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The Effects of Urate Lowering Therapy on Inflammation, Endothelial Function, and Blood Pressure (SURPHER) Study Design and Rationale

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Abstract

Background—The association between hyperuricemia and hypertension is controversial. Animal models, epidemiological data, and small clinical trials have favored a causative role for hyperuricemia in hypertension but more studies are necessary to elucidate putative mechanisms, population susceptibility, and potential for urate-lowering therapies (ULT) to decrease blood pressure (BP).

Purpose—To describe the background and design of the Serum Urate Reduction to Prevent Hypertension (SURPHER) study.

Methods—SURPHER is a single center, double-blinded, crossover trial in which participants are randomly assigned to allopurinol (300 mg) or placebo. Enrollment focused on adults 18 – 40 yrs old with baseline systolic blood pressure ≥ 120 and < 160 mmHg or diastolic blood pressure ≥ 80 and < 100 mmHg, and serum urate ≥ 5.0 mg/dL or ≥ 4.0 mg/dL for men or women, respectively. SURPHER recruitment targeted participants without chronic kidney disease (estimated glomerular filtration rate > 60 ml/min/1.73m²), and without prior diagnosis of gout or use of ULT to treat gout. The primary outcome is change from baseline in blood pressure assessed by 24 hour ambulatory blood pressure monitoring and mechanistic outcomes include changes in flow-mediated dilation and C-reactive protein levels.

Results—Since June 16, 2014 until present, SURPHER is recruiting participants in the city of Birmingham, Alabama.

Limitations—The study aims to enroll otherwise healthy young adults for a pharmacological intervention study with multiple study-related procedures. Challenges related to recruitment are anticipated and multiple strategies for increasing recruitment and retention have been implemented.

Keywords

hyperuricemia; prehypertension; urate lowering therapy (ULT); uric acid; serum urate; hypertension; blood pressure; allopurinol; endothelial function

1. Introduction, Background, and Rationale

High serum urate concentration is a well-established causative factor for the development of gouty arthritis. There is growing interest in a role for serum urate as contributing factor for the development or worsening of vascular, cardiac, and renal disease. The association between serum urate levels and blood pressure (BP) has been described for many decades.^{1–13} Evidence from animal studies provides strong support for this association.¹⁴ The contribution of serum urate in to hypertension is further supported by evidence from small clinical trials demonstrating antihypertensive benefit of urate lowering therapy (ULT) in adolescence.^{15,16} Mechanisms involved in the development of hypertension include the renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system, and endothelin-nitric oxide system. It is of particular note that transport of serum urate into human endothelial cells via the urate anion transporter-1 (URAT-1) results in reduced nitric oxide levels in endothelial cells and concurrent activation of the RAAS.^{17, 18–20} the resultant endothelial dysfunction is an independent risk factor for cardiovascular events via promotion of both atherosclerosis and hypertension.^{21–23} endothelial dysfunction and increased plasma renin activity have been associated with increases in serum urate.^{19,20} Administration of allopurinol, a first-line ULT, to treat hyperuricemia has been shown to improve endothelial function in both animal and human studies.^{19, 24–26} Moreover, higher serum urate concentrations are positively associated with increased levels of inflammatory markers, such as C-reactive protein (CRP). Improvements in endothelial function and BP control have been associated with decreases in C-reactive protein levels, suggesting another potential vasoprotective mechanism for ULT.^{27,28} Our previous double-blinded, crossover trial has demonstrated that administration of allopurinol (200 mg daily) for one month significantly reduced clinic and ambulatory BP among adolescents with hypertension and hyperuricemia when compared with placebo.¹⁵ We also showed that the uricosuric drug probenecid reduced BP in both children and adolescents, thus supporting the hypothesis that reduction of serum urate levels by mechanisms other than xanthine-oxidase inhibition is associated with BP reduction.²⁹ Our group also described an increased risk for incident hypertension among young adults with serum urate level ≥ 5.0 mg/dL for men, and ≥ 4.0 mg/dL for women followed over 20 years.³⁰ Finally, it is well established that hypertension disproportionately affects African-Americans³¹ and that African-Americans (AAs) have differential responses to hypertension therapies.³² The main objective of the Serum Urate

Reduction to Prevent Hypertension (SURPHER) study is to determine if ULT is associated with reductions in BP in young adults, paying special attention to ethnic and gender differences in the response. Mechanisms mediating this effect, particularly endothelial dysfunction and markers of inflammation, will be examined. We hypothesize that BP in AAs is more sensitive to variations in serum urate and that this may be an additional mechanism to explain the higher prevalence of hypertension among young AAs. We further hypothesize that any relationship between inflammatory marker levels, serum urate, and BP would be optimally tested in a younger population with a lower prevalence of vascular damage.^{33 34}

