:

Dose-adjusted Tacrolimus Concentration = Tacrolimus Concentration / Tacrolimus Dose of that Day.

Switching of Tacrolimus Manufacturer

The study medication of the BID Immediate-release tacrolimus can be from different manufacturers. A manufacturer switch is counted if the manufacturer(s) of record (based on NDC codes) at one dispensing date are not completely same as those of the previous dispensing.

Compliance

The two common measures of medication adherence using refill data are the medication possession ratio (MPR) and proportion of days covered (PDC). Although the MPR has been used to define medication adherence in kidney transplantation, the PDC seems to be the estimator of choice for CMS and pharmacies. This is due to the fact that the PDC estimator is considered a more conservative measure of refill record-based adherence.

PDC is the percentage of days covered with medication, calculated based on dispensing days from the pharmacy, as shown in the following formula:

$$\text{PDC} = \left(\frac{\text{Number of days in period "covered"}}{\text{Number of days in period}}\right) \times 100\%$$

PDC threshold for compliance is typically set at 80% of days covered. This means that someone missing 5 or fewer days' worth of medication in an average month is considered compliant.

Steps to calculate individual PDC based on dispensing data are provided below:

- Step 1: Determine subject's measurement period, defined as the index prescription date until graft loss, death or end of 12-month follow-up period. Index prescription date can be the first fill date after discharge from the hospital for kidney transplantation.
- Step 2: Within the measurement period, count the days the subject was covered (2-daily, immediate release) based on the prescription fill date and days of supply. If prescriptions for the same drug overlap, then adjust the prescription start date to be the day after the previous fill has ended.
- Step 3: Divide the number of covered days found in Step 2 by the number of days found in Step 1. Multiply this number by 100 to obtain the PDC (as a percentage) for each subject.
- Step 4: Count the number of subjects who had a PDC greater than 80% and then divide by the total number of eligible subject.

MPR is a ratio between number of days of medication supply and number of days in the refill interval. MPR is calculated as:

$$\text{MPR} = \left(\frac{\text{Total number of days of medication supplied}}{\text{Number of days in period}}\right) \times 100\%$$

The number of days in period (e.g., 1 year) is the sum of the number of days from first dispensing (ie., first fill date after discharge from the hospital for kidney transplantation) up to the date of last dispensation plus the number of days' supply obtained at the last dispensation (e.g., 30 days). Any prescription days' supply that spans the 1-year observation period will be truncated at 365 days. MPR threshold for compliance is typically set at 80%.

For this study, the study drug dispense data is only available for the BID Immediate-Release Tacrolimus group. PDC, MPR, and their compliance rates can only be calculated for that treatment group.

Previous and concomitant medication

Previous medication is defined as medications which started and stopped prior to transplant.

Concomitant medication is defined as medications with at least one dose taken between the date of transplant (inclusive) and the date of last dose (inclusive) of study drug.

7 STATISTICAL METHODOLOGY

7.1 General Considerations

All numeric and categorical efficacy and safety endpoints will be descriptively summarized. For continuous variables, descriptive statistics will include the number of subjects (n), mean,

standard deviation, median, minimum and maximum. Frequencies and percentages will be displayed for categorical data.

Summaries based on the FAS, mFAS, PPS, and BAS (e.g. disposition, baseline and efficacy data) will be presented by planned treatment group, unless specifically stated otherwise. Unless otherwise stated, all analyses will be performed using data pooled across sites. Subject listings of all data from the CRFs as well as any derived variables will be presented.

All statistical comparisons will be made using two sided tests at the $\alpha = 0.05$ significance level unless specifically stated otherwise. All null hypotheses will be of no treatment difference, all alternative hypotheses will be two-sided, unless specifically stated otherwise. P-values will not be adjusted for multiple comparisons. In the event that any analysis set sample size falls below 15 in any treatment group, descriptive statistics will exclusively be reported with no p-values or statistical inferences. In addition, logistic regression analysis and other regression model analysis as planned in the following sections will not be performed for outcome variables that have an event rate of less than 10% (i.e. reported by fewer than 57 subjects).

