A Multicenter Clinical Trial of Allopurinol to Prevent GFR Loss in Type 1 Diabetes

Statistical Analysis Plan
Prepared by PERL DCC

August 3, 2019

Contents

1. Overview 3
2. Schema 4
3. Rationale for Adjustments of Statistical Analysis Plan as Compared to Protocol (Version 10, approved by DSMB on March 6, 2018) 5
3.1. Specifying primary and secondary estimands 5
3.2. Simplified model for the primary efficacy analysis using a multiple imputation approach 5
3.3. Revised cut-points for variables used in subgroup analyses 6
3.4. Calculations of visit windows for the analytical dataset 6
3.5. Additional analysis assessing an effect of post-randomization serum uric acid changes on iGFR values at Visit 17 7
3.6. Additional analysis assessing treatment effect on time to 40% eGFR decrease 7
3.7. Additional analysis assessing time to doubling of serum creatinine, end-stage renal disease (ESRD), or cardiovascular/renal death 7
3.8. Modifying definition of per-protocol analysis set 7
4. Study Aim 8
5. Study Estimands 8
5.1. Primary estimand for iGFR at Visit 17 endpoint 9
5.2. Secondary estimands 9
5.2.1. Estimand for iGFR at the end of the 3-year treatment period (Visit 16, before the washout) a secondary endpoint 9
5.2.2. Estimand for iGFR time trajectory estimated from repeated iGFR measurements 10
5.2.3. Estimand for estimated Glomerular Filtration Rate (eGFR) at 4 months after randomization (Visit 7) 10
5.2.4. Estimand for estimated GFR (eGFR) time trajectory 10
5.2.5. Estimand for time to doubling of serum creatinine or end-stage renal disease (ESRD) 11
5.2.6. Estimand for urinary AER at the end of the two-month wash-out period (Visit 17) 11
5.2.7. Estimand for urinary AER during the last three months of the treatment period (Visits 15 and 16) 12
5.2.8. Estimand for the time to fatal or non-fatal cardiovascular events endpoint 12
6. Analytical Strategy 13
6.1. Study populations 13

6.2. Blinded data review 14
6.3. Visit windows 14
6.4. Baseline covariates 16
6.5. Missing values 16
6.6. Analysis for the primary estimand 17
6.6.1. Primary analysis for the primary estimand 17
6.6.2. Secondary analysis for the primary estimand 18
6.7. Analyses for secondary estimands 19
6.7.1. iGFR at the end of the 3-year treatment period (Visit 16, before the washout) 19
6.7.2. iGFR time trajectory estimated from repeated iGFR measurements 19
6.7.3. eGFR at 4 months after randomization (Visit 7) 20
6.7.4. eGFR time trajectory 20
6.7.5. Time to serum creatinine doubling or ESRD 21
6.7.6. Urinary AER at the end of the wash-out period 21
6.7.7. Urinary AER during the last three months of the treatment period (Visits 15 and 16). 22
6.7.8. Time to fatal or non-fatal cardiovascular events 22
6.8. Other analyses 23
6.8.1. Subgroup analyses 23
6.8.2. Analyses of safety outcomes 24
6.8.3 Analyses of other measures 24
6.8.4. Analysis of the effect of post-randomization sUA changes on iGFR value at Visit 17 25
6.8.5. Time to 40% eGFR decrease 26
6.8.6. Time to doubling of serum creatinine, end-stage renal disease (ESRD), or cardiovascular/renal death 26
6.9. Model assumptions and alternative analyses 27
ACKNOWLEDGEMENTS 28
REFERENCES 29
APPENDIX I. Study Objective, Study Design, Outcomes & Statistical Analysis and Data Management Sections from Protocol 30

1. Overview

DESIGN:

- Multicenter, double-blind, placebo-controlled, parallel-group randomized clinical trial.
- N=530 total number of subjects

STUDY POPULATION:

- Type 1 Diabetes
- Inclusion/Exclusion Criteria listed in the Study Protocol

STUDY TREATMENTS:

- Oral allopurinol or placebo administered for 3 years followed by a 2-month drug washout

PRIMARY OUTCOME MEASURE:

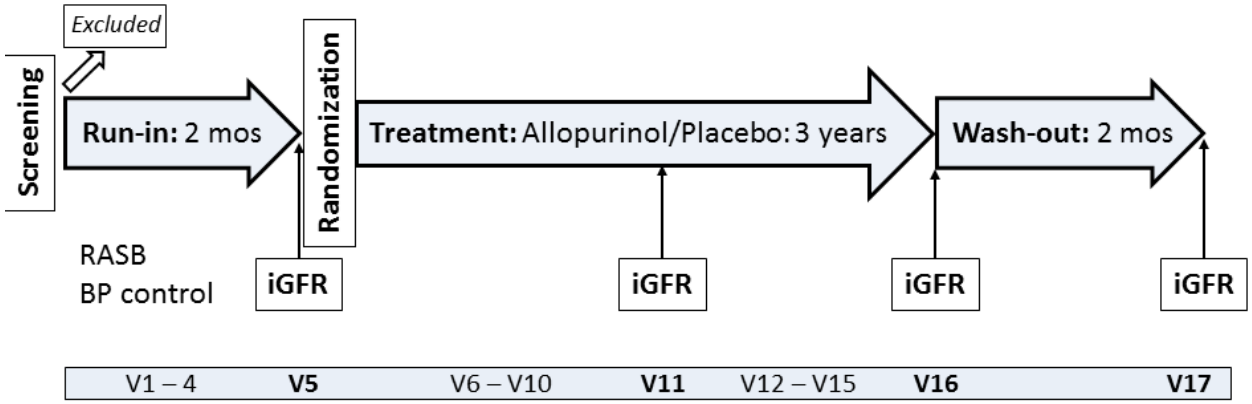
- iGFR at the end of the 2-month wash-out period following the 3-year intervention

STATISTICAL ANALYSIS PLAN:

- This plan will be finalized prior to the database lock and unblinding of treatment groups

2. Schema

Figure 2.1. PERL Study Schema



3. Rationale for Adjustments of Statistical Analysis Plan as Compared to Protocol (Version 10, approved by DSMB on March 6, 2018)

Changes from the protocol-specified definitions of aims, outcomes, and statistical analytical approaches are outlined below. These changes reflect internal discussions since the initiation of the study that have not been incorporated as protocol amendments, but were discussed during the preparation of the Statistical Analysis Plan. These changes and the rationale for their implementation are documented herein and represent changes made prior to the database lock and unblinding of the study.

3.1. Specifying primary and secondary estimands

RATIONALE:
In the study protocol, we describe the analysis populations (section 11.1) and methods to deal with incomplete data (section 11.5); however, we do not explicitly specify estimands of interest. To meet recently proposed guidelines in the "ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials" (August 30, 2017) and to elucidate the target of our research questions, we formally define estimands that have led us to our decisions in terms of conducting the study and selecting analytical approaches.

3.2. Simplified model for the primary efficacy analysis using a multiple imputation approach

RATIONALE:
The primary efficacy analysis presented in Section 11.3 of the study protocol was based on a linear model for correlated errors using all available iGFR measures (including those at baseline, 80, 156, and 164 weeks, respectively) as the dependent variable.

To effectively address missing values in baseline covariates and the need to consider iGFR values that were not measured after end stage renal disease (ESRD) as an unfavorable outcome, direct likelihood based methods are difficult to implement. For this reason, we have decided to perform the primary efficacy analysis using a *multiple imputation* (MI) approach. To perform the MI analysis, we define both *imputation* and *substantive* models. We note that in the substantive model, iGFR at baseline is no longer included as a dependent variable.

PROTOCOL:
We specify the model as follows "… perform the analysis by means of a linear model for correlated errors with general/unstructured covariance matrix using all available iGFR measures (including those at baseline, 80, 156, and 164 weeks, respectively) as the dependent variable."

SAP:
We specify the model as follows "… perform the analysis using a *multiple imputation* approach with a substantive model defined by means of a linear model for correlated errors with general/unstructured covariance matrix using all post-baseline iGFR measures (including those at 80, 156, and 164 weeks, respectively) as the dependent variable.

3.3. Revised cut-points for variables used in subgroup analyses

RATIONALE:
The protocol specified cut-points for subgroup analyses based on educated guesses about the distributions of variables. After investigating baseline distributions of age and AER in pooled analyses, we changed the cut-points for these variables to achieve better balance in subgroup sample size: (1) for age from 40 to 50 years (median age 52 years), (2) for iGFR from 70 to 60 ml/min/1.73 m^2 and (3) for AER from 300 to 30 mg/24h (median AER 42 mg/24h).

PROTOCOL:
To investigate the possible presence of heterogeneity in the response to allopurinol, subgroup analyses (based on the primary efficacy analysis described in section 11.3, with the inclusion of an interaction term of the treatment group by the subgroup variable) will be performed by age groups (≤40 and >40 yrs.), …, baseline iGFR (≤70 and >70 ml/ min/1.73m^2) … AER at baseline (≤300 and >300 mg/24 hr.), and ….

SAP:
To investigate the possible presence of heterogeneity in the response to allopurinol, subgroup analyses (based on the primary efficacy analysis described in section 11.3, with the inclusion of an interaction term of the treatment group by the subgroup variable) will be performed by age groups (≤50 and >50 yrs.), …, baseline iGFR (≤60 and >60 ml/ min/1.73m2) AER at baseline (≤30 and > 30 mg/24 hr.), and ….

3.4. Calculations of visit windows for the analytical dataset

RATIONALE:
Per-protocol windows for scheduling Visits 6-16 are calculated relative to the Visit 5 date. In early versions of the Study Protocol (versions 5.0 and 6.0), randomization was performed at a study visit (Visit 5) and consequently the visit date and randomization date were equivalent. Starting with Study Protocol, version 7.0, Visit 5 became a phone call visit and randomization did not necessarily occur on the date of the phone call. For analytical purposes (see SAP Section 6.3), visit windows will be calculated relative to randomization date.

PROTOCOL:
Visit 1 will be considered as Time 0 for scheduling Visits 2-5, Visit 5 will be considered as Time 0 for scheduling Visit 6-16, Visit 16 as Time 0 for scheduling Visit 17.

SAP:
Visit 1 will be considered as Time 0 for scheduling Visits 2-5, Visit 5 will be considered as Time 0 for scheduling Visit 6-16, Visit 16 as Time 0 for scheduling Visit 17.
For analytical purposes, the randomization date will be considered as Time 0 for calculating windows for Visits 6-16.

3.5. Additional analysis assessing an effect of post-randomization serum uric acid changes on iGFR values at Visit 17

RATIONALE:
Following internal discussion on the importance of the relationship between serum uric acid (sUA) and iGFR measures, we added this analysis.

PROTOCOL:
Not applicable

SAP:
Details are provided in SAP Section 6.8.4.

3.6. Additional analysis assessing treatment effect on time to 40% eGFR decrease

RATIONALE:
Following internal discussion on the importance of the recently proposed measure of kidney decline, namely 40% eGFR decrease, we added this analysis.

PROTOCOL:
Not applicable

SAP:
Details are provided in SAP Section 6.8.5.

3.7. Additional analysis assessing time to doubling of serum creatinine, end-stage renal disease (ESRD), or cardiovascular/renal death

RATIONALE:
Following internal discussion on the importance of the recently proposed measure of kidney decline, namely using cardiovascular/renal death as part of the composite endpoint definition, we added this analysis.

PROTOCOL:
Not applicable

SAP:
Details are provided in SAP Section 6.8.6.

3.8. Modifying definition of per-protocol analysis set

RATIONALE:
Following internal discussion we modified the per-protocol definition as follows.

PROTOCOL:

- ***Per Protocol:*** … The per protocol population will exclude subjects … as well as data points for which the cumulative exposure to the study medication from

randomization to that time point was less than 80% of the theoretical full exposure (see Section 11.1 in the protocol).

SAP:

- ***Per Protocol:*** … The per protocol population will exclude subjects … for whom the average drug exposure was less than 80% (see Section 11.1 in the protocol).

4. Study Aim

The study aim is to determine whether lowering serum UA by means of oral allopurinol is effective in preventing or slowing decline of renal function in T1D patients with history and/or presence of microalbuminuria or moderate macroalbuminuria, or with ongoing GFR loss regardless of history or presence of albuminuria, who have only mildly or moderately impaired kidney function.

5. Study Estimands

This section describes the primary and secondary estimands for corresponding endpoints and variables of interest. We follow ICH-E9 (R1) recommendations and specify estimands in terms of four attributes defining the treatment effect of interest:

A1. The target population
A2. The variable (or endpoint) to be obtained for each patient that is required to address scientific question of interest
A3. Strategies for addressing intercurrent events
A4. The population summary for the variable (endpoint), that provides a basis for a comparison between treatment conditions.

In Table 5.1. we include various intercurrent events that occurred in the PERL study and divide them into three groups, based on their implications for subsequent data collection of the endpoint of interest.

Table 5.1. Groups of intercurrent (IC) events in PERL study

Group of IC events	**IC event**	**Implications for Post-IC data**
Group A	Non-adherence to study drug schedule	Post-IC data are collected, but their interpretation may be affected depending on the estimand of interest
	Permanent discontinuation of study drug	
	Use of prohibited medication	
	Missed scheduled visit	
Group B	ESRD treatment (hemodialysis or transplant for ESRD subjects)	Post-IC data do not contain any relevant information about estimands of interest and for this reason they are not collected
Group C	Early discontinuation from the study	Post-IC data cannot be collected
	Terminal event, i.e. death	

5.1. Primary estimand for iGFR at Visit 17 endpoint

This is the de-facto (effectiveness) estimand of the primary endpoint – iGFR at Visit 17 – that quantifies a treatment effect due to the initially randomized treatments as actually taken, i.e., the treatment of allopurinol versus placebo without a confounding effect of treatment for ESRD subjects. The four attributes of this estimand are as follows:

A1. Target population: Individuals with T1D meeting the inclusion/exclusion criteria specified in the Study Protocol.

A2. The variable (or endpoint): The primary endpoint is the measured glomerular filtration rate (GFR) based on plasma disappearance of non-radioactive iohexol (iGFR) at the end of the 2-month wash-out period (Visit 17 at Week 164) following the 3-year intervention. The rationale of measuring the primary outcome at the end of the wash-out period is to test allopurinol for *durable* effects on the natural history of kidney disease, independent from any transient, hemodynamic effect that the medication may have on GFR. iGFR is calculated from blood samples drawn at baseline and 120, 150, 180, 210, and 240 minutes after an i.v. bolus of iohexol, adjusting for body surface area.

A3. Strategies for different groups of IC events: Depending on IC event group membership (see Table 5.1), the variable of interest, in this case iGFR values, collected after an IC event in

- Group A will be considered as directly interpretable. Effectively, IC events in this group are ignored, which is consistent with the ITT principle.
- Group B are assumed to follow a hypothetical scenario, in which variable of interest after developing ESRD takes on biologically plausible values that are not confounded by IC event i.e. by ESRD treatment.
- Group C are assumed to conform to a hypothetical scenario in which post-IC values of the variable of interest (or endpoint) have a similar distribution to other non-ESRD subjects

A4. Population summary to compare treatments: Population-average treatment effect on iGFR at V17.

5.2. Secondary estimands

5.2.1. Estimand for iGFR at the end of the 3-year treatment period (Visit 16, before the washout) a secondary endpoint

This is de-facto (effectiveness) estimand for the iGFR at Visit 16 endpoint with the following attributes:

A1. Target population: T1D (inclusion/exclusion criteria specified in the Study Protocol)

A2. Variable of interest (endpoint): iGFR calculated at the end of the 3-year intervention (at Visit 16, last visit before washout)

A3. Strategies for different groups of IC events: The same as those used for primary estimand (see Section 5.1)

A4. Population summary to compare treatments: Population-average treatment effect on iGFR at V16

5.2.2. Estimand for iGFR time trajectory estimated from repeated iGFR measurements

This is de-facto (effectiveness) estimand for repeated iGFR measures with the following attributes:

A1. Target population: Individuals with T1D meeting the inclusion/exclusion criteria specified in the Study Protocol.

A2. Variables of interest: Repeated measures of iGFR at Visits 11, 16, 17.

A3. Strategies for different groups of IC events: The same as those used for primary estimand (see Section 5.1)

A4. Population summary to compare treatments: Population-average treatment effect on the slope of iGFR trajectory

5.2.3. Estimand for estimated Glomerular Filtration Rate (eGFR) at 4 months after randomization (Visit 7)

This is de-facto (effectiveness) estimand for eGFR at 4 months after randomization with the following attributes:

A1. Target population: Individuals with T1D meeting the inclusion/exclusion criteria specified in the Study Protocol.

A2. Variable of interest (endpoint): eGFR at 4 months after randomization as estimated from serum creatinine and cystatin C using the CKD-EPI SCr and the CKD-EPI SCr-SCysC equations (Inker et al, 2012, Fan et al, 2015). This endpoint is employed to measure a transient, hemodynamic effect that the study medication may have on GFR.

A3. Strategies for different groups of IC events: The same as those used for primary estimand (see Section 5.1)

A4. Population summary to compare treatments: Population-average treatment effect on eGFR at 4 months after randomization.

5.2.4. Estimand for estimated GFR (eGFR) time trajectory

This is de-facto (effectiveness) estimand for eGFR trajectory with the following attributes:

A1. Target population: Individuals with T1D meeting the inclusion/exclusion criteria specified in the Study Protocol.

A2. Variable of interest (endpoint): Repeated eGFR measures at all post-randomization visits (Visit 6 through 17) as estimated from repeated serum creatinine and cystatin C measurements using the CKD-EPI SCr and the CKD-EPI SCr-SCysC equations.

A3. Strategies for different groups of IC events: The same as those used for primary estimand (see Section 5.1)

A4. Population summary to compare treatments: Population-average treatment effect on the slope of post-randomization eGFR trajectory

5.2.5. Estimand for time to doubling of serum creatinine or end-stage renal disease (ESRD)

This is de-facto (effectiveness) estimand for doubling of serum creatinine or developing ESRD with the following attributes.

A1. Target population: Individuals with T1D meeting the inclusion/exclusion criteria specified in the Study Protocol.

A2. Variable of interest (endpoint): This secondary endpoint is defined as a composite of two events: (1) doubling to serum creatinine, and (2) ESRD. Time to event is defined as time from randomization to the first event (one of the events defined above) or censoring (lost-to-follow-up, withdrawal, death, and study completion without experiencing the event).

A3. Strategies for different groups of IC events: Depending on IC event group membership (see Table 5.1) variable of interest/endpoint values, collected after IC event in

- Group A will be considered as directly interpretable. Effectively IC events in this group are ignored, which is consistent with the ITT principle.

- Groups B and C are assumed to conform a hypothetical scenario in which the variable of interest/endpoint values have a similar distribution to subjects not experiencing the IC event.

A4. Population summary to compare treatments: Hazard ratio for allopurinol versus placebo.

5.2.6. Estimand for urinary AER at the end of the two-month wash-out period (Visit 17)

This is de-facto (effectiveness) estimand for AER at V17 with the following attributes.

A1. Target population: Individuals with T1D meeting the inclusion/exclusion criteria specified in the Study Protocol.

A2. Variable of interest (endpoint): Geometric mean of two urinary AER measures obtained at Visit 17.

A3. Strategies for different groups of IC events: Depending on IC event group membership (see Table 5.1) variable of interest/endpoint values, collected after IC event in

- Group A will be considered as directly interpretable. Effectively IC events in this group are ignored, which is consistent with the ITT principle.
- Groups B and C are assumed to conform a hypothetical scenario in which the variable of interest/endpoint values have a similar distribution to subjects not experiencing the IC event.

A4. Population summary to compare treatments: Population-average treatment effect on AER at V17 expressed as a ratio of geometric means.

5.2.7. Estimand for urinary AER during the last three months of the treatment period (Visits 15 and 16)

This is de-facto (effectiveness) estimand for AER at Visit 15 and 16 with the following attributes:

A1. Target population: Individuals with T1D meeting the inclusion/exclusion criteria specified in the Study Protocol.

A2. Variable of interest (endpoint): Geometric mean of urinary AER measures at Visit 15 and Visit 16.

A3. Strategies for different groups of IC events: Depending on IC event group membership (see Table 5.1) variable of interest/endpoint values, collected after IC event in

- Group A will be considered as directly interpretable. Effectively IC events in this group are ignored, which is consistent with ITT principle.
- Groups B and C are assumed to conform a hypothetical scenario in which the variable of interest/endpoint values have a similar distribution to subjects not experiencing the IC event.

A4. Population summary to compare treatments: Population-average treatment effect on AER during last three months of the treatment expressed as a ratio of geometric means.

5.2.8. Estimand for the time to fatal or non-fatal cardiovascular events endpoint

This is de-facto (effectiveness) estimand for fatal and non-fatal cardio-vascular events with the following attributes:

A1. Target population: Individuals with T1D meeting the inclusion/exclusion criteria specified in the Study Protocol.

A2. Variable of interest (endpoint): This secondary endpoint is defined as a composite of multiple events: (1) Cardiovascular disease (CVD) death (ICD-10 code I10 to I74.9), (2) Myocardial infarction, (3) Stroke (ischemic or hemorrhagic), (4) Coronary artery bypass grafting, or (5) Percutaneous coronary intervention. Time to fatal or non-fatal cardiovascular events is defined as the time from randomization to the first event (one of the events defined above) or censoring (lost-to-follow-up, withdrawal, non-CVD death, and study completion without experiencing the event).

A3. Strategies for different groups of IC events: Depending on IC event group membership (see Table 5.1) variable of interest/endpoint values, collected after IC event in

- Group A will be considered as directly interpretable. Effectively IC events in this group are ignored, which is consistent with ITT principle.
- Groups B and C (except CVD death) are assumed to conform a hypothetical scenario in which variable of interest/endpoint values have similar distribution to subjects not experiencing IC event.

A4. Population summary to compare treatments: Hazard ratio for allopurinol versus placebo

6. Analytical Strategy

In the initial analysis of the primary outcome we will present iGFR univariate statistics by Treatment Groups at each study visit (V4, V11, V16 and V17).

No formal interim analyses of the primary endpoint will be conducted, therefore the nominal α level to be used at the final analysis will be 0.05 for the primary endpoint. All other secondary outcomes will also be tested at the 0.05 level, with no adjustment for multiplicity. Many of the models used in the analyses include baseline covariates, such as stratifying variables (serum uric acid (sUA), HbA1C, clinical site), iGFR, albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who qualified by eGFR slope and were normoalbuminuric at baseline), AER, and time, and time by treatment interaction. If there are problems with fitting these models, due, for example, to lack of convergence to optimal values, covariates will be eliminated from the models in the following order: baseline AER, albuminuria status, serum uric acid (sUA), HbA1c, and clinical site. More detailed information about these covariates is included in Section 6.4.

6.1. Study populations

Two study populations will be defined for the purpose of data analysis:

- ***Intention to Treat (ITT):*** The ITT analysis set consists of all subjects enrolled in PERL, randomized to study medication.
- ***Per Protocol:*** The per protocol analysis set will consist of a subset of ITT subjects. The per protocol population will exclude subjects with major protocol deviations (defined as receiving the wrong study medication) as well as subjects for whom the average drug exposure is less than 80% (see Section 11.1 in the protocol).

To account for missing values in any specific analysis, all subjects meeting the study population definitions will be included and analyzed using (1) multiple imputation techniques (see Section 6.4), or (2) appropriate analytical approaches that allow for missing values under plausible missing data mechanisms, such as linear mixed-effects models that allow values of the dependent variable to be missing under missing at random (MAR) mechanism.

Long study follow-up results in missing values for the outcomes and precludes strict adhering to ITT principle. To mitigate this issue we will follow four strategies proposed by I.R. White et al (2011):

1. Attempt to follow up all randomized participants, even if they withdraw from allocated treatment.
2. Perform a main analysis of all observed data that are valid under a plausible assumption about missing data.
3. Perform sensitivity analyses to explore the effect of departures from the assumptions made in the main analysis.
4. Account for all ITT study population participants, at least in the sensitivity analyses.

6.2. Blinded data review

Prior to unmasking the study and starting any formal analysis, data will be reviewed in a blinded fashion by computing summary statistics for primary and secondary outcomes, and baseline covariates. This will allow the identification of unusual values and/or patterns of missing values for key variables that need to be queried. In addition, such blinded data review will allow the writing committee to assess the format of data presentation. Note that the blinded data review incorporates real data but *random* treatment assignment (i.e., investigators do not receive data summarized by actual treatment group, rather they review data on two randomly formed groups). All decisions will be made and documented in this SAP document prior to database lock and unblinding.

6.3. Visit windows

To provide scheduling flexibility to study sites and participants, visits were required to occur within a protocol-defined window rather than on a specific date. The protocol-defined visit windows are summarized in the tables 6.3.1 and 6.3.2 below. For analytic purposes, the visit windows defined in the protocol will be expanded in order to eliminate gaps between them. This will ensure that all observations, including those that may have occurred outside a protocol-specified time window, will be associated with the most appropriate visit and therefore properly included in the analysis. If multiple observations occur within a window, the one closest to the visit target date will be utilized. If two observations are equi-distant from the target date, the first one will be utilized.

As iGFR is the primary and key secondary endpoint, the protocol allowed for repeats of the iGFR procedure in order to achieve qualified iGFR values. Also, the procedure required a longer visit, so it was more difficult to schedule. Thus, we allowed wider windows for iGFR visits (V11, V16 and V17) to ensure that all qualified iGFRs are analyzed. In addition to avoid over-writing iGFR visits with a non-iGFR (V6-V10, V12-V15) visit, and vice-versa the aforementioned procedure will be performed separately for non-iGFR (Table 6.3.1) and iGFR visits (Table 6.3.2).

Table 6.3.1. PERL windows for post-randomization non-IGFR visits.

Visit	Lower Boundary of Window (Week, Excluding First day)	Per protocol Target Date window in weeks	Upper Boundary of Window (Week, Including last day)	Time since randomization attributed to visit window (in weeks)
Visit Windows relative to Randomization Date				
Visit 6	0	4 [3-5]	10	5
Visit 7	10	16 [14-20]	24	17
Visit 8	24	32 [30-34]	40	32
Visit 9	40	48 [46-50]	56	48
Visit 10	56	64 [62-66]	72	64
Visit 12	88	96 [94-98]	104	96
Visit 13	104	112 [110 -114]	120	112
Visit 14	120	128 [126 -130]	135	128
Visit 15	135	142 [140 -146]	150	142

All intervals (target dates and lower/upper window boundaries) for visits 6 through 16 are calculated relative to the randomization date. The interval for Visit 17 is calculated relative to Visit 16. Most post-randomization visits are 16 weeks apart, with the exception of Visits 6 and 7 and Visits 16 to visit 17. For the purpose of selected analyses (sections 6.7.2 and 6.7.4) involving multiple imputations, we included in the last column of Tables 6.3.1 and 6.3.2 a time since randomization associated with a corresponding visit window. Entries in this column are based approximately on the mid-points between target dates.

Table 6.3.2. PERL windows for post-randomization iGFR visits.

Visit	Lower Boundary of Window (Week, Excluding First day)	Per protocol Target Date window in weeks	Upper Boundary of Window (Week, Including last day)	Time since randomization associated with visit window (in weeks)
Visit Windows relative to Randomization Date				
Visit 11 (iGFR)	53	80 [78-84]	97	80
Visit 16 (iGFR)	149	156 [154 -160]	178	164
Visit Window relative to V16				
Visit 17 (iGFR)	0	8 [6–12]	20	174

6.4. Baseline covariates

The following is a description of the baseline covariates that will be used in the various analyses outlined in the remainder of Section 6.

- Stratifying variables
    - serum uric acid (sUA) at baseline with 2 levels (≤6.0 and > 6.0 mg/dl)
    - glycated hemoglobin (HbA1c) at baseline with two levels (≤7.8 and >7.8%)
    - clinical site/study center with 16 levels (based on main sites with satellite sites collapsed into main sites)
- Baseline iGFR measured at Visit 4
- Baseline eGFR measured at Visit 4
- Treatment group with two levels (Allopurinol, Placebo)
- Albuminuria status with 2 levels (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who qualified by eGFR slope and were normoalbuminuric at baseline)
- Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale

As of Dec. 17, 2018, we have complete data available for baseline serum uric acid, treatment group, and study center. The number of missing values for the other baseline covariates is 1 for iGFR, 2 for HbA1c, and 17 for albuminuria status. Missing baseline data will be imputed according to the approach described in Section 6.5. When creating covariates for analytical and imputation purposes, we will aggregate clinical sites with a small number of randomized subjects, such as Edmonton (site #11, n=3) and Vancouver (site #16, n=11) will be combined with Calgary (site #10, n=20) in the same geographic region. Similarly, Spokane (site #15, n=5) will be combined with Seattle (site #13, n=35).

6.5. Missing values

Missing values both for baseline characteristics and for outcomes/endpoints of interest are inevitable, especially in studies with longer follow-up. To effectively address missing values that occurred for baseline covariates and for post-randomization variables of interest, and the necessity to consider post-ESRD iGFR values as an unfavorable outcome, models involving direct likelihood methods are difficult to implement. For this reason, we will perform the analyses using a *multiple imputation* (MI) approach consisting of three steps:

Step 1. Using an *imputation* model, create multiple datasets with missing values imputed
Step 2. Fit *substantive models* described in Sections 6.6 and 6.7 using imputed datasets created in Step 1
Step 3. For each substantive model, combine the results obtained in Step 2 for the inference using Rubin's rule (Rubin, 1987).

To create imputed datasets in Step 1, we will employ multivariate imputation by means of fully conditional specification (FCS) method introduced by van Buuren et al, 2006. This method is especially attractive in our case because it handles non-monotone patterns of missingness, and arbitrary types of imputed variables, i.e. both continuous and categorical. The imputation model will include baseline covariates listed in Section 6.4. In addition, to make imputation model more general than substantive models, we will include HbA1c at Visit 1 and geometric mean of AER at Visit 3 and 4 expressed on logarithmic base to 10 scale predictive of other baseline covariates.

We will also include eGFR at all post-randomization visits, i.e., Visit 6-17, iGFR at Visits 11, 16 and 17, AER at Visits 15, 16 and 17 expressed on logarithmic base to 10 scale. Imputation of baseline variables will be performed starting with variables having the lowest number of missing values. Variables measured longitudinally, i.e., eGFR and iGFR, will also be modeled sequentially in order determined by visit number. To preserve different response patterns in the study treatment groups (i.e., treatment group by study visit interaction) imputations will be performed separately in each group. Resulting data will consist of 25 imputed datasets. We note that the FCS method imputes data under the missing at random (MAR) assumption, e.g., the probability that the iGFR/eGFR value is missing depends on *observed* rather than *unobserved* values of the variable. Although we consider the MAR assumption to be sensible for our study, it does not apply for post-ESRD iGFR/eGFR values. To model *post-ESRD eGFR* measures as a deviation from the MAR assumption, we will impute these values using a controlled imputation technique, specifically the delta-adjustment approach (O'Kelly, Ratitch, 2014). This technique will impute post-ESRD eGFR values on average at 7 ml/min/1.73m^2, with a small variation around it, that is consistent with: (1) attributing to missing post-ESRD eGFR values the value representing 'the worst case scenario', (2) assigning a biologically acceptable value, and (3) including the 'absorbing state' feature of ESRD. We note that eGFR measures are taken at every visit and are used to determine time of developing ESRD. For this reason pre-ESRD eGFR values are highly predictive of post-ESRD iGFR values. In addition, we note that post-ESRD iGFR and eGFR values lie in a very narrow range and they are effectively interchangeable. For these reasons, we will impute post-ESRD iGFR values by using corresponding post-ESRD eGFR imputed values as a proxy. We note that the imputation of *eGFR* and *iGFR* values for subjects who *did not develop* ESRD and have low values to start with may lead to imputed values lower than 15 ml/min/1.73m^2, which is biologically implausible. For this reason, imputed values of *eGFR* and *iGFR* for subjects who *did not develop* ESRD will be truncated at 15 ml/min/1.73m^2. Similarly, log(AER) base 10 imputed values for these subjects will be truncated at a lower limit of detection of -0.60206 (= $\log_{10}(0.25)$).

6.6. Analysis for the primary estimand

In this Section we describe primary and secondary analyses aligned with the primary estimand defined in Section 5.1. These models will be employed as substantive models (see Step 2 of multiple imputation approach described in Section 6.5).

6.6.1. Primary analysis for the primary estimand

The goal of the primary analysis for the primary estimand is to test the null hypothesis of the difference in means between treatment arms in the primary endpoint (iGFR at the end of the 2-month wash-out period [Visit 17] following the 3-year intervention) being equal to zero. The analysis will be performed in a multiple imputation framework on the intention-to-treat (ITT) population and will employ a linear model for correlated errors with general/unstructured covariance matrix (Molenberghs and Verbeke, 2005; Galecki and Burzykowski, 2013) as a substantive model. For each time t ($t = 1, 2, 3$) corresponding to post-randomization iGFR visits, i.e. visits V11 (80 weeks), V16 (156 weeks), and V17 (164 weeks after randomization) the model equation is specified as:

$$iGFR_{it} = \beta_{0t} + \beta_{1t} TRT_i + \mathbf{x}_i'\boldsymbol{\beta} + \epsilon_{it}, \qquad (6.1)$$

where $iGFR_{it}$ is the value of iGFR at time t for subject i ($i = 1, \dots, 530$). Fixed effects β_{0t}, β_{1t} for $t = 1, 2, 3$ denote visit-specific intercepts and treatment effects. TRT_i is

treatment group (equal to 1 for the allopurinol and 0 for placebo). Stratifying variables (serum uric acid, HbA1c, study center), and baseline covariates: albuminuria status, AER, iGFR for subject i are included in a vector $\mathbf{x}_i$ of p covariates $(x_1, \ldots, x_p)$ and associated fixed effects are stored in vector $\boldsymbol{\beta} = (\beta_1, \ldots, \beta_p)$. We assume that residual errors ϵ_{it} $(t = 1, 2, 3)$ for subject i are normally distributed with zero mean and 3x3 general/unstructured variance-covariance matrix. The model specified in (6.1) will yield the estimates of visit-specific treatment effects $\beta_{11}, \beta_{12}, \beta_{13}$ for all three visits V11, V16 and V17. In the context of the primary analysis of the primary endpoint, we are interested in parameter β_{13}, representing treatment effect at Visit 17 adjusted for stratifying variables and baseline covariates. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom.

Estimand	Primary estimand defined in Section 5.2.1
Analysis	Primary Analysis of the Primary Endpoint: iGFR at the end of the 2-month wash-out period (Visit 17)
Analysis Set	ITT Population
Methods	Linear model for repeated measures with correlated errors using multiple imputation technique.
Dependent Variable	iGFR measured at Visits V11 (80 weeks) , V16 (156 weeks) and V17 (164 weeks after randomization)
Model	Fixed effects: • Visit-specific intercepts corresponding to V11, V16, V17 • Visit-specific treatment effects corresponding to V11, V16, V17 • Stratifying variables: sUA, HbA1c, study center • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline iGFR
Results	• Least square iGFR means *at Visit 17* by treatment group. • Estimate of treatment effect *at Visit 17* adjusted for baseline covariates • 95% confidence interval for treatment effect • P-value for treatment effect

We will assess the impact of deviation from the MAR assumption on the robustness of the results through a *sensitivity analysis.* For the primary estimand, it will be performed within the same multiple imputation framework; however we will employ marginal delta-adjusted method and apply it to Visit 17 with adjustments in allopurinol arm increasing by one unit of iGFR value until the MAR results are overturned, that is, we will use so called tipping point approach (O'Kelly, Ratitch, 2014).

6.6.2. Secondary analysis for the primary estimand

The following secondary analyses will be performed to assess how alternative assumptions of the primary endpoint (as defined above) and alternative approaches for handling missing data may affect the conclusions of the analysis:

1. Analysis of covariance using iGFR values at Visit 17 as the dependent variable and treatment effect as a covariate of primary interest. The same baseline covariates, as in the primary analysis of the primary endpoint, stored in vector $\boldsymbol{x}_i$ (see Equation. 6.1) will be used in the model.

2. Performing an analysis identical to the primary one (same endpoint and substantive model) using the per-protocol analysis set rather than the ITT analysis set.

6.7. Analyses for secondary estimands

In this section we present analyses aligned with secondary estimands defined in section 5.2. Analyses will be performed using the multiple imputation technique, except those involving time-to event endpoints (sections 6.7.5, 6.7.8).

6.7.1. iGFR at the end of the 3-year treatment period (Visit 16, before the washout)

In this section we describe the analysis (estimator) aligned with the estimand for iGFR at the end of the 3-year treatment period (Visit 16, before the washout) endpoint defined in Section 5.2.1.

The least square means at Visit 16, estimate of treatment effect *at Visit 16* adjusted for baseline covariates, their 95% confidence interval and P-value will be obtained as part of the primary analysis of the primary estimand (Equation (6.1) in section 6.1.1). In the context of this secondary endpoint, we are interested in the fixed effect β_{12} , which represents the treatment effect at Visit 16 adjusted for stratifying variables and baseline covariates.To assess the hemodynamic/transient effect of the allopurinol, we will estimate the contrast $\beta_{12} - \beta_{13}$ between the treatment effect at Visit 16 (before washout) compared to that at Visit 17 (after washout).

6.7.2. iGFR time trajectory estimated from repeated iGFR measurements

This analysis is aligned with the estimand defined in Section 5.2.2

Estimand	See section. 5.2.2 for definition
Analysis	Analysis of the Secondary Endpoint: iGFR time trajectory estimated from repeated iGFR measurements.
Analysis Set	ITT Population
Methods	Linear mixed-effects model for longitudinal iGFR measures using multiple imputation technique.
Dependent Variable	iGFR measured at Visits V11 (80 weeks) , V16 (156 weeks) and V17 (164 weeks after randomization)
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Time since randomization (in years) associated visit windows defined in section 6.3 • Time by treatment group interaction • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline iGFR Subject-specific random effects • Random intercept for iGFR • Random slope for iGFR
Results	• iGFR slope estimates and 95%CIs by treatment group • Estimate of a treatment effect measured as a difference between average slopes of iGFR versus time for allopurinol and placebo groups adjusted

	for stratifying variables and baseline covariates • 95% confidence interval for treatment effect • P-value for treatment effect

6.7.3. eGFR at 4 months after randomization (Visit 7)

Estimand	See section 5.2.3 for definition
Analysis	Analysis of the Secondary Endpoint: eGFR at 4 months after randomization (Visit 7).
Analysis Set	ITT Population
Methods	Linear model using multiple imputation technique.
Dependent Variable	eGFR measured at Visit V7 (16 weeks after randomization)
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline eGFR
Results	• Least square eGFR means *at Visit 7* by treatment group. • Estimate of treatment effect *at Visit 7* adjusted for stratifying variables and baseline covariates • 95% confidence interval for treatment effect • P-value for treatment effect

6.7.4. eGFR time trajectory

Estimand	See section 5.2.4 for definition
Analysis	Analysis of the Secondary Endpoint: eGFR time trajectory estimated from repeated eGFR measurements using multiple imputation technique..
Analysis Set	ITT Population
Methods	Linear mixed-effects model for longitudinal eGFR measures using multiple imputation technique.
Dependent Variable	Post-randomization eGFR measured from Visits V6 through V17
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Time since randomization in (in years) associated visit windows defined in section 6.3 • Time by treatment group interaction • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline eGFR Subject-specific random effects • Random intercept for eGFR • Random slope for eGFR
Results	• eGFR slope estimates and 95%CIs by treatment group • Estimate of a treatment effect measured as a difference between average eGFR versus time slopes for allopurinol and placebo groups adjusted for stratifying variables and baseline covariates • 95% confidence interval for treatment effect • P-value for treatment effect

6.7.5. Time to serum creatinine doubling or ESRD

Estimand	See section 5.2.5 for definition
Analysis Set	ITT Population
Methods	Proportional hazards model for interval censored data.
Dependent Variable	Time to composite endpoint of serum creatinine doubling or ESRD
Proportional Hazards Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline iGFR
Results	• N(%) of subjects with doubled serum creatinine or ESRD during the course of the study • Hazard ratio of allopurinol to placebo • 95% confidence interval for hazard ratio • P-value for treatment effect

6.7.6. Urinary AER at the end of the wash-out period

Estimand	See section 5.2.6 for definition
Analysis Set	ITT Population
Methods	Linear model using multiple imputation technique.
Dependent Variable	Two AER measures obtained at Visit 17 and summarized using the geometric mean expressed on logarithm base to 10 scale
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log base to 10 scale • Baseline iGFR
Results	• Predicted urinary AERs *at Visit 17* by treatment group obtained by antilog transformation applied to corresponding least square means • Estimate of treatment effect *at Visit 17* expressed on percent change scale using antilog transformation. • 95% confidence interval for treatment effect expressed on percent change scale using antilog transformation. • P-value for treatment effect

6.7.7. Urinary AER during the last three months of the treatment period (Visits 15 and 16)

Estimand	See Section 5.2.7 for definition
Analysis Set	ITT Population
Methods	Linear model using multiple imputation technique.
Dependent Variable	Two AER measures obtained at Visit 15 and 16 are summarized using the geometric mean expressed on logarithmic scale
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log base to 10 scale • Baseline iGFR
Results	• Predicted AERs at the end of treatment period by treatment group obtained by antilog transformation applied to corresponding least square means. • Estimate of treatment effect at the end of treatment period adjusted for baseline covariates expressed on percent change scale using antilog transformation. • 95% confidence interval for treatment effect expressed on percent change using antilog transformation. • P-value for treatment effect

6.7.8. Time to fatal or non-fatal cardiovascular events

Estimand	See Section 5.2.8 for definition
Analysis Set	ITT Population
Methods	Cox proportional hazards model.
Dependent Variable	Time to composite endpoint: fatal or non-fatal cardiovascular events
Cox Model	Fixed effects: • Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log base to 10 scale • Baseline iGFR
Results	• N(%) of subjects with fatal or non-fatal cardiovascular events during the course of the study • Hazard ratio of allopurinol to placebo • 95% confidence interval for hazard ratio • P-value for treatment effect

6.8. Other analyses

6.8.1. Subgroup analyses

To investigate the possible presence of heterogeneity in the response to allopurinol, subgroup analyses (based on the primary efficacy analysis described in subsection 6.6.1, with the inclusion of appropriate interaction terms with the subgroup variable) will be performed by age groups (≤50 and >50 yrs), gender, racial/ethnic group, HbA1c (≤7.8 and >7.8%), serum uric acid (≤6.0 and > 6.0 mg/dl), baseline iGFR (≤60 ml/min and > 60). ml/min/1.73m^2), AER at baseline (≤50 and >50 mg/24 hr), and albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who did qualify by eGFR slope and were normoalbuminuric at baseline).

An example of such subgroup analysis for age groups (≤50 and >50 yrs) is provided below. Similar to Equation (6.1) for each time t ($t = 1, 2, 3$), corresponding to visits V11, V16, V17, we specify the model:

$$iGFR_{it} = \beta_{0t} + \beta_{1t} TRT_i + \beta_{2t} AGE_i + \beta_{3t} AGE_i \times TRT_i + \boldsymbol{x}_i'\boldsymbol{\beta} + \epsilon_{it}, \qquad (6.2)$$

where $iGFR_{it}$ is the value of iGFR at time t for subject i $(i = 1, \ldots, 530)$. Fixed effects $\beta_{0t}, \beta_{1t}, \beta_{2t}, \beta_{3t}$ for $t = 1, 2, 3$ denote visit-specific intercepts, treatment effects, age effects and age by treatment interactions, respectively. TRT_i is treatment group (equal to 1 for the allopurinol and 0 for placebo). AGE_i indicates age group (≤50 and >50 yrs). Stratifying variables, and baseline covariates albuminuria status, AER, iGFR for subject i are included in a vector of covariates $\boldsymbol{x}_i$ and associated fixed effects are stored in vector $\boldsymbol{\beta}$. We assume that residual errors ϵ_{it} $(t = 1, 2, 3)$ for subject i are normally distributed with zero mean and 3x3 general/unstructured variance-covariance matrix. The model specified in (6.2) will yield the estimates of visit-specific treatment by age interaction effects β_{31}, β_{32}, β_{33} for all three visits V11, V16 and V17. In the context of subgroup analysis, we are interested in β_{33}, which represents treatment by age interaction at Visit 17 adjusted for baseline covariates.

Analysis	Subgroup Analysis of the Primary Endpoint: iGFR at the end of the 2-month wash-out period (Visit 17) by Age group
Analysis Set	ITT Population
Methods	Linear model for repeated measures with correlated errors
Dependent Variable	iGFR measured at Visits V11 (80 weeks) , V16 (156 weeks) and V17 (164 weeks after randomization)
Model	Fixed effects: • Visit-specific intercepts, age effects, treatment effects and age by treatment interaction effects • Stratifying variables: sUA, HbA1c , Study center • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log base to 10 scale • Baseline iGFR
Results	• Estimate of age by treatment interaction *at Visit 17* adjusted for baseline covariates • 95% confidence interval for age by treatment effect interaction *at Visit 17* • P-value for treatment effect

6.8.2. Analyses of safety outcomes

Safety measures are assessed during three periods of the study: run-in (Visits 1-5), on-treatment (after Visit 5 through Visit 16), and off-treatment washout (after Visit 16 through Visit 17). Safety will be summarized overall (treatment and off-treatment combined) and by period, depending on the safety outcome of interest during that period.

- Percentage of subjects with and number of SAEs, time to first SAEs during on-treatment period and overall by MedDRA System Organ Class and by MedDRA Preferred Term Categories.
- Percentage of subjects with and number of permanent discontinuations of study medication because of adverse effects on-treatment period and overall.
- Percentage of subjects with and number of AEs, overall and by severity and by relatedness to study medication during on-treatment period and overall.
- Percentage and number of subjects with skin rash during on-treatment period and overall.

For dichotomous safety outcomes, the proportion of subjects experiencing adverse outcomes (AEs, SAEs) will be summarized by treatment group and compared by means of odds ratios and 95% CIs. Poisson regression models will be used for safety outcomes (e.g., SAEs and AEs) with multiple recurrences per patient, with the logarithm of the period of observation from the time of study medication used as the offset. Time to first SAE will be analyzed using Kaplan-Meier methods to estimate the SAE-free distributions for each treatment group. This analysis will employ the ITT analysis set. No imputation for missing data will be used.

6.8.3 Analyses of other measures

In addition to primary, secondary, and safety measures, the following additional outcomes will be analyzed to help with the interpretation of study results:

- Descriptive statistics for body weight, blood pressure, serum creatinine, HbA1c, and serum uric acid at each post-baseline visit and their changes from baseline, by treatment group in the ITT population. No imputation for missing data will be employed.
- Percentage of subjects receiving adequate study medication exposure (i.e., allopurinol or placebo) independent of adverse events. This is defined as the actual total dose during the 156-week dosing period, as determined from the dispensed dosage and pill counts, divided by the expected total dose defined by the eGFR-adjusted protocol-described dosing regimen, without consideration for temporary or permanent discontinuations or reductions owing to adverse events. The proportion of subjects receiving the presumed adequate study medication exposure is defined as the number of subjects who had at least 80% and no more than 120% of the intended study medications during the entire dosing period, independent of adverse events, among all randomized subjects. No imputation for missing data will be employed.

6.8.4. Analysis of the effect of post-randomization sUA changes on iGFR value at Visit 17

The analysis outlined below will be performed using linear model with correlated errors. In addition to fixed effects associated with baseline covariates, we will include fixed effects associated with visit-specific effects of another covariate, namely the average sUA change from baseline over the initial post-randomization period (Visits 6-10) on iGFR values. We note that this covariate is created based on sUA values that precede iGFR measures (our dependent variable) and in this way we attempt to mitigate the impact of the bidirectional relationship between concurrent measures of sUA and iGFR.

Analysis	Analysis of the effect of post-randomization average changes of sUA values (V6-V10) relative to sUA at baseline on iGFR value at Visit 17
Analysis Set	ITT Population
Methods	Linear model with correlated errors for longitudinal iGFR measures
Dependent Variable	iGFR measured at Visits V11 (80 weeks), V16 (156 weeks) and V17 (164 weeks after randomization)
Model	Fixed effects associated with: • Visit-specific intercepts corresponding to V11, V16, V17 • Visit-specific effects of sUA change on iGFR at V11, V16, V17 • Stratifying variables: sUA, HbA1c, study center • Albuminuria status at baseline with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline iGFR
Results	• Estimate of an effect of an average sUA changes from baseline on iGFR value at Visit 17, adjusted for stratifying variables and baseline covariates • 95% confidence interval for sUA changes effect • P-value for sUA changes effect

6.8.5. Time to 40% eGFR decrease

Analysis	Analysis of time to 40% eGFR decrease from randomization
Analysis Set	ITT Population
Methods	Proportional hazards model for interval censored data.
Dependent Variable	Time to endpoint of 40% eGFR decrease from randomization
Proportional Hazards Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale
Results	• N(%) of subjects with 40% eGFR decrease during the course of the study • Hazard ratio of allopurinol to placebo • 95% confidence interval for hazard ratio • P-value for treatment effect

6.8.6. Time to doubling of serum creatinine, end-stage renal disease (ESRD), or cardiovascular/renal death

Variable of interest (endpoint) is defined as a composite of three events: (1) doubling to serum creatinine, (2) ESRD, or (3) cardiovascular/renal death. Time to event is defined as time from randomization to the first event (one of the events defined above) or censoring (lost-to-follow-up, withdrawal, death other than due to cardiovascular/renal cause, and study completion without experiencing the event).

Analysis Set	ITT Population
Methods	Proportional hazards model for interval censored data.
Dependent Variable	Time from randomization to composite endpoint of serum creatinine doubling, ESRD or cardiovascular/renal death
Proportional Hazards Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline iGFR
Results	• N(%) of subjects with doubled serum creatinine, ESRD or cardiovascular/renal death during the course of the study • Hazard ratio of allopurinol to placebo • 95% confidence interval for hazard ratio • P-value for treatment effect

6.9. Model assumptions and alternative analyses

Model assumptions will be thoroughly checked for individual and systematic departures, using informal, e.g. inspection of residuals, and formal methods such as methods based on likelihood displacement. If individual outliers are detected, their influence will be evaluated using influence diagnostics methods based on comparing estimates from models fitted to data with and without outlying values. Whenever we are not successful in fitting the parametric model (linear or non-linear), then non-parametric analyses and/or transformation of the variables involved in the analysis will be considered.

ACKNOWLEDGEMENTS

We thank Drs. Tom Greene, Charity Moore, Geert Molenberghs, and Rod Little for their comments and suggestions.

REFERENCES

Fan, L., Levey, A.S., Gudnason, V., Eiriksdottir, G., Andresdottir, M.B., Gudmundsdottir, H., Indridason, O.S., Palsson, R., Mitchell, G. and Inker, L.A., 2015. Comparing GFR estimating equations using cystatin C and creatinine in elderly individuals. *Journal of the American Society of Nephrology*, *26*(8), pp.1982-1989.

Gałecki, A. and Burzykowski, T., 2013. *Linear mixed-effects models using R: A step-by-step approach*. Springer Science & Business Media.

International Conference on Harmonization, ICH E9 (R1): Addendum on Estimands and Sensitivity Analyses in Clinical Trials to the guideline on statistical principles for clinical trials. Step 2b, 30 August 2017.

Inker, L.A., Schmid, C.H., Tighiouart, H., Eckfeldt, J.H., Feldman, H.I., Greene, T., Kusek, J.W., Manzi, J., Van Lente, F., Zhang, Y.L. and Coresh, J., 2012. Estimating glomerular filtration rate from serum creatinine and cystatin C. *New England Journal of Medicine*, *367*(1), pp.20-29.

Molenberghs, G. and Verbeke, G., 2005. *Models for Discrete Longitudinal Data*. Springer Science & Business Media.

O'Kelly, M. and Ratitch, B., 2014. *Clinical trials with missing data: A guide for practitioners*. John Wiley & Sons.

Rubin, D. B. (1987), *Multiple Imputation for Nonresponse in Surveys*, New York: John Wiley & Sons.

van Buuren, S., Brand, J. P. L., Groothuis-Oudshoorn, C. G. M., and Rubin, D. B. (2006), "Fully Conditional Specification in Multiple Imputation," *Journal of Statistical Computation and Simulation*, 76, 1049–1064

van Buuren, S. (2007), "Multiple Imputation of Discrete and Continuous Data by Fully Conditional Specification," *Statistical Methods in Medical Research*, 16, 219–242.

White, Ian R., Nicholas J. Horton, James Carpenter, and Stuart J. Pocock. "Strategy for intention to treat analysis in randomised trials with missing outcome data." *BMJ* 342 (2011): d40.

APPENDIX I. Study Objective, Study Design, Outcomes & Statistical Analysis and Data Management Sections from Protocol

In this appendix, selected sections (from protocol, version 10, approved by DSMB on March 6th, 2018) are included for reference. The following sections/figures from the study protocol are included:

- 2. Study objective
- 3. Study design
- 7.1. Primary outcomes
- 7.2. Secondary outcomes
- Schedule of events (original figure on p. 27 in the study protocol)
- 9. Safety assessments
- 10. Adverse event reporting
- 11. Statistical analysis

2. STUDY OBJECTIVE

To determine whether lowering serum UA by means of oral allopurinol is effective in preventing or slowing decline of renal function in T1D patients with microalbuminuria or moderate macroalbuminuria who still have only mildly or moderately impaired kidney function.

3. STUDY DESIGN

The study will be a multi-center, double-blind, placebo-controlled, parallel-group randomized clinical trial including a total of 480 patients with type 1 diabetes (T1D) who are at high risk for GFR loss because of increased albuminuria and a relatively high serum UA (≥ 4.5 mg/dl), but have only mildly or moderately decreased renal function.

7. STUDY OUTCOMES

7.1. Primary outcome

The primary outcome will be the iGFR at the end of the 2-month wash-out period following the 3-year treatment period, measured by the plasma clearance of non-radioactive iohexol (iGFR) and adjusted for the iGFR at baseline. The rationale of measuring the primary outcome at the end of the wash-out period is to test allopurinol for permanent effects of on the natural history of kidney disease, independent from any transient, hemodynamic effect that the medication may have on GFR. Plasma iohexol clearance has been shown to provide accurate and reproducible GFR measurements.[30,31] It is highly correlated with inulin clearance (the gold standard to measuring GFR)[32] and is a safe, cost-effective method to test hundreds of patients enrolled in multicenter clinical trials.[33] The method consists of injecting a 5 mL bolus of Iohexol (Omnipaque, 300 mg iodine/mL) and drawing blood samples at baseline and 120, 150, 180, 210, and 240 minutes after the injection. Plasma concentrations of iohexol at different time points are measured by HPLC and used to calculate the plasma clearance of iohexol (Cl=Dose/AUC, where AUC is the area under the plasma concentration time curve), which is taken after appropriate body surface area corrections as a measure of GFR. [30,31]

7.2. Secondary outcomes

1. Iohexol-clearance GFR at the end of the 3-year treatment period (before the washout).
2. Iohexol-clearance GFR time trajectory estimated from periodical iohexol-GFR measurements.
3. Estimated (eGFR) at 4 months estimated from serum creatinine and cystatin C and adjusted for the eGFR at baseline.
4. Estimated GFR (eGFR) time trajectory estimated from quarterly serum creatinine and cystatin C measurements using the CKD-EPI SCr and the CKD-EPI SCr-SCysC equations.34,35
5. Time to doubling of baseline serum creatinine value or ESRD (eGFR ≤ 15 ml/min/1.73 m2, institution of dialysis, kidney transplantation).
6. Geometric mean of two AER measurements at the end of the 2-month wash-out period following the 3-year treatment period, adjusted for the mean urinary AER at baseline. Urinary AER will be determined in timed overnight urine collections brought by study participants to regular clinic visits, and expressed in ·g/minute and as urinary albumin/creatinine ratios.
7. Geometric mean of urinary AER during the last three months of the treatment period (Visits 15 and 16), adjusted for the mean urinary AER at baseline.
8. Time to fatal or non-fatal cardiovascular events, defined as the composite of CVD death (ICD-10 code I10 to I74.9), myocardial infarction, stroke (ischemic or hemorrhagic), coronary artery bypass grafting, or percutaneous coronary intervention.

Figure 1. Schedule of events

Year	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3	3	3
Week	-12	-9	-7	-3		0	4	16	32	48	64	80	96	112	128	142	156	164
Visit #	**1**	**2**	**3**	**4**	**4a***	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
^Type of Visit: In-Person Visit Required (V); Phone Call (C) ; Other Visit (In-Person or Remote Visit, O)	O	V	O	V	V	C	O	O	O	O	O	V	O	O	O	O	V	V
EVENT						Allopurinol or placebo											Wash-out	
	Screen	Run-in				RANDO 100 mg	200-400 mg											EOS
Informed Consent	x	x																
Demographics	x																	
Initial Medical Hx		x																
Interval Medical Hx and BP Control			x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Concomitant Meds	x	x	x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Blood Pressure and Measurements	x	x	(x)	x	(x)		(x)	(x)	(x)	(x)	(x)	x	(x)	(x)	(x)	(x)	x	x
ECG Report		x		x	(x)							x					x	
Physical Exam		x		(x)	(x)							x					x	
Skin Assessment				x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Eligibility	x			x	(x)	x												
Randomization						x												
Family History				x	(x)													
RAS and BP Med Log		x	x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
IGFR Procedure				x	(x)							x					x	x
PERL Study Drug Prescription						x	x	x	x	x	x	x	x	x	x	x		
Study Drug Compliance							x	x	x	x	x	x	x	x	x	x	x	
CENTRAL LAB																		
Serum uric acid, serum creat, cystatin C	x		x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Urine ACR/AER	x		x	x	(x)		.	x		x		x		x		x	x	x
HbA1c	x			x	(x)			x	x	x	x	x	x	x	x	x	x	x
HLA B*58:01				x	(x)													
iGFR				x	(x)							x					x	x
NIDDK Repository: serum, plasma, urine				x								x					x	x
LOCAL LAB																		
Pregnancy test serum HCG	x		x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Pregnancy test urine dipstick		x		x	(x)							x					x	x
ALT, K, CBC, serum creatinine, urine	x		x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Protocol Deviation		x	x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Adverse Events		x	x	x	(x)	x	x	x	x	x	x	x	x	x	x	x	x	x

*****If normal blood pressure control is not achieved at Visit 4, the run-in period may be extended for two more weeks after which participants will be examined as in Visit 4 (Visit 4A). In this event, the GFR measurement scheduled for Visit 4 will be conducted at Visit 4A.

^ Study visits will be generally conducted at the Study Sites or their Satellites. "In-Person Visits" (V) are required for Visit 2 and all visits requiring iohexol-GFR measurements. If a participant lives far from a study site or satellite, or travel impediments are present, other (O) visits may be conducted remotely or in-person. For any given study visit to be conducted remotely, a Phone Visit and a Remote Biospecimen Collection will be both required; a Phone Visit is performed by the study coordinator using the telephone or other media such as Skype to collect results of study procedures that do not require physical interactions (e.g., collection of medical history), and a Remote Biospecimen Collection is performed at a clinical laboratory close to where participants live.

Note: (x) indicates an optional assessment; For "BP and Measurements", (x) indicates an optional assessment only if the patient is NOT seen in-person.

9. SAFETY ASSESSMENTS

9.1. Demographic data/medical history

After collecting a detailed medical history at Visit 1, this information will be updated at each visit through a structured interview, with a special emphasis on skin symptoms and signs such as rash, itching and exfoliation and on pregnancy in females. Participants will be instructed to communicate any change in their health status and intervening hospitalizations to the study coordinator in-between visits. In particular, they will be instructed to discontinue study medication and immediately contact the study coordinator if they develop a suspicious skin rash, swelling of the lips or mouth, arthralgias, and/or jaundice, which may indicate a hypersensitivity reaction to allopurinol. Fever and chills should also be reported but would not require cessation of medication prior to discussion with study personnel.

9.2 Skin exam

The skin of study participants will be examined for the presence of any kind of rash at each in-person visit. Participants will be instructed to carry-out periodical skin self-exams. If skin abnormalities are reported to the study personnel during the phone visits or on any other occasion, participants will be asked to immediately report to the study site, their PCP's office, or other local healthcare facilities for an in-person skin exam. Suspicion of drug allergy or Stevens-Johnson Syndrome SJS would require immediate discontinuation of study medication and dermatologic consultation.

9.3. Vital signs

Blood pressure and heart rate will be recorded at each in-person visit. BP readings at home will be reviewed during each phone visits; if abnormal values are reported, participants will be asked to visit the study site, their PCP's office, or other local healthcare facilities to have their BP measured.

9.4. Clinical laboratory tests

Serum ALT, creatinine and K^+, and CBC will be monitored and a pregnancy test, if a female of child bearing potential, performed at each visit. Participants who are started for the first time on RAS blockers as part of this study will have their serum K^+ and creatinine measured at a local laboratory after 2 weeks of full dose RASB treatment (i.e., after Visit 3). HbA1c will be measured at Visits 1, 4, and 7-17. An ECG will be performed at Visits 2, 4, 11, and 16.

9.5. Management of uric acid levels

Study participants and study personnel, other than the DCC and the study pharmacists, will be masked as to the uric acid levels obtained during the study. The patients' physicians will receive written requests to refrain from measuring uric acid levels during the time of the patients' participation in the study, except as is mandatory for the patient's wellbeing, e.g., in the treatment of malignancy or diagnosis of a clinical syndrome highly likely to represent gout. If gout is diagnosed, open-label treatment with allopurinol will become indicated. In such case, the study drug will be discontinued but the patient will remain in the study and will continue to be followed as if he/she was taking the study medication. If uric acid lowering for malignancy treatment is required, the patient will receive open-label treatment until such time as return to study drug is deemed clinically reasonable by their physician.

10. ADVERSE EVENT REPORTING

10.1. Definitions

An Adverse Event (AE) is any untoward medical occurrence in a study participant regardless of its relationship to study treatment. A treatment-emergent AE is an adverse event occurring during the period between the first dose and 30 days after the final dose of the study medication. A Serious Adverse Event (SAE) is any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongation of an existing hospitalization, results in persistent or significant disability, or is a congenital anomaly/birth defect. Important medical events that do not fall into the above categories may also be considered an SAE when, based on medical judgment, such events may jeopardize the patient's safety and require medical/surgical intervention to prevent one of the outcomes listed in the SAE definition. The term SAE is not intended as a measure of severity or intensity. All AE's/SAE's that occur after the time of informed consent will be reported.

A Suspected Adverse Reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. An Unexpected Adverse Event or Unexpected Suspected Adverse Reaction is an adverse event or suspected adverse reaction that is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected", as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation. An Expected Adverse Event or Expected Adverse Reaction is any adverse experience that has been identified in nature or severity in the current investigator brochure and/or protocol.

10.2. Adverse Events Reporting

All AEs will be reported on the Adverse Events form that will be completed by the study staff, who are masked as to study treatment assignment, at each regular follow-up visits. This will insure that AEs are ascertained in an unbiased manner using the same standardized methodology for participants in both treatment arms. Forms will include standardized questions relating to specific events of import in diabetic patients on either of the study treatment arms as well as any significantly abnormal physical finding identified on examination and any significantly abnormal laboratory results obtained on the patient between visits or at the time of the visit. AEs reported or ascertained between clinic visits will be captured and reported at the time of the next schedule visit. Pre-existing conditions (that is, any condition that was known to be present prior to the signing of informed consent or was identified during the screening procedures at Visit 1) will not be considered or recorded as AEs unless the condition worsens in

intensity or frequency after Visit 1. Likewise, continuing AEs will not be reported as AEs at subsequent visits unless they increase in severity or frequency between visits, they result in criteria for a SAE, and/or they resolve between visits. Each site will be responsible for reporting all AE's to their IRB according to its AE reporting policy and procedures.

10.3. Assessment of causality and severity

The seriousness of adverse events will be ascertained by the study staff according to the criteria listed in 10.1 and the need for further evaluation, follow-up, or referral. The relationship between study participation and AEs will be determined according to the following criteria:

A. Not related – temporal relationship of the onset of the event, relative to study participation, is not reasonable or another cause can by itself explain the occurrence of the event.

B. Possibly related – temporal relationship of the onset of the event, relative to study participation, is reasonable but the event could have been due to another, equally likely cause.

C. Probably related – temporal relationship of the onset of the event, relative to study participation, is reasonable and the event is more likely explained by the study treatment than by another cause.

D. Definitely related – temporal relationship of the onset of the event, relative to study participation, is reasonable and there is no other cause to explain the event.

10.4. Serious adverse events reporting

See Section 15 – Data and Safety Monitoring Plan.

11. STATISTICAL ANALYSIS

This section presents a summary of the planned statistical analyses. A statistical analysis plan (SAP) will be written for the study that contains detailed descriptions of the analyses to be performed. The SAP will be written prior to database lock.

11.1. Analysis population

For most of the analyses, including the primary efficacy analysis described in section 11.3, an intention to treat (ITT) analytical approach will be employed. Accordingly, the population for statistical analysis will consist of all randomized study participants considered in their original randomization group, regardless of treatment discontinuation or loss to follow-up.

Selected secondary efficacy analyses will be performed using a per-protocol analytical approach. In this case, the analysis population will consist of the ITT population excluding data points which 1. had cumulative exposure to the study medication from randomization that was less than 80% of the theoretical full exposure; or 2. during major protocol deviations (e.g., treatment with prohibited medications), which could affect primary outcome.

11.2. Initial data analysis

The initial data analysis will be performed to detect any differences in distributions of characteristics measured at baseline, 4, 20, 36, and 38 months (0, 16, 80, 156, and 164 weeks, respectively) between study groups. The number of patients screened, enrolled, and completing the study will be summarized within and across study centers. Measures of central tendency (means, medians) and variability (standard deviations, ranges) will be estimated from the data for continuous variables. Frequency distributions will be provided for categorical data. This preliminary analysis step will provide us with insight into data, distributions of the variables considered, and will allow us to find additional invalid values not detected earlier during data validation.

11.3. Primary efficacy analysis

For the primary endpoint (iGFR at the end of the 2-month wash-out period following the 3-year intervention), we will follow the recommendations by Carpenter et al[38,39] and perform the analysis by means of a linear model for correlated errors with general/unstructured covariance matrix using all available iGFR measures (including those at baseline, 80, 156, and 164 weeks, respectively) as the dependent variable. By conditioning on the baseline iGFR measure we will also effectively use this variable as a covariate. Treatment group, study center, stratifying variables, albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who did qualified by eGFR slope and were normoalbuminuric at baseline), baseline AER, time, and time by treatment interaction will also be included as covariates in the model. Three features make this analytical approach especially attractive:

1. If there is no dropout (a very unlikely case), the estimate of the treatment effect at the end of the 2-month wash-out period following the 3-year intervention and its precision obtained using this approach will be exactly the same as those based on a classical approach employing an analysis of covariance (ANCOVA) model with treatment group, study center, iGFR and AER/ACR measured at baseline included as covariates.
2. If the iGFR measure at the end of the wash-out period is missing, we will be able to efficiently use the information contained in the intermediate iGFR measurements obtained at 80 and 156 weeks, by virtue of them being correlated with the GFR measurement at washout. Estimate of the treatment effect obtained this way is valid under the missing at random (MAR) assumption. This is in contrast to the ANCOVA approach, which would lead to the loss of this information and would require a more stringent assumption about the mechanism of data missingness, i.e. a missing completely at random (MCAR) mechanism.
3. The underlying analytical framework allows the use of all post-randomization data and is well suited to investigate the reason for withdrawal, for example to study whether participants having low iGFR values are more likely to withdraw.

Calculations will be performed using SAS PROC/MIXED. Results of the analysis will be expressed in terms of point estimate and its corresponding 95% confidence interval for the treatment effect at the end of the 2-month wash-out period following the 3-year treatment and will be accompanied by the corresponding p value.

11.4. Secondary efficacy analyses

1. The effect of treatment on the iGFR at the end of the 3-year treatment period (before the washout) will be evaluated using the same analytical approach employed for the primary outcome.

2. The effect of treatment on the eGFR at 4 months after randomization will be evaluated using the same analytical approach employed for the primary outcome.
3. The iGFR and eGFR time trajectories, estimated from periodical iGFR measures and quarterly serum creatinine and cystatin C measurements using the CKD-EPI SCr and the CKD-EPI SCr-SCysC equations[34,35], respectively, will be analyzed using linear mixed-effects models.[40-42] The main objective of the analysis will be to construct confidence interval for the effect of the intervention over three years of observation (treatment main effect) and investigate whether the effect of the intervention changes with time (time by treatment interaction).
4. Time to serum creatinine doubling or ESRD in the two treatment groups is subject to censoring due to dropouts or reaching the end of study before the participant experiences the event. Survival time will be defined as the time from randomization to the event (the first of serum creatinine doubling from baseline or occurrence of ESRD, defined as eGFR $\leq$ 15 ml/min/1.73 m^2, hemodialysis, or kidney transplant) or, for participants who did not experienced an event, to the last study visit. Data will be summarized by means of Kaplan-Meier survival curves and by providing the proportions of participants surviving without events at 1, 2, 3 years, and at the end of the wash-out period along with their 95% CIs. Given the potentially small number of events, differences between study groups will be tested by means of the log rank test or by means of simple Cox regression models including a limited number of predictors in addition to treatment group.
5. The effect of treatment on the AER at the end of the wash-out period, based on the geometric mean of two AER measured at this time point and adjusted for the geometric mean of AER at baseline (Visit 3 and 4), will be investigated in a linear regression model framework as in the case of the primary outcome.
6. The effect of treatment on the AER at the end of the treatment period, based on the geometric mean of the AER measures at visit 15 and 16 adjusted for the geometric mean of AER at baseline (Visit 3 and 4) will be investigated as in #5.
7. Time to fatal or non-fatal cardiovascular events will be analyzed as proposed for time to serum creatinine doubling or ESRD.
8. We will perform a per-protocol analysis (as defined in 11.1) for the primary efficacy endpoint (iGFR at the end of the 2-month wash-out period following the 3-year intervention).

11.5. Incomplete data

Missing values represent a potential source of bias. Efforts will be made to keep all participants in the study. If this is not feasible, at least some information regarding the status at the end of the trial will be obtained. For randomized patients, the number of completing and dropouts will be summarized. This procedure will help to compare characteristics of the participants' groups who drop out from the study with those who completed the study by treatment group, within and across study centers. The models considered in the proposal allow for a missing at random (MAR) mechanism. MAR means that the missing values mechanism can be explained by observed data and does not depend on the *unobserved* values of outcome measures. The differences in distributions between characteristics of the groups may indicate potential sources of bias due to missing values. For instance, some patients may dropout from

the study due to *unobserved* factors related to the intervention itself. If we suspect such bias is present, the methods discussed in this section, assuming (MAR), are not applicable. We will incorporate plausible missing values mechanism into the model as discussed in Little[43] and investigate how such mechanism may affect the estimates of treatment effect. To this end, sensitivity analyses will be conducted involving selection and/or pattern-mixture models[44] with an appropriate submodel used to describe dropout.

11.6. Pilot participants

All pilot participants who were already randomized to allopurinol or placebo during the pilot will be included in the final analysis of the pivotal trial. Those who do not consent to the pivotal trial will be treated as having dropped from the study at a time corresponding to their last pilot visit. Sensitivity analyses will be performed to investigate whether results may be potentially affected by the roll-over of pilot subjects in the pivotal trial.

11.7. Model assumptions and alternative analyses

Model assumptions will be thoroughly checked for individual and systematic departures, using informal, e.g. inspection of residuals, and formal methods such as score test for extra parameter or methods based on likelihood displacement. If individual outliers are detected, their influence will be evaluated using influence diagnostics methods based on comparing estimates from models fitted to data with and without outlying values. Whenever we are not successful in fitting the parametric model (linear or non-linear), then non-parametric analyses and/or transformation of the variables involved in the analysis will be considered. To investigate the potential hemodynamic influence of allopurinol on treatment effect, in addition to the aforementioned analyses, we will consider models including the post-randomization measure of GFR at 4 months as an additional covariate. To investigate the possible presence of heterogeneity in the response to allopurinol, subgroup analyses (based on the primary efficacy analysis described in section 11.3, with the inclusion of an interaction term of the treatment group by the subgroup variable) will be performed by age groups (≤40 and >40 yrs), gender, racial/ethnic group, HbA1c (≤7.8 and >7.8%), serum uric acid (≤6.0 and > 6.0 mg/dl), baseline iGFR (≤70 ml/min and >70 ml/min/1.73m^2), AER at baseline (≤300 and >300 mg/24 hr), and albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who did qualify by eGFR slope and were normoalbuminuric at baseline). To investigate possible influence of using selected covariates on the treatment effect estimate in the models considered in Section 11, we will perform appropriate sensitivity analyses. These additional analyses will be considered as strictly exploratory.

11.8. Safety analyses

Adverse events will be independently reviewed by an independent data safety monitoring board (DSMB, see Sections 15 and 16). All safety data will be available in data listing in the clinical protocol report. Data will be described in terms of descriptive statistics and presented by treatment group. Presentation will include graphs (scatterplots, boxplots, histograms), measures of central tendency (mean, median) and variability (confidence intervals) for continuous variables and frequency tables for categorical variables.

11.9. Interim analysis

No formal interim analyses of efficacy to stop for benefit or futility are planned, given the timing of the primary endpoint.

11.10. Sample size

Since a variance-covariance matrix for the iGFR measures is not available and this matrix is essential in order to perform formal power calculations for a model with correlated errors, we performed alternative power calculations based on an intent-to-treat analysis within an ANCOVA framework. Specifically, we assumed that the primary hypothesis is tested in the following model:

M1: iGFR at washout = iGFR at baseline + treatment group

Compared to the model that will be used in the primary analysis, model M1 is simplified in two aspects. First, it does not use information from iGFR values measured at intermediate time points. Second, it does not include covariates such as the stratifying variables (HbA1c and UA) or other GFR predictors such as baseline AER. Both of these aspects may lead to loss of precision of the treatment effect estimate. Consequently, our sample size calculations should be considered as conservative.

The hypothesis being tested, i.e. the effect of treatment on iGFR at washout, corresponds to testing whether the treatment group factor in Model M1 is significant. The choice of the ANCOVA model for the purpose of power calculations is sensible, as residuals from a univariate model involving baseline iGFR as covariate fitted to data from RASS study conform to normal distribution. Sample size calculations were performed based on Cohen[45] and making the following assumptions:

1. Postulated effect on iGFR at washout (·) = 3 ml/min/1.73 m^2. We deem this effect to be clinically meaningful and attainable. It is clinically meaningful because it would translate on average into a 10-year delay in the progression to ESRD. It is attainable because it is smaller than the difference in 3-year GFR that we observed in the JKS between subjects with serum UA ≥ 4.5 mg/dl compared to those with levels below this value. The postulated effect was based on the following changes in GFR levels in the two treatment groups:
   a. Untreated group = 3 ml/min/1.73 m^2 per year. This estimate is based on data from the Joslin Kidney Study (JKS), in which the median GFR loss among 43 subjects meeting the above criteria was 3.1 ml/min/1.73 m^2 per year, with 70% of subjects having a GFR loss >1.5 ml/min/1.73 m^2 per year. Also, among 116 subjects from Steno who met the albuminuria and GFR criteria, but for whom serum uric acid values were not available, the median GFR loss was 3.3 ml/min/1.73 m^2 per year, with 71% of subjects having a GFR loss >1.5 ml/min/1.73 m^2 per year.
   b. Treated group = 2 ml/min/1.73 m^2 per year. The average GFR loss in the JKS subjects with serum UA <4.5 mg/dl was 1.5 ml/min per year. On this basis, we conservatively assumed that the allopurinol treatment, if effective, would decrease the GFR loss to 2 ml/min per year (a 33% decrease compared to the untreated group).
2. Standard deviation (SD) of residual error = 10.1 ml/min/1.73 m^2. This was estimated based on the root-mean-squared error from a regression model with eGFR at 3 yrs as the dependent variable and baseline eGFR as the independent variable fitted to data concerning T1D patients from the Joslin Kidney Study meeting the PERL inclusion criteria.

Assuming a two-sided alpha error equal to 0.05, the effective sample size needed to detect the pre-specified treatment effect (▪▪= 3 ml/min/1.73 m^2) at washout adjusted for baseline iGFR with 80% power is equal to n=180 per group. To take into account the anticipated overall dropout rate (up to 5%/yr or 15% over the entire duration of the study) and drug discontinuation or non-compliance in the treatment group (up to 2%/yr or 6% over the entire duration of the study), and to maintain the desired power of at least 80%, it will be necessary to recruit n=240 subjects per group. In Table 1, we show the power of the proposed sample size for Model M1 under different dropout and non-compliance scenarios. We also provide the corresponding power for a model (Model M2) including the two stratifying variables (Hb1Ac and UA) and baseline AER as covariates to illustrate the effect of adding these variables to Model M1. In this analysis, we assumed that adding these covariates reduces the residual variance by 10%, which corresponds to these covariates explaining merely 4% of the total iGFR variation over and above the variability explained by iGFR at baseline. As shown in Table 1, once these covariates are accounted for, power is expected to exceed the conservative estimates provided by Model M1 and reach almost 90% for 15% dropout and 6% non-compliance rates.

Table 1. Power to detect treatment effect for two ANCOVA models under different drop-out and non-compliance scenarios.

Overall Dropout (%)	Non-compliance (%)	Model	
		M1	M2
9	0	.87	.92
12	0	.86	.91
15	0	.85	.90
9	6	.83	.89
12	6	.82	.88
15	6	.80	.87

A multicenter clinical trial of allopurinol to prevent GFR loss in type 1 diabetes

Statistical Analysis Plan
Prepared by PERL DCC

February 22, 2017

Contents

1. Overview 4

2. Schema 5

3. Rationale for Adjustments of Statistical Analysis Plan as Compared to Protocol (Version 9, approved by DSMB on August 16th, 2016) 6

3.1. Use of modified Intention-to-Treat (ITT) Analysis Population 6

3.2. Simplified model for the primary efficacy analysis 6

4. Study Aim 8

5. Study Endpoints and Other Outcomes 9

5.1. Primary Endpoint 9

5.2. Secondary Outcome Measures 9

5.2.1. Secondary endpoint: iGFR at the end of the 3-year treatment period (Visit 16, before the washout) 9

5.2.2. Secondary endpoint: iGFR time trajectory estimated from repeated iGFR measurements 9

5.2.3. Secondary endpoint: Estimated (eGFR) at 4 months after randomization (Visit 7) 9

5.2.4. Secondary endpoint: Estimated GFR (eGFR) time trajectory 9

5.2.5. Secondary endpoint: Time to doubling of serum creatinine or end-stage renal disease (ESRD) 9

5.2.6. Secondary endpoint: Urinary AER at the end of the two-month wash-out period (Visit 17) 10

5.2.7. Secondary endpoint: Urinary AER during the last three months of the treatment period (Visits 15 and 16) 10

5.2.8. Secondary endpoint: Time to fatal or non-fatal cardiovascular events 10

5.3. Safety measures 10

5.4. Other measures 10

6. Analytical Strategy 12

6.1. Study populations 12

6.2. Blinded Data Review 13

6.3. Visit Windows 13

6.4. Covariates 14

6.5. Analysis of the Primary Endpoint 14

6.5.1. Primary Analysis of the Primary Endpoint 14

6.5.2. Secondary Analysis of the Primary Endpoint 15

6.6. Analyses of Secondary Endpoints 16

6.6.1. iGFR at the end of the 3-year treatment period (Visit 16, before the washout) 16

6.6.2. iGFR time trajectory estimated from repeated iGFR measurements 16

6.6.3. eGFR at 4 months after randomization (Visit 7) 16

6.6.4. eGFR time trajectory 17

6.6.5. Time to serum creatinine doubling or ESRD 17

6.6.6. Urinary AER at the end of the wash-out period 18

6.6.7. Urinary AER during the last three months of the treatment period (Visits 15 and 16) 18

6.6.8. Time to fatal or non-fatal cardiovascular events 19

6.7. Subgroup Analyses 19
6.8. Analyses of Safety Outcomes 20
6.9. Model assumptions and alternative analyses 21
7. Table, Listing and Figure Shells 22
7.1. Analyses of Primary and Secondary Outcomes 31
7.2. Analyses of Safety Outcomes 35
REFERENCES 40
APPENDIX I. Study Objective, Study Design, Outcomes & Statistical Analysis and Data Management Sections from Protocol 41

1. Overview

DESIGN:

- Multicenter, double-blind, placebo-controlled, parallel-group randomized clinical trial.
- N=530 total number of subjects

STUDY POPULATION:

- Type 1 Diabetes
- Inclusion/Exclusion Criteria listed in the Study Protocol

STUDY TREATMENTS:

- Oral allopurinol or placebo administered for 3 years followed by a 2-month washout

PRIMARY OUTCOME MEASURE:

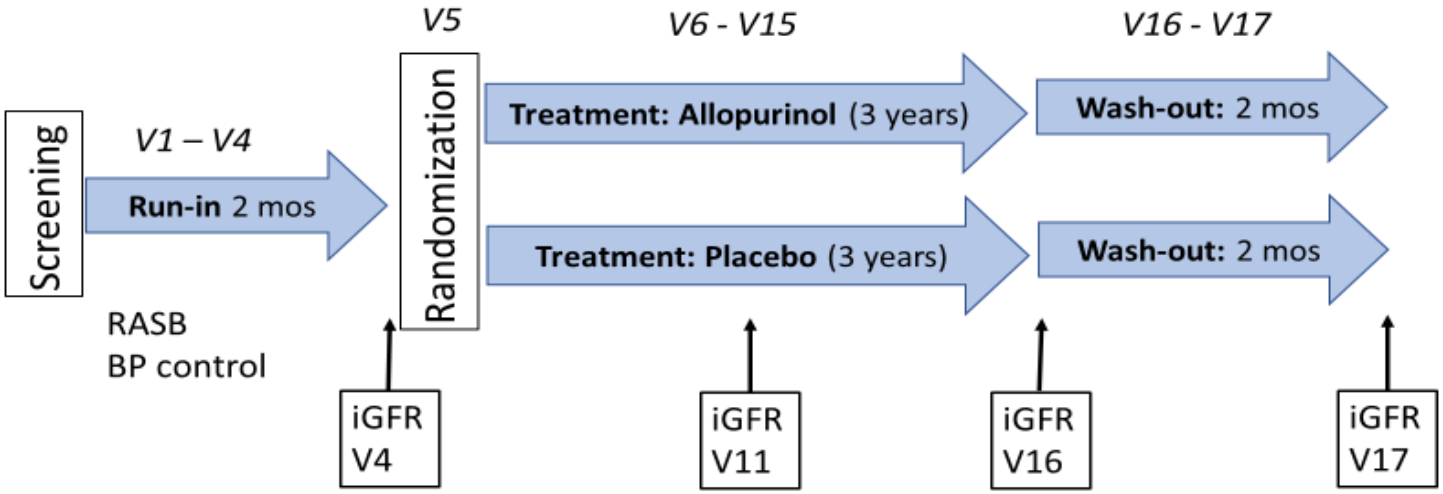
- iGFR at the end of the 2-month wash-out period following the 3-year intervention

STATISTICAL ANALYSIS PLAN:

- This plan will be finalized prior to the database lock and unblinding of treatment groups

2. Schema

Figure 2.1. PERL Study Schema



3. Rationale for Adjustments of Statistical Analysis Plan as Compared to Protocol (Version 9, approved by DSMB on August 16th, 2016)

Changes from the protocol-specified definitions of aims, outcomes, and statistical analytical approaches are outlined below. These changes reflect internal discussions since the design of the study that have not been incorporated yet as protocol amendments, but were discussed during the preparation of the Statistical Analysis Plan. These changes and the rationale for their implementation are documented herein and represent changes made prior to the database lock and unblinding of the study.

3.1. Use of modified Intention-to-Treat (ITT) Analysis Population

RATIONALE:
Given that some of the randomized subjects did not receive *any* study medication, data analysis will be based on the concept of *modified* ITT (mITT) population rather than ITT population.

PROTOCOL:
We define the ITT analysis population as "… all randomized study participants considered in their original randomization group, regardless of treatment discontinuation or loss to follow-up."

SAP:
We define the mITT analysis population as "… all randomized study participants considered in their original randomization group, regardless of treatment discontinuation or loss to follow-up who received *at least one dose* of study medication."

3.2. Simplified model for the primary efficacy analysis

RATIONALE:
The primary efficacy analysis presented in Section 11.2 of the study protocol was based on a linear model for correlated errors using all available iGFR measures (including those at baseline, 80, 156, and 164 weeks, respectively) as the dependent variable. The iGFR at baseline was included among the dependent variables to effectively adjust the treatment effect for baseline iGFR in the presence of a considerable number of missing values. Since iGFR values at baseline are missing for only two randomized subjects, which can be imputed in the analyses, we have decided to adjust for baseline iGFR in a standard way by including it as a covariate. Please note that both modeling approaches/specifications are *equivalent* if there are no missing iGFR values at baseline.

PROTOCOL:
We specify the model as follows "… perform the analysis by means of a linear model for correlated errors with general/unstructured covariance matrix using all available iGFR measures (including those at baseline, 80, 156, and 164 weeks, respectively) as the dependent variable. By conditioning on the baseline iGFR measure we will also effectively use this variable as a covariate. Treatment group, study center, stratifying variables, albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who did

qualified by eGFR slope and were normoalbuminuric at baseline), baseline AER, time, and time by treatment interaction will also be included as covariates in the model."

SAP:
We specify the model as follows "… perform the analysis by means of a linear model for correlated errors with general/unstructured covariance matrix using all available post-randomization iGFR measures (including 80, 156, and 164 weeks, respectively) as the dependent variable. At any given visit, iGFR in this model depends on treatment group, study center, stratifying variables, iGFR at baseline, albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who did qualified by eGFR slope and were normoalbuminuric at baseline), and baseline AER."

4. Study Aim

The study aim is to determine whether lowering serum UA by means of oral allopurinol is effective in preventing or slowing decline of renal function in T1D patients with history and/or presence of microalbuminuria or moderate macroalbuminuria, or with ongoing GFR loss regardless of history or presence of albuminuria, who have only mildly or moderately impaired kidney function.

5. Study Endpoints and Other Outcomes

This section describes the primary and secondary efficacy outcomes, as well as safety and other outcomes, that will be included in the primary manuscript. Derivation of the endpoints and other outcomes from the data collected in the Case Report Forms will be described in detail in the Derived Dataset Requirements document.

5.1. Primary Endpoint

The primary endpoint is iohexol-plasma disappearance GFR (iGFR) at the end of the 2-month wash-out period (Visit 17 at Week 164) following the 3-year intervention. The rationale of measuring the primary outcome at the end of the wash-out period is to test allopurinol for *permanent* effects on the natural history of kidney disease, independent from any transient, hemodynamic effect that the medication may have on GFR. iGFR is calculated from blood samples drawn at baseline and 120, 150, 180, 210, and 240 minutes after an i.v. bolus of iohexol, adjusting for body surface area. If there are fewer than five measures or the other quality criteria described in the protocol are not met, the iGFR value is not used in the analysis.

5.2. Secondary Outcome Measures

5.2.1. Secondary endpoint: iGFR at the end of the 3-year treatment period (Visit 16, before the washout)

iGFR calculated at the end of the 3-year intervention (at Visit 16, last visit before washout) as measured by the plasma disappearance of non-radioactive iohexol.

5.2.2. Secondary endpoint: iGFR time trajectory estimated from repeated iGFR measurements

Repeated measures of iGFR at Visits 11, 16, 17.

5.2.3. Secondary endpoint: Estimated (eGFR) at 4 months after randomization (Visit 7)

eGFR at 4 months after randomization as estimated from serum creatinine and cystatin C using the CKD-EPI SCr and the CKD-EPI SCr-SCysC equations (Fan et al, 2015). This endpoint is employed to measure a transient, hemodynamic effect that the study medication may have on GFR.

5.2.4. Secondary endpoint: Estimated GFR (eGFR) time trajectory

Repeated eGFR measures at all post-randomization visits (Visit 6 through 17) as estimated from repeated serum creatinine and cystatin C measurements using the CKD-EPI SCr and the CKD-EPI SCr-SCysC equations.

5.2.5. Secondary endpoint: Time to doubling of serum creatinine or end-stage renal disease (ESRD)

This secondary endpoint is defined as a composite of two events: (1) ESRD, defined as eGFR ≤15 ml/min/1.73 m^2, institution of chronic dialysis treatment or kidney transplantation, or (2) Doubling of serum creatinine levels as compared to baseline levels. Time to doubling of serum creatinine or ESRD is defined as the time from randomization to the first event (doubling of serum creatinine or ESRD) or censoring (lost-to-follow-up, withdrawal, and study completion without experiencing the event).

5.2.6. Secondary endpoint: Urinary AER at the end of the two-month wash-out period (Visit 17)

Geometric mean of two urinary AER measures obtained at Visit 17.

5.2.7. Secondary endpoint: Urinary AER during the last three months of the treatment period (Visits 15 and 16)

Geometric mean of urinary AER measures at Visit 15 and Visit 16.

5.2.8. Secondary endpoint: Time to fatal or non-fatal cardiovascular events

This secondary endpoint is defined as a composite of multiple events: (1) Cardiovascular disease (CVD) death (ICD-10 code I10 to I74.9), (2) Myocardial infarction, (3) Stroke (ischemic or hemorrhagic), (4) Coronary artery bypass grafting, or (5) Percutaneous coronary intervention. Time to fatal or non-fatal cardiovascular events is defined as the time from randomization to the first event (one of the events defined above) or censoring (lost-to-follow-up, withdrawal, non-CVD death, and study completion without experiencing the event).

5.3. Safety measures

Safety measures are assessed during three periods of the study: run-in (Visits 1-5), treatment (after Visit 5 through Visit 16), and off-treatment washout (after Visit 16 through Visit 17). Safety will be summarized overall (treatment and off-treatment combined) and by period, depending on the safety outcome of interest during that period.

- Percentage of subjects with SAEs, number of SAEs, time to first SAEs during on-treatment period.
- Percentage of subjects with and number of permanent discontinuations of study medication because of adverse effects.
- Percentage of subjects with and number of AEs, overall and by severity and by relatedness to study medication.
- Percentage of subjects with skin rash during on-treatment period.

5.4. Other measures

In addition to primary, secondary, and safety measures, the following additional outcomes will be analyzed to help with the interpretation of study results.

- Body weight, blood pressure, serum creatinine, HbA1c, and serum uric acid at each post-baseline visit and their changes from baseline
- Percentage of subjects receiving adequate study medication exposure (i.e., allopurinol or placebo) independent of adverse events. This is defined as the actual total dose during the 156-week dosing period, as determined from the dispensed dosage and pill counts, divided by the expected total dose defined by the eGFR-adjusted protocol-described dosing regimen, without consideration for temporary or permanent discontinuations or reductions owing to adverse events. The proportion of subjects receiving the adequate intended study medication exposure is defined as the number of subjects who had at least 80% and no more than 120% of the intended study medications during the entire dosing period, independent of adverse events, among all randomized subjects.

6. Analytical Strategy

In the initial analysis of the primary outcome we will present iGFR univariate statistics by Treatment Groups at each study visits (V4, V11, V16 and V17).

No formal interim analyses of the primary endpoint will be conducted, therefore the nominal α level to be used at the final analysis will be 0.05 for the primary endpoint. All other secondary outcomes will also be tested at the 5% level, with no adjustment for multiplicity. Many of the models used in efficacy analyses include baseline covariates, such as stratifying variables (serum uric acid (sUA), HbA1C, clinical site), iGFR, albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who qualified by eGFR slope and were normoalbuminuric at baseline), AER, and time, and time by treatment interaction. If there are problems with fitting these models, due, for example, to lack of convergence to optimal values, covariates will be eliminated from the models in the following order: baseline AER, albuminuria status, clinical site, serum uric acid (sUA), and HbA1c. More detailed information about these covariates is included in Section 6.4.

6.1. Study populations

Two study populations will be defined for the purpose of data analysis:

- ***Modified Intention to Treat (mITT):*** The mITT analysis set consists of all subjects enrolled in PERL, randomized to study medication, and receiving at least one dose of study medication.
- ***Per Protocol:*** The per protocol analysis set will consist of a subset of mITT subjects. The per protocol population will exclude subjects with major protocol deviations (defined as receiving the wrong study medication) as well as data points for which the cumulative exposure to the study medication from randomization to that time point was less than 80% of the theoretical full exposure (see Section 11.1 in the protocol).

To account for missing values in any specific analysis, all subjects meeting the study population definitions will be included in the analysis using (1) appropriate analytical approaches that allow for missing values under plausible missing data mechanisms, or (2) analytical methods (defined within specific analyses) that allows the imputation of missing outcomes.

Since the mITT approach can result in the need to analyze data with missing values of the outcomes (or covariates), we will follow four strategies proposed by I.R. White et al (2011):

1. Attempt to follow up all randomized participants, even if they withdraw from allocated treatment.
2. Perform a main analysis of all observed data that are valid under a plausible assumption about missing data.
3. Perform sensitivity analyses to explore the effect of departures from the assumptions made in the main analysis.
4. Account for all mITT study population participants, at least in the sensitivity analyses.

Note that this approach is tailored to mITT population and deviates slightly from the “all randomized participants” suggested by White.

6.2. Blinded Data Review

Prior to unmasking the study and starting any formal analysis, data will be reviewed in a blinded fashion by computing summary statistics for primary and secondary outcomes, and baseline covariates. This will allow the identification of unusual values and/or patterns of missing values for key variables that need to be queried. In addition, such blinded data review will allow the writing committee to assess the format of data presentation. Note that the blinded data review incorporates real data but *random* treatment assignment (i.e., investigators do not receive data summarized by actual treatment group, rather they review data on two randomly formed groups). All decisions will be made and documented in this SAP document prior to database lock and unblinding.

6.3. Visit Windows

To provide scheduling flexibility to study sites and participants, visits were required to occur within a protocol-defined window rather than on a specific date. The protocol-defined visit windows are summarized in the table below. For analytic purposes, the visit windows defined in the protocol will be expanded in order to eliminate gaps between them. This will ensure that all observations, including those that may have occurred outside a protocol-specified time window, will be associated with the most appropriate visit and therefore properly included in the analysis. If multiple observations occur within a window, the one closest to the visit target date will be utilized. If two observations are equi-distant from the target date, the first one will be utilized.

Table 6.3.1. PERL windows for post-randomization visits.

Visit	Lower Boundary of Window (Week, Excluding First day)	Per protocol Target Date window in weeks	Upper Boundary of Window (Week, Including last day)
Visit Windows Relative to Visit 5 Date			
Visit 6	0	4 [3-5]	10
Visit 7	10	16 [14-20]	24
Visit 8	24	32 [30-34]	40
Visit 9	40	48 [46-50]	56
Visit 10	56	64 [62-66]	72
Visit 11	72	80 [78-84]	88
Visit 12	88	96 [94-98]	104
Visit 13	104	112 [110 -114]	120
Visit 14	120	128 [126 -130]	135
Visit 15	135	142 [140 -146]	149

Visit	Lower Boundary of Window (Week, Excluding First day)	Per protocol Target Date window in weeks	Upper Boundary of Window (Week, Including last day)
Visit 16	149	156 [154 -160]	160
Visit Window relative to V16			
Visit 17	0	8 [6–12]	16

All intervals (target dates and lower/upper window boundaries) for visits 6 through 16 are calculated relative to the Visit 5 date. The interval for Visit 17 is calculated relative to Visit 16. Lower and upper boundaries are based on the mid-points between target dates. Most post-randomization visits are 16 weeks apart, with the exception of Visits 6 and 7 and Visits 16 to visit 17.

6.4. Covariates

The following is a description of the covariates that will be used in the various analyses outlined in the remainder of Section 6.

- Stratifying variables
  - serum uric acid (sUA) at baseline with 2 levels (≤6.0 and > 6.0 mg/dl)
  - glycated hemoglobin (HbA1c) at baseline with two levels (≤7.8 and >7.8%)
  - clinical site/study center with 16 levels (based on main sites with satellite sites collapsed into main sites)
- Baseline iGFR measured at Visit 4
- Baseline eGFR measured at Visit 4
- Treatment group with two levels (Allopurinol, Placebo)
- Albuminuria status with 2 levels (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who qualified by eGFR slope and were normoalbuminuric at baseline)
- Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale

At the time of writing, we have complete data available for serum uric acid, treatment group, and study center. The number of missing values for the other baseline covariates is 2 for iGFR, 6 for HbA1c, and 2 for albuminuria status. Given the small number of missing values for baseline covariates, we will employ single-value stochastic regression model imputation (Van Buuren, 2012).

6.5. Analysis of the Primary Endpoint

6.5.1. Primary Analysis of the Primary Endpoint

The goal of the primary analysis will be to test the null hypothesis of the difference between treatment arms in the primary endpoint (iGFR at the end of the 2-month wash-out period [Visit 17] following the 3-year intervention) being equal to zero. The analysis will be performed on the modified intention-to-treat (mITT) population and will employ a linear

model for correlated errors with general/unstructured covariance matrix (Molenberghs and Verbeke, 2005; Galecki and Burzykowski, 2013). For each time t ($t = 1, 2, 3$) corresponding to post-randomization iGFR visits, i.e. visits V11 (80 weeks), V16 (156 weeks), and V17 (164 weeks after randomization) the model equation is specified as:

$$iGFR_{it} = \beta_{0t} + \beta_{1t} TRT_i + \mathbf{x}_i'\boldsymbol{\beta} + \epsilon_{it}, \qquad (6.1)$$

where $iGFR_{it}$ is the value of iGFR at time t for subject i ($i = 1, \dots, 530$). Fixed effects β_{0t}, β_{1t} for $t = 1, 2, 3$ denote visit-specific intercepts and treatment effects. TRT_i is treatment group (equal to 1 for the allopurinol and 0 for placebo). Stratifying variables (serum uric acid, HbA1c, study center), and baseline covariates: albuminuria status, AER, iGFR for subject i are included in a vector $\mathbf{x}_i$ of p covariates $(x_1, \dots, x_p)$ and associated fixed effects are stored in vector $\boldsymbol{\beta} = (\beta_1, \dots, \beta_p)$. We assume that residual errors ϵ_{it} $(t = 1, 2, 3)$ for subject i are normally distributed with zero mean and 3x3 general/unstructured variance-covariance matrix. The model specified in (6.1) will yield the estimates of visit-specific treatment effects $\beta_{11}, \beta_{12}, \beta_{13}$ for all three visits V11, V16 and V17. In the context of the primary analysis of the primary endpoint, we are interested in parameter β_{13}, representing treatment effect at Visit 17 adjusted for stratifying variables and baseline covariates.

Analysis	Primary Analysis of the Primary Endpoint: iGFR at the end of the 2-month wash-out period (Visit 17)
Analysis Set	mITT Population
Methods	Linear model for repeated measures with correlated errors
Dependent Variable	iGFR measured at Visits V11 (80 weeks) , V16 (156 weeks) and V17 (164 weeks after randomization)
Model	Fixed effects: • Visit-specific intercepts corresponding to V11, V16, V17 • Visit-specific treatment effects corresponding to V11, V16, V17 • Stratifying variables: sUA, HbA1c, study center • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline iGFR
Results	• Predicted iGFR means *at Visit 17* for an exemplary subject by treatment group. • Estimate of treatment effect *at Visit 17* adjusted for baseline covariates • 95% confidence interval for treatment effect • P-value for treatment effect

6.5.2. Secondary Analysis of the Primary Endpoint

The primary analysis of the primary endpoint will be performed under the missing at random (MAR) assumption, i.e. the probability that the iGFR is missing depends on *observed* rather than *unobserved* values of the dependent variable. Although we consider the MAR assumption to be sensible for our study, the following sensitivity analyses will be performed to assess how alternative definitions of the primary endpoint (as defined above) and alternative approaches for handling missing data may affect the conclusions of the analysis:

1. Analysis of covariance using iGFR values at Visit 17 as the dependent variable and treatment effect as a covariate of primary interest. The same baseline covariates, as in the primary analysis of the primary endpoint, stored in vector $\boldsymbol{x}_i$ (see Equation. 6.1) will be used in the model.
2. Performing an analysis identical to the primary one (same endpoint and model) using the per-protocol analysis set rather than the mITT analysis set.

6.6. Analyses of Secondary Endpoints

6.6.1. iGFR at the end of the 3-year treatment period (Visit 16, before the washout)

The predicted means at Visit 16, estimate of treatment effect *at Visit 16* adjusted for baseline covariates, their 95% confidence interval and P-value will be obtained as part of the primary analysis of the primary endpoint (Equation (6.1) in section 6.1.1). In the context of this secondary endpoint, we are interested in fixed effect β_{12} , which represents treatment effect at Visit 16 adjusted for stratifying variables and baseline covariates.

6.6.2. iGFR time trajectory estimated from repeated iGFR measurements

Analysis	Analysis of the Secondary Endpoint: iGFR time trajectory estimated from repeated iGFR measurements
Analysis Set	mITT Population
Methods	Linear mixed-effects model for longitudinal iGFR measures
Dependent Variable	iGFR measured at Visits V11 (80 weeks) , V16 (156 weeks) and V17 (164 weeks after randomization)
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Time since randomization in days • Time by treatment group interaction • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline iGFR Subject-specific random effects • Random intercept for iGFR • Random slope for iGFR
Results	• iGFR slope estimates and 95%CIs by treatment group • Estimate of a treatment effect measured as a difference between average slopes of iGFR versus time for allopurinol and placebo groups adjusted for stratifying variables and baseline covariates • 95% confidence interval for treatment effect • P-value for treatment effect

6.6.3. eGFR at 4 months after randomization (Visit 7)

Analysis	Analysis of the Secondary Endpoint: eGFR at 4 months after randomization (Visit 7)

Analysis Set	mITT Population
Methods	Linear model
Dependent Variable	eGFR measured at Visit V7 (16 weeks after randomization)
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline eGFR
Results	• Predicted eGFR means *at Visit 7* for an exemplary subject by treatment group. • Estimate of treatment effect *at Visit 7* adjusted for stratifying variables and baseline covariates • 95% confidence interval for treatment effect • P-value for treatment effect

6.6.4. eGFR time trajectory

Analysis	Analysis of the Secondary Endpoint: eGFR time trajectory estimated from repeated eGFR measurements
Analysis Set	mITT Population
Methods	Linear mixed-effects model for longitudinal eGFR measures
Dependent Variable	Post-randomization eGFR measured from Visits V6 through V17
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Time since randomization in days • Time by treatment group interaction • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline eGFR Subject-specific random effects • Random intercept for eGFR • Random slope for eGFR
Results	• eGFR slope estimates and 95%CIs by treatment group • Estimate of a treatment effect measured as a difference between average eGFR versus time slopes for allopurinol and placebo groups adjusted for stratifying variables and baseline covariates • 95% confidence interval for treatment effect • P-value for treatment effect

6.6.5. Time to serum creatinine doubling or ESRD

Analysis	Analysis of the Secondary Endpoint: Time to composite endpoint of serum creatinine doubling or ESRD
Analysis Set	mITT Population
Methods	Cox proportional hazards model

Dependent Variable	Time to composite endpoint of serum creatinine doubling or ESRD
Cox Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale • Baseline iGFR
Results	• N(%) of subjects with doubled serum creatinine or ESRD during the course of the study • Hazard ratio of allopurinol to placebo • 95% confidence interval for hazard ratio • P-value for treatment effect

6.6.6. Urinary AER at the end of the wash-out period

Analysis	Analysis of the Secondary Endpoint: AER at the end of the wash-out period Visit 17
Analysis Set	mITT Population
Methods	Linear model
Dependent Variable	Two AER measures obtained at Visit 17 and summarized using the geometric mean expressed on logarithm base to 10 scale
Model	Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log base to 10 scale • Baseline iGFR
Results	• Predicted urinary AERs *at Visit 17* for an exemplary subject by treatment group • Estimate of treatment effect *at Visit 17* expressed on percent change scale using antilog transformation. • 95% confidence interval for treatment effect expressed on percent change scale using antilog transformation. • P-value for treatment effect

6.6.7. Urinary AER during the last three months of the treatment period (Visits 15 and 16)

Analysis	Analysis of the Secondary Endpoint: AER at the end of the treatment period (Visits V15 and V16)
Analysis Set	mITT Population
Methods	Linear model
Dependent Variable	Two AER measures obtained at Visit 15 and 16 are summarized using the geometric mean expressed on logarithmic scale
Model	Fixed effects associated with:

	• Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log base to 10 scale • Baseline iGFR
Results	• Predicted AERs at the end of treatment period for an exemplary subject by treatment group. • Estimate of treatment effect at the end of treatment period adjusted for baseline covariates expressed on percent change scale using antilog transformation. • 95% confidence interval for treatment effect expressed on percent change using antilog transformation. • P-value for treatment effect

6.6.8. Time to fatal or non-fatal cardiovascular events

Analysis	Analysis of the Secondary Endpoint: Time to fatal or non-fatal cardiovascular events
Analysis Set	mITT Population
Methods	Cox proportional hazards model
Dependent Variable	Time to composite endpoint: fatal or non-fatal cardiovascular events
Cox Model	Fixed effects: • Fixed effects associated with: • Stratifying variables: sUA, HbA1c, study center • Treatment group • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log base to 10 scale • Baseline iGFR
Results	• N(%) of subjects with fatal or non-fatal cardiovascular events during the course of the study • Hazard ratio of allopurinol to placebo • 95% confidence interval for hazard ratio • P-value for treatment effect

6.7. Subgroup Analyses

To investigate the possible presence of heterogeneity in the response to allopurinol, subgroup analyses (based on the primary efficacy analysis described in subsection 6.6.1, with the inclusion of appropriate interaction terms with the subgroup variable) will be performed by age groups (≤40 and >40 yrs), gender, racial/ethnic group, HbA1c (≤7.8 and >7.8%), serum uric acid (≤6.0 and > 6.0 mg/dl), baseline iGFR (≤70 ml/min and >70). ml/min/1.73m^2), AER at baseline (≤300 and >300 mg/24 hr), and albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who did qualify by eGFR slope and were normoalbuminuric at baseline).

An example of such subgroup analysis for age groups (≤40 and >40 yrs) is provided below. Similar to Equation (6.1) for each time t ($t = 1, 2, 3$), corresponding to visits V11, V16, V17, we specify the model:

$$iGFR_{it} = \beta_{0t} + \beta_{1t} TRT_i + \beta_{2t} AGE_i + \beta_{3t} AGE_i \times TRT_i + \boldsymbol{x}_i'\boldsymbol{\beta} + \epsilon_{it}, \qquad (6.2)$$

where $iGFR_{it}$ is the value of iGFR at time t for subject i *(*$i = 1, \ldots, 530$*)*. Fixed effects $\beta_{0t}, \beta_{1t}, \beta_{2t}, \beta_{3t}$ for $t = 1, 2, 3$ denote visit-specific intercepts, treatment effects, age effects and age by treatment interactions, respectively. TRT_i is treatment group (equal to 1 for the allopurinol and 0 for placebo). AGE_i indicates age group (≤40 and >40 yrs). Stratifying variables, and baseline covariates albuminuria status, AER, iGFR for subject i are included in a vector of covariates $\boldsymbol{x}_i$ and associated fixed effects are stored in vector $\boldsymbol{\beta}$. We assume that residual errors ϵ_{it} ($t = 1, 2, 3$) for subject i are normally distributed with zero mean and 3x3 general/unstructured variance-covariance matrix. The model specified in (6.2) will yield the estimates of visit-specific treatment by age interaction effects β_{31}, β_{32}, β_{33} for all three visits V11, V16 and V17. In the context of subgroup analysis, we are interested in β_{33}, which represents treatment by age interaction at Visit 17 adjusted for baseline covariates.

Analysis	Subgroup Analysis of the Primary Endpoint: iGFR at the end of the 2-month wash-out period (Visit 17) by Age group
Analysis Set	mITT Population
Methods	Linear model for repeated measures with correlated errors
Dependent Variable	iGFR measured at Visits V11 (80 weeks) , V16 (156 weeks) and V17 (164 weeks after randomization)
Model	Fixed effects: • Visit-specific intercepts, age effects, treatment effects and age by treatment interaction effects • Stratifying variables: sUA, Hba1c, Study center • Albuminuria status with 2 levels • Baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log base to 10 scale • Baseline iGFR
Results	• Estimate of age by treatment interaction *at Visit 17* adjusted for baseline covariates • 95% confidence interval for age by treatment effect interaction *at Visit 17* • P-value for treatment effect

6.8. Analyses of Safety Outcomes

For dichotomous safety outcomes, the proportion of subjects experiencing adverse outcomes (AEs, SAEs) will be summarized by treatment group and compared by means of Fisher's exact tests. Poisson regression models will be used for safety outcomes (e.g., SAEs and AEs) with multiple recurrences per patient, with logarithm of the period of observation from the time of study medication used as the offset. Time to first SAE will be analyzed using Kaplan-Meier methods to estimate the SAE-free distributions for each treatment group. This analysis will employ the mITT analysis set.

6.9. Model assumptions and alternative analyses

Model assumptions will be thoroughly checked for individual and systematic departures, using informal, e.g. inspection of residuals, and formal methods such as methods based on likelihood displacement. If individual outliers are detected, their influence will be evaluated using influence diagnostics methods based on comparing estimates from models fitted to data with and without outlying values. Whenever we are not successful in fitting the parametric model (linear or non-linear), then non-parametric analyses and/or transformation of the variables involved in the analysis will be considered.

7. Table, Listing and Figure Shells

Figure 7.1. Time from Randomization to End of Study by Treatment Group

Kaplan-Meier plot of time from randomization to End of Study (death, withdrawal or lost-to-follow-up) in months
Y axis label = % of Subjects (100%, 90%, … , 10%, 0%)
X axis label = Time (months) post-randomization (0, 6, 12, 18, 24, 36 months)

Figure 7.2. Consort diagram describing the trial.

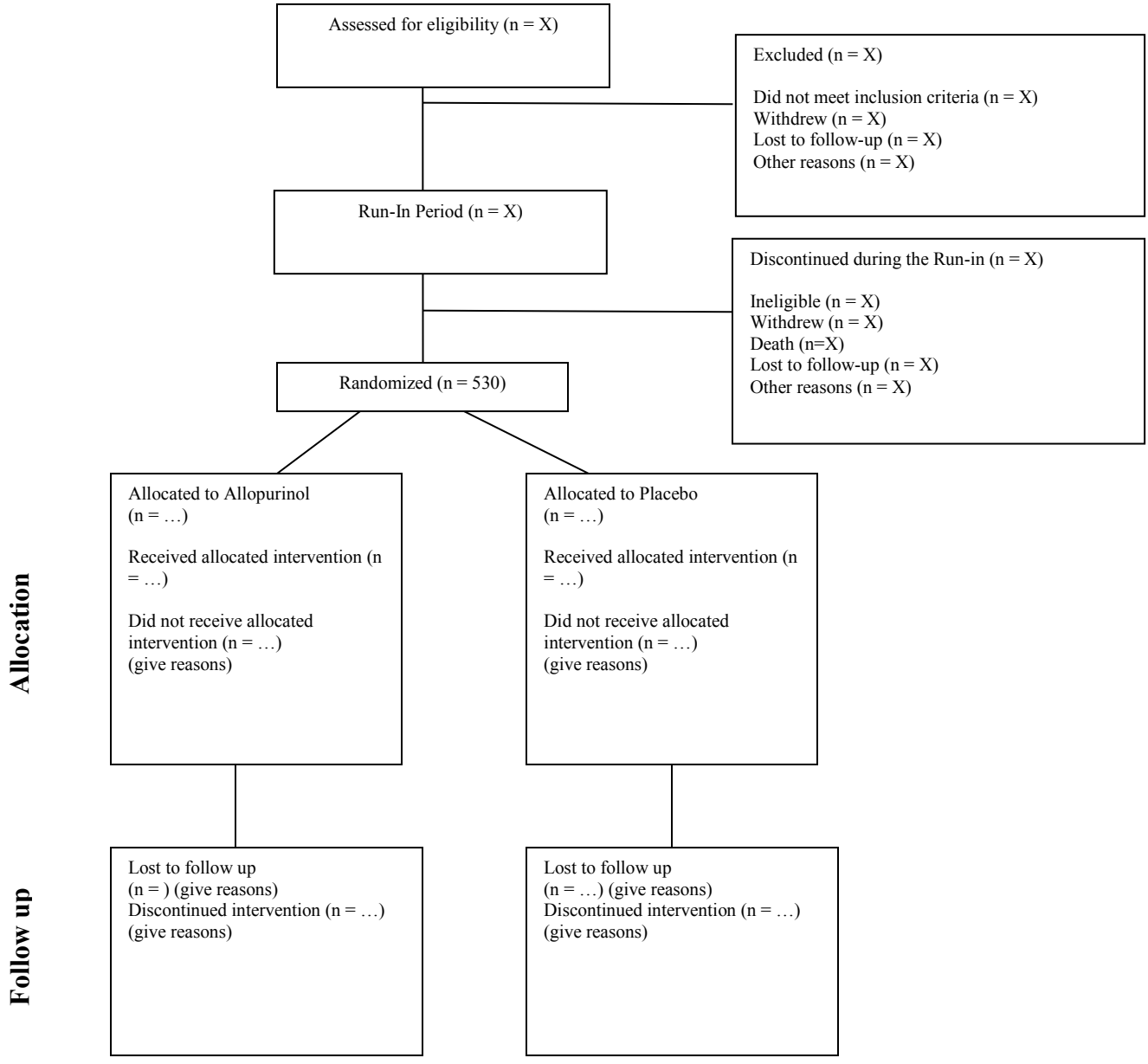


Table 7.1. Patients disposition by Treatment Group. All Subjects

	N (%)	
SCREENING PERIOD (at Visit 1)	N	
Discontinuations after Visit 1 and before Visit 2	xx.x%	
Screen Failure (Ineligible for Run-in)	xx.x%	
Withdrawal	xx.x%	
Lost to Follow-up	xx.x%	
Other	xx.x%	
Eligible for Run-in	xx.x%	
RUN-IN PERIOD (at Visit 2 through Visit 4)	N	
Discontinued during Run-in (at Visit 2 through Visit 4)	N	
Ineligible	xx.x%	
Death	xx.x%	
Lost to Follow-up	xx.x%	
Withdrawal	xx.x%	
Other	xx.x%	
RANDOMIZED (Visit 5)	xx.x%	
POST-RANDOMIZATION PERIOD (Visits 6-17)	**Treatment Group**	
	Allopurinol N=	**Placebo N=**
Randomized	n	n
Post-Randomization Discontinuations	n	n
Death	n	n
Lost to Follow-up	n	n
Withdrew Consent	n	n
Other	n	n
Post-Randomization Discontinuations /# Randomized (%)	xx.x%	xx.x%
Completed Study	n	n
Completed Study (%)	xx.x%	xx.x%

Table 7.2. Patient Follow-Up by Treatment Group. All Subjects.

Time Point, n (%)	Treatment Group		Total
	Allopurinol N=	Placebo N=	
Screened (Visit 1)			N
Randomization			N/N_S (xx%)
Visit 6	N_Visit 6/N* (xx%)	N_Visit 6/N* (xx%)	N_Visit 6/N* (xx%)
Visit 7	N_ Visit 7/N* (xx%)	N_ Visit 7/N* (xx%)	N_ Visit 7/N* (xx%)
Visit 8	N_ Visit 8/N* (xx%)	N_ Visit 8/N* (xx%)	N_ Visit 8/N* (xx%)
Visit 9	N_ Visit 9/N* (xx%)	N_ Visit 9/N* (xx%)	N_ Visit 9/N* (xx%)
Visit 10	N_ Visit 10/N* (xx%)	N_ Visit 10/N* (xx%)	N_ Visit 10/N* (xx%)
Visit 11	N_ Visit 11/N* (xx%)	N_ Visit 11/N* (xx%)	N_ Visit 11/N* (xx%)
Visit 12	N_Visit 12/N (xx%)	N_Visit 12/N (xx%)	N_Visit 12/N (xx%)
Visit 13	N_ Visit 13/N* (xx%)	N_ Visit 13/N* (xx%)	N_ Visit 13/N* (xx%)
Visit 14	N_ Visit 14/N* (xx%)	N_ Visit 14/N* (xx%)	N_ Visit 14/N* (xx%)
Visit 15	N_ Visit 15/N* (xx%)	N_ Visit 15/N* (xx%)	N_ Visit 15/N* (xx%)
Visit 16	N_ Visit 16/N* (xx%)	N_ Visit 16/N* (xx%)	N_ Visit 16/N* (xx%)
Visit 17	N_ Visit 17/N* (xx%)	N_ Visit 17/N* (xx%)	N_ Visit 17/N* (xx%)
Completed Across All Visits	Σ N_i	Σ N_i	Σ N_i
% Completed of Total	Σ N_i /n =xx%	Σ N_i /n =xx%	Σ N_i /n =xx%
Analysis Sets for Primary Endpoint:			
mITT Analysis Set[1]	N	N	n
Per Protocol Analysis Set[2]	N	N	n

[1] modified intention to treat (mITT) analysis set will consist of all subjects enrolled in PERL, randomized to study medication who received *at least one dose* of study medication.

[2] per-protocol analysis set will consist of a subset of mITT subjects. The per-protocol analysis set will exclude data points which 1. had cumulative exposure to the study medication from randomization less than 80% of the theoretical full exposure; or 2. with major protocol deviations (e.g., treatment with prohibited medications).

Note: N* = number assessable at this point, i.e., denominator reflects loss from WD, LFU & deaths;

Table 7.3. Demographics and Baseline Characteristics by Treatment Group. ITT Analysis Set.

Variable Statistic or Category	Treatment Group	
	Allopurinol N=	**Placebo N=**
Age (years)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
BMI (kg/m^2) (Visit 4)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
Diabetes duration (years)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
SBP (mm Hg) (Visit 4)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
DBP (mm Hg) (Visit 4)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
BP (Visit 4), n (%)		
SBP > 140 or DBP > 90 mm Hg	xx (xx%)	xx (xx%)

SBP ≤140 and DBP <=90 mm Hg	xx (xx%)	xx (xx%)
Missing	xx (xx%)	xx (xx%)
On RASB at Visit 2	xx (xx%)	xx (xx%)

Table 7.3. Demographics and Baseline Characteristics by Treatment Group. ITT Analysis Set. (continued)

Variable Statistic or Category	Treatment Group	
	Allopurinol N=	**Placebo N=**
Gender, n (%)		
Male	xx (xx%)	xx (xx%)
Female	xx (xx%)	xx (xx%)
Ethnicity, n (%)		
Hispanic or Latino	xx (xx%)	xx (xx%)
Not Hispanic or Latino	xx (xx%)	xx (xx%)
Unknown	xx (xx%)	xx (xx%)
Race, n (%)		
American Indian or Alaska Native	xx (xx%)	xx (xx%)
Asian	xx (xx%)	xx (xx%)
Black or African American	xx (xx%)	xx (xx%)
Native Hawaiian or Other Pacific Islander	xx (xx%)	xx (xx%)
White	xx (xx%)	xx (xx%)
Multi-Race	xx (xx%)	xx (xx%)
Other, Unknown, or not reported	xx (xx%)	xx (xx%)

Table 7.4. Baseline Laboratory Values by Treatment Group. ITT Analysis Set.

Variable Statistic or Category	Treatment Group	
	Allopurinol N=	Placebo N=
HbA1c (Visit 1) (%)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
HbA1C (Visit 1), n (%)		
≤7.8%	xx (xx%)	xx (xx%)
>7.8%	xx (xx%)	xx (xx%)
Missing	xx (xx%)	xx (xx%)
Serum Uric Acid (mg/dL) (Visit 4)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
Serum Uric Acid (Visit 4), n (%)		
≤6 mg/dL	xx (xx%)	xx (xx%)
> 6 mg/dL	xx (xx%)	xx (xx%)
Missing	xx (xx%)	xx (xx%)
eGFR (ml/min/1.73 m^2) (Visit 4)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
iGFR (ml/min/1.73 m^2) (Visit 4)		
Mean (SD)	xx (xx%)	xx (xx%)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx (xx%)	xx (xx%)
N missing	xx (xx%)	xx (xx%)
AER (ug/min) (Geometric mean for Visits 3 and 4)		
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x

AER, n (%) (Geometric mean for Visits 3 and 4)		
<20 ug/min	xx (xx%)	xx (xx%)
20-199 ug/min	xx (xx%)	xx (xx%)
≥200 ug/min	xx (xx%)	xx (xx%)
Missing	xx (xx%)	xx (xx%)

Table 7.4. Baseline Laboratory Values by Treatment Group. ITT Analysis Set. (continued)

Variable Statistic or Category	Treatment Group	
	Allopurinol N=	Placebo N=
Potassium (mmol/L) (Visit 4)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
Hemoglobin (gm/dl) (Visit 4)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
Platelets (mmol) (Visit 4)		
Mean (SD)	xx.x (xx.xx)	xx.x (xx.xx)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx, xx	xx, xx
N missing	x	x
White Blood Cell (cells/mcL) (Visit 4)		
Mean (SD)	xx (xx%)	xx (xx%)
Median (Q1, Q3)	xx.x (xx.x, xx.x)	xx.x (xx.x, xx.x)
Min, Max	xx (xx%)	xx (xx%)
N missing	xx (xx%)	xx (xx%)

7.1. Analyses of Primary and Secondary Outcomes

P1. Primary Analysis of the Primary Endpoint: iGFR at the end of the 2-month wash-out period (Visit 17). mITT Analysis Set[1].

Variable	Treatment Group	
	Allopurinol N=	**Placebo N=**
iGFR Predicted Means at the end of the 2-month wash-out period (Visit 17) (95% CI)[2]	**x.xx (x.xx, x.xx)**	**x.xx (x.xx, x.xx)**
Treatment Effect at Visit 17 (95% CI)	**x.xx (x.xx, x.xx)**	
p-value	**0.xxxx**	

[1] Results are obtained from a linear model with correlated errors. The dependent variable is iGFR measured at visits V11, V16 and V17. Treatment effect at V17 was adjusted for stratifying variables (serum uric acid, HbA1c, study center), albuminuria status, baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale, baseline iGFR.

[2]Predicted means are calculated for an exemplary subject assuming that he/she is albuminuric from the Joslin site, with uric acid posited at 6 mg/dL, HbA1c at 8%, baseline AER geometric mean at 80 μg/min and baseline iGFR at 70 ml/min/1.73 m^2. Covariate values for this exemplary subject were set close to their median values for continuous variables and most frequent categories for categorical variables

S_1. iGFR at the end of the 3-year treatment period (Visit 16, before the washout). mITT Analysis Set.[1]

Variable	Treatment Group	
	Allopurinol N=	**Placebo N=**
iGFR Predicted Means at Visit 16, before the wash-out period (95% CI)[2]	**x.xx (x.xx, x.xx)**	**x.xx (x.xx, x.xx)**
Treatment Effect at Visit 16 (95% CI)	**x.xx (x.xx, x.xx)**	
p-value	**0.xxxx**	

[1] Results are obtained from a linear model with correlated errors employed for the Primary Analysis of the Primary Endpoint.

[2]Predicted means are calculated for an exemplary subject assuming that he/she is albuminuric from the Joslin site, with serum uric acid posited at 6 mg/dL, HbA1c at 8%, baseline AER geometric mean at 80 mg/min and baseline iGFR at 70 ml/min/1.73 m^2. Covariate values for exemplary subject were set close to their median values for continuous and most frequent categories for categorical variables.

S_2. iGFR time trajectory estimated from repeated iGFR measurements. mITT Analysis Set.[1]

Variable	Treatment Group	
	Allopurinol N=	**Placebo N=**
iGFR slope (95% CI)	**x.xx (x.xx, x.xx)**	**x.xx (x.xx, x.xx)**
Treatment Effect (difference between Allopurinol versus Placebo iGFR slopes) (95% CI)	**x.xx (x.xx, x.xx)**	
p-value	**0.xxxx**	

[1] iGFR slope estimates and 95%CIs are obtained from a linear mixed-effects model for repeated iGFR measures. Fixed effects included stratifying variables: serum uric acid, HbA1c, study center, time since randomization in days, time by treatment group interaction, albuminuria status, baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale, baseline iGFR. Random effects included subject-specific intercepts and slopes for iGFR

S_3. eGFR at 4 months after randomization (Visit 7). mITT Analysis Set[1]**.**

Variable	Treatment Group	
	Allopurinol N=	**Placebo N=**
eGFR Predicted Means at Visit 7 (95% CI)[2]	**x.xx (x.xx, x.xx)**	**x.xx (x.xx, x.xx)**
Treatment Effect at Visit 7 (95% CI)	**x.xx (x.xx, x.xx)**	
p-value	**0.xxxx**	

[1]Results are obtained using a linear model with independent residual errors. Fixed effects, included stratifying variables (serum uric acid, HbA1c, study center), treatment group, albuminuria status, baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale, baseline eGFR.

[2]Predicted means are calculated for an exemplary subject assuming that he/she is albuminuric from the Joslin site, with serum uric acid posited at 6 mg/dL, HBA1c at 8%, baseline AER geometric mean at 80 mg/min and baseline eGFR at 70 ml/min/1.73 m^2. Covariate values for this exemplary subject were set close to their median values for continuous variables and most frequent categories for categorical variables

S_4. eGFR time trajectory. mITT Analysis Set.[1]

Variable	**Treatment Group**	
	Allopurinol N=	**Placebo N=**
eGFR slope (95% CI)	**x.xx (x.xx, x.xx)**	**x.xx (x.xx, x.xx)**
Treatment Effect (difference between Allopurinol versus Placebo eGFR slopes) (95% CI)	**x.xx (x.xx, x.xx)**	
p-value	**0.xxxx**	

[1] eGFR slope estimates and 95% CIs are obtained from a linear mixed-effects model for repeated eGFR measures. Fixed effects included stratifying variables: serum uric acid, HbA1c, study center, time since randomization in days, time by treatment group interaction, albuminuria status, baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale, baseline eGFR. Random effects included subject-specific intercepts and slopes for eGFR.

S_5. Time to serum creatinine doubling or ESRD. mITT Analysis Set.

Variable	**Treatment Group**	
	Allopurinol N=	**Placebo N=**
N (%) of subjects with doubled serum creatinine or ESRD during the course of the study	**Xx (xx.x%)**	**Xx(xx.x%)**
Adjusted Hazard Ratio (95% CI)[1]	**x.xx (x.xx, x.xx)**	
p-value[1]	**0.xxxx**	

[1]based on Cox Proportional Hazards Model. Fixed effects, included stratifying variables (serum uric acid, HbA1c, study center), treatment group, albuminuria status, baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale, and baseline iGFR.

S_6. Urinary AER at the end of the wash-out period. mITT Analysis Set.[1]

Variable	**Treatment Group**	
	Allopurinol N=	**Placebo N=**
Predicted Urinary AER at the end of the wash-out period (95% CI)[2]	**x.xx (x.xx, x.xx)**	**x.xx (x.xx, x.xx)**
Treatment Effect at Visit 17 expressed as % difference (95% CI)	**xx.x% (xx.x%, xx.x%)**	
p-value	**0.xxxx**	

[1]Results are obtained using a linear model with independent residual errors. The dependent variable is geometric mean of two AER measures obtained at Visit 17 expressed on log base to 10 scale. Fixed

effects, included stratifying variables (serum uric acid, HbA1c, study center), treatment group, albuminuria status, baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale, and baseline iGFR.

[2]Predicted urinary AER values are calculated using antilog transformation for an exemplary subject assuming that he/she is albuminuric from the Joslin site, with serum uric acid posited at 6 mg/dL, HbA1c at 8%, baseline AER geometric mean at 80 mg/min and baseline iGFR at 70 ml/min/1.73 m^2. Covariate values for exemplary subject were set close to their median values for continuous variables and most frequent categories for categorical variables

S_7. Urinary AER during the last three months of the treatment period (Visits 15 and 16). mITT Analysis Set.[1]

Variable	Treatment Group	
	Allopurinol N=	Placebo N=
Predicted Urinary AER during the last three months of the treatment period (95% CI)[2]	x.xx (x.xx, x.xx)	x.xx (x.xx, x.xx)
Treatment Effect expressed as % difference (95% CI) between treatment groups	xx.x% (xx.x%, xx.x%)	
p-value	0.xxxx	

[1]Results are obtained using a linear model with independent residual errors. The dependent variable is geometric mean of two AER measures obtained at Visits 15 and 16 expressed on log base to 10 scale. Fixed effects, included stratifying variables (serum uric acid, HbA1c, study center), treatment group, albuminuria status, baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale, and baseline iGFR.

[2]Predicted urinary AER values are calculated using antilog transformation for an exemplary subject assuming that he/she is albuminuric from the Joslin site, with uric acid posited at 6 mg/dL, glycated hemoglobin at 8%, baseline AER geometric mean at 80 mg/min and baseline iGFR at 70 ml/min/1.73 m^2. Covariate values for this exemplary subject were set close to their median values for continuous variables and most frequent categories for categorical variables

S_8. Time to fatal or non-fatal cardiovascular events. mITT Analysis Set.

Variable	Treatment Group	
	Allopurinol N=	Placebo N=
N (%) of Subjects with fatal or non-fatal CVD during the course of the study	xx (xx.x%)	xx (xx.x%)
Adjusted Hazard Ratio (95% CI)[1]	x.xx (x.xx, x.xx)	
p-value[1]	0.xxxx	

[1]based on Cox Proportional Hazards Model. Fixed effects, included stratifying variables (serum uric acid, HbA1c, study center), treatment group, albuminuria status, baseline AER geometric mean (at Visits 3 and 4 combined) expressed on log to base 10 scale, and baseline iGFR.

7.2. Analyses of Safety Outcomes.

Table S1: Subjects Discontinuing Study Medication because of Severe Adverse Events by Treatment Group. mITT Subjects.

	Treatment Group		
	Allopurinol N=	Placebo N=	**Total**
# of subjects d/c treatment	X	X	X
# of Subjects	X	X	X
% of subjects d/c treatment	x.x%	x.x%	x.x%
p-value*	x.xxxx		

*p-value from Fisher's exact test comparing percentage of subjects with discontinuing treatment by treatment group

Table S2: SAEs by Treatment Group, Regardless of Relatedness to Intervention. mITT Subjects.

	Treatment Group		
	Allopurinol N=	Placebo N=	**Total**
# of SAE's	X	X	X
# of subjects with SAE's	X	X	X
# of Subjects	X	X	X
SAE's per subject	x.xx	x.xx	x.xx
% of subjects with SAE's	x.x%	x.x%	x.x%
p-value*	x.xxxx		

*p-value from Fisher's exact test comparing percentage of subjects with SAEs by treatment group

Table S3: Summary of Number of SAEs per Subject in the Pre- and Post-Randomization Periods for Non-Randomized and Randomized Subjects. mITT Subjects.

Number of SAEs per Subject	Allopurinol N=	Placebo N=	Total N=
1	N (xx.x%)	N (xx.x%)	N
2	N (xx.x%)	N (xx.x%)	N
3	N (xx.x%)	N (xx.x%)	N
≥4	N (xx.x%)	N (xx.x%)	N
Relative Risk (95% CI)[1]	x.xx (x.xx, x.xx)		

Number of SAEs per Subject	Allopurinol N=	Placebo N=	Total N=
p-value[1]	0.xxxx		

[1]Based on Poisson regression model with treatment as a covariate and follow-up time as an offset

Table S4: SAEs by Treatment Group, Regardless of Relatedness to Intervention. mITT Subjects.

	Treatment Group		
BODY SYSTEM	Allopurinol N=	Placebo N=	**TOTAL**
Congenital	N	N	N
Gastrointestinal	N	N	N
Hepatic	N	N	N
Immunological	N	N	N
Infectious	N	N	N
Metabolic	N	N	N
Miscellaneous	N	N	N
Neoplastic	N	N	N
Neurological	N	N	N
Nutritional	N	N	N
Orthopedic	N	N	N
Pulmonary	N	N	N
Surgical	N	N	N
Total SAEs	N	N	N
Total Subjects with SAEs	N	N	N
Total Subjects Randomized	N	N	N
% with SAEs	xx.x%	xx.x%	xx.x%
p-value*	0.xxxx		

*p-value from Fisher's exact test comparing percentage of subjects with SAEs by treatment group

Table S5: Subjects Discontinuing Study Medication because of Adverse Events by Treatment Group. mITT Subjects.

	Treatment Group		
	Allopurinol N=	Placebo N=	**Total**
# of subjects d/c treatment	X	X	X
# of Subjects	X	X	X
% of subjects d/c treatment	x.x%	x.x%	x.x%
p-value*	x.xxxx		

*p-value from Fisher's exact test comparing percentage of subjects with discontinuing treatment by treatment group

Table S6: AEs by Treatment Group, Regardless of Relatedness to Intervention. mITT Subjects.

	Treatment Group		
	Allopurinol N=	Placebo N=	**Total**
# of AE's	X	X	X
# of subjects with AE's	X	X	X
# of Subjects	X	X	X
AE's per subject	x.xx	x.xx	x.xx
% of subjects with AE's	x.x%	x.x%	x.x%
p-value*	x.xxxx		

*p-value from Fisher's exact test comparing percentage of subjects with AEs by treatment group

Table S7: Summary of Number of AEs per Subject in the Pre- and Post-Randomization Periods for Non-Randomized and Randomized Subjects. mITT Subjects.

Number of AEs per Subject	Allopurinol N=	Placebo N=	Total N=
1	N (xx.x%)	N (xx.x%)	N
2	N (xx.x%)	N (xx.x%)	N
3	N (xx.x%)	N (xx.x%)	N
≥4	N (xx.x%)	N (xx.x%)	N
Relative Risk (95% CI)[1]	x.xx (x.xx, x.xx)		
p-value[1]	0.xxxx		

[1]Based on Poisson regression model with treatment as a covariate and follow-up time as an offset

Table S8: AEs by Treatment Group, Regardless of Relatedness to Intervention. mITT Subjects.

	Treatment Group		
BODY SYSTEM	Allopurinol N=	Placebo N=	**TOTAL**
Congenital	N	N	N
Gastrointestinal	N	N	N
Hepatic	N	N	N
Immunological	N	N	N
Infectious	N	N	N
Metabolic	N	N	N
Miscellaneous	N	N	N
Neoplastic	N	N	N
Neurological	N	N	N
Nutritional	N	N	N
Orthopedic	N	N	N
Pulmonary	N	N	N
Surgical	N	N	N
Total AEs	N	N	N
Total Subjects with AEs	N	N	N
Total Subjects Randomized	N	N	N
% with AEs	xx.x%	xx.x%	xx.x%
p-value*	0.xxxx		

*p-value from Fisher's exact test comparing percentage of subjects with AEs by treatment group

Table S9: Skin reaction Adverse Event by Treatment Group. mITT Subjects.

	Treatment Group		
	Allopurinol N=	Placebo N=	TOTAL
Stevens-Johnson Syndrome (SJS)	x/x (xx%)	x/x (xx%)	x/x (xx%)
Skin rash	x/x (xx%)	x/x (xx%)	x/x (xx%)
Subjects with SJS or skin rash	X	x	N
Total Subjects with Skin reaction Assessed	x	x	N
% with skin reaction AEs	xx.x%	xx.x%	xx.x%
p-value*	0.xxxx		

*p-value from Fisher's exact test comparing percentage of subjects with expected AEs by treatment group

Figure S1a. Time to first SAE during On-Study Drug Period, with Log-Rank Test to Compare Treatment Groups. mITT Subjects.
Kaplan Meier curve of time from randomization to first SAE by treatment group; subjects censored at earliest of death, withdrawal, lost-to-follow-up, or end of study medication provided subject didn't have an SAE.
Y axis label = % of Subjects (100%, 90%, … , 10%, 0%) without SAE
X axis label = Time (days) post-randomization (0, 6, 12, 18, 24, 30, 36 months)

Figure S1b. Time to first SAE during Off-Study Drug Period, with Log-Rank Test to Compare Treatment Groups. mITT Subjects.
Kaplan Meier curve of time from end of study medication to first SAE by treatment group; subjects censored at earliest of death or completion of study (withdrawal, lost-to-follow-up or end of study).
Y axis label = % of Subjects (100%, 90%, … , 10%, 0%) without SAE
X axis label = Time (months) post-treatment (0, 6, 12, 18, 24, 30, 36 months)

Additional Descriptive Statistics by Treatment Group
Table A1a. Comparison of iGFR by Treatment Groups. mITT Analysis Set.

Visit	Treatment Group		LSMean Treatment Difference (95% CI)	p-value
	Allopurinol N=	Placebo N=		
iGFR at Visit 4				
N	xx	xx		
Mean (SD)	xx.x (x.xx)	xx.x (x.xx)		
Min, Max	xx.x, xx.x	xx.x, xx.x		
N missing	xx	xx		
iGFR at Visit 11				0.xxxx
N	xx	xx		
Mean (SD)	xx.x (x.xx)	xx.x (x.xx)	x.x	
Min, Max	xx.x, xx.x	xx.x, xx.x	(x.x, x.x)	
N missing	xx	xx		
iGFR at Visit 16				0.xxxx
N	xx	xx		
Mean (SD)	xx.x (x.xx)	xx.x (x.xx)	x.x	
Min, Max	xx.x, xx.x	xx.x, xx.x	(x.x, x.x)	
N missing	xx	xx		
iGFR Visit 17				0.xxxx
N	xx	xx		
Mean (SD)	xx.x (x.xx)	xx.x (x.xx)	x.x	
Min, Max	xx.x, xx.x	xx.x, xx.x	(x.x, x.x)	
N missing	xx	xx		

REFERENCES

Fan, L., Levey, A.S., Gudnason, V., Eiriksdottir, G., Andresdottir, M.B., Gudmundsdottir, H., Indridason, O.S., Palsson, R., Mitchell, G. and Inker, L.A., 2015. Comparing GFR estimating equations using cystatin C and creatinine in elderly individuals. *Journal of the American Society of Nephrology*, *26*(8), pp.1982-1989.

Gałecki, A. and Burzykowski, T., 2013. *Linear mixed-effects models using R: A step-by-step approach*. Springer Science & Business Media.

Molenberghs, G. and Verbeke, G., 2005. *Models for Discrete Longitudinal Data*. Springer Science & Business Media.

Van Buuren, S., 2012. *Flexible imputation of missing data*. CRC press.

White, Ian R., Nicholas J. Horton, James Carpenter, and Stuart J. Pocock. "Strategy for intention to treat analysis in randomised trials with missing outcome data." *BMJ* 342 (2011): d40.

APPENDIX I. Study Objective, Study Design, Outcomes & Statistical Analysis and Data Management Sections from Protocol

In this appendix, selected sections (from protocol, version 9, approved by DSMB on August 16th, 2016) are included for reference. The following sections/figures from the study protocol are included:

- 2. Study Objective
- 3. Study design
- 7.1. Primary outcomes
- 7.2. Secondary outcomes
- Schedule of events (original figure on p. 27 in the study protocol)
- 9. Safety assessments
- 10. Adverse Event Reporting
- 11. Statistical Analysis

2. STUDY OBJECTIVE

To determine whether lowering serum UA by means of oral allopurinol is effective in preventing or slowing decline of renal function in T1D patients with microalbuminuria or moderate macroalbuminuria who still have only mildly or moderately impaired kidney function.

3. STUDY DESIGN

The study will be a multi-center, double-blind, placebo-controlled, parallel-group randomized clinical trial including a total of 480 patients with type 1 diabetes (T1D) who are at high risk for GFR loss because of increased albuminuria and a relatively high serum UA (≥ 4.5 mg/dl), but have only mildly or moderately decreased renal function.

7. STUDY OUTCOMES

7.1. Primary outcome

The primary outcome will be the iGFR at the end of the 2-month wash-out period following the 3-year treatment period, measured by the plasma clearance of non-radioactive iohexol (iGFR) and adjusted for the iGFR at baseline. The rationale of measuring the primary outcome at the end of the wash-out period is to test allopurinol for permanent effects of on the natural history of kidney disease, independent from any transient, hemodynamic effect that the medication may have on GFR. Plasma iohexol clearance has been shown to provide accurate and reproducible GFR measurements.[30,31] It is highly correlated with inulin clearance (the gold standard to measuring GFR)[32] and is a safe, cost-effective method to test hundreds of patients enrolled in multicenter clinical trials.[33] The method consists of injecting a 5 mL bolus of Iohexol (Omnipaque, 300 mg iodine/mL) and drawing blood samples at baseline and 120, 150, 180, 210, and 240 minutes after the injection. Plasma concentrations of iohexol at different time points are measured by HPLC and used to calculate the plasma clearance of iohexol (Cl=Dose/AUC, where AUC is the area under the plasma concentration time curve), which is taken after appropriate body surface area corrections as a measure of GFR. [30,31]

7.2. Secondary outcomes

1. Iohexol-clearance GFR at the end of the 3-year treatment period (before the washout).
2. Iohexol-clearance GFR time trajectory estimated from periodical iohexol-GFR measurements.
3. Estimated (eGFR) at 4 months estimated from serum creatinine and cystatin C and adjusted for the eGFR at baseline.
4. Estimated GFR (eGFR) time trajectory estimated from quarterly serum creatinine and cystatin C measurements using the CKD-EPI SCr and the CKD-EPI SCr-SCysC equations.34,35
5. Time to doubling of baseline serum creatinine value or ESRD (eGFR ≤ 15 ml/min/1.73 m2, institution of dialysis, kidney transplantation).
6. Geometric mean of two AER measurements at the end of the 2-month wash-out period following the 3-year treatment period, adjusted for the mean urinary AER at baseline. Urinary AER will be determined in timed overnight urine collections brought by study participants to regular clinic visits, and expressed in ·g/minute and as urinary albumin/creatinine ratios.
7. Geometric mean of urinary AER during the last three months of the treatment period (Visits 15 and 16), adjusted for the mean urinary AER at baseline.
8. Time to fatal or non-fatal cardiovascular events, defined as the composite of CVD death (ICD-10 code I10 to I74.9), myocardial infarction, stroke (ischemic or hemorrhagic), coronary artery bypass grafting, or percutaneous coronary intervention.

Figure 1. Schedule of Events

Year	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3	3	3
Week	-12	-9	-7	-3		0	4	16	32	48	64	80	96	112	128	142	156	164
Visit #	**1**	**2**	**3**	**4**	**4a***	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
^Type of Visit: In-Person Visit Required (V); Phone Call (C) ; Other Visit (In-Person or Remote Visit, O)	O	V	O	V	V	C	O	O	O	O	O	V	O	O	O	O	V	V
EVENT							Allopurinol or placebo										Wash-out	
	Screen	Run-in				RANDO 100 mg	200-400 mg											EOS
Informed Consent	x	x																
Demographics	x																	
Initial Medical Hx		x																
Interval Medical Hx and BP Control			x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Concomitant Meds	x	x	x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Blood Pressure and Measurements	x	x	(x)	x	(x)		(x)	(x)	(x)	(x)	(x)	x	(x)	(x)	(x)	(x)	x	x
ECG Report		x		x	(x)							x					x	
Physical Exam		x		(x)	(x)							x					x	
Skin Assessment				x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Eligibility	x			x	(x)	x												
Randomization						x												
Family History				x	(x)													
RAS and BP Med Log		x	x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
IGFR Procedure				x	(x)							x					x	x
PERL Study Drug Prescription						x	x	x	x	x	x	x	x	x	x	x		
Study Drug Compliance							x	x	x	x	x	x	x	x	x	x	x	
CENTRAL LAB																		
Serum uric acid, serum creat, cystatin C	x		x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Urine ACR/AER	x		x	x	(x)		.	x		x		x		x		x	x	x
HbA1c	x			x	(x)			x	x	x	x	x	x	x	x	x	x	x
HLA B*58:01				x	(x)													
iGFR				x	(x)							x					x	x
NIDDK Repository: serum, plasma, urine				x								x					x	x
LOCAL LAB																		
Pregnancy test serum HCG	x		x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Pregnancy test urine dipstick		x		x	(x)							x					x	x
ALT, K, CBC, serum creatinine, urine	x		x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Protocol Deviation		x	x	x	(x)		x	x	x	x	x	x	x	x	x	x	x	x
Adverse Events		x	x	x	(x)	x	x	x	x	x	x	x	x	x	x	x	x	x

*****If normal blood pressure control is not achieved at Visit 4, the run-in period may be extended for two more weeks after which participants will be examined as in Visit 4 (Visit 4A). In this event, the GFR measurement scheduled for Visit 4 will be conducted at Visit 4A.

^ Study visits will be generally conducted at the Study Sites or their Satellites. "In-Person Visits" (V) are required for Visit 2 and all visits requiring iohexol-GFR measurements. If a participant lives far from a study site or satellite, or travel impediments are present, other (O) visits may be conducted remotely or in-person. For any given study visit to be conducted remotely, a Phone Visit and a Remote Biospecimen Collection will be both required; a Phone Visit is performed by the study coordinator using the telephone or other media such as Skype to collect results of study procedures that do not require physical interactions (e.g., collection of medical history), and a Remote Biospecimen Collection is performed at a clinical laboratory close to where participants live.

Note: (x) indicates an optional assessment; For "BP and Measurements", (x) indicates an optional assessment only if the patient is NOT seen in-person.

9. SAFETY ASSESSMENTS

9.1. Demographic Data/Medical History

After collecting a detailed medical history at Visit 1, this information will be updated at each visit through a structured interview, with a special emphasis on skin symptoms and signs such as rash, itching and exfoliation and on pregnancy in females. Participants will be instructed to communicate any change in their health status and intervening hospitalizations to the study coordinator in-between visits. In particular, they will be instructed to discontinue study medication and immediately contact the study coordinator if they develop a suspicious skin rash, swelling of the lips or mouth, arthralgias, and/or jaundice, which may indicate a hypersensitivity reaction to allopurinol. Fever and chills should also be reported but would not require cessation of medication prior to discussion with study personnel.

9.2 Skin exam

The skin of study participants will be examined for the presence of any kind of rash at each in-person visit. Participants will be instructed to carry-out periodical skin self-exams. If skin abnormalities are reported to the study personnel during the phone visits or on any other occasion, participants will be asked to immediately report to the study site, their PCP's office, or other local healthcare facilities for an in-person skin exam. Suspicion of drug allergy or Stevens-Johnson Syndrome SJS would require immediate discontinuation of study medication and dermatologic consultation.

9.3. Vital Signs

Blood pressure and heart rate will be recorded at each in-person visit. BP readings at home will be reviewed during each phone visits; if abnormal values are reported, participants will be asked to visit the study site, their PCP's office, or other local healthcare facilities to have their BP measured.

9.4. Clinical Laboratory Tests

Serum ALT, creatinine and K^+, and CBC will be monitored and a pregnancy test, if a female of child bearing potential, performed at each visit. Participants who are started for the first time on RAS blockers as part of this study will have their serum K^+ and creatinine measured at a local laboratory after 2 weeks of full dose RASB treatment (i.e., after Visit 3). HbA1c will be measured at Visits 1, 4, and 7-17. An ECG will be performed at Visits 2, 4, 11, and 16.

9.5. Management of Uric Acid Levels

Study participants and study personnel, other than the DCC and the study pharmacists, will be masked as to the uric acid levels obtained during the study. The patients' physicians will receive written requests to refrain from measuring uric acid levels during the time of the patients' participation in the study, except as is mandatory for the patient's wellbeing, e.g., in the treatment of malignancy or diagnosis of a clinical syndrome highly likely to represent gout. If gout is diagnosed, open-label treatment with allopurinol will become indicated. In such case, the study drug will be discontinued but the patient will remain in the study and will continue to be followed as if he/she was taking the study medication. If uric acid lowering for malignancy treatment is required, the patient will receive open-label treatment until such time as return to study drug is deemed clinically reasonable by their physician.

10. ADVERSE EVENT REPORTING

10.1. Definitions

An Adverse Event (AE) is any untoward medical occurrence in a study participant regardless of its relationship to study treatment. A treatment-emergent AE is an adverse event occurring during the period between the first dose and 30 days after the final dose of the study medication. A Serious Adverse Event (SAE) is any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongation of an existing hospitalization, results in persistent or significant disability, or is a congenital anomaly/birth defect. Important medical events that do not fall into the above categories may also be considered an SAE when, based on medical judgment, such events may jeopardize the patient's safety and require medical/surgical intervention to prevent one of the outcomes listed in the SAE definition. The term SAE is not intended as a measure of severity or intensity. All AE's/SAE's that occur after the time of informed consent will be reported.

A Suspected Adverse Reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. An Unexpected Adverse Event or Unexpected Suspected Adverse Reaction is an adverse event or suspected adverse reaction that is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected", as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation. An Expected Adverse Event or Expected Adverse Reaction is any adverse experience that has been identified in nature or severity in the current investigator brochure and/or protocol.

10.2. Adverse Events Reporting

All AEs will be reported on the Adverse Events form that will be completed by the study staff, who are masked as to study treatment assignment, at each regular follow-up visits. This will insure that AEs are ascertained in an unbiased manner using the same standardized methodology for participants in both treatment arms. Forms will include standardized questions relating to specific events of import in diabetic patients on either of the study treatment arms as well as any significantly abnormal physical finding identified on examination and any significantly abnormal laboratory results obtained on the patient between visits or at the time of the visit. AEs reported or ascertained between clinic visits will be captured and reported at the time of the next schedule visit. Pre-existing conditions (that is, any condition that was known to be present prior to the signing of informed consent or was identified during the screening procedures at Visit 1) will not be considered or recorded as AEs unless the condition worsens in intensity or frequency after Visit 1. Likewise, continuing AEs will not be reported as AEs at

subsequent visits unless they increase in severity or frequency between visits, they result in criteria for a SAE, and/or they resolve between visits. Each site will be responsible for reporting all AE's to their IRB according to its AE reporting policy and procedures.

10.3. Assessment of Causality and Severity

The seriousness of adverse events will be ascertained by the study staff according to the criteria listed in 10.1 and the need for further evaluation, follow-up, or referral. The relationship between study participation and AEs will be determined according to the following criteria:

A. Not related – temporal relationship of the onset of the event, relative to study participation, is not reasonable or another cause can by itself explain the occurrence of the event.

B. Possibly related – temporal relationship of the onset of the event, relative to study participation, is reasonable but the event could have been due to another, equally likely cause.

C. Probably related – temporal relationship of the onset of the event, relative to study participation, is reasonable and the event is more likely explained by the study treatment than by another cause.

D. Definitely related – temporal relationship of the onset of the event, relative to study participation, is reasonable and there is no other cause to explain the event.

10.4. Serious Adverse Events Reporting

See Section 15 – Data and Safety Monitoring Plan.

11. STATISTICAL ANALYSIS

This section presents a summary of the planned statistical analyses. A statistical analysis plan (SAP) will be written for the study that contains detailed descriptions of the analyses to be performed. The SAP will be written prior to database lock.

11.1. Analysis Population

For most of the analyses, including the primary efficacy analysis described in section 11.3, an intention to treat (ITT) analytical approach will be employed. Accordingly, the population for statistical analysis will consist of all randomized study participants considered in their original randomization group, regardless of treatment discontinuation or loss to follow-up.

Selected secondary efficacy analyses will be performed using a per-protocol analytical approach. In this case, the analysis population will consist of the ITT population excluding data points which 1. had cumulative exposure to the study medication from randomization that was less than 80% of the theoretical full exposure; or 2. during major protocol deviations (e.g., treatment with prohibited medications), which could affect primary outcome.

11.2. Initial Data Analysis

The initial data analysis will be performed to detect any differences in distributions of characteristics measured at baseline, 4, 20, 36, and 38 months (0, 16, 80, 156, and 164 weeks, respectively) between study groups. The number of patients screened, enrolled, and completing the study will be summarized within and across study centers. Measures of central tendency (means, medians) and variability (standard deviations, ranges) will be estimated from the data for continuous variables. Frequency distributions will be provided for categorical data. This preliminary analysis step will provide us with insight into data, distributions of the variables considered, and will allow us to find additional invalid values not detected earlier during data validation.

11.3. Primary Efficacy Analysis

For the primary endpoint (iGFR at the end of the 2-month wash-out period following the 3-year intervention), we will follow the recommendations by Carpenter et al[38,39] and perform the analysis by means of a linear model for correlated errors with general/unstructured covariance matrix using all available iGFR measures (including those at baseline, 80, 156, and 164 weeks, respectively) as the dependent variable. By conditioning on the baseline iGFR measure we will also effectively use this variable as a covariate. Treatment group, study center, stratifying variables, albuminuria status (subjects who qualified by ACR or AER or were albuminuric at baseline vs. subjects who did qualified by eGFR slope and were normoalbuminuric at baseline), baseline AER, time, and time by treatment interaction will also be included as covariates in the model. Three features make this analytical approach especially attractive:

1. If there is no dropout (a very unlikely case), the estimate of the treatment effect at the end of the 2-month wash-out period following the 3-year intervention and its precision obtained using this approach will be exactly the same as those based on a classical approach employing an analysis of covariance (ANCOVA) model with treatment group, study center, iGFR and AER/ACR measured at baseline included as covariates.
2. If the iGFR measure at the end of the wash-out period is missing, we will be able to efficiently use the information contained in the intermediate iGFR measurements

obtained at 80 and 156 weeks, by virtue of them being correlated with the GFR measurement at washout. Estimate of the treatment effect obtained this way is valid under the missing at random (MAR) assumption. This is in contrast to the ANCOVA approach, which would lead to the loss of this information and would require a more stringent assumption about the mechanism of data missingness, i.e. a missing completely at random (MCAR) mechanism.

3. The underlying analytical framework allows the use of all post-randomization data and is well suited to investigate the reason for withdrawal, for example to study whether participants having low iGFR values are more likely to withdraw.

Calculations will be performed using SAS PROC/MIXED. Results of the analysis will be expressed in terms of point estimate and its corresponding 95% confidence interval for the treatment effect at the end of the 2-month wash-out period following the 3-year treatment and will be accompanied by the corresponding p value.

11.4. Secondary Efficacy Analyses

1. The effect of treatment on the iGFR at the end of the 3-year treatment period (before the washout) will be evaluated using the same analytical approach employed for the primary outcome.
2. The effect of treatment on the eGFR at 4 months after randomization will be evaluated using the same analytical approach employed for the primary outcome.
3. The iGFR and eGFR time trajectories, estimated from periodical iGFR measures and quarterly serum creatinine and cystatin C measurements using the CKD-EPI SCr and the CKD-EPI SCr-SCysC equations[34,35], respectively, will be analyzed using linear mixed-effects models.[40-42] The main objective of the analysis will be to construct confidence interval for the effect of the intervention over three years of observation (treatment main effect) and investigate whether the effect of the intervention changes with time (time by treatment interaction).
4. Time to serum creatinine doubling or ESRD in the two treatment groups is subject to censoring due to dropouts or reaching the end of study before the participant experiences the event. Survival time will be defined as the time from randomization to the event (the first of serum creatinine doubling from baseline or occurrence of ESRD, defined as eGFR $\leq$ 15 ml/min/1.73 m^2, hemodialysis, or kidney transplant) or, for participants who did not experienced an event, to the last study visit. Data will be summarized by means of Kaplan-Meier survival curves and by providing the proportions of participants surviving without events at 1, 2, 3 years, and at the end of the wash-out period along with their 95% CIs. Given the potentially small number of events, differences between study groups will be tested by means of the log rang test or by means of simple Cox regression models including a limited number of predictors in addition to treatment group.
5. The effect of treatment on the AER at the end of the wash-out period, based on the geometric mean of two AER measured at this time point and adjusted for the geometric mean of AER at baseline (Visit 3 and 4), will be investigated in a linear regression model framework as in the case of the primary outcome.
6. The effect of treatment on the AER at the end of the treatment period, based on the geometric mean of the AER measures at visit 15 and 16 adjusted for the geometric mean of AER at baseline (Visit 3 and 4) will be investigated as in #5.

7. Time to fatal or non-fatal cardiovascular events will be analyzed as proposed for time to serum creatinine doubling or ESRD.
8. We will perform a per-protocol analysis (as defined in 11.1) for the primary efficacy endpoint (iGFR at the end of the 2-month wash-out period following the 3-year intervention).

11.5. Incomplete Data

Missing values represent a potential source of bias. Efforts will be made to keep all participants in the study. If this is not feasible, at least some information regarding the status at the end of the trial will be obtained. For randomized patients, the number of completing and dropouts will be summarized. This procedure will help to compare characteristics of the participants' groups who drop out from the study with those who completed the study by treatment group, within and across study centers. The models considered in the proposal allow for a missing at random (MAR) mechanism. MAR means that the missing values mechanism can be explained by observed data and does not depend on the *unobserved* values of outcome measures. The differences in distributions between characteristics of the groups may indicate potential sources of bias due to missing values. For instance, some patients may dropout from the study due to *unobserved* factors related to the intervention itself. If we suspect such bias is present, the methods discussed in this section, assuming (MAR), are not applicable. We will incorporate plausible missing values mechanism into the model as discussed in Little[43] and investigate how such mechanism may affect the estimates of treatment effect. To this end, sensitivity analyses will be conducted involving selection and/or pattern-mixture models[44] with an appropriate submodel used to describe dropout.

11.6. Pilot participants

All pilot participants who were already randomized to allopurinol or placebo during the pilot will be included in the final analysis of the pivotal trial. Those who do not consent to the pivotal trial will be treated as having dropped from the study at a time corresponding to their last pilot visit. Sensitivity analyses will be performed to investigate whether results may be potentially affected by the roll-over of pilot subjects in the pivotal trial.

11.7. Model assumptions and alternative analyses

Model assumptions will be thoroughly checked for individual and systematic departures, using informal, e.g. inspection of residuals, and formal methods such as score test for extra parameter or methods based on likelihood displacement. If individual outliers are detected, their influence will be evaluated using influence diagnostics methods based on comparing estimates from models fitted to data with and without outlying values. Whenever we are not successful in fitting the parametric model (linear or non-linear), then non-parametric analyses and/or transformation of the variables involved in the analysis will be considered. To investigate the potential hemodynamic influence of allopurinol on treatment effect, in addition to the aforementioned analyses, we will consider models including the post-randomization measure of GFR at 4 months as an additional covariate. To investigate the possible presence of heterogeneity in the response to allopurinol, subgroup analyses (based on the primary efficacy analysis described in section 11.3, with the inclusion of an interaction term of the treatment group by the subgroup variable) will be performed by age groups (≤40 and >40 yrs), gender, racial/ethnic group, HbA1c (≤7.8 and >7.8%), serum uric acid (≤6.0 and > 6.0 mg/dl), baseline iGFR (≤70 ml/min and >70 ml/min/1.73m^2), AER at baseline (≤300 and >300 mg/24 hr), and albuminuria status (subjects who qualified by ACR or AER or were albuminuric at

baseline vs. subjects who did qualify by eGFR slope and were normoalbuminuric at baseline). To investigate possible influence of using selected covariates on the treatment effect estimate in the models considered in Section 11, we will perform appropriate sensitivity analyses. These additional analyses will be considered as strictly exploratory.

11.8. Safety Analyses

Adverse events will be independently reviewed by an independent data safety monitoring board (DSMB, see Sections 15 and 16). All safety data will be available in data listing in the clinical protocol report. Data will be described in terms of descriptive statistics and presented by treatment group. Presentation will include graphs (scatterplots, boxplots, histograms), measures of central tendency (mean, median) and variability (confidence intervals) for continuous variables and frequency tables for categorical variables.

11.9. Interim Analysis

No formal interim analyses of efficacy to stop for benefit or futility are planned, given the timing of the primary endpoint.

11.10. Sample Size

Since a variance-covariance matrix for the iGFR measures is not available and this matrix is essential in order to perform formal power calculations for a model with correlated errors, we performed alternative power calculations based on an intent-to-treat analysis within an ANCOVA framework. Specifically, we assumed that the primary hypothesis is tested in the following model:

M1: iGFR at washout = iGFR at baseline + treatment group

Compared to the model that will be used in the primary analysis, model M1 is simplified in two aspects. First, it does not use information from iGFR values measured at intermediate time points. Second, it does not include covariates such as the stratifying variables (HbA1c and UA) or other GFR predictors such as baseline AER. Both of these aspects may lead to loss of precision of the treatment effect estimate. Consequently, our sample size calculations should be considered as conservative.

The hypothesis being tested, i.e. the effect of treatment on iGFR at washout, corresponds to testing whether the treatment group factor in Model M1 is significant. The choice of the ANCOVA model for the purpose of power calculations is sensible, as residuals from a univariate model involving baseline iGFR as covariate fitted to data from RASS study conform to normal distribution. Sample size calculations were performed based on Cohen[45] and making the following assumptions:

1. Postulated effect on iGFR at washout ($\cdot$) = 3 ml/min/1.73 m^2. We deem this effect to be clinically meaningful and attainable. It is clinically meaningful because it would translate on average into a 10-year delay in the progression to ESRD. It is attainable because it is smaller than the difference in 3-year GFR that we observed in the JKS between subjects with serum UA $\geq$ 4.5 mg/dl compared to those with levels below this value. The postulated effect was based on the following changes in GFR levels in the two treatment groups:
   a. Untreated group = 3 ml/min/1.73 m^2 per year. This estimate is based on data from the Joslin Kidney Study (JKS), in which the median GFR loss among 43 subjects meeting the above criteria was 3.1 ml/min/1.73 m^2 per year, with 70% of subjects having a GFR loss >1.5 ml/min/1.73 m^2 per year. Also, among 116

subjects from Steno who met the albuminuria and GFR criteria, but for whom serum uric acid values were not available, the median GFR loss was 3.3 ml/min/1.73 m^2 per year, with 71% of subjects having a GFR loss >1.5 ml/min/1.73 m^2 per year.

b. Treated group = 2 ml/min/1.73 m^2 per year. The average GFR loss in the JKS subjects with serum UA <4.5 mg/dl was 1.5 ml/min per year. On this basis, we conservatively assumed that the allopurinol treatment, if effective, would decrease the GFR loss to 2 ml/min per year (a 33% decrease compared to the untreated group).

2. Standard deviation (SD) of residual error = 10.1 ml/min/1.73 m^2. This was estimated based on the root-mean-squared error from a regression model with eGFR at 3 yrs as the dependent variable and baseline eGFR as the independent variable fitted to data concerning T1D patients from the Joslin Kidney Study meeting the PERL inclusion criteria.

Assuming a two-sided alpha error equal to 0.05, the effective sample size needed to detect the pre-specified treatment effect (‥= 3 ml/min/1.73 m^2) at washout adjusted for baseline iGFR with 80% power is equal to n=180 per group. To take into account the anticipated overall dropout rate (up to 5%/yr or 15% over the entire duration of the study) and drug discontinuation or non-compliance in the treatment group (up to 2%/yr or 6% over the entire duration of the study), and to maintain the desired power of at least 80%, it will be necessary to recruit n=240 subjects per group. In Table 1, we show the power of the proposed sample size for Model M1 under different dropout and non-compliance scenarios. We also provide the corresponding power for a model (Model M2) including the two stratifying variables (Hb1Ac and UA) and baseline AER as covariates to illustrate the effect of adding these variables to Model M1. In this analysis, we assumed that adding these covariates reduces the residual variance by 10%, which corresponds to these covariates explaining merely 4% of the total iGFR variation over and above the variability explained by iGFR at baseline. As shown in Table 1, once these covariates are accounted for, power is expected to exceed the conservative estimates provided by Model M1 and reach almost 90% for 15% dropout and 6% non-compliance rates.

Table 1. Power to detect treatment effect for two ANCOVA models under different drop-out and non-compliance scenarios.

Overall Dropout (%)	Non-compliance (%)	Model	
		M1	M2
9	0	.87	.92
12	0	.86	.91
15	0	.85	.90
9	6	.83	.89
12	6	.82	.88
15	6	.80	.87

Summary of Changes to the Statistical Analysis Plan

The first statistical analysis plan for the PERL status (version dated February 22, 2017) was prepared for inclusion as an appendix to the NIH application for renewal of the grant supporting this clinical trial. This SAP was approved by the Steering Committee but was not reviewed by NIDDK or the PERL DSMB.

A subsequent SAP version (dated May 14, 2019) was prepared as the trial was approaching completion. This was reviewed and approved by the Steering Committee, NIDDK, and the DSMB. The main changes in this version as compared to the previous one (dated February 22, 2017 were):

- Section 3. Rationale for adjustments of the SAP as compared to Protocol
    - Addition and justification of several adjustments to the analyses detailed in the Protocol (v10, dated March 6, 2018).
- Section 5. Study estimands
    - Re-framing of data analysis in terms of "Study estimands", in order to follow ICH-E9 (R1) recommendations.
- Section 6. Analytical strategy:
    - 6.1. Study population.
        - Revision of the definition of the ITT and per protocol populations.
        - Addition of details regarding the imputation methods.
    - 6.3. Visit Windows.
        - Specification of wider windows for V11, V16, and V17 to ensure that all iGFR are analyzed.
        - Addition of procedure to avoid temporal overlap of iGFR and non-iGFR visits.
        - Creation of separate tables for non-iGFR and iGFR visit windows (Tables 6.3.1 and 6.3.2).
        - Addition of "Time since randomization attributed to visit window" to tables with visit windows.
    - Section 6.4. Baseline Covariates
        - Addition of methods to impute missing values for baseline covariates.
    - Section 6.5 (New) Missing values
        - Description of methods to account for missing values.
    - Section 6.6. Analysis of the primary estimand
        - Miscellaneous changes to make the analysis consistent with the re-framing in terms of Study estimands and for the more detail description of imputation methods.
        - Addition of the tipping point sensitivity analysis to check the robustness of the MAR assumption.
    - Section 6.7. Analysis of secondary estimands
        - Miscellaneous changes to make analysis consistent with the re-framing in terms of Study estimands and for the more detail description of imputation methods.
    - Section 6.8. Other analyses
        - Section 6.8.1 changes in cut-offs for stratified analyses to make them consistent with changes made in Section 3.

        - Section 6.8.2. Addition of details about the metrics used to evaluate safety.
        - Section 6.8.3. Addition of details on the additional analyses introduced in Section 3 (effect of post-randomization serum urate changes on iGFR at V17, effect of allopurinol on time to 40% eGFR decrease, effect of allopurinol on composite of serum creatinine doubling, ESRD, and CVD/renal death.
- Section 7. Mock Tables and Figures – Deleted.

Minor revisions were made to the SAP on August 3, 2019, right before the lock of the study database. These changes included:

- Section 6. Analytical Strategy
    - Section 6.4. Baseline Covariates
        - Clarification about the aggregation of sites with small numbers of randomized individuals within the baseline covariate "Clinical site".
    - Section 6.5. Missing values
        - Clarification about the imputation of eGFR and iGFR values in subjects who started with low GFR values and did not develop ESRD.
    - 6.6. Analysis of the primary estimand.
        - Addition of Kenward-Roger approximation to estimate degrees of freedom.