2. Methods

2.1. Study Organization

The SURPHER organizational structures and responsibilities are similar to many previous single center clinical trials. The study is sponsored by National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) (F130408004) as part of the funded University of Alabama at Birmingham (UAB) Center of Research Translation in Gout and Hyperuricemia (CoRT) (P50AR060772). SURPHER is registered at [clinicaltrials.gov](https://clinicaltrials.gov/show/NCT02038179) (<https://clinicaltrials.gov/show/NCT02038179>) and the study has been approved by the UAB Institutional Review Board (IRB).

2.2. Study Design Decisions

2.2.1. Overall Study Design—SURPHER is a single center double-blinded, crossover trial in which participants are randomly assigned to 300 mg of allopurinol as ULT or placebo (Figure 1). Change from baseline in BP assessed by 24 hours ambulatory blood pressure monitoring (ABPM) is the primary study outcome. We plan to recruit and randomize 112 participants from the Birmingham, Alabama, USA metro area into the study. The sample will include 56 African-Americans and 56 participants of other races/ethnicities.

2.2.2. Eligibility, Recruitment, Retention, and Adherence—The SURPHER inclusion/exclusion criteria are presented in (Table 1). Three major inclusion criteria include 1) pre-hypertension or stage I hypertension defined as the following after the mean of two clinic measurements: systolic blood pressure (SBP) ≥ 120 and <160 mmHg or; diastolic blood pressure (DBP) ≥ 80 and <100 mmHg; 2) a serum urate ≥ 5.0 mg/dL for men or ≥ 4.0 mg/dL for women; and 3) are between 18–40 years old. Two major exclusion criteria include 1) any current pharmacological treatment for hypertension excluding the calcium channel blockers; 2) prior diagnosis of gout or past use of ULT for gout. Patient recruitment began on July 14th 2014 and is ongoing. Recruitment efforts include posting of flyers in university, metro, and community areas frequented by our target demographic of young adults. Announcements and profiles for the study have also been placed on the websites researchmatch.com, craigslist.com, and facebook.com. Other recruitment efforts include direct mailing advertisement through databases of individuals interested in research studies, advertisements in local area newspapers, and radio stations. Participants are also being recruited directly from outpatient care facilities.

Attention to adherence and retention planning began before enrollment commenced. During screening and an initial placebo run-in phase, individuals who demonstrate a significant risk for non-adherence to study medication or for completing study visits are excluded from trial participation. Once participants enroll, a baseline assessment of adherence using standardized, valid adherence measures in conjunction with assessment of behavioral “red flags” by clinic staff allows for early identification of potential problems so that study resources could be devoted to improving adherence and retention for these individuals. Throughout the study, participants are called at 2 weeks intervals to encourage study compliance and to ask about any adverse events. Continuous monitoring of adherence is considered a critical factor for identifying low adherence and enabling clinic staff to intervene and address problems as they develop.

2.3. Intervention

The study visits and procedures are shown in the Table 2. Study outcomes are assessed during the 2nd, 3rd, 4th, and 5th visits. An initial telephone screen is performed with all potential participants. If deemed potentially eligible for the study, participants are scheduled for a study visit, during which the study objectives are explained and an initial informed consent for the screening evaluation is signed. Participants who meet enrollment criteria are asked to participate in a single-blind, 2-week run-in period during which they receive a daily dose of placebo. Upon completion of study run-in and assessment of adherence to placebo, participants are randomized at visit 2 (week 3) to receive allopurinol 300 mg or placebo. The random permuted blocks strategy will be used in this study to randomly allocate equal number of participants into two crossover arms. Specifically, this randomization scheme consists of 3 steps: 1) generate a sequence of blocks with variable sizes and each block contains the same number of A/B and B/A, indicating crossover arms; 2) the sequence (A/B, B/A) are permuted in each block; and 3) the blocks are permuted. The permutation will be done by computer-generated random numbers. This method will assure the balance between crossover arms throughout the investigation. The variable block will help prevent research staff from anticipating subsequent crossover arm allocations. The sequence of treatment assignment is determined by a random number generator held by the pharmacist who maintains the master list and stores the randomization files securely. Upon completion of visit 3, participants enter a 4-week washout period before crossing over to the other study arm. This 4-week washout interval was chosen to allow for complete dissipation of any persistent effects from allopurinol, which upon being converted to oxypurinol, has a half-life of 18 to 30 hours.² This approach is supported by published interventional studies examining the effect of allopurinol on cardiovascular disease.¹⁵ No residual urate-lowering effect or xanthine-oxidase inhibition is expected beyond 2 weeks after the last administered dose of allopurinol, more so in individuals with normal renal function. However, the potential carry-over effects will be examined before testing the efficacy. For a 2-by-2 crossover design with continuous outcomes, such as this study, the potential carry-over effects will be tested by a two sample t test that has commonly been used in trials with similar designs.

2.4. Hypotheses and choice of outcomes

The overall aim and main study hypothesis of SURPHER is to determine whether, and by what mechanism, ULT with allopurinol reduces BP in young adults with pre-hypertension or stage I hypertension defined as SBP ≥ 120 and <160 mmHg or DBP ≥ 80 and <100 mmHg.²⁹ A secondary hypothesis is that these ULT-mediated BP reductions will correlate positively with improvement in flow-mediated dilation as an index of endothelial function, and reduction in CRP as a marker of inflammation. We also hypothesize that ULT-mediated BP reduction is more pronounced among African-American participants than in Caucasians and other races. Changes in BP as measured by ABPM is primary outcome; secondary outcomes are changes in flow-mediated dilation (FMD) and high sensitivity C-reactive protein (hsCRP).

3. Measurement, Ascertainment, and Follow-up

3.1. Clinic Blood Pressure Measurement

Clinic BP is measured by trained personnel after at least 5 minutes of quiet rest in the sitting position with the back supported using an automated BP monitor (Model HEM-705CP, Omron Healthcare, Inc., Vernon Hills, IL, 60061, USA) with the arm supported at heart level. An appropriate sized cuff is used with a cuff bladder encircling at least 80% of the arm. Three BP readings are taken at intervals of 2 minutes, and the second and third readings are averaged. The BP is measured in both arms, and the arm with the higher BP is used for further BP measurements. All BP measurements are performed according to guidelines.³⁵

3.2. Biochemical Assessment

Blood samples are collected for complete blood counts, serum urate levels, liver function tests, creatinine levels, glomerular filtration rate (GFR), high sensitivity CRP levels, urine pregnancy and cotinine tests, and serum and DNA banking.

3.3. Ambulatory Blood Pressure Monitoring

All patients undergo 24-hour ABPM with an automated, noninvasive, oscillometric device (Spacelabs Healthcare, Inc., 35301 SE Center Street, Snoqualmie, WA 98065, and U.S.A.)^{36,37} an appropriate sized cuff is used with a cuff bladder encircling at least 80% of the arm, according to guidelines.³⁵ The first measurement is obtained in the clinic to ensure proper function. Recordings are made every 20 minutes for the daytime (awake) and every 30 minutes for the nighttime (asleep) for a 24-hour period. Awake and asleep periods are defined individually according to the patient's self-reported data. Valid 24-hour ABPM is defined as $>80\%$ recorded successful measurements. Normal ABP is defined as mean 24-hour BP $<130/80$ mmHg with a daytime (awake) BP of $<135/80$ mmHg and a nighttime (asleep) BP of $<120/70$ mmHg by ABPM according to guidelines.^{36,37}

3.4. Assessment of Vascular Endothelial Function

At visits 2, 3, 4, and 5, vascular endothelial function is measured by flow-mediated dilation (FMD) of the brachial artery performed by the same physician who is blinded to treatment assignment.^{38–41} all medications are withheld the morning of the testing. In women

participants, FMD is performed within the first 7 days after onset of menstrual cycle to avoid known cyclic variations in FMD.^{38,39} after an 8-hour fasting period, FMD is measured by high-resolution ultrasound with an 11-3 MHz linear-array probe (Philips HP Agilent Technologies, Sonos 5500, Andover, MA, 01810). Prior to testing, participants are asked to rest for 10 minutes in the supine position in a quiet, ambient room according to guidelines. Ultrasound of the brachial artery is taken at approximately 5 cm proximal from the elbow in longitudinal section on the right arm. The probe is maintained in a fixed position at a fixed angle, and baseline artery diameter is recorded for 1 minute. Blood flow is estimated by pulsed Doppler velocity, and baseline artery diameter is assessed with simultaneous ECG recording for baseline assessment at every study visit.

Reactive hyperemia is induced by inflating a BP cuff around the forearm to 50 mmHg above resting systolic BP. The cuff remains inflated for a five-minute period, and then it is rapidly deflated. The longitudinal image of the brachial artery is recorded continuously from 30 seconds before to 3 minutes after cuff deflation. A mid-artery pulsed Doppler signal is obtained at an $< 60^\circ$ angle upon immediate cuff release and no later than 15 seconds after cuff deflation to assess hyperemic velocity parameters. Ultrasound images are recorded, 30 seconds before cuff deflation, and 3 minutes after cuff deflation. The five largest diameters after deflation are averaged, and FMD is expressed as the percentage increase in diameter from the baseline average measurement to the peak average dilation. Brachial arterial flow-mediated dilation related parameters are analyzed with edge detection software and Doppler flow analyzer (Vascular Research Tools 5 Software, Medical Imaging Applications, LLC, Coralville, IA). The calculation of FMD as a percentage change utilizes the peak diameter in response to reactive hyperemia in relation to the baseline diameter, and is calculated utilizing the following equation: Flow mediated dilation (%) = (Peak diameter-Baseline diameter)/Baseline diameter, and when multiplied by 100, FMD is expressed as a percent change in vessel caliber. An increase in the % change is considered positive. Mean and standard deviations will be calculated at the beginning and end of allopurinol and placebo phases for FMD.

4. Quality Control

All study visits and procedures are performed at facilities dedicated to clinical research studies. All data for are collected by study staff in accordance with Code of Federal Regulations (CFR) title 21 Part 11 and meets all regulatory requirements. We use electronic case report forms for all data entry that are entered into the Research Electronic Data Capture (REDCap) system by the study coordinators. All REDCap data are exported into SAS files and stored on a secure and encrypted server/hard drive. The data are reviewed by a study coordinator every 2 weeks for completeness and validity (e.g., no abnormal lab value, typographical errors). All efforts are made to ensure the data are in compliance with local IRB policies, Good Clinical Practice (GCP) guidelines, and federal regulations.

4.1. Statistical analysis, sample size, and power considerations

The demographic and clinic covariates (e.g., age, race, comorbidities, etc.) and baseline measures (e.g., hsCRP, systolic and diastolic BP on ABPM, and endothelial function) will be

summarized as mean and standard deviation for continuous variables and proportion for categorical variables. The comparisons between randomization assignments will be conducted by two sample t test or nonparametric Wilcoxon test for continuous variables after checking the normality assumption, and Chi square test for categorical variables.. The primary analyses of the study will be to compare the change in average ABPM-measured ambulatory SBP (primary endpoint), DBP, and mean arterial pressure in participants between the active treatment (allopurinol) and placebo phases at weeks 3, 6, 11, and 14. A paired t test or a linear mixed model, the standard methods for crossover design,⁵² will be used to evaluate the primary outcome. Analyses will be changes in ABPM-measured BP while awake, BP while asleep, and BP loads. Table 3 shows the variables that will be analyzed from the ABPM studies. Exploratory analyses will be changes in pulse pressure, changes in dipping with continuous and categorical patterns (Table 3), changes in BP variability, and changes in heart rate variability. Similarly, the changes of endothelial function and hsCRP in participants during the different study phases will also be analyzed. For variables with skewed distribution, such as hsCRP, log-transformation will be applied. Considering the crossover design, the lack of carryover effect on the relative treatment responses will be initially confirmed with standard methods. Continuous outcomes (such as the mean of each patient's changes in BP between pretreatment and placebo and pretreatment and allopurinol) will be analyzed by paired t test and Hotelling's T² test for repeated measurements. Dipping patterns will be assigned to one of four categories at the beginning and end of the treatment phases. Comparisons will be between individuals to test what proportion of participants were dippers (> 10 %) or non-dippers (< 10 %) at the end of each treatment phase. A McNemar's test to compare proportions will be used to test the null hypothesis that the proportions are equal. ABPM studies with more than 80% successful reads (14 daytime readings and 5 nighttime readings) will be considered satisfactory for analysis. With 112 participants, we will have 90% statistical power to detect a difference in SBP and DBP of 3.7 mmHg and 2.0 mmHg with standard deviations of 8.5mmHg⁴² and 6.3 mmHg⁴³ respectively. Assuming the standard deviation of the difference is 2.5% as reported⁴⁴, we will have 90% power to be able to detect a difference in the increase of FMD of 1.48% for allopurinol versus placebo in African-Americans compared to other participants. We will also have 90% statistical power to detect a differential reduction in log transformed hsCRP of 32 log mg/L (e.g., a 10 log mg/L reduction in whites and a 42 log mg/L reduction in African-Americans). All power calculations assume a two-tailed test with significance level of 0.05. Statistical analyses will use an intent-to-treat method which analyzes every subject strictly by their randomized treatment assignment independent of issues of noncompliance, protocol deviations, withdrawal, and / or other events after randomization. Randomized participants may miss study visits, resulting in missing data. Excluding those participants from the analyses would violate the intention to treat principle and lead to biased estimates. Multiple imputation will be used to address this concern and has proven to be efficient at handling missing data and providing unbiased estimates⁴⁵. All the analyses will be conducted using SAS software (SAS 9.4, Cary, NC) by statisticians masked to randomization assignment.

4.2. Safety Monitoring

A safety officer was mandated by the study's funding agency to provide independent oversight and act in an advisory capacity to the NIAMS to monitor research participant safety and data quality as well as to alert the Institute to potential issues in either of these areas. As an additional safety measure, we convened a Data Safety and Monitoring Board (DSMB) for monitoring of study procedures. The DSMB is comprised of a rheumatologist, a nephrologist, and a biostatistician who are independent of the study, the study investigators, and of University of Alabama at Birmingham.

4.3. Safety assessments

All adverse events (AEs) will be collected, analyzed, and monitored using the Adverse Event Reporting Form as well as the AE Tracking Log in REDCap. The study AEs definitions are detailed in Table 4. All laboratory abnormalities identified in the protocol as critical to participant safety are reported. All AEs experienced by the participant from the time of study drug administration through the end of the study are reported, as outlined in the protocol. We will collect AEs during the study visits at weeks 3, 6, 9, and 14. Participants also will be instructed to report AEs and SAEs to the study team immediately after their occurrence. Safety assessments will be conducted during both study periods in subjects independent of their initial allocation (which is unknown to the study investigators). Additionally, the study coordinators will inquire about AEs during interim phone calls between the visits at week 4, 5, 7, 9, 12, and 13. All adverse events will be reported to the local IRB, our study DSMB and the Safety Officer at the time of continuing review. All serious adverse events (SAEs) will be reported to the safety officer and the DSMB within 48 hours of the PI becoming aware of the event. A single occurrence of Stevens Johnson Syndrome, regardless of severity, will be considered a Serious Adverse Event (SAE) and will trigger a study stop. This event requires expedited reporting to NIAMS and the Safety Officer within 48 hours. All study staff members will inform the PI about any unanticipated problems involving risks to the participants. If any protocol changes are needed, the PIs will submit a modification request to the IRB and NIH. Protocol changes will not be implemented prior to IRB and NIH approval unless necessary to eliminate apparent immediate hazards to research participants. In such case the IRB will be promptly informed of the change following implementation (within 1 week).

Of special interest is the development of allopurinol hypersensitivity syndrome (AHS), a very rare life threatening idiosyncratic reaction that is commonly preceded by rash. The most recent published findings report an incidence rate of 0.69 per 1000 person-years for severe AHS cutaneous reactions.⁴⁶ During the course of this study we will expose a total of 112 (<30 participants/year) ambulatory young adults aged 18–40, with normal kidney function, to allopurinol. The rate of AHS in younger ambulatory patients with normal kidney function has not been established but is likely lower than in individuals with gout and other medical comorbidities. AHS has mainly been observed in individuals that are HLAB5801 positive (primarily individuals of Han Chinese or Thai descent).⁴⁷ As recommended in the recently released American College of Rheumatology guidelines, we will test for HLAB5801 during initial screening in individuals who self-identify as being of Han Chinese or Thai descent.⁴⁷

5. Discussion

The SURPHER Study is a NIH funded prospective, randomized, double-blinded, placebo-controlled trial with crossover to study the safety and effectiveness of 300 mg of allopurinol in patients between the ages of 18–40 years with pre- or stage I hypertension.

We chose a crossover design since it is a more efficient approach as each participant can serve as their own control, allowing for a more reasonable sample size for our federally funded study, while simultaneously controlling for imbalance between the groups. In addition, prior studies have successfully used a crossover design to answer similar research questions in adolescents.¹⁵ The study aims to recruit 112 participants from the community and measure ABPM, FMD and inflammatory markers. The study is unique in it being the first randomized controlled trial to study the effects of ULTs in young adults with hypertension, attempting to elucidate the mechanisms underlying any observed effect of urate-lowering on BP and differential responses to ULT by race/ethnicity. This latter point is particularly relