STUDY PROTOCOL

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

Version 10.0

Approved by the DSMB

March 6, 2018

Table of Contents

GENERAL INFORMATION

Funding Agencies

- National Institute of Diabetes and Digestive and Kidney Diseases
- Juvenile Diabetes Research Foundation (JDRF)

Program Officers

Teresa Jones, MD
Division of Diabetes, Endocrinology, & Metabolic Diseases
NIDDK, National Institutes of Health
6707 Democracy Blvd. Room 609
Bethesda, MD 20892-5460
Phone: (301) 435-2996
Fax: (301) 480-3503
jonester@mail.nih.gov

Afshin Parsa, MD, MPH
Program Director, Clinical Kidney Genomics, PKD and CKD NIDDK/NIH
6707 Democracy Blvd. Room 6139
Bethesda, MD 20892-5460
Tel: (301) 827-1375
afshin.parsa@nih.gov

Marlon Pragnell, PhD
Senior Scientist, Translational Development
Juvenile Diabetes Research Foundation
26 Broadway
New York, NY 10004
Phone: (212) 479-7690
Fax: (212) 785-9609
mpragnell@jdrf.org

Grant Numbers

NIH UC4 DK101108-01
JDRF 17-2012-377
ClinicalTrials.gov NCT02017171

Sponsor for IND #115313

Alessandro Doria, MD PhD MPH
Joslin Diabetes Center and Harvard Medical School
One Joslin Place
Boston, MA 02215
Phone: (617) 309-2406
Fax: (617) 309-2667
alessandro.doria@joslin.harvard.edu

Principal Investigators

Alessandro Doria, MD PhD MPH
Joslin Diabetes Center and Harvard Medical School
One Joslin Place
Boston, MA 02215
Phone: (617) 309-2406
Fax: (617) 309-2667
alessandro.doria@joslin.harvard.edu

S. Michael Mauer, MD
University of Minnesota
2450 Riverside Ave.
East Building, MB681
Minneapolis, MN 55454
Phone: (612) 626-2720
Mobile: (612) 703-5884
Fax: (612) 626-2791
mauer002@umn.edu

Directors of Clinical Sites

Maria Luiza Caramori, MD PhD
University of Minnesota
420 Delaware Street SE, MMC 101
Minneapolis, MN, 55455
Phone: (612) 625-7189
Fax: (612) 626-3133
caram001@umn.edu

Sylvia Rosas, MD
Joslin Diabetes Center and Harvard Medical School
One Joslin Place
Boston, MA 02215
Phone: (617) 309-2477
Fax: (617) 309-3403
Sylvia.Rosas@joslin.harvard.edu

Sarit Polsky, MD, MPH
Barbara Davis Center for Diabetes
University of Colorado at Denver - Anschutz Medical Campus
1775 Aurora Court, Mail Stop A140
Aurora, CO 80045~~P~~
Phone: (303) 724-8575
Fax: (303) 724-6784
sarit.polsky@ucdenver.edu

Bruce Perkins, MD MPH
Mount Sinai Hospital
University of Toronto
Room L5-210, Mail Box 16
60 Murray St.
Toronto, ON
Canada, M5G 1X5
Phone: (416) 586-8763
Fax: (647) 826-1528
bruce.perkins@mtsinai.ca

Ronnie Aronson, MD
LMC Diabetes and Endocrinology
1929 Bayview Avenue, Suite 107
Toronto, ON Canada M4G 3E8
Ph: (416) 646-2929
Fax: (416) 645-2930
Email: Ronnie.Aronson@LMC.CA

Rodica Pop-Busui, MD PhD

University of Michigan
Division of Metabolism, Endocrinology and Diabetes
5329 Brehm Tower
1000 Wall Street
Ann Arbor MI 48105
Phone: (734) 763-3056
rpbusui@umich.edu

Peter Rossing, MD
Steno Diabetes Center
Niels Steensens Vej 1
DK-2820 Gentofte
Denmark
Phone: +45 4443-7310
pro@steno.dk

Mark Molitch, MD
Division of Endocrinology, Metabolism and Molecular Medicine
Northwestern University Feinberg School of Medicine
645 N. Michigan Avenue, Suite 530
Chicago, IL 60611
Phone: (312) 503-4130
Fax: (312) 926-8693
molitch@northwestern.edu

Jill Crandall, MD
Albert Einstein College of Medicine
Jack and Pearl Resnick Campus
1300 Morris Park Avenue
Belfer Building, Room 702
Bronx, NY 10461
Phone: (718) 430-3765
Fax: (718) 430-8557
jill.crandall@einstein.yu.edu

Janet McGill, MD
Washington University School of Medicine
660 S. Euclid, Campus Box 8127
St. Louis, MO 63110
Phone: (314) 362-8681
Fax: (314) 362-4833
hmcgill@dom.wustl.edu

Ronald J. Sigal, MD, MPH
Cumming School of Medicine, University of Calgary
1820 Richmond Road SW
Calgary, Alberta, Canada T2T 5C7
Phone: (403) 955-8327
Fax: (403) 955-8249
rsigal@ucalgary.ca

Peter Senior, MD
Alberta Diabetes Institute
2-004 Li Ka Shing Center for Health Research Innovation
Edmonton, Alberta, Canada T6G2E1
Phone: (780) 407-1480

Fax: (780) 492-9555
petersenior@ualberta.ca

Guillermo E. Umpierrez, MD
Emory University School of Medicine
101 Wodruff Circle, 1st Floor, RM 1311
Atlanta, GA 30322
Phone: (404) 778-1663
geumpie@emory.edu

Ian de Boer, MD, MS
University of Washington Medical Center
Box 359606
325 9th Avenue, Room 3NJ357
Seattle, WA 98104
Phone: (206) 616-5403
Fax: (206) 685-9399IDeBoer@Nephrology.washington.edu

Ildiko Lingvay, MD, MPH, MSCS
University of Texas Southwestern Medical Center
5323 Harry Hines Blvd., U9.134E
Dallas, TX 75390-9302
Phone: (214) 648-2779
Fax: (214) 648-2885
ildiko.lingvay@utsouthwestern.edu

Katherine R. Tuttle, MD
Providence Medical Research Center
104 W. 5th Avenue, Suite 350E
Spokane, WA 99204
Phone: (509) 474-4345
Fax: (509) 474-4325
katherine.tuttle@providence.org

Tom Elliott MBBS, FRCPC
BC Diabetes
4102-2775 Laurel Street
Vancouver, BC V5Z 1M9
Phone: (604) 683-3734 ext. 1001
Fax: (604) 628-3821
telliott@bcdiabetes.ca

Global Clinical Coordinator

Debbie Conboy, RN CDE
Joslin Diabetes Center
One Joslin Place, Room 445
Section on Genetics & Epidemiology
Boston, MA 02215
Phone: (617) 309-4343
Fax: (617) 309-4170
debbie.conboy@joslin.harvard.edu

Data Coordinating Center

Co-Director: Andrzej Galecki, MD PhD
Division of Geriatrics, Medical School
Department of Biostatistics, School of Public Health

University of Michigan
300 North Ingalls
Ann Arbor, MI 48109-2009
Phone: (734) 936-2138
Fax: (734) 936-2116
agalecki@umich.edu

Co-Director: Cathie Spino, ScD
Statistical Analysis of Biomedical and Educational Research (SABER) unit
University of Michigan
M4507 SPH II
1415 Washington Heights
Ann Arbor, Michigan 48109-2029
Phone: (734) 615-5469
Fax: (734) 647-3711
spino@umich.edu

Central Laboratory

Advanced Research and Diagnostics Laboratory
Director: Amy Karger, MD, PhD
University of Minnesota
Mayo Mail Code 609, Room Mayo D211
420 Delaware Street, SE
Minneapolis, MN 55455
Ph: (612) 624-2150
Fax: (612) 625-1121
karge026@umn.edu

PROTOCOL SUMMARY

Study Title	A multicenter clinical trial of allopurinol to prevent GFR loss in type 1 diabetes
Study Phase	Phase 3
Objectives	To determine whether lowering serum uric acid by means of allopurinol early in the course of kidney disease may be effective in preventing or slowing the decline of renal function in T1D patients.
Study Design	Multicenter, double-blind, placebo-controlled, parallel-group randomized clinical trial.
Participating Centers	Joslin Diabetes Center (Boston), University of Minnesota (Minneapolis), University of Colorado (Denver), University of Michigan (Ann Arbor), University of Toronto (Toronto), Northwestern University (Chicago), Albert Einstein College of Medicine (New York), Steno Diabetes Center (Copenhagen, Denmark), University of Washington (Seattle), University of Calgary (Calgary), University of Alberta (Edmonton), Emory University (Atlanta), Washington University (St. Louis), University of Texas Southwestern (Dallas), Providence Medical Research Center (Spokane), BC Diabetes (Vancouver).
Subject Population	480 T1D subjects.

Inclusion criteria:

1. Male or female T1D patients.

2. T1D continuously treated with insulin within one year from diagnosis. If the onset was after age 35, the presence of one or more of the following will also be required:
   - documentation of the presence of circulating T1D-associated autoantibodies at diagnosis or at any other time
   - history of hospitalization for DKA
   - plasma C-peptide below the limit of detection with standard assay (with concurrent blood glucose >100 mg/dl)

3. Duration of T1D ≥ 8 years.

4. Age 18-70 years.

5. History or presence of microalbuminuria or moderate macroalbuminuria, or evidence of declining kidney function regardless of history or presence of albuminuria and/or RAS Blocker treatment. Micro- or moderate macroalbuminuria will be defined as at least two out of three consecutive urinary albumin excretion rates [AERs] or albumin creatinine ratios [ACRs] taken at any time during the two years before screening or at screening in the 30-5000 mg/24 hr (20-3333 µg/min) or 30-5000 mg/g range, respectively, if not on RASB agents, or in the 18-5000 mg/24 hr (12-3333 µg/min) or 18-5000 mg/g range, respectively, if on RASB agents); Evidence of declining kidney function will be defined as an eGFR (CKD-EPI) decline ≥3.0 ml/min/1.73 m^2/year, estimated from the slope derived from all the available serum creatinine measurements (including the one at screening assessment) from the previous 3 years. If at least 3 serum creatinine measures are not available in the previous 3 years, then the slope can be derived from creatinine values from the previous 5 years.

6. Estimated GFR (eGFR) based on serum creatinine between 40 and 99.9 ml/min/1.73 m^2 at screening. The upper and the lower limits should be

decreased by 1 ml/min/1.73 m^2 for each year over age 60 (with a lower limit of 35 ml/min/1.73m^2) and by 10 ml/min/1.73 m^2 for strict vegans.

7. Serum UA (UA) ≥ 4.5 mg/dl at screening.

8. Valid baseline (Visit 4) iGFR measurement.

OR

9. Being an active participant in the PERL Pilot Study.

Exclusion criteria:

1. History of gout or xanthinuria or other indications for uric acid lowering therapy such as cancer chemotherapy.

2. Recurrent renal calculi.

3. Use of urate-lowering agents within 2 months before screening.

4. Current use of azathioprine, 6-mercaptopurine, didanosine, warfarin, tamoxifen, amoxicillin/ampicillin, or other drugs interacting with allopurinol.

5. Known allergy to xanthine-oxidase inhibitors or iodine containing substances.

6. HLA B*58:01 positivity (tested before randomization).

7. Renal transplant.

8. Non-diabetic kidney disease.

9. SBP>160 or DBP >100 mmHg at screening or SBP>150 or DBP>95 mmHg at the end of the run-in period.

10. Cancer treatment (excluding non-melanoma skin cancer treated by excision) within two years before screening.

11. History of clinically significant hepatic disease including hepatitis B or C and/or persistently elevated serum liver enzymes at screening and/or history of HBV/HCV positivity.

12. History of acquired immune deficiency syndrome or human immunodeficiency virus (HIV) infection.

13. Hemoglobin concentration <11 g/dL (males), <10 g/dL (females) at screening.

14. Platelet count <100,000/mm^3 at screening.

15. History of alcohol or drug abuse in the past 6 months.

16. Blood donation in the 3 months before screening.

17. Breastfeeding or pregnancy or unwillingness to be on contraception throughout the trial.

18. Poor mental function or any other reason to expect patient difficulty in

	complying with the requirements of the study. 19. Serious pre-existing medical problems other than diabetes, e.g. congestive heart failure, pulmonary insufficiency.
Study Duration	9-week run-in period, during which RAS inhibition will be introduced and/or standardized, if indicated, and BP normalized, if elevated above 140/90 mmHg, followed by a 3-year treatment period and then by a 2-month wash-out period.
Study Treatment, Dosage, and Route of Administration	After the run-in period, eligible subjects will be randomized in a 1 to 1 ratio to receive placebo or oral allopurinol at a dose of 100 mg per day for 4 weeks and then at a dose ranging from 200 to 400 mg per day depending on kidney function.
Efficacy Assessments	Primary outcome measure: GFR 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. Secondary outcome measures: 1. iGFR the end of the 3-yr treatment period (before the washout period) adjusted for the iGFR at baseline. 2. iGFR time trajectory estimated from periodical iGFR measurements. 3. eGFR at 4 months estimated from serum creatinine and cystatin C and adjusted for the eGFR at baseline. 4. eGFR time trajectory estimated from quarterly serum creatinine and cystatin C measurements (eGFR). 5. Time to serum creatinine doubling or end stage renal disease (ESRD). 6. AER at the end of the 2-month wash-out following the 3-yr treatment period, adjusted for the AER at baseline. 7. AER at the end of the 3-yr treatment period, adjusted for the AER at baseline. 8. Time to fatal or non-fatal cardiovascular events.
Safety Assessment	Examination for skin rash, measurements of liver enzymes, serum creatinine, and CBC, carried out 1 month after randomization and every 3-4 months thereafter.
Statistical Methods	The majority of data analyses, including the primary analysis, will be performed according to an intention-to-treat approach. Differences between treatment arms in the primary outcome will be tested for significance by means of a linear model with correlated errors. Intervention effects on other secondary outcomes will be tested by mixed-effect models (GFR time trajectory), ANCOVA (AER), and survival analysis (time to serum creatinine doubling/ESRD and CVD events).
Date of protocol	February 22, 2018

ABBREVIATIONS

AE: Adverse Event

ACE: Angiotensin Converting Enzyme

ACR: Albumin Creatinine Ratio

AER: Albumin Excretion Rate

ALT: Alanine Transaminase

ARB: Angiotensin Receptor Blocker

CBC: Complete Blood Count

CKD: Chronic Kidney Disease

CRF: Case Report From

CVD: Cardiovascular Disease

DBP: Diastolic Blood Pressure

DCC: Data Coordinating Center

DMC: Drug Monitoring Committee

DN: Diabetic Nephropathy

DSMB: Data and Safety Monitoring Board

ESRD: End Stage Renal Disease

GFR: Glomerular Filtration Rate

ITT: Intention to Treat

HbA1c: Glycated Hemoglobin A1C

HBV: Hepatitis B Virus

HCV: Hepatitis C Virus

HIV: Human Immunodeficiency Virus

IRB: Institutional Review Board

MAP: Mean Arterial Pressure

NO: Nitric Oxide

PERL: Preventing Early Renal Function Loss in Diabetes Consortium

RAS: Renin Angiotensin System

RASB: Renin Angiotensing System Blocker

SAE: Severe Adverse Event

SBP: Systolic Blood Pressure

SC: Steering Committee

SOP: Standard Operating Procedure

T1D: Type 1 Diabetes

UA: Uric Acid

1. INTRODUCTION

Diabetic nephropathy (DN) is the long-term complication of T1D that imposes the highest social and economic burden. After 40 years of diabetes, about one in three patients with T1D has developed kidney abnormalities, which frequently progress to end stage renal disease (ESRD).[1] Despite improvements during the past 20 years in glycemic and blood pressure control, and the introduction of 'renoprotective' drugs such as renin–angiotensin system (RAS) blockers, the overall incidence of DN is not declining.[2-4] Thus, DN remains one of the most important causes of excess morbidity and mortality in patients with diabetes mellitus, and novel therapies to complement and increase the therapeutic effects of glycemic control and RAS inhibition are urgently needed.

DN has been traditionally viewed as a multi-stage process, in which an initial clinical phase characterized by increased urinary excretion of small amounts of albumin (microalbuminuria) is followed by excretion of larger amounts of proteins (overt proteinuria), which then ushers in progressive decline in renal function ultimately leading to end-stage renal disease (ESRD).[1] This paradigm, however, is changing with the demonstration in prospective studies that, in a substantial proportion of T1D patients, renal function starts to decline before the onset of overt proteinuria.[5-7] These findings indicate that T1D patients should be screened for GFR loss when albumin excretion rate (AER) is still in the microalbuminuria range, and that interventions aimed at preventing ESRD should be started at these earlier stages. The earlier the rate of GFR loss is reduced through appropriate interventions, the longer will be the delay of ESRD.

Mounting evidence from epidemiological studies indicates that serum UA levels are strong risk factors for the development of chronic kidney disease and loss of kidney function among persons with T1D. Prospective data from the Second Joslin Kidney Study (JKS) identified elevated baseline serum UA as one of the strongest independent predictors of early GFR loss among T1D persons with microalbuminuria and normal renal function at baseline.[8] The unadjusted odds ratio of developing increased GFR loss was 1.5 (95% CI 1.3-1.9, p=0.0002) for each mg/dl increase in serum UA. This translates into a ~2.4-fold increase in the risk of early GFR loss for UA levels ≥ 4.5 mg/dl as compared to UA levels <4.5 mg/dl. The magnitude of this effect did not significantly change after adjustment for urinary AER, gender, HbA1c, or, importantly, baseline GFR. The U. of Colorado group also found that serum UA predicted the transition from normoalbuminuria to micro- or macro-albuminuria as well as the progression of subclinical atherosclerosis in the CACTI study.[9,10] As in the JKS, the effect of UA was not influenced by adjustment for other baseline variables. An association between UA and development of persistent macroalbuminuria has also been reported by the Steno group. It is very important to note that, in that study, the UA levels shortly after the onset of T1D was a significant independent predictor of macroalbuminuria 18 years later (hazard ratio 1.90 per mg/dl increase in UA level; p=0.04)[11], this being suggestive of a pathogenetic role.

The prospective nature of these findings and their robustness after adjustment for potential confounders strongly support the concept that moderately elevated serum UA has a pathogenetic role in DN development and in the deterioration of kidney function observed in T1D. Consistent with this hypothesis, hyperuricemia has predicted chronic renal failure in population-based studies[12-14] and mild UA elevation has been shown to cause renal disease in animal models.[15,16] Alterations of nitric oxide (NO) pathways and induction of pro-inflammatory cytokines[17,18], and increased oxidative stress resulting from the generation of UA by xanthine oxidase[19,20] have been proposed as being responsible for these effects. Two small clinical trials have recently provided proof of concept data for translating these findings into a novel intervention by showing that the urate-lowering agent allopurinol was effective in slowing the progression of non-diabetic renal disease among hyperuricemic as well as normouricemic individuals with moderately reduced GFR.[21,22] A beneficial effect of urate-lowering drugs on the progression of kidney disease has also been observed in animal models.[23] These findings, along with the observational data discussed above, strongly suggest that **lowering serum UA levels may prevent or slow the loss of kidney function among diabetic subjects.**

To test this hypothesis, we have established a consortium of investigators from academic centers where large rosters of T1D patients are available along with long-standing expertise in the study of diabetic complications, especially DN, and in DN clinical trials. Included in this initiative are the Joslin Diabetes Center, the Universities of Minnesota, Colorado, Toronto, Michigan, Washington (Seattle), Texas Southwestern, Calgary, and Alberta Northwestern University, Washington University (St. Louis), Emory University, Albert Einstein College of Medicine, BC Diabetes, Providence Medical Research Center, and Steno Diabetes Center in Copenhagen, Denmark. The Consortium, led by Dr. Alessandro Doria from the Joslin Kidney Study, and by Dr. Michael Mauer, who recently led the Renin Angiotensin System Study (RASS) clinical trial, has been named PERL (**P**reventing **E**arly **R**enal Function **L**oss in Diabetes) to emphasize the Consortium's focus on intervening early in the course of kidney disease, when renal damage is most likely to be able to be arrested or reversed and interventions are more likely to be effective.

PERL has designed the present 3-year clinical trial to test whether the uric acid lowering drug allopurinol can preserve kidney function among type 1 diabetic patients. In preparation for this trial, the Consortium has been conducting a pilot study to determine the study feasibility and establish study procedures. Funded by JDRF (JDRF file # 17-2012-377), the pilot study has a comparable design as the pivotal trial, but a smaller size and shorter duration. As of February 1, 2014 a total of 31 pilot study subjects have been randomized to allopurinol or placebo. Upon activation of the present trial, pilot participants will be re-consented and rolled-over to the present study at a time point corresponding to their next scheduled visit. The first 7 visits have identical timing in the two protocols. Visit schedules slightly differ after that, but given the current follow-up status, it will be possible to transfer all pilot participants to the pivotal trial before the timing diverges.

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.

4. PARTICIPATING CENTERS

The study will involve 16 centers that are part of the PERL Consortium:

- Joslin Diabetes Center (Boston)
- University of Minnesota (Minneapolis)
- University of Colorado (Barbara Davis Center for Childhood Diabetes, Denver)
- University of Michigan (Ann Arbor)
- University of Toronto (Toronto)
- Northwestern University (Chicago)
- Albert Einstein College of Medicine (New York)

- Steno Diabetes Center (Copenhagen, Denmark)
- Washington University (St. Louis, MO)
- University of Calgary (Calgary, Alberta, Canada)
- University of Alberta (Edmonton, Alberta, Canada)
- Emory University (Atlanta)
- University of Washington (Seattle)
- University of Texas Soutwestern (Dallas)
- Providence Medical Research Center (Spokane)
- BC Diabetes (Vancouver)

4.1 Location of study visits

Study visits will be generally conducted at the Study Sites or their Satellites (hereby referred to as "In-Person Visits"). However, if a participant lives far from a study site or satellite, or travel impediments are present, visits V1, V3, V5-10, and V12-15 may be conducted remotely (Visit 2 and all the visits including an iohexol-GFR measurement, i.e., V4, V11, V16, V17, will always be done "In-person"). In the case of remote visits, study procedures that do not require physical interactions (e.g., collection of medical history, compliance issues) will be carried out over the phone or other media such as Skype (hereby referred to as "Phone Visits"). Blood draws and urine collections scheduled at the time of Phone Visits will be performed at local facilities close to where participants live (hereby referred to as "Remote Biospecimen Collections"). For any given study visit to be conducted remotely, a Phone Visit and a Remote Biospecimen Collection will be both required. Phone Visits and Remote Biospecimen Collections will be conducted according to the following protocol:

<u>Phone Visits</u>

- Phone Visits will be scheduled based on the same calendar and time windows used for In-Person Visits (see paragraph 8.1 and Fig 1).
- All Phone Visits will be carried out by the same trained study personnel performing the In-Person Visits according to the same standards as those in place for In-Person Visits.
- If Visit 1 is a Phone Visit, a copy of the informed consent form (ICF) will be mailed, faxed, or sent electronically to the study subject before the visit. After reviewing the ICF content with the study personnel over the phone/Skype, subjects who agree to participate in the study will be invited to mail, fax, or send electronically a signed copy of the ICF back to the study site. Phone Visit 1 and any other study activity will take place only after the signed ICF has been received by the study site.
- Study procedures that may be carried out during Phone Visits include:
    - Collection of demographic data.
    - Collection of medical history.
    - Collection of family history.
    - Review of concomitant medications.
    - Evaluation of eligibility.
    - Randomization.
    - Review of RASB medication and BP control.

- Study drug prescription and instructions.
- Review of study drug compliance.
- Review of adverse events.
- Any other study procedure that can be carried out by talking on the phone.

- Study procedures will be carried out according to the same protocol as the corresponding In-Person Visits and as described in the Manual of Operations.
- All study material that would be provided to participants at In-Person Visits (e.g., urine collection instructions, urine containers, study drug instruction, BP monitoring logs) will be mailed, faxed, or sent electronically to participants right after the Phone Visit. In addition, specific instructions will be provided for presentation to the local lab for specimen collection, handling and tube labeling for specimens requiring shipment to the Study Site or Central lab. Pre-addressed shipping containers will also be provided.
- Following a Phone Visit, participants may be invited to an In-Person Visit at the Study Site, at their PCP's office, or at other local healthcare facilities if procedures that require physical interactions are deemed to be necessary (e.g., BP measurement to confirm the self-report of elevated BP values, physical exam to confirm the self report of skin rash). Sites for remote in-person visits will be chosen by the Study Site based on the participant's preference, logistic and financial considerations, and site's qualifications. Study personnel will discuss study requirements with the remote site health providers and operators and will provided with written instructions on how to carry out the procedures that will be conducted at their locations and report the results to the Study Site.