Unless otherwise specified, all data processing, summarization, and analyses will be performed using SAS® Version 9.4 or higher.

A hard database lock will occur prior to the final analysis.

7.2 Study Population

7.2.1 Disposition of Subjects

The following subject data will be summarized overall and by treatment group. Unless otherwise noted, the data will be summarized using the randomized treatment group:

- Number of subjects who provided informed consent, who discontinued before randomization, who were randomized, who received a transplant, and who had at least one post-baseline assessment;
- Number and percentage of subjects in each analysis set;
- Number and percentage of subjects who discontinued (actual) tacrolimus treatment for any reason, with primary reason for treatment discontinuation presented; and
- Number and percentage of subjects excluded from the PPS by reason for exclusion.

A subject listing for early discontinuations and reasons for discontinuation will be provided.

7.2.2 Protocol Deviations

Protocol deviations as defined in the study protocol (Section 8.1.6 Protocol Deviations) will be assessed for all randomized subjects. The number and percentage of subjects with major protocol deviations will be summarized by criterion, (randomized) treatment group, and overall. Protocol deviations will also be summarized by study site. Subjects with more than one major deviation from the same criterion will be summarized once for the corresponding criterion. Any subjects who have more than one major deviation will be counted once in the overall summary. A data listing will be provided for all deviations by site and subject.

All deviations will be assessed as either major or minor according to a final review meeting prior to unblinding.

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.

7.2.3 Demographic and Other Baseline Characteristics

Demographic and other baseline characteristics will be summarized using descriptive statistics, presented overall and by randomized treatment group.

Number and percentage of subjects randomized in each site will be presented by treatment group for the SAF.

7.2.3.1 Recipient Demographics

Descriptive statistics for age (years), age categories (<=17, 19-34, 35-49, >=50), weight (kg), body mass index (BMI, kg/m^2) and height (cm) will be presented. Frequency tabulations for sex, ethnicity, age group (defined in section 7.8) and race will be presented. This will be done for the non-randomized subjects, as well as for the SAF, FAS, mFAS, PPS, and BAS by treatment group. A listing of demographic characteristics will also be provided.

7.2.3.2 Donor Demographics and Baseline Characteristics

Descriptive statistics for donor demographic and baseline characteristics of age (years), weight (kg), height (cm), BMI (kg/m^2), KDPI will be presented. Frequency tabulations for age groups (<=17, 18-34, 35-49, >=50), extended criteria donor (ECD), race, ethnicity, Living/Deceased donor, deceased donor type, cause of death of deceased donor, Donation after Circulatory Death (DCD), will be presented. This will be done for the FAS, mFAS, SAF, PPS, and BAS by treatment group. A listing of demographic characteristics will also be provided.

7.2.3.3 Recipient Baseline Disease Characteristics

Baseline characteristics, including primary etiology of end of stage renal disease (ESRD), duration of ESRD prior to transplantation (months), on dialysis prior to current transplant, duration of dialysis prior to transplant, type of dialysis, weight before transplant, and reason for dialysis will be summarized using the FAS by treatment group.

7.2.3.4 Baseline Viral Serology

The number and percent of subjects for each recipient/donor pair with the following results at baseline: (Positive – Positive, Positive – Negative, Negative – Positive, Negative – Negative)

for viral serology test Hepatitis B Core Antibody IgG and Hepatitis C Antibody will be summarized using the FAS by treatment group.

All baseline viral serology data will be listed.

7.2.3.5 Transplant Related Information

The following transplant-related information will be summarized using the FAS by treatment group:

- Number and percent of previous kidney transplants
- Reason for most recent graft loss
- Type of transplant
- ABO compatibility for each recipient/donor pair
- Calculated Panel reactive antibody (cPRA)
- Organ storage method
- Any complications in organ preservation or transplant procedure?
- Cold ischemic time
- Warm ischemic time
- Test used for recipient histocompatibility
- Crossmatch results
- Pre-transplant donor kidney biopsy performed (yes/no)

7.2.3.6 DSA Testing

The following transplant-related information will be summarized using the FAS by treatment group:

- Method used
- Any evidence of DSA positivity
- Single Antigen Bead Testing performed

7.2.3.7 Medical History

Targeted diabetes medical history and non-diabetic medical history will be collected. The number and percent of subjects with each targeted diabetes medical history condition will be summarized using the SAF by treatment group. All non-diabetic medical history conditions are coded in MedDRA version 20.1, and will be summarized by System Organ Class (SOC) and Preferred Term (PT) as well as by PT alone, by treatment group for the SAF. All medical history data will be listed.