Remote Biospecimen Collections

- Local sites for Remote Biospecimen Collections will be chosen by the Study Site based on the participant's preference, logistic and financial considerations, and site's qualifications.
- Specific instructions will be provided for presentation to the local sites for collection, handling and tube labeling for specimens requiring shipment to the Study Site or Central Laboratory. Pre-addressed shipping containers will also be provided along with an inventory sheet for faxing to the Study Site or Central Laboratory and inclusion with the shipment.
- Blood samples for local lab tests (serum creatinine, K, and ALT, CBC, pregnancy tests) will be processed and analyzed at the facilities where samples are collected or shipped to commercial laboratories or to the Central Laboratory for testing. Results will be transmitted to the Study Site by fax or other secure methods.
- Blood and urine samples for central lab tests (serum creatinine, Cystatin C, uric acid, HbA1c, urinary ACR and AER) will be mailed to the Central Lab or to the Study Site where they will be processed, aliquoted, and forwarded to the Central Lab. Blood tubes and urine containers will be provided by the Study Site.

5. SUBJECT SELECTION

5.1. Inclusion Criteria

1. Male or female T1D patients between 18 and 70 years of age, inclusive.
2. T1D continuously treated with insulin within one year from diagnosis. If the onset was after age 35, documentation of the presence of one or more of the following will also be required:

   a. documentation of the presence of circulating T1D-associated autoantibodies at diagnosis or at any other time
   b. history of hospitalization for DKA
   c. plasma C-peptide below the limit of detection with standard assay (with concurrent blood glucose >100 mg/dl)
3. Duration of T1D ≥ 8 years;
4. History or presence of microalbuminuria or moderate macroalbuminuria, or evidence of declining kidney function regardless of history or presence of albuminuria and/or RAS Blockage. Micro- or moderate macroalbuminuria will be defined as at least two out of three consecutive urinary albumin excretion rates [AERs] or albumin creatinine ratios [ACRs] taken during the two years before screening or at screening in the 30-5000 mg/24 hr (20-3333 μg/min) or 30-5000 mg/g range, respectively, if not on RASB agents, or in the 18-5000 mg/24 hr (12-3333 μg/min) or 18-5000 mg/g range, respectively, if on RASB. Evidence of declining kidney function will be defined as an eGFR (CKD-EPI) decline ≥3.0 ml/min/1.73 m^2/year, estimated from all the available creatinine measurements (including the one at screening assessment) from the previous 3 years. If at least 3 serum creatinine measures are not available in the previous 3 years, then the slope can be derived from creatinine values from the previous 5 years.
5. Estimated GFR (eGFR) based on serum creatinine between 40 and 99.9 ml/min/1.73 m^2 at screening. The upper and the lower limits should be decreased by 1 ml/min/1.73 m^2 for each year over age 60 (with a lower limit of 35 ml/min/1.73m^2) and by 10 ml/min/1.73 m^2 for strict vegans.
6. Serum UA ≥ 4.5 mg/dl at the screening visit.
7. Willing to comply with schedule of events and protocol requirements, including written informed consent.
8. Valid baseline (Visit 4) iohexol GFR measurement prior to randomization.

   OR
9. Being an active participant in the PERL Pilot Study.

5.2. Exclusion Criteria

1. History of gout requiring allopurinol therapy or xanthinuria or other indications for uric acid lowering therapy such as cancer chemotherapy or extremely high serum uric acid values (>12 mg/dl).
2. Recurrent renal calculi (history of more than one episode).
3. Use of urate-lowering agents within 2 months before screening.
4. Current use of azathioprine, 6-mercaptopurine, didanosine, warfarin, tamoxifen, amoxicillin/ampicillin, or other drugs interacting with allopurinol.
5. Known allergy to xanthine-oxidase inhibitors or iodine containing substances.
6. HLA B*58:01 genotype (determined prior to randomization) indicating increased risk of Stevens-Johnson syndrome in response to allopurinol.
7. Renal transplant.
8. Non-diabetic kidney disease as indicated by medical history and/or laboratory findings.
9. SBP>160 or DBP >100 mmHg at screening or SBP>150 or DBP>95 mmHg at the end of the run-in period.

10. Cancer treatment (excluding non-melanoma skin cancer treated by excision) within two years before screening.
11. History of clinically significant hepatic disease including hepatitis B or C and/or ALT (SGPT) >2.50 x ULN at screening and/or history of HBV/HCV antibody positivity.
12. History of acquired immune deficiency syndrome or human immunodeficiency virus (HIV) infection.
13. Hemoglobin concentration <11 g/dL (males), <10 g/dL (females) at screening.
14. Platelet count <100,000/mm3 at screening.
15. Ongoing alcohol or drug abuse or history of treatment for these conditions in the past 6 months.
16. Blood donation in the 3 months before screening (subjects become eligible once 3 months have elapsed since the last donation).
17. Breastfeeding or pregnancy or unwillingness to be on contraception if still fertile.
18. Poor mental function or any other reason to expect patient difficulty in complying with the requirements of the study.
19. Serious pre-existing medical problems other than diabetes, e.g. congestive heart failure, pulmonary insufficiency.

5.3. Prohibited Medications and Restrictions

- Allopurinol and other urate lowering agents (e.g., probenecid, rasburicase rys) for the treatment of gout. Patients treated with uric acid lowering agents for elevated uric acid levels with no history of gout can, with the agreement of their treating physician, undergo a 2 month washout of uric acid lowering medication and then be tested to determine if uric acid entry criteria are met.
- Herbal supplements that may have urate lowering actions (e.g., Devil's Claw or Harpagophytum procumbens, Indigenous cinnamon or Cinnamomum osmophloeum, Skunkvine or Paederia scandens or Paederia foetida)
- Azathioprine
- 6-Mercaptopurine
- Didanosine
- Warfarin
- Tamoxifen
- Amoxicillin/ampicillin
- Any other drug for which there is evidence of interaction with allopurinol
- Dual RASB therapy (i.e., another RASB medication in addition to that already in use)
- Non-RASB antihypertensives that are not listed in the PERL approved menu of antihypertensive drugs, unless these were in use before joining the study.

5.4. Randomization Procedures

After the run-in period (described in Section 8.3) and with a valid baseline iohexol GFR measurement prior to randomization, participants will be randomized in a 1 to 1 ratio to receive either oral allopurinol or placebo. Randomization will be stratified by center, uric acid (≤6.0 vs. >6.0 mg/dl), and HbA1c (≤7.8 vs. >7.8%). Randomization will be performed using permuted blocks, with a block size that is known only to the DCC. After a participant has been randomized, the clinical site will send a study medication request to the research pharmacy, including the participant's address, so that the study medication can be directly mailed to the participant. Clinical sites will not have access to the treatment

assignment (see 6.2., Blinding Procedures). This will be directly communicated or made electronically available to the pharmacy by the DCC.

5.5. Discontinuation of study drug

5.5.1. Reasons for discontinuation

The study drug will be *temporarily* discontinued if a participant:

- Has clinically significant persistent changes from baseline based on laboratory safety assessment results (the response to discontinuation will be monitored to assess whether the drug can be re-instituted, see next paragraph on permanent discontinuations).
- Requires treatment with allopurinol or medications that make allopurinol contraindicated (see 5.5.2 and 9.5).
- Becomes pregnant or breastfeeding (see 5.5.2)

Whenever the reason for temporary discontinuation of the study drug ceases to exist, the study medication will be resumed with the consensus of the drug monitoring committee, according to the following procedures:

- If the study medication was discontinued because of a suspected drug reaction or the participant was off-medication for 3 months or longer, the study drug will be re-started at a dosage of 100 mg for 4 weeks, which will then be increased to the full dosage appropriate for the eGFR. (see 6.1.2)
- If the study medication was not discontinued because of a drug reaction and the participant was off-medication for less than 3 months, the study medication will be re-started, at the full dosage appropriate for the eGFR.

The study drug will be *permanently* discontinued if a participant:

- Experiences an SAE related to the study drug or an intolerable AE such as a persistent allergy or rash.
- Has clinically significant persistent changes from baseline based on laboratory safety assessment results which do not respond to temporary 2-week discontinuation of study drug and re-institution of drug at ½ of the initial dose.
- Develops end-stage renal disease (confirmed eGFR ≤15 ml/min/1.73 m^2 in the absence of acute kidney injury [AKI], institution of chronic dialysis treatment or kidney transplantation) or iGFR decreases by 50% from one measurement to the next or serum creatinine levels double over any 12 month interval in the post-randomization period. If any of these renal function changes prove to be temporary, the study medication could be resumed as described above with the consensus of the drug monitoring committee.

5.5.2. Handling of study drug discontinuation

- Date and reason for drug discontinuation will be recorded on the relevant Case Report Form.
- All study discontinuations decided by a clinical site will have to be reviewed and approved by the Drug Monitoring Committee within 10 days from their start.
- If the study drug is discontinued due to treatment with medications that make allopurinol contraindicated (e.g. amoxicillin/ampicillin) or due to pregnancy/breastfeeding, the possibility of resuming the study drug will be evaluated by the Drug Monitoring Committee once those medications have been discontinued or pregnancy/breastfeeding has ended.

- If the study drug is temporarily discontinued and then re-instated, the end-date of the intervention will remain the same as if the study drug had not been discontinued. All visits will be carried out as scheduled while the study drug is temporarily discontinued.
- Unless a participant withdraws consent all participants that are permanently discontinued from study drug or who discontinue study medication on their own will be followed for the full study period (i.e., 164 weeks, including the washout period) and all data will be collected as scheduled.
- If a participant reaches ESRD as defined above under 5.5.1,, he/she will be permanently discontinued from the study and invited to participate in a study close-out call or visit to be held within three months from the occurrence of ESRD. Data collection at this call or visit will be limited to standard adverse event reporting. In addition, sites should continue to contact participants who have reached end-stage renal disease to determine their final status until 3 years and 2 months after randomization. Major attempts will be made to schedule an end-of-study assessment for all participants who are lost to follow-up during the course of the study.

5.5.3. Replacements

Participants that withdraw consent from the study during the Run-in period (i.e., before randomization) or do not qualify for study continuation at the end of the Run-in period will be replaced until the target number of randomized study participants is reached. Participants that withdraw consent from the study or discontinue the study drug after randomization will not be replaced.

5.5.4. Termination of Study

Premature termination of this clinical trial may occur because of a regulatory authority decision, drug safety problems as determined by the Data Safety Monitoring Board (DSMB), or at the discretion of the funding agency (NIDDK).

6. STUDY TREATMENTS

6.1. Study Drug Description, Dosage, Administration, and Accountability

6.1.1. Description

Eligible study subjects who agree to participate in the study will all be randomized to receive placebo or allopurinol – a serum UA lowering medication that has been on the market since 1964 as the main drug for the therapy of symptomatic hyperuricemia and for the prophylaxis of gout in cancer patients receiving chemotherapy. Allopurinol is an inhibitor of xanthine oxidase, which is responsible for the conversion of hypoxanthine to xanthine and of xanthine to UA. It is metabolized to the corresponding xanthine analogue, oxypurinol (alloxanthine), which is also an inhibitor of xanthine oxidase. At the average dosage (300 mg/day), allopurinol causes a 30-40% reduction in serum UA[24-26], but up to a 60% reduction can be obtained using the maximum dosage of 600 mg.[27] While allopurinol is mostly used in individuals with gout and very high UA levels, several studies have shown that it is also effective at lower UA levels[27-29].

Because of its rapid oxidation to its active metabolite oxypurinol, allopurinol has a short plasma half-life (~1-2 hrs). However, since oxypurinol has a longer half-life (~15 hrs), effective xanthine oxidase inhibition can be maintained over 24 hrs with a single daily dose of allopurinol. Since both allopurinol and oxypurinol are eliminated through the kidneys, patients with impaired renal function require lower doses than those with normal renal function. A common rule of thumb is to use 75% of the dosage in individuals with eGFR in the 50-90 ml/min range, and 50% of the dosage in individuals in the 10-50 ml/min range.

6.1.2. Dosage

After an initial four weeks where all participants randomized to allopurinol will take 100 mg per day, the allopurinol dosage will vary from 200 to 400 mg per day based on eGFR levels. Participants will take 400 mg per day if their eGFR is ≥50 ml/min/1.73 m^2, 300 mg per day if their eGFR is in the 25 to <50 ml/min/1.73 m^2 range, and 200 mg per day if the eGFR is in the 15 to <25 ml/min/1.73 m^2 range. Allopurinol will be continued at this dosage throughout the study unless the eGFR changes, in which case the dosage will be modified to that appropriate for the new eGFR class.

All participants, whether they are randomized to allopurinol or placebo, will be given four tablets per day to be taken orally following breakfast. Tablets will be provided in four vials (A, B, C, and D) or in blister packs, in which each blister contains the four tablets for a given day. If the medication is provided in bottles, participants randomized to allopurinol will receive a dosage of 100 mg as a 100 mg tablet (from vial A) plus three placebo tablets (from vials B, C, D), 200 mg as two 100 mg (from vials A and C) and two placebo tablets (from vials B and D), 300 mg as three 100 mg (from vials A, B, C) and one placebo tablet (from vial D), 400 mg as four 100 mg tablets (from vials A, B, C, D). Subjects randomized to placebo will be given four placebo tablets (from vials A, B, C, D). If the medication is provided in blister packs, each blister will contain the four tablets for a given day, with the same proportion of active and placebo tablets described above for each allopurinol dosage and for placebo.

The dose adjustment will be carried out as follows:

1. At each follow-up visit, a study drug requisition will be sent by the clinical site to the research pharmacy indicating the study ID, name, and address of the participant, the most recent eGFR value (CKD-EPI), calculated using a recent local lab creatinine value, and the number of days to be covered by the drug supply.
2. At the pharmacy, a clinical pharmacist will determine the allopurinol dose (ranging from 0 to 400 mg) that should be given at that time according to the study protocol given the participant's treatment assignment and the most recent eGFR value (CKD-EPI) calculated using a recent local lab serum creatinine value.
3. The research pharmacy will mail the new batch of study medication directly to the study participant.
4. At some sites the study medication may be dispensed directly to the study participant at a relevant in person study visit or by mail from the site following a relevant in-person or phone study visit.
5. Participants will be instructed to immediately inform the clinical site upon receipt of the new tablets and mail the pill bottles or blister packs with the tablets remaining from the previous prescription in a provided pre-addressed mailer, to the clinical site for drug accounting and compliance assessment.

6.1.3. Compliance and accountability

Skills will be taught and reinforced at each visit with regard to scheduling and administration of pills at home and while traveling. Methods (e.g. record-keeping) will be taught to help participants monitor tablet usage and enhance compliance. To complement the regular compliance interventions at the scheduled visits, study information and motivational materials (postcards, newsletters, etc.) will be mailed. In addition, at midpoint between clinic visits, participants will be phoned by the clinic staff to review pill-taking. Patients will be provided with random but known numbers of excess medications, providing extras in case of pill loss. Adherence will be monitored by instructing participants to expect extra pills and to mail the pill bottles or the blister packs with the tablets remaining from the previous prescription to the study center upon receipt of a new batch of tablets. The number of extra pills included in each supply of medications will be decided by the pharmacist, who will keep a record of it and will transmit this information to the Study Site. Personnel at the Study Site will enter this

information in the appropriate electronic Case Report Form along with the expected number of pills used during the period covered by the supply and the number of unused pills returned by the participant. These data will be used to analyze compliance. If poor adherence is noticed, measures will be taken to increase compliance, such as explaining the purpose of the study again, providing pill reminders, and more frequently contacting the study subject by phone. Participants at each visit will be asked about their perceived compliance and about any difficulties with taking the study medications, but the individualized strategies to improve compliance will not be openly linked to the pill counts, i.e. participants will not be informed of the results of pill counting. Participants showing poor compliance will not be withdrawn from the study.

6.2. Blinding Procedures

Study participants, the investigators and research staff at the Clinical Sites, and the PERL co-PIs (Drs. Doria and Mauer) will be blinded to treatment assignment whereas the Data Coordinating Center Co-Directors and staff and the pharmacy personnel will have access to this information. Serum uric acid values, from which the treatment assignment might be inferred, will not be transmitted to the Clinical Sites by the central or local laboratory and will not be available for viewing in the study database. Should unblinding of a study participant be necessary because of an emergency, the site personnel will login to the password-protected electronic database application that will provide the treatment assignment. Audit procedures will ensure that the name of the individual associated with the login will be communicated to the Data Coordinating Center project manager and Co-Directors. As an additional safety measure, the personnel at the Clinical Sites will be provided with telephone numbers to contact the Data Coordinating Center and/or Pharmacy personnel having access to the treatment assignment on a 24-7 basis. If unblinding occurs, the circumstances that led to it will be reviewed and reported.

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]

7.1.1. iGFR quality assurance

It is of the foremost importance that reliable iGFR measurements are obtained. To maximize accuracy and precision, the following procedure will be in place.

1. Personnel performing the iohexol clearance test will undergo a standardized training program administered under the Site Directors' supervision through in-person meetings or on-line modules. All clinical site staff will complete the online knowledge testing in order to perform the tests.
2. Participants will be instructed to discontinue non-steroidal anti-inflammatory drugs (NSAIDs) for at least 3 days and avoid large protein meals for one day prior to the test, since these

could influence GFR. They will also be instructed to aim for a fasting glycemia between 90 and 160 mg/dl on the day of the test. Before the test, participants will have a light breakfast at the clinic along with their morning insulin. The insulin dose will be adjusted to keep their blood glucose in the 90-160 mg/dl range. If blood glucose is outside this range right before or during the test, small amounts of intravenous insulin (if blood glucose is too high) or orange juice/milk (if glucose is too low) may be administered to bring blood glucose levels within the desired interval.

3. In the case of extreme deviations from the target blood glucose values, the test may be rescheduled to another day (within 2 weeks). The test will also be postponed in the case of recent febrile illness, diarrhea or vomiting, dehydration, poor fluid intake, recent intake of nephrotoxic drugs such as NSAIDS, urinary tract infection, or a positive pregnancy test.

7.1.2. iGFR quality control

The quality of iGFR results will be monitored by:

1. Systematically checking for deviations from the study protocol, such as deviations from the target blood draws time points during the test or the presence of medical conditions that should have prompted a postponement of the test.
2. Calculating the R-square (R2) of the regression between log-iohexol values and time. iGFR tests will be defined as technically acceptable if the R2 is >0.90. R2 calculations will be performed by the DCC using all 5 time points of the iGFR test.

7.1.3. Technically unacceptable iGFR measures

If an iGFR test is deemed to be technically unacceptable according to the above QC criterion ($R^2 \leq 0.90$), or a study protocol deviation is suspected, the following procedures will be followed:

1. Source documents related to the test in question will be reviewed to verify whether there was a protocol deviation or a technical error in the iGFR procedure (e.g., presence of contraindications to iGFR, swapping of tubes, wrong collection times, typos, etc.). In the case of an R2≤0.90, the iohexol measurements will be repeated by the central laboratory.
2. If a technical error is found and the error can be rectified, or the new laboratory measures yield an R2>0.90, the iGFR value will be recalculated after the appropriate corrections are made. The study site or the central laboratory, as applicable, will be alerted about the error and measures aimed at improving iGFR quality will be implemented.
3. If the error is confirmed and cannot be fixed, or no error can be found, the iGFR will be dropped and will be repeated within 4 weeks from when the iGFR results become available.
4. If the repeated test is technically unacceptable, or the test cannot be repeated within 4 weeks for logistical reasons, the iGFR value at that time point will be considered as missing for the analysis of the primary outcome. It is therefore critical that every effort be made to obtain this repeat iGFR measure.

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.

8. STUDY PROCEDURES

8.1. Schedule of Events

The schedule of events that will take place in the proposed study is outlined in Figure 1. Visits will be frequent during the Run-In period and during the first 30 days after randomization in order to escalate the allopurinol dosage and closely monitor the occurrence of AEs. After that, participants will be seen every 3-4 months to monitor their UA levels, renal function, occurrence of AEs, and medication compliance and, if necessary, to perform interventions to improve compliance. 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. The study windows that define when study visits may occur are noted in Figure 1 and differ by type of visit. Visits 2, 3, and 6 will be carried out within 6 business days (before or after) from their scheduled dates; visits 11, 16, and 17 within 2 weeks before and 4 weeks after their scheduled dates; visit 4A (if necessary) within 1 week before and 3 weeks after its scheduled date, and all other visits within 2 weeks (before or after) from their schedule dates. Additional blood or urine samples may be required in between visits if clinically significant changes are observed in blood or urine measurements that need to be confirmed or otherwise monitored. iGFR measurements may be repeated for medical reasons or technical problems (see 7.1). Safety laboratory tests (CBC, serum creatinine, K^+, ALT, and pregnancy tests in women) will be performed by local laboratories. Outcome variables (plasma iohexol, serum creatinine and cystatin C, urinary AER), HbA1c, and serum uric acid will be measured by the Central Laboratory at the University of Minnesota, directed by Dr. Amy Karger.

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.

8.2. Screening and Enrollment in the Run-in Period (Visit 1)

Subjects who have a confirmed history of micro- or macroalbuminuria (at least two out of three consecutive urinary AER or ACR in micro- or macroalbuminuria range as defined in Section 5.1) will not need to bring a sample of urine to Visit 1. Subjects who have incomplete or no previous evidence of micro- or macroalbuminuria or have unknown albuminuria status, will be mailed two containers before Visit 1 along with instructions for collecting two samples of urine from their first morning void and bringing it to the visit to confirm the presence of micro- or macro-albuminuria. During **Visit 1**, subjects will undergo the following procedures:

- Obtain written informed consent.
- Collect prior and concomitant medications, and demographic information.
- Measure weight and height.
- Measure vital signs.
- Perform pregnancy test in women of childbearing potential.
- Review the fetal risks of RAS blockade.
- Collect samples for clinical laboratory assessment.
- Provide a container and instructions for an overnight urine collection to be made immediately before Visit 3 if subject qualifies for the study.
- Upon receipt of laboratory measurements, confirm that inclusion/exclusion criteria are met.

The Screening Visit can be repeated after 4 weeks if the circumstances that led to the exclusion of a participant are deemed to have possibly changed.

Patients on losartan with uric acid levels between 4.2-4.4mg/dl at initial screening may, with the agreement of the patient and their PCP, be switched to another angiotensin receptor blocker (ARB) and have their uric acid level rechecked in one month.

8.3. Run-in Period (Visits 2, 3, and 4)

Starting at **Visit 2**, eligible subjects who agree to participate in the study will enter a run-in period of 9 weeks (see note at the end of this section for exceptions to this duration). During this visit, subjects will undergo the following procedures:

- Obtain written informed consent to enter run-in period (if the consent at V1 was only for screening).
- Review the fetal risks of RAS blockade.
- Collect medical history.
- Perform ECG.
- Collect concomitant medications.
- Measure weight and height.
- Measure vital signs.
- Physical Examination
- Obtain a urine pregnancy test

RAS antagonist treatment will be standardized, and BP, if elevated (>140/90 mm Hg), normalized. Letters will be written to the participants' physicians informing them about the study and notifying them of the study's protocol RAS blocker requirements and blood pressure goals. The letter will propose active participation of the participants' physicians in blood pressure management with the

availability of advice from the PERL site physicians and, if needed, the PERL Drug monitoring Committee for out of range blood pressure values during the course of the study. The run-in period will start at **Visit 2**. If a participant is already on a RAS Blocker, its dose will be increased, if necessary, to make it at least equivalent to ramipril 10 mg (if on ACE inhibitor [ACEI]) or irbesartan 300 mg (if on an angiotensin receptor blocker [ARB]), if acceptable to the patient's primary physician, if tolerated and if not contraindicated (see below). Participants who were not taking a RAS Blocker will be prescribed and instructed to start taking 10 mg of ramipril daily or 300 mg of irbesartan daily (if ramipril is contraindicated or has side effects) or another ACE inhibitor or ARB at equivalent doses if there are impediments to the use of ramipril or irbesartan. Participants who have contraindications to RAS blockers (e.g., SBP<100 mmHg, K^+>5.5 mEq) or do not have evidence or history of micro- or macroalbuminuria (as defined in 5.1.4), are normotensive, and are not being treated with RASB or other anti-hypertensive agents will not be treated with these drugs, as this represents the standard of care.

Participants who are placed for the first time on RAS blockers as part of this study will start with half a dose; if there are no side effects, this will be increased to a full dose at Visit 3 and their serum K^+ and creatinine measured at a local laboratory after 2 weeks. RAS blockers will be immediately discontinued in the case of allergic reactions or angioedema or the suspicion of pregnancy. If pregnancy is confirmed, the patient will remain off RAS blockade until the pregnancy and breast-feeding are completed. Their dose will be decreased to half if symptomatic hypotension (SBP<100%) or intractable cough develops, followed by their discontinuation. For persistent cough with ramipril or other ACEI, irbesartan or another ARB will be prescribed in substitution of the ACEI.

In the case of hyperkalemia (K+ >6.0 mEq) or serum creatinine elevation (>30% increase over baseline values), the participant will be asked to immediately obtain a confirmatory lab value at their local lab or clinical site and then discontinue the RAS blocker while awaiting this confirmatory result. If confirmed, the participant will resume RAS blockade at half dose 72 hours later and will have repeat labs one week later. If the problem persists, RAS blockade will be discontinued for the remainder of the trial and BP managed by alternate drugs (see below). If not confirmed, the participant will resume RAS blockade at their usual dose and have a repeat lab check one week later. These same steps will be taken if hyperkalemia develops during the trial.

Participants will continue to take any other antihypertensive drug that they may have been taking before study entry. Participants will be provided with a blood pressure monitoring device (if they do not already have access to one), will be trained on its use, and will be instructed to periodically monitor their blood pressure at home and to record the results into a BP diary, and to communicate them to study personnel if values are abnormal.

If hypotension develops (SBP<100 or significant lightheadedness), the dosage of non-RAS antagonist antihypertensive drugs will be progressively reduced until discontinuation, followed by a reduction of RAS blockers to half the dose and their discontinuation if the problem persists. If BP is found to be elevated (>140/90 mm Hg) on three consecutive occasions, the dosage of existing non-RAS antagonists antihypertensive drugs will be maximized, followed, if necessary, by the introduction of antihypertensive drugs of a different class. These will be chosen in collaboration with the other health care providers that are involved in managing the participant's anti-hypertensive therapy[33]. If the goal of BP ≤140/90 is not achieved with these drugs, a Drug Monitoring Committee conference call will be convened to consider the possibility of causes of hypertension other than diabetic nephropathy and discuss alternative therapeutic approaches. BP will continue to be monitored and the anti-hypertensive therapy to be adjusted in a similar way throughout the study.

After 2 weeks of run-in, participants will come in for **Visit 3** during which they will undergo the following procedures:

- Obtain interval medical history (with special emphasis on CVD events).
- Review concomitant medications and AEs

- Review RASB and BP therapy.
- Collect samples for clinical laboratory assessments as outlined in Figure 1.
- Perform pregnancy test in women of childbearing potential.
- Be provided with a container and instructions for an overnight urine collection to be made immediately before Visit 4.

After 6 weeks of run-in, participants will come in for **Visit 4** during which they will undergo the following procedures:

- Obtain interval medical history (with special emphasis on CVD events).
- Conduct a physical exam (if deemed to be required by the study physician)
- Review concomitant medications and AEs
- Review BP therapy.
- Review the fetal risks of RAS blockade.
- Measure height, weight and vital signs.
- Perform ECG.
- Collect samples for clinical laboratory assessments (including HLA B*58:01) as outlined in Figure 1.
- Perform pregnancy test in women of childbearing potential.
- Measure iohexol GFR.

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. Participants whose SBP is >150 or whose DBP is >95 mmHg at the end of the run-in period will be discontinued from the study (prior to randomization).

IMPORTANT: Visit 2 and Visit 3 can be skipped, i.e., a participant can move directly from Visit 1 to Visit 4, if the following criteria are met at Visit 1:

1. The participant is eligible based on the results of Visit 1 assessments, including laboratory values;
2. Blood pressure is <140/90 mmHg;
   AND
3. The participant meets one of the following criteria:
   - Has been treated with a RASB for at least two months at a dose at least equivalent to Ramipril 10 mg or Irbesartan 300 mg;
   - Has contraindications to RASB;
   - Does not have evidence or history of micro- or macroalbuminuria (as defined in 5.1.4) and is not being treated with RASB or other anti-hypertensive agents.

If the above criteria are met and Visits 2 and 3 are skipped, Visit 4 will be scheduled 3 weeks after Visit 1 with a window of 2 weeks before and 3 weeks after the target date. The collection of medical history and the physical exam scheduled at Visit 2 will be conducted at Visit 4.

8.4. Enrollment in the Study and Randomization (Visit 5)

- At the end of the run-in period, eligibility will be re-assessed based on the BP measures obtained at Visits 4 or 4A (if applicable), HLA-based genetic susceptibility to allopurinol skin reactions[36,37] (tested at Visit 4)and a valid baseline iGFR measurement. Participants who are eligible for randomization based on those measures (SBP ≤ 150 and DBP ≤95 mmHg) and a negative HLA B*58:01 test will be telephoned by the study coordinator to discuss how the

study medication should be taken and its potential side effects.

- Immediately after the phone call, the participants will be randomized.
- Immediately after randomization the first batch of study medication will be mailed to the participant by the research pharmacy along with written instructions on how to take it. Participants will be instructed to notify the study personnel by phone and start taking the study medication as soon as they receive it.
- If the participant is positive for HLA-based genetic susceptibility to allopurinol skin reactions, or acceptable BP measurements, or a valid iGFR measurement cannot be obtained, he/she will be discontinued from the study prior to randomization.

8.5. Treatment Period (Visits 6 to 15)

During the treatment period, the following procedures will be completed at each visit for each participant:

- Obtain interval medical history (with special emphasis on BP control and CVD events).
- Review of concomitant medications and AEs.
- Review RASB and BP therapy.
- Measure height, weight, and vital signs according to the schedule outlined in Figure 1
- Inspect for skin rash.
- Conduct a physical exam (Visit 11).
- Perform ECG according to the schedule outlined in Figure 1 (Visit 11).
- Collect samples for clinical laboratory assessments and for storage of serum, plasma and urine for later biomarker research according to the schedule outlined in Figure 1.
- Perform pregnancy test in women of childbearing potential.
- Measure GFR by means of plasma disappearance of non-radioactive iohexol, iGFR at Visit 11.
- Provide a container and instructions for an overnight urine collection whenever an AER measurement is scheduled at the following visit.

In the days immediately after each visit, upon completion of serum creatinine measurements, participants will receive a new batch of study medication by mail from the research pharmacy. Upon receipt of the new tablets, participants will be instructed to immediately mail the pill bottles or the blister packs with the tablets remaining from the previous prescription to the study center for drug accounting and compliance assessment (see 6.1.2). A pre-stamped and addressed envelope will be provided to participants for this purpose.

At some sites the study medication may be dispensed directly to the study participant at a relevant in person study visit or by mail from the site following a relevant in-person or phone study visit.

8.6. End of Intervention (Visit 16)

At the end of the treatment period (Visit 16), the following procedures will be completed for each participant:

- Obtain interval medical history (with special emphasis on CVD events).
- Review of concomitant medications and AEs.
- Review RASB and BP therapy.
- Collect unused study medication and document compliance.

- Measure height, weight and vital signs.
- Inspect for skin rash.
- Conduct a physical exam.
- Perform ECG.
- Collect samples for clinical laboratory assessments as outlined in Figure 1 and for storage for later biomarker research.
- Perform pregnancy test in women of childbearing potential.
- Measure iGFR.
- Provide containers and instructions for 2 overnight urine collections to be made immediately before Visit 17.

Participants will be instructed to stop taking the study medication and to mail the pill bottles or the blister packs with the tablets remaining from the last prescription to the study center if they did not already bring the unused study medication at the visit. The RAS and BP therapy will be continued as before until the closing visit (Visit 17). The importance of coming back in 8 weeks for the closing visit (Visit 17) will be emphasized.

8.7. End of Wash-out Period (Visit 17)

After the end of the treatment period, participants will enter an 8-week wash-out period at the end of which the following procedures will be completed:

- Obtain medical history.
- Review of concomitant medications and AEs.
- Measure height and weight and vital signs.
- Inspect for skin rash.
- Collect samples for clinical laboratory assessments as outlined in Figure 1 and for storage for later biomarker research.
- Perform pregnancy test in women of childbearing potential.
- Measure iGFR.

8.8. RAS blocking and anti-hypertensive therapy after completion of the study

When the participant completes the study, control of the RAS-blocking and anti-hypertensive therapy will be relinquished to the participants' physicians, who will decide whether or not to continue the therapy established during the study. Participants will continue the anti-hypertensive therapy established during the study until they see their physicians.

8.9. Future biomarker studies

Plasma, serum, and urine specimens and DNA will be stored the Advanced Research and Diagnostics Laboratory at the University of Minnesota and the NIDDK Central Repository for possible future studies of biomarkers of kidney disease in diabetes or other diabetic complications. Twelve ml of plasma, 12 mL of serum, and 24 ml of urine will be collected at Visit 4, 11, 16, and 17, with one quarter of the aliquots of each stored at the University of Minnesota and three quarters of the aliquots sent to the NIDDK Central Repository for storage. Ten ml of whole blood will be obtained at Visit 3. This will be used for white blood cell DNA extraction and subsequent storage. Altogether, the stored plasma and serum aliquots will correspond to about 210 ml of blood collected for this purpose over the entire duration of the study. Participants will be allowed to elect to participate in the study while not having any or one or more of these samples stored, if they so choose.

8.10. Early Withdrawal

Unless the participant withdraws consent, all randomized participants will be followed for the full study period (through week 164) and all data will be collected as scheduled.

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 results in criteria for a SAEs, 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 ≥ 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

12. DATA COLLECTION AND QUALITY ASSURANCE

Comprehensive data coordinating center (DCC) functions for this clinical trial, including clinical monitoring, database development, web-based data entry and management, as well as the creation and export of study reports for the DSMB will be provided by the University of Michigan Statistical Analysis of Biomedical and Education Research (SABER) group. Housed in the top nationally ranked Department of Biostatistics, SABER, in its 13-year existence, has served as the DCC for over 50 studies, including multiple NIH-sponsored networks.

The DCC will use OpenClinica® (OpenClinica Clinical Trial Software; OpenClinica, LLC, Waltham, MA), a clinical trial software platform for electronic remote (i.e., site-based entry) data capture and clinical data management, as the basis for our custom-designed data entry and management system. We expect that the majority of data will be collected via Case Report Forms (CRFs); however, other data sources, such as laboratory data from the central laboratory, may be used. In these circumstances, the DCC will also utilize electronic data transfer. Protocols for the transfer of data, with careful attention to data integrity, will be written by experienced programmers and stored in the OpenClinica database or data mart.

The DCC has established a set of standard operating procedures (SOPs) governing the processes used to ensure patient privacy and data confidentiality, including the use of anonymous participant IDs on CRFs and in reports. In addition to clinical study databases, the UM DCC has also incorporated MEDdra® [www.meddramsso.com/] and database into our systems to have the capacity to code adverse events and illnesses by body organ system, respectively. OpenClinica® enables compliance with Good Clinical Practice (GCP) and regulatory requirements by providing differentiated user roles and privileges, password and user authentication security, electronic signatures, SSL encryption, and comprehensive auditing to record and monitor access and data changes.

12.1. Case Report Forms

Study information will be collected for each participant by study staff using standardized electronic Case Report Forms (CRFs). CRFs will be developed by the DCC, modeling their formats on the CRFs developed for the RASS clinical trial[33], to which the study group has access through Dr. Mauer. CRFs will not report information about treatment assignment, in order to maintain blinding of study site. Forms will be stored at a secure location at the clinical sites.

12.2. Quality Control and Quality Assurance

DCC staff will prepare data management and clinical monitoring plans. The clinical monitoring plan will detail procedures to assess accuracy of the database relative to source documents, as well as site adherence to regulatory and study procedures. Emphasis will be placed on the process of consenting subjects, compliance with regulatory requirements and study protocol, values of key endpoints, and identification of SAEs that may not have been reported. The data management plan will describe the front-and back-end edit checks, as well as forms tracking procedures, that will be implemented to ensure timely and high-quality data collection. It will also define the periodic reports that will be shared with site coordinators and PIs that summarize site performance. The clinical monitoring and data management procedures will be consistent with the International Conference on Harmonisation (ICH E6) standards for Good Clinical Practice (GCPs).[46]

12.2.1. Clinical monitoring

During trial conduct, the DCC will conduct periodic monitoring visits to ensure that the protocol and GCPs are being followed. The monitors may review source documents to confirm that the data recorded on CRFs is accurate. The investigator and institution will allow DCC monitors direct access to source documents to perform this verification. It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and that sufficient time is devoted to the process.

Clinical monitoring will be conducted through approximately yearly on-site visits by qualified personnel. In the first year, site initiation visits will be conducted to review the protocol, verify that the Site Director and his/her collaborators receive all necessary trial documents for a proper trial conduct, and review the procedures related to CRFs completion and query resolution. Investigators will be instructed about the importance of recording accurate and clean data, avoiding protocol violations, and retaining participants in the study. Follow-up monitoring visits will involve the verification of source documents and reporting of adverse events. Monitors will also verify that an informed consent form is on file for each subject screened, with appropriately dated signatures and all pages present, that all of the inclusion and exclusion criteria were met for each subject enrolled into the study, and that all the withdrawals and dropouts of enrolled participants from the trial are reported and explained in the appropriate CRFs. Accrual and retention rates will also be monitored and if these fall appreciably below the projected levels, attempts will be made to identify the reasons. At the end of each monitoring visit, a clinical monitoring report will be prepared by the DCC Clinical Monitor and sent to the site PI and to a NIDDK representative with recommendations to correct problems and/or improve the trial quality.

12.2.2. Statistical monitoring

Clinical monitoring will be complemented by statistical central monitoring, as described by Venet et al.[47]. Such a statistical approach to central monitoring relies on assessing the clinical data for departures from expected patterns (e.g., baseline variables in our randomized trial should be comparable between treatment arms for each site; visit days should be randomly distributed over the week), and assessment of a greater number of data values than those associated with site performance metrics.

The statistical monitoring plan will be incorporated into the larger data management plan, and will identify the specific descriptive statistics, graphical presentations, and formal hypothesis tests to be performed and at what frequency. Pattern recognition may require a sufficient number of participants followed for a sufficient amount of time in order to be valid.

12.2.3. Laboratory quality monitoring

The Central Laboratory at the U. of Minnesota uses internal quality control methods to assess assay precision and drift as well external quality control (e.g., proficiency testing) to assess accuracy. The laboratory is well versed in both aspects and adheres to rigid performance standards. For all analytes, enough commercial lyophilized control material is typically purchased prior to beginning the study, so that a single control pool lot can be used throughout. Control pool mean and between-day SD's for each analyte are routinely established at a minimum of two different analyte concentrations on a minimum of one hundred different analytical batches. In the unlikely event that a new control pool lot becomes necessary during the study, both the new and old control pool lots will be run for a minimum of 20 days to establish a target mean for the new pool. The target SD's are not changed if the new and old lots of control material are the same "matrix" (e.g., lyophilized human serum). An in-house control pool is also typically prepared from the exact same specimen type (e.g., 0.5-mL aliquots of pooled serum or EDTA-plasma from several individual donors), kept at -70°C, and incorporated as an internal control in all analytical batches throughout the study. This sort of control is very useful since occasionally the commercial pools of lyophilized serum have matrix effects that make assessment of the true accuracy of the given assay difficult to assess. Accuracy is assessed by comparison of external quality control proficiency testing results. For most of the assays, the laboratory participates in either the College of American Pathologists' Surveys Program (uric acid, creatinine, ALT, glycosylated hemoglobin, urine albumin, urine creatinine) or by sample exchange in a survey program with another laboratory (iohexol). The laboratory is CLIA certified.

12.3. Study Record Retention

The source documents will be stored for at least 10 years after the study ends.

12.4. Data and Biosample Archiving in the NIDDK Central Repository

In agreement with NIDDK's policy on the sharing of data from large, NIDDK-sponsored, multi-site studies, the data and biosamples collected in the course of the trial will be archived in anonymized form in the NIDDK Central Repository for future distribution to the scientific community. All samples and data transferred to the Repository will be under the custodianship of the NIDDK, although the study's Steering Committee will have proprietary control of and exclusive access to the sample and data for an agreed-upon period of time before these are made available to the wider scientific community.

13. PROTECTION OF HUMAN SUBJECTS

13.1. Characteristics of the study population

Participants will be patients who have had T1D for 8 years or more and who will be 18-70 years old at entry into the study. We anticipate that there will be approximately an equal number of males and females. There will be no selection criterion based on race, although most patients will be of Western background and European extraction, given the demographics of the cities in which the centers are located and the fact that T1D is 30-40% less common among Blacks and Hispanics than among Whites. Inclusion and exclusion criteria are as noted above. Female patients of child-bearing potential will be included in the study but only if pregnancy is not planned during the time frame of the study. Women who become pregnant during the study will be discontinued from the study medication, if they had already been randomized, and from RAS blockers until pregnancy and breast feeding are complete; iGFR will not be obtained during or for 6 months after pregnancy is completed. Individuals younger than

18 will not be included since kidney complications are rare before this age. Patients will be tested for HLA-based genetic susceptibility to Stevens-Johnson Syndrome and excluded if this is found.

13.2. Sources of research material

1. Specimens on patients obtained specifically for research purposes.
    a. Renal function studies requiring multiple blood specimens drawn from an indwelling IV over 4-6 hours for measurement of glomerular filtration rate at yearly intervals.
    b. Collection of urine for measurement of urinary albumin and creatinine at 3-4 month intervals.
    c. Blood for measurement of serum UA, creatinine, and liver enzymes, and WBC at quarterly intervals.
2. Specimens or measures obtained quarterly as component of routine patient care
    a. HbA1c
    b. Blood pressure
    c. Height and weight
3. Patient and family medical information.

All study participants will be assigned a unique study identifier and in no publications or public presentations will information be available which could identify individual study participants or their families.

13.3. Plans for recruitment of subjects and consent procedures.

Potential participants will be sought (1) from among the patients attending the study centers (including the satellite centers) involved in the study, (2) by placing advertisements at other health care facilities and in newspapers or other media, and (3) by soliciting referrals from other health care providers. At each clinical site, potential candidates will be identified and contacted according to the procedures established by the local IRBs in compliance with local laws protecting patient confidentiality. Invitation letters to patients attending the study centers will clearly offer the possibility to opt out of any further contacts with the study. Patients who agree to participate will be screened by means of a telephone or in-person interview to determine whether exclusion criteria apply. Subjects who respond to advertisements will undergo the same screening interview. Subjects who pass this initial screening will be given or mailed an informed consent form and will be invited to come to the clinic for a screening visit (Visit 1) during which a final eligibility determination will be made on the basis of a detailed medical history and laboratory tests. Written consent will be obtained on that occasion from all subjects undergoing the screening visit after explaining again the purpose and procedures of the study. In the initial contact and again at the time of the screening visit, study subjects will be encouraged to ask questions and they will be reassured that they may withdraw from the study at any time. Written consent will be obtained again at V2 if the consent at V1 was only for the screening procedures.

13.4. Potential Risks

13.4.1. Risks associated with screening procedures and blood tests

After participating in the screening tests and procedures, or after the run-in period, subjects may find out that they are not eligible to participate in the study. In that case, they will be told the reasons for their ineligibility and will be given the results of clinically approved tests such as serum UA, serum creatinine, urinary albumin/creatinine ratio, and ALT. The results of the test for genetically increased risk of allopurinol-induced SJS will also be given to their physician, if the subject agrees with this. Thus, they may learn about as yet unknown health problems such as anemia or liver disease, more advanced kidney disease, or the need for allopurinol avoidance. This and/or the exclusion from the study may

cause psychological distress. The drawing of blood samples may cause some pain and discomfort and hematoma formation at the site of venipuncture. The total amount of blood taken for the entire study will be about 520 mL (16 ml at Visits 1, 3, 6-10, and 12-15; 67 ml at Visits 4, 11, 16, and 17). At the dose used in the study, there are no known risks to the infusion of the substance used for the measurement of renal function other than the very small risk of allergic reactions (<0.5%), diminished by the exclusion of patients with a history of iodine allergy and by having appropriate treatment drugs for allergic reactions on hand.

13.4.2. Risks associated with allopurinol treatment

Allopurinol has been used for several decades for the long-term therapy of symptomatic gout. The risks associated with its use are low and include:

a. Skin rashes, usually pruritic maculopapular skin eruptions, sometimes scaly or exfoliative, are the most commonly reported adverse effect of allopurinol. Skin reactions were observed in the past in up to 3% of treated patients, but more recent data suggest that their frequency is now less than 1% (www.drugs.com/pro/allopurinol.html) perhaps more likely due to changes in the filler compounds rather than the actual drug. Rashes may be followed by more severe hypersensitivity reactions such as exfoliative lesions and the Stevens-Johnson syndrome (erythema multiforme major), which can be fatal. Although such occurrence is very rare, in the order of 1 in 10,000[48], treatment with allopurinol will be immediately discontinued if a rash develops and will not be reinstated. As noted, those with HLA-based genetic susceptibility to allopurinol-related SJS will be screened out. About 0.7% of Whites and 2-3% of African Americans and Asians are carriers of such genetic susceptibility.

b. An increased frequency of acute gout attacks has been reported during the early stages of allopurinol administration, possibly resulting from the mobilization of urates from tissue deposits causing fluctuations in serum UA levels. Early studies estimated the risk of such events to be about 6%, but an analysis of current usage suggests that the risk has now decreased to less than 1% (www.drugs.com/pro/ allopurinol.html). The risk is expected to be even lower in this study population since individuals with a previous history of gout will be excluded and UA levels will be on average lower than in patients usually taking allopurinol for elevated UA levels.

c. Reversible liver damage as well as asymptomatic rises in liver enzymes has been observed in 1-2% of patients taking allopurinol. Some very rare cases of irreversible liver damage have been observed in the context of the Stevens-Johnson syndrome.

d. Bone marrow depression has been reported in patients receiving allopurinol, most of whom received concomitant drugs with the potential for causing this reaction. Bone marrow depression has been rarely observed in patients receiving allopurinol alone.

e. Experience with allopurinol during human pregnancy is limited because women of reproductive age rarely require this treatment. Given this paucity of data, the study will consider it unsafe for the fetus or the mother to receive this drug. Allopurinol has been found in the milk of a mother on this drug and, therefore, will not be taken by nursing mothers.

13.4.3. Risks associated with RAS blocker treatment

Treatment with RAS blockers (either ACE inhibitors such as ramipril or angiotensin receptor blockers such as irbesartan) is currently the standard of care for diabetic individuals who have micro- or macroalbuminuria. The risk associated with the use of these drugs during the trial will not be greater than the risks participants would face outside the trial by being treated with these agents. These risks include allergic reactions, hyperkalemia, hypotension, increased serum creatinine, persistent cough (with ramipril), liver damage, bone marrow depression, and fetal and neonatal morbidity and death when RAS

blockers are taken during pregnancy. The occurrence of these adverse events will be monitored during the trial.

13.5. Procedures for protecting against and minimizing potential risks

a. General

The patients are under constant medical supervision. They are told that the data which are collected will be used for scientific report, but they will not be identified in such reports.

b. Specific

1. Regular pregnancy tests and education regarding fetal risk of the study drug will be provided to female patients of child bearing age.
2. Quarterly measures of liver enzyme and white blood cell count will allow for early detection of liver injury or leucopenia potentially representing drug toxicity.
3. Quarterly measures of serum creatinine will allow titration of allopurinol in relation to kidney function to avoid excessive dosage of the medication.
4. IV's for kidney function studies will be placed by trained skilled clinical research nurses or technicians or by experienced physicians.
5. Blood drawing for laboratory studies will be performed by trained skilled phlebotomy personnel at the respective institutions, thus limiting the risk of discomfort or local hematoma formation.
6. Participants will be advised not to donate blood throughout the time they are in the study.
7. Regarding possible drug toxicity:
    a. To avoid fetal risks from the study drug, patients planning pregnancies will not be included. Sexually active female patients will be instructed to immediately discontinue study drugs and RAS blockers if a menstrual period is missed by more than two weeks and, if found to be pregnant, the study medication and RAS blocker will be discontinued and not resumed until pregnancy and nursing are completed. Pregnancy tests will be done on all women of child-bearing potential at each visit.
    b. Subjects with known allergy to xanthine-oxidase inhibitors will be excluded from the study. Patients will be instructed to immediately report skin reactions and allergic symptoms and to immediately stop the study medication should these occur. Patients will be given antihistamines for symptom relief. A small supply of antihistamines to be used in such an event will be supplied to each patient. Should an allergic reaction or skin rash occur, the study drug will be permanently discontinued.
    c. To minimize the risk of gout attacks, subjects with a gout history will be excluded from the study and the allopurinol dosage in those enrolled in the study will be gradually increased over several weeks. Should a gout attack occur, this will be treated with colchicine or anti-inflammatory agents according to current standards of care by study personnel. Study uric acid levels <2.0 mg/dl will be flagged by the DCC and reported to the appropriate study pharmacist who will initiate a 50% dose reduction in study drug at the next quarterly visit. In order to avoid gout attacks, if uric acid levels exceed 12 mg/dl this will be flagged by the DCC and the center informed and open-label allopurinol will be started and titrated with the goal of bringing and keeping serum uric acid below 7.0 mg/dl. Participants will continue to be followed according to the study protocol and will be analyzed according to their blinded treatment groups.
    d. Primary care physicians will be notified (with the participants' permission) of the patients' participation in the trial, so that they avoid the prescription of drugs interacting with allopurinol or notify the study personnel that treatment with such drugs is necessary.

e. Participants will be reminded at each visit to immediately to notify the study personnel if they start a new drug, so that possible interactions with allopurinol can be identified at once and appropriate precautions can be taken including discontinuation of the study drug.

f. Subjects taking drugs known to interact with allopurinol in causing bone marrow depression will be excluded from the study. White blood cell counts will be done before the study drugs are prescribed, and quarterly thereafter. The study drug should be temporarily discontinued should evidence of bone marrow depression (WBC<3500/mm^3) be present and confirmed. WBC should be repeated two weeks after study drug discontinuation. If WBC recovers, consider re-challenging and repeating WBC two weeks after drug re-introduction. In addition, if WBC is confirmed to be <2500/mm^3 and/or ANC is <1000/mm^3, the event also needs to be reported as an AE. The Drug Monitoring Committee will review each case and decide whether a referral to a hematologist is warranted and whether study treatment can be reinstated after blood values have returned to normal. If drugs potentially causing bone marrow depression in combination with allopurinol are begun after entry into the trial, observations for this side effect will be intensified or, if recommended by the Drug Monitoring Committee, study drug may be interrupted.

g. To minimize the risk of allergic reactions during the iGFR measurement, subjects with a history of iodine allergy will be excluded from the study.

h. To minimize the risk of liver injury, subjects with clinically significant hepatic disease and/or elevated liver enzymes above 2.5 x the upper limit of normal at the screening visit will be excluded from the study. In those subjects that are enrolled in the study, liver enzyme levels will be monitored at each follow-up visit. If levels are abnormal, the measurement will be repeated and if values are confirmed to be elevated the study drug will be discontinued. The Drug Monitoring Committee will review each case and decide whether a referral to a hepatologist is warranted and whether study treatment can be reinstated after enzyme values have returned to normal on the recommendation of a hepatologist.

i. To minimize the impact of blood draws, participants with low hemoglobin levels (<11 g/dL in males, <10 g/dl in females) will be excluded. Subjects will be advised not to donate blood while participating in the study and for two months after their participation has ended. If they have just donated blood, their screening for the study will be delayed by 3 months. Hemoglobin levels will be monitored quarterly.

j. Most of the participants will already be on RAS Blockers. For those who were not previously taking these medications, risks will be minimized by not prescribing RAS Blockers to participants who have contraindications to these drugs and by prescribing an ARB whenever ACE inhibitors are contraindicated. If adverse events develop that are deemed to be related to the use of RAS blockers, the dose of these drugs will be decreased, followed by their discontinuation if the problem persists (see 8.2. Run-in period), thus adhering to current standards of care. If receiving discontinuation of RAS blockade becomes necessary, BP will be managed by alternate drugs as described above.

k. Blood pressure will be measured quarterly with the goal of maintaining BP ≤140mmHg systolic and ≤90 mmHg diastolic. If elevated, a recheck will be performed within 2 weeks and if still elevated additional antihypertensive non-RAS blockers will be added in collaboration with the participants' physicians. Failure to achieve satisfactory BP control within 2 months would lead to a case review by the Drug Monitoring Committee.

l. Participants with a decrease in both iGFR (meeting the R^2 criterion described in 7.1.2) and eGFR from one measurement to the following one corresponding to a GFR decline >20% per year will be referred to a nephrologist to investigate the causes of such rapid loss of kidney function. If a decrease of such magnitude is observed for the iGFR but is less than a 20%

decline per year for the eGFR, the iGFR measurement will be repeated. If the >20% per year iGFR decrease is confirmed, the participant will be referred to a nephrologist for further evaluation. In this case, the first iGFR value will be used for the analysis of the primary outcome. If the >20% per year iGFR decrease is not confirmed, the participant will not be referred to a nephrologist. The first iGFR test will be reviewed to verify whether medical conditions that should have prompted a test postponement, such as dehydration or recent use of NSAID (see 7.1.1.3), were present. In that case, the repeated iGFR value will be used for the primary outcome analysis. Otherwise, the first iGFR value will be used.

m. Data monitoring will be performed on a regular basis. Data entry computers will be programmed to flag any parameters outside clinically acceptable ranges.

c. Protection of confidentiality

All data, forms, and specimens will be labeled with each study participant's unique study identifier. All data transferred to the Data Coordinating Center for accumulation in the central database or to the NIDDK Central Repository will identify study participants only with their unique study identifier. Each study center will maintain a file on each study participant that includes personal identifiers, linking name and contact information to the unique study ID. These data will not be entered into the study data management system. Participants' names and addresses will be shared with the Pharmacy along with selected laboratory results (serum creatinine and, if needed, uric acid) for the purpose of adjusting the dosage and mailing the study medication. Identifiers may also be shared with the local laboratories if required by the laboratory ordering procedures. Study participants' files will be kept in secure locations and the clinical center will be responsible for taking every other reasonable measure (those set by the state, the site, and the study) to ensure and maintain record confidentiality and patient privacy. Participants will be given the opportunity to decide whether or not the clinical information gained from the study should be shared with their health care providers. Participants will be made aware that, despite these measures, confidentiality cannot be totally ensured. Each site will adhere as required by law to regulatory oversight by federal and state agencies that have authority over the conduct of clinical research such as the Department of Health and Human Services, the Food and Drug Administration, the National Institutes of Health, the Office of Human Research Protection, the Department of Social Services and the Data Safety Monitoring Board.

d. Risk-benefit ratio

If urate-lowering therapy is demonstrated to be effective in preventing or slowing early GFR decline, the reduction in morbidity and mortality resulting from the prevention or delay of ESRD would have a major impact on the lives of T1D patients as well as on society at large, significantly reducing the human suffering and financial costs associated with this condition. Also, demonstrating a causal link between serum UA and kidney damage in T1D would prompt further research on the molecular mechanisms responsible for this link, which could lead to the development of further interventions to prevent renal disease in T1D. Overall, the risks to study participants are deemed reasonable in relation to the anticipated benefit of identifying an effective therapy for early GFR loss in type 1 diabetes.

13.6. Incentives/remuneration

If allowed by local regulations, participants will be reimbursed for the time and effort associated with participating in this study. Reimbursement amounts will be decided locally. Payments will be made at each visit. Participants with financial hardships deriving, for example, from loss of income or child care costs, may be reimbursed for such costs on a case by case basis. Participants who do not complete the whole study will only be reimbursed for the visits they completed. Transportation costs (e.g., parking or public transportation) may be reimbursed according to local policies. Remote site participant costs associated with travel to a Main Site will be reimbursed according to Federal travel, hotel, and per diem rates (gsa.gov).

13.7. Institutional Review Board

The protocol and informed consent forms and subsequent modifications will be reviewed and approved by the Human Subject Committees at all the centers involved in the study for compliance with applicable standards/regulations.

14. DATA AND SAFETY MONITORING PLAN

The Data and Safety Monitoring Plan for this study includes the following elements:

1. A *Data Safety Monitoring Board (DSMB),* including outside experts in the design and conduct of clinical trials and in diabetic nephropathy, will be established by NIH. The purpose of the DSMB is to assure independent review as to whether study patients are exposed to unreasonable risk because of study participation, and to monitor study progress and integrity The DSMB will receive detailed data from the Data Coordinating Center as frequently as deemed appropriate by the board, including summary tabulations and narratives of adverse events, and will meet periodically with the Study Investigators and the Data Coordinating Center personnel. They will have full access to all data, and their recommendations and input will be given high priority and will be incorporated into the study protocol. To this end, the DSMB will meet separately, "*in camera*" (Closed Sessions), with the Co-Director of the Data Coordinating Center, Dr. Andrzej Galecki, to review all adverse event data in relation to the randomized treatment groups in order to detect any increased frequency of significant adverse events which could be study drug related, and decide whether continuation of the trial is warranted.
2. *IRB monitoring* will be in place from:
    - Joslin Diabetes Center
    - University of Minnesota
    - University of Colorado
    - University of Michigan
    - Northwestern University
    - University of Toronto
    - Albert Einstein University
    - Washington University
    - Steno Diabetes Center
    - University of Calgary
    - University of Alberta
    - Emory University
    - University of Washington
    - University of Texas Southwestern
    - Providence Medical Research
    - BC Diabetes
3. *SAE reporting.*

All adverse events are reported to the DCC by completion of the Adverse Events Form. All SAEs as defined previously will require expedited event notification within 72 hours of occurrence or identification to the DCC. The DCC will promptly notify the study PIs, who may convene a Drug

Monitoring Committee (DMC) conference to acquire further information about the event and take appropriate actions concerning the study medication (see Section 15.1).

An independent physician not involved in the study will serve as the Medical Safety Officer, reviewing all SAEs promptly after being reported in the database by the clinical sites. Based on the clinical site report and any additional input from the DMC, the Medical Safety Officer will prepare a preliminary SAE narrative report (in cases where the SAE is not resolved) for each SAE which will be distributed to the PIs, NIDDK Program Director, DSMB Chair, clinical site director, and appropriate DCC staff. Once the SAE is resolved, a final SAE narrative report is generated by the Medical Safety Officer. This report will be sent to the clinical site PI to review for accuracy and completeness. Following review by the clinical site PI, the Medical Safety Officer will send the final SAE narrative report to the PIs, NIDDK Program Director, DSMB Chair, clinical site director, and appropriate DCC staff. All SAE narrative reports, both preliminary and final, will be reviewed by the DSMB during their regularly scheduled meetings or on an expedited basis as determined by the NIDDK Program Director, who will solicit the input of the Chair of the DSMB as needed. The FDA definitions and requirements for expedited reporting will be used to determine if any individual SAE warrants notification to the FDA and to the IRBs of all participating PERL clinical sites.

The clinical site at which the SAE occurred is responsible for expedited reporting of the SAE to their respective IRB. Each site is responsible to report all AE's to their IRB according to its AE reporting policy and procedures.

On behalf of the NIDDK, the Data Coordinating Center will submit an expedited safety report to the FDA for all serious unexpected suspected adverse reactions (SUSARs). That is, when the SAE is unexpected and may be related to the study drug based on evidence of causality. This report will include information on frequency of similar events along with a narrative of similar events to provide context for the individual report. Copies of the expedited safety report will be provided to the PIs, NIDDK, DSMB, and site investigators.

4. When collecting data on participants, adequate safety levels will be set for flagging test results. When these levels are reached, the Data Coordinating Center will notify the appropriate clinic that an abnormal result has been received. Detailed follow-up procedures will be set in the Manual of Operations that will be followed by the clinic when any abnormal results are received.
5. Monthly conference calls will be scheduled for the Steering Committee (SC) and the Trial Coordinators. Subject participation and compliance will be discussed in detail during these calls. A clinical psychology expert in the behavioral and compliance aspects of clinical trials, Dr. William Robiner from the University of Minnesota, will be included in the Trial Coordinator calls when discussing participant compliance issues.
6. A Drug Monitoring Committee (DMC) consisting of the PERL Center Directors and PIs, a research pharmacist, and the Project Manager will discuss any serious medication related problem that a participant has. Changes in study medication dose, medication discontinuation and medication re-institution will be included in these discussions.
7. Twice a year, the Study Group will meet face-to-face with the Data Coordinating Center personnel for a 1½ day meeting to discuss the study in detail and any problems that may have occurred. The Trial Coordinators will hold a separate ½ day meeting with the Data Coordinating Center prior to the SC meeting and any issues needing discussion will be presented at that time and carried from and to the main Study Group meeting for discussion and resolution.

15. STUDY ADMINISTRATION

15.1. Organization

The major organizational components of the study are:

- The *Study Group* is composed of all investigators and study staff from the Clinical Sites, the Data Coordinating Center, and the Central Laboratory. The Study Group is responsible for the conduct of the study.
- The *Steering Committee* is responsible for the design of the study and provides guidance to its execution. Members are the co-Chairs of the PERL Consortium (Drs. Mauer and Doria), the Directors of the Clinical Sites (Drs. Caramori, Rosas, Polsky, Perkins, Pop-Busui, Molitch, Crandall, Rossing, Sigal, Senior, Umpierrez, De Boer, Lingvay, Tuttle, Aronson and Elliott), the Directors of the Data Coordinating Center (Drs. Galecki and Spino), and the Director of the Central Laboratory (Dr. Karger), the NIH program officers (Drs. Jones and Parsa), and the JDRF program officer (Dr. Pragnell).
- The *Executive Committee* will consist of the two PIs, Drs Doria and Mauer, the DCC leaders, Drs Galecki and Spino, the Project Manager, the Lead Clinical Coordinator, and the NIH officers. The EC will have at least monthly conference calls to discuss the overall conduct of the study and set the agendas for the Clinical Coordinators and Steering Committee conference calls. The EC will be responsible for the overall quality of the study, the setting of broad policy directions, and will address major budgetary issues, including, if necessary, reallocation of funds based on developed parameters of need and performance.
- The *Drug Monitoring Committee* is responsible for the oversight of the study drug administration as well as the RAS blocking and antihypertensive therapy during the trial. Members are Dr. Doria, Dr. Mauer, the PIs of the clinical sites, the Project Manager, the Lead Clinical Coordinator, and a research pharmacist. The participation of one of the PIs and 5 of the 16 Center Directors will be sufficient for making decisions.
- The *Clinical Sites* are located at the Joslin Diabetes Center, the University of Minnesota, the University of Colorado (Barbara Davis Center for Childhood Diabetes), the University of Michigan, Northwestern University, Albert Einstein College of Medicine, Washington University (St. Louis), the University of Toronto, the Steno Diabetes Center (Denmark), the University of Calgary (Calgary, Alberta, Canada), University of Alberta (Edmonton, Alberta, Canada), Emory University, the University of Washington (Seattle), University of Texas Southwestern, Providence Medical Research, and BC Diabetes are responsible for recruiting study participants and implementing the protocol.
- The *Data Coordinating Center (DCC),* based at the University of Michigan, is directed by Drs. Galecki and Spino and is responsible for managing the trial on a day-to-day basis, monitoring enrollment, retention, and protocol adherence and for collecting, monitoring, editing, and analyzing data from the Clinical Sites.
- The *Central Laboratory*, located at the University of Minnesota, is directed by Dr. Karger, and is responsible for all blood and urine tests.
- The *Data Safety Monitoring Board (DSMB)* will be composed of to-be-named outside experts in the design and conduct of clinical trials and in diabetic nephropathy. The board will be responsible for reviewing the study documents, monitoring study progress and participant safety.

Monthly conference calls will be scheduled for the Steering Committee and the trial coordinators to discuss subject participation and compliance. Twice a year, the Steering Committee, the Data Coordinating Center, and the trial coordinators will meet for two days to discuss the study progress. Dr. Robiner, the study psychologist, will attend this meeting annually.

A study website will be maintained where all study meetings and phone call minutes will be maintained and where an updated version of the Manual of Operations will be available.

15.2. Protocol Deviations, Violations, and Amendments

A Protocol Deviation is defined as any change, divergence, or departure from the approve study protocol that does not affect the participant's safety, rights, welfare or the integrity of the study and its resultant data. A Protocol Violation is defined as a protocol deviation that may affect the participant's rights, safety, or wellbeing and/or the completeness, accuracy, and reliability of the study data. Deviation will be reported to the IRB at the time of continuing review whereas violations will be reported as soon as study personnel are aware of the event. The PI will keep an internal protocol deviation and violation log that will be forwarded to the IRB at the time of continuing review. Adoption of protocol amendments will require three-fifths majority approval by members of the Steering Committee. The amended protocol is resubmitted to the IRB.

15.3. Financial Disclosure

On an annual basis or whenever there is a significant change in status, participating investigators will be required to disclose any financial or related interest that could present an actual conflict of interest or could be perceived as presenting a conflict of interest. The Steering Committee will determine (1) if the disclosed interest could directly and significantly affect the performance of study responsibility and, (2) the management, reduction, or elimination of the conflict.

15.4. Publications

It is anticipated that this research may lead to oral and written presentations including one or more jointly-authored publications. The contribution of investigators will be acknowledged in accordance with scientific custom in all published and oral communications concerning this study and its results.

16. REFERENCES

1. Krolewski AS, Warram JH. Epidemiology of late complications of diabetes: A basis for the development and evaluation of preventive program. in *Joslin's Diabetes Mellitus*. Lippincott, Williams & Wilkins, New York (2005).

2. U S Renal Data System. *USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States.*National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD (2010).

3. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA* 305, 2532-2539, 2011.

4. Rosolowsky ET, Skupien J, Smiles AM, Niewczas M, Roshan B, Stanton R, Eckfeldt JH, Warram JH, Krolewski AS. Risk for ESRD in type 1 diabetes remains high despite renoprotection. *J Am Soc Nephrol* 22, 545-553, 2011.

5. Caramori ML, Fioretto P, Mauer M. Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. *Diabetes* 52, 1036-1040, 2003.

6. Perkins BA, Ficociello LH, Ostrander BE, Silva KH, Weinberg J, Warram JH, Krolewski AS. Microalbuminuria and the risk for early progressive renal function decline in type 1 diabetes. *J Am Soc Nephrol* 18, 1353-1361, 2007.

7. Premaratne E, MacIsaac RJ, Finch S, Panagiotopoulos S, Ekinci E, Jerums G. Serial measurements of cystatin C are more accurate than creatinine-based methods in detecting declining renal function in type 1 diabetes. *Diabetes Care* 31, 971-973, 2008.

8. Ficociello LH, Rosolowsky ET, Niewczas MA, Maselli NJ, Weinberg JM, Aschengrau A, Eckfeldt JH, Stanton RC, Galecki AT, Doria A, Warram JH, Krolewski AS. High-normal serum uric acid increases risk of early progressive renal function loss in type 1 diabetes: results of a 6-year follow-up. *Diabetes Care* 33, 1337-1343, 2010.

9. Jalal DI, Rivard CJ, Johnson RJ, Maahs DM, McFann K, Rewers M, Snell-Bergeon JK. Serum uric acid levels predict the development of albuminuria over 6 years in patients with type 1 diabetes: findings from the Coronary Artery Calcification in Type 1 Diabetes study. *Nephrol Dial Transplant* 25, 1865-1869, 2010.

10. Rodrigues TC, Maahs DM, Johnson RJ, Jalal DI, Kinney GL, Rivard C, Rewers M, Snell-Bergeon JK. Serum uric acid predicts progression of subclinical coronary atherosclerosis in individuals without renal disease. *Diabetes Care* 33, 2471-2473, 2010.

11. Hovind P, Rossing P, Tarnow L, Johnson RJ, Parving HH. Serum uric acid as a predictor for development of diabetic nephropathy in type 1 diabetes: an inception cohort study. *Diabetes* 58, 1668-1671, 2009.

12. Iseki K, Ikemiya Y, Inoue T, Iseki C, Kinjo K, Takishita S. Significance of hyperuricemia as a risk factor for developing ESRD in a screened cohort. *Am J Kidney Dis* 44, 642-650, 2004.

13. Domrongkitchaiporn S, Sritara P, Kitiyakara C, Stitchantrakul W, Krittaphol V, Lolekha P, Cheepudomwit S, Yipintsoi T. Risk factors for development of decreased kidney function in a southeast Asian population: a 12-year cohort study. *J Am Soc Nephrol* 16, 791-799, 2005.

14. Zhang L, Zuo L, Xu G, Wang F, Wang M, Wang S, Lv J, Liu L, Wang H. Community-based screening for chronic kidney disease among populations older than 40 years in Beijing. *Nephrol Dial Transplant* 22, 1093-1099, 2007.

15. Kang DH, Nakagawa T, Feng L, Watanabe S, Han L, Mazzali M, Truong L, Harris R, Johnson RJ. A role for uric acid in the progression of renal disease. *J Am Soc Nephrol* 13, 2888-2897, 2002.

16. Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, Lan HY, Kivlighn S, Johnson RJ. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension* 38, 1101-1106, 2001.

17. Johnson RJ, Segal MS, Srinivas T, Ejaz A, Mu W, Roncal C, Sanchez-Lozada LG, Gersch M, Rodriguez-Iturbe B, Kang DH, Acosta JH. Essential hypertension, progressive renal disease, and uric acid: a pathogenetic link? *J Am Soc Nephrol* 16, 1909-1919, 2005.

18. Mazzali M, Kanellis J, Han L, Feng L, Xia YY, Chen Q, Kang DH, Gordon KL, Watanabe S, Nakagawa T, Lan HY, Johnson RJ. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. *Am J Physiol Renal Physiol* 282, F991-F997, 2002.

19. Desco MC, Asensi M, Marquez R, Martinez-Valls J, Vento M, Pallardo FV, Sastre J, Vina J. Xanthine oxidase is involved in free radical production in type 1 diabetes: protection by allopurinol. *Diabetes* 51, 1118-1124, 2002.

20. Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev* 58, 87-114, 2006.

21. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. *Am J Kidney Dis* 47, 51-59, 2006.

22. Goicoechea M, de Vinuesa SG, Verdalles U, Ruiz-Caro C, Ampuero J, Rincon A, Arroyo D, Luno J. Effect of allopurinol in chronic kidney disease progression and cardiovascular risk. *Clin J Am Soc Nephrol* 5, 1388-1393, 2010.

23. Sanchez-Lozada LG, Tapia E, Soto V, Avila-Casado C, Franco M, Wessale JL, Zhao L, Johnson RJ. Effect of febuxostat on the progression of renal disease in 5/6 nephrectomy rats with and without hyperuricemia. *Nephron Physiol* 108, 69-78, 2008.

24. Becker MA, Schumacher HR, Jr., Wortmann RL, MacDonald PA, Eustace D, Palo WA, Streit J, Joseph-Ridge N. Febuxostat compared with allopurinol in patients with hyperuricemia and gout. *N Engl J Med* 353, 2450-2461, 2005.

25. Feig DI, Soletsky B, Johnson RJ. Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. *JAMA* 300, 924-932, 2008.

26. Schumacher HR, Jr., Becker MA, Wortmann RL, MacDonald PA, Hunt B, Streit J, Lademacher C, Joseph-Ridge N. Effects of febuxostat versus allopurinol and placebo in reducing serum urate in subjects with hyperuricemia and gout: a 28-week, phase III, randomized, double-blind, parallel-group trial. *Arthritis Rheum* 59, 1540-1548, 2008.

27. Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. *Lancet* 375, 2161-2167, 2010.

28. Gibson T, Rodgers V, Potter C, Simmonds HA. Allopurinol treatment and its effect on renal function in gout: a controlled study. *Ann Rheum Dis* 41, 59-65, 1982.

29. Ettinger B, Tang A, Citron JT, Livermore B, Williams T. Randomized trial of allopurinol in the prevention of calcium oxalate calculi. *N Engl J Med* 315, 1386-1389, 1986.

30. Gaspari F, Perico N, Matalone M, Signorini O, Azzollini N, Mister M, Remuzzi G. Precision of plasma clearance of iohexol for estimation of GFR in patients with renal disease. *J Am Soc Nephrol* 9, 310-313, 1998.

31. O'Reilly PH, Brooman PJ, Martin PJ, Pollard AJ, Farah NB, Mason GC. Accuracy and reproducibility of a new contrast clearance method for the determination of glomerular filtration rate. *Br Med J (Clin Res Ed)* 293, 234-236, 1986.

32. Gaspari F, Perico N, Remuzzi G. Measurement of glomerular filtration rate. *Kidney Int Suppl* 63, S151-S154, 1997.

33. Mauer M, Zinman B, Gardiner R, Suissa S, Sinaiko A, Strand T, Drummond K, Donnelly S, Goodyer P, Gubler MC, Klein R. Renal and retinal effects of enalapril and losartan in type 1 diabetes. *N Engl J Med* 361, 40-51, 2009.

34. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, III, Feldman HI, Kusek JW, Eggers P, Van LF, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 150, 604-612, 2009.

35. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, Rossert J, Van Lente F, Bruce RD, III, Zhang YL, Greene T, Levey AS. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis* 51, 395-406, 2008.

36. Lonjou C, Borot N, Sekula P, Ledger N, Thomas L, Halevy S, Naldi L, Bouwes-Bavinck JN, Sidoroff A, de TC, Schumacher M, Roujeau JC, Hovnanian A, Mockenhaupt M. A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* 18, 99-107, 2008.

37. Jung JW, Song WJ, Kim YS, Joo KW, Lee KW, Kim SH, Park HW, Chang YS, Cho SH, Min KU, Kang HR. HLA-B58 can help the clinical decision on starting allopurinol in patients with chronic renal insufficiency. *Nephrol Dial Transplant* 26, 3567-3572, 2011.

38. Carpenter J, Pocock S, Lamm CJ. Coping with missing data in clinical trials: a model-based approach applied to asthma trials. *Stat Med* 21, 1043-1066, 2002.

39. Carpenter J, Kenward MG. Missing data in randomised controlled trials - a practical guide. *Birmingham: National Institute for Health Research* Publication RM03/JH17/MK, 2008.

40. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 38, 963-974, 1982.

41. Verbeke, G. & Molenberghs, G. *Linear mixed models for longitudinal data* 1-568 Springer Verlag, New York, NY (2000).

42. West, B. T., Welch, K. B., & Galecki, A. T. *Linear mixed models: a practical guide using statistical software*Chapman & Hall/CRC Press,(2007).

43. Little, R. J. A. & Rubin, D. B. *Statistical Analysis with Missing Data* Wiley & Sons, Inc., New York (2002).

44. Molenberghs, G. & Kenward, M. G. *Missing Data in Clinical Studies*Wiley & Sons, Inc., New York (2007).

45. Cohen, J. *Statistical power analysis for the behavioral sciences*Erlbaum, Hillsdale, NJ (2013).

46. ICH. Guideline for Good Clincial Practice in *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human Use*. (1996).

47. Venet D, Doffagne E, Burzykowski T, Beckers F, Tellier Y, Genevois-Marlin E, Becker U, Bee V, Wilson V, Legrand C, Buyse M. A statistical approach to central monitoring of data quality in clinical trials. *Clin Trials* 9, 705-713, 2012.

48. Roujeau JC, Kelly JP, Naldi L, Rzany B, Stern RS, Anderson T, Auquier A, Bastuji-Garin S, Correia O, Locati F, . Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. *N Engl J Med* 333, 1600-1607, 1995.