7.2.4 Previous and Concomitant Medications

Previous medications will be coded using the version September 2017 of the World Health Organization Drug Dictionary (WHO-DD), and will be included in data listings.

Concomitant medications will be summarized for each treatment group by therapeutic subgroup (ATC 2nd level) and chemical subgroup (ATC 4th level) for the SAF. Subjects taking the same medication multiple times will be counted once per medication.

Immunosuppression medications including mycophenolic acid (MMF, EC-MPS, or generic total daily dose [mg]), corticosteroids usage (total daily dose [mg]), and antithymocyte globulin (total daily dose [mg]) will be summarized.

Separate listings for concomitant and immunosuppression medications will be provided.

7.3 Study Drugs

7.3.1 Exposure

The following information on drug exposure according to the details in Section 6.5 will be presented for each treatment group for the SAF:

- Descriptive statistics for cumulative amount of the drug that a subject was exposed to and average daily dose; and
- Number and percent of subjects with dose increases, decreases, or interruption by treatment group.

Duration of exposure will be summarized in two ways.

- Descriptive statistics will be presented by treatment group.
- Exposure time will be categorized according to the following categories by treatment group:
    - less than 7 days
    - at least 7 days, less than 14 days
    - at least 14 days, less than 28 days
    - at least 28 days, less than 42 days
    - at least 42 days, less than 60 days
    - at least 60 days, less than 91 days
    - at least 91 days, less than 182 days
    - at least 182 days, less than 364 days
    - 364 days or more
    - Unknown.

    Counts and percentages of subjects in each of these categories will be summarized for each treatment group for the SAF.

Additionally, descriptive statistics (counts and percentages) will be used to assess for the BID Immediate-release Tacrolimus treatment group for the SAF the distribution of use for the different tacrolimus formulations in the study (i.e., generic formulations of immediate release tacrolimus) and the frequency of manufacturer switching for the study.

7.3.2 Treatment Compliance

Treatment Compliance is only available for the BID Immediate-release tacrolimus treatment group. See section 6.5. All analyses planned in the Protocol to compare compliance between treatment groups will not be conducted.

PDC, MPR rates and their compliance values will be descriptively summarized for the BID Immediate-release Tacrolimus treatment group.

7.3.3 Tacrolimus Trough Concentrations

Since tacrolimus trough concentration is not assessed at scheduled visits and each subject may have different numbers of assessments. Following analyses will be conducted:

1) Based on all tacrolimus trough concentration assessments from D30 to Day 365, the number of assessments, the mean and median trough level of each subject will be calculated and summarized by treatment group.
2) Number of excursions of tacrolimus trough concentrations above 120% or below 80% of the average trough level of each subject will be tabulated as a proportion of the all tacrolimus trough concentrations reported during the study: proportion of tacrolimus trough concentrations above 120% or below 80% of the average trough level = 100 x (number of tacrolimus trough concentrations above 120% or below 80% of the average trough level/total number of tacrolimus trough concentrations).
3) IPV estimated from CV, geometric CV, and SD, and Dose-adjusted IPVs (see section 6.5) will be summarized by treatment group. The summaries will be provided for SAF and subgroups of subjects with TG, microcirculatory inflammantion, or BPAR.
4) IPVs will also be visually presented in Box plots.

Mean tacrolimus level, number of excursions of tacrolimus trough concentrations above 120% or below 80% of the average trough level, and IPV estimated by CV% will also be used as covariates in the risk factor analysis. See section 7.4.2.

IPVs will also be estimated from the mixed model and functional regression model (Kim et al., 2019). The methods and results will be presented in a separate report, and are not covered by this SAP.

7.4 Analysis of Efficacy

The analysis of Efficacy will be conducted using the mFAS, with sensitivity analyses using the FAS and PPS. All efficacy analyses related to central pathology findings will be conducted using BAS. Risk factor analysis will be conducted for both mFAS and PPS.

7.4.1 Analysis of Primary Endpoint(s)

7.4.1.1 Primary Analysis of Primary Endpoint

The primary analysis will be performed on the primary composite endpoint of combined incidence of either De Novo DSA or IA on molecular blood profiling at one year. Both De novo DSA (Positive/Negative) and IA (Present/Absent) will be considered binary. DSA positivity is assessed by the Transplant Immunology Lab of Northwestern University.

In the case where MFI signal is greater than 1000, and DSA is suspected, but the formation of De Novo DSA cannot be confirmed due to inadequate donor typing, the formation of De Novo DSA will be labeled indeterminate. If subject discontinues the study between D0 and D30, and no testing results are available, then De Novo DSA will be assumed to be negative. If subject does not have a specimen collection, then De Novo DSA will be assumed missing and will be excluded from primary efficacy analysis, but will be assumed to be negative in

sensitivity analysis. The table below shows the logic set up for actual assessment of study composite endpoint.

De Novo DSA	IA	Study Composite Endpoint
Positive	Present	Positive
Positive	Not Present	Positive
Positive	Unknown	Positive
Negative	Present	Positive
Negative	Not Present	Negative
Negative	Unknown	Indeterminate
Indeterminate	Present	Positive
Indeterminate	Not Present	Indeterminate
Indeterminate	Unknown	Indeterminate

The proportion of subjects meeting the primary composite endpoint definition will be summarized by treatment group. The difference in the proportion of subjects meeting the primary composite endpoint definition and 95% confidence interval will also be provided.

The primary analysis of the treatment group comparison will be based on fitting a multivariable binary logistic regression model with randomized treatment group (Astagraf XL group versus twice-daily, immediate-release tacrolimus group), pre-defined randomization stratification factors (planned use of Campath [Yes/No], levels of KDPI as defined in Section 4.5 of the protocol, and HLA Class II mismatch), recipient age, gender, and race, and pre-transplant cPRA included in the model. Investigational site will be included as a random effect. The limited numbers of subjects per center, however, may make it impracticable to include all sites in the statistical model. If reasonable, data from low enrolling sites (e.g., < 5 subjects) will be combined. The decision to include sites and how to combine sites, if necessary, will be made prior to database unblinding.

Adjusted (common) odds ratio (OR: Astagraf XL group versus immediate-release tacrolimus group) and two-sided exact 95% CI will be provided as measures of strength of association and precision, respectively. The null hypothesis of no treatment effect can be expressed as H_0: OR = 1 and will be tested at the ≤ 0.05 level using the Wald's chi-square statistic on one degree of freedom. Odds ratio will also be estimated for the covariates included in the model.

Subjects with primary endpoint unknown, undetermined, or missing will be excluded from the primary analysis.

The hypothesis for the comparison is as follows:

H_0: The proportion of subjects with the primary endpoint is the same between subjects receiving Astagraf XL and subjects receiving immediate-release tacrolimus.

H_1: The proportion of subjects with the primary endpoint is not the same between subjects receiving Astagraf XL and those receiving immediate-release tacrolimus.

7.4.1.2 Sensitivity Analyses of Primary Endpoint

A sensitivity analysis, based on missing data imputation, will be included for the mFAS. Subjects that have missing or incomplete 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.

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

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

Additional sensitivity analysis under different assumptions may be conducted in the future to address specific research questions.

7.4.2 Analysis of Secondary Endpoints

Risk factor analysis will be conducted in two parts. First, is to identify risk factors for outcomes based on factors measured at time of transplantation (baseline) including the pre-defined stratification factors. Last, is to identify risk factors for outcomes based on factors that are measured post-randomization and therefore, are subject to change e.g., average daily dose of MPA treatment or cumulative dose of corticosteroids. All models will include randomized treatment, irrespective of statistical significance. Models to assess risk factors for binary or categorical variables will be done using logistic regression. Odds ratio and 95% CI will be used as measures of strength of association and precision, respectively.

Risk factor analyses will be conducted for eight binary variables: IFTA, IFTA and Inflammation, BPAR, the four-part traditional composite endpoint, early MMF interruption/discontinuation, and eGFR status based on three thresholds 30, 40, and 50. The biopsy data are based on the central pathology results over the course of one year plus 2 months window. eGFR status variables are based on the assessment at Day 365 visit.

Each risk factor analysis model will include treatment group and a main factor: either De novo DSA, IA, or the composite of De novo DSA and IA. Following additional covariates will be considered for the two types of models, and a multicollinearity assessment will be proceeded for the final variable selection:

Risk factors measured at time of transplantation include:

- Cold ischemic time
- Warm ischemic time

- KDPI% <= 50% (yes/no)
- KDPI% > 50% (yes/no)
- HLA Class II mismatch (yes/no)
- Donor status (living/deceased)
- Donation after circulatory death (yes/no)
- Donor Age
- Donor-Recipient sex mismatch (match/mismatch)
- Donor-Recipient race mismatch (match/mismatch)
- Preservation by cold storage (yes/no)

Post randomization risk factors include:

- Cumulative Dose of Corticosteroids
- Average daily dose of MPA
- Mean dose level of tacrolimus
- Mean tacrolimus trough level
- Number of excursions below 80% or above 120% of the average tacrolimus trough level
- IPV estimated by CV%
- Post-transplant BK virus infection

Following secondary endpoints will be summarized with frequencies and percentages by treatment group. The incidence of events will be analyzed for treatment group differences. Analysis of treatment group differences will be implemented from logistic regression model with the same covariates of primary efficacy analysis.

1. De novo DSA formation
2. IA
3. C1q-binding DSA
4. DSA IgG_3 isotype
5. Persistence of DSA
6. Persistence of IA
7. TG (cg>0)
8. Microcirculatory Inflammation (g+ptc>2)
9. IFTA
10. IFTA and Inflammation (IFTA and ci>0)
11. Acute and chronic forms of ABMR
12. eGFR at various thresholds (less than 30, 40, 50 mL/min/1.73 m2 at one year; and a five-point decline from Day 30 to Day 365)
13. Graft loss
14. Mortality

15. BPAR
16. The four-part traditional composite endpoint

Separate risk factor analyses will be conducted for BID Immediate-release Tacrolimus treatment group only. Manufacturer will be included as additional covariate.

Ordinal assessments of antibody peak strength (weak, moderate, and strong) will be summarized based on the results over the course of one year using frequencies and percentages. Analysis of treatment group difference will be done using Fisher's Exact Test.

The continuous endpoint of Peak MFI score will be summarized with descriptive statistics based on the results over the course of one year by antibody.

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 Fisher's Exact Test. 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 performed using Fisher's Exact test.

Assessments of association between De novo DSA and IA will be analyzed with logistic regression models using DSA formation as the response and IA as predictor using the same covariates detailed in Section 7.4.1.1.

Mean of eGFR by treatment group will be plotted across visits.

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). Logistic model with the same covariates of the primary analysis will be conducted for treatment group differences with the incidence of DSA or TG as the response and treatment group as the predictor. The analysis will be performed using the biopsy analysis set.

7.4.4 Analysis of Other Variables

Not applicable

7.5 Analysis of Safety

All analysis of safety will be presented by treatment group for SAF, unless specified otherwise.

7.5.1 Adverse Events

The coding dictionary for this study will be MedDRA. All AEs will be summarized by SOC and PT.

The following lists the TEAE summaries that will be provided:

- Number of TEAEs,
- Number and percentage of subjects with TEAEs,

- Number and percentage of TEAEs by relationship,
- Number and percentage of subjects with TEAEs by relationship,
- Number and percentage of TEAEs by intensity,
- Number and percentage of subjects with TEAEs by intensity,
- Number and percentage of TEAEs occurring in at least 3.5% of any treatment group,
- Number and percentage of subjects with TEAEs occurring in at least 3.5% of any treatment group,
- Exposure adjusted incidence rate (EAIR) of TEAEs,
- Number of TEAEs leading to permanent discontinuation of study drug,
- Number and percentage of subjects with TEAEs leading to permanent discontinuation of study drug,
- Number of serious TEAEs,
- Number and percentage of subjects with serious TEAEs,
- Number and percentage of serious TEAEs by relationship,
- Number and percentage of subjects with serious TEAEs by relationship,
- Exposure adjusted incidence rate (EAIR) of serious TEAEs,
- Number of deaths.

The number and percentage of subjects with TEAEs, as classified by PT only, will be summarized for each treatment group.

If a TEAE has missing grade of intensity or relationship, the highest level of assessment, i.e. Severe for intensity and Probable for relationship, will be used for the table summary. The data listings will still present original missing assessments.

7.5.2 Clinical Laboratory Evaluation

The baseline renal function (serum creatinine, eGFR) will be defined as the value measured at the Day 30 visit. For all other data, the baseline value will be defined as the last observation collected prior to reperfusion of the transplanted kidney.

Quantitative clinical laboratory variables, i.e. hematology and biochemistry, will be summarized using mean, standard deviation, minimum, maximum and median for each treatment group at each visit. Additionally, a within-subject change will be calculated as the post-baseline measurement minus the baseline measurement and summarized in the same way.

Urinalysis and coagulation results will be presented in data listing.

7.5.2.1 Liver Enzymes and Total Bilirubin

The following potentially clinically significant (PCS) criteria for liver tests – defined as Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), total bilirubin (TBL), Aspartate Transaminase (AST) and their combination are defined. The subject's highest value during the investigational period will be used.

Parameter	Criteria
ALT	> 3xULN

	> 5xULN
	> 10xULN
	> 20xULN
AST	> 3xULN
	> 5xULN
	> 10xULN
	> 20xULN
ALT or AST	> 3xULN
Total Bilirubin	> 2xULN
ALP	> 1.5xULN
ALT and/or AST AND Total Bilirubin (*)	(ALT and/or AST > 3xULN) and total bilirubin > 2xULN

(*) Combination of values measured within same sample

The PCS of liver enzymes and total Bilirubin data will be summarized and listed.

7.5.3 Vital Signs

Vital signs will not be collected for this study.

7.5.4 Electrocardiograms (ECGs)

ECG data will not be collected as stand-alone data. Any abnormal ECG results will be reported as AEs. No separate ECG results will be presented.

7.5.5 Other Safety-Related Observations

For the plasma exchange therapy with reason of Post-Transplant AMR Treatment, the number and percentage of subjects who received therapy, and the total number of sessions will be reported by treatment group.

The number and percent of subjects who undergo any antibody removal therapy or get any transfusions during the study will be reported by treatment group

7.6 Analysis of PK

Not applicable

7.7 Analysis of PD

Not applicable

7.8 Other Analyses

Not applicable

7.9 Interim Analysis

An interim analysis was conducted after 50% of control subjects had completed one year of therapy, following the procedures as described in the protocol and SAP version 1. The results of the interim analysis lead to the following decision:

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

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

7.10.1 Missing Data

Generally, all missing efficacy endpoints (e.g. DSA, IA, pathological findings, eGFR) will not be imputed. Only for the sensitivity analyses, e.g. the sensitivity analysis of primary endpoint (section 7.4.1.2), the indetermined endpoint will be imputed as Negative.

In AE summary tables, the missing AE severity and relationship to study drug will be replaced with the highest level of severity and relationship: “Severe” and “Probable”, respectively. In AE data listing, original missing data will be displayed.

As a general principle, no imputation of missing data for other variables will be done.

7.10.2 Outliers

Unless otherwise specified, all values will be included in the analyses.

7.10.3 Visit Windows

Visit windows are defined in Section 2 (Flow Chart and Visit Schedule). For all data collected by visit, the assessments will be presented per the scheduled visits. For data collected by date (e.g. start/stop dates) or collected at unscheduled visits, following rules are applied:

- Study treatment and Tacrolimus trough level – All data from Day 30 to Day 365 will be used in calculation. In the calculation of IPVs, only data after Day 42 (i.e. 6 weeks) will be used.
- Central pathology – All data from Day 0 to Day 426 (Month 14 = 365 + 61) will be included in the calculation of endpoints related to central pathology, e.g. IFTA, Acute Rejection, etc.

- Serum creatinine and eGFR – If the scheduled Day 30 assessment is missing, it will be imputed using the unscheduled assessment within Day 30 +/- 14 window that is closest to Day 30; If the scheduled Day 365 assessment is missing, it will be imputed using the unscheduled assessment within Day 365 +/- 61 window that is closest to Day 365.
- All other primary and secondary endpoints will use the data up to Day 386.
- Death, graft loss, immunosuppressant total dose, and other safety endpoints - Day 365 will be used as cut point for the one-year analysis.

8 DOCUMENT REVISION HISTORY

Version	Date	Changes	Comment/rationale for change
1.00	12-JUL-2018	NA	NA
2.00	25-OCT-2019	1. Define a biopsy set	To facilitate analyses of biopsy based endpoints by selecting subjects who undergo at least one kidney biopsy.
		2. Define protocol deviation based on ICH guidance. Clarify PPS definition.	To follow ICH guidance on defining protocol deviations and a PPS. PPS is further clarified as subjects of the mFAS who do not experience any protocol deviation criteria defined in Section 7.2.2. This will allow for a more meaningful comparison of the results between mFAS and PPS since mFAS is the analysis set for primary efficacy analyses.
		3. Revise the plan for risk factor analysis	To prevent from overfitting of logistic regression models when the expected number of incidences is very small. A large number of pre-specified risk factors and predictors can potentially affect accuracy of the estimated parameters in the case of low event rates.
		4.Define prior medication	To be more clinically relevant, previous medication is defined as medications which started and stopped prior to transplant.
		5. Time-to-first occurrence for DSA, HLA-DQ DSA, C11-binding DSA, DSA IgG_3 type, IA, TG, etc.	When it is not possible to define the exact date of occurrence due to the way the data is collected, the analysis of time-to-first occurrence cannot provide exact information.
		6. Revise the analysis of tacrolimus dose and trough concentration	To provide clarification and details.
		7. Revise the analysis of plasma exchange therapy	Given that the low number of incidence is expected, assessing the treatment differences are not practically applicable. Descriptive statistics will be summarized.
		8. Add the estimation exposure-adjusted incidence rate (EAIR) for TEAEs and SAEs in the safety analysis plan	The crude percent estimation of AEs can result in the increased percent rates for TEAEs and SAE particularly in the later phase of the study.
		9. Exploratory endpoints and analyses	The exploratory endpoints and analyses have been adjusted based on available data, to more closely address the clinical and research questions.

9 REFERENCES

1. Tambur AR, Herrera ND, Haarberg KM, Cusick MF, Gordon RA, Leventhal JR, et al. Assessing antibody strength: comparison of MFI, C1q, and titer information. Am J Transplant. Epub 2015 Apr 30. doi 10.1111/ajt.13295.
2. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, PF et al. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant*. 2008 Apr;8(4):1-8.
3. Kim J, Wilson S, Undre N, Fei S, Kristy R, Schwartz J. A Novel, Dose-Adjusted Tacrolimus Trough Concentration (TTC) Model for Prediction and Variance Estimation. Am J Transplant. 2017;17 (suppl 3).
4. Kim, J., A. Maity, and A. M. Staicu (2016). General functional concurrent model. Unpublished manuscript (under review).
5. Scheipl, F., A.-M. Staicu, and S. Greven (2015). Functional additive mixed models. Journal of Computational and Graphical Statistics 24, 477-501.
6. Shuker et al. (2015) Intra-patient variability in tacrolimus exposure: Causes, consequences for clinical management. Transplantation Reviews. 29: 78-84.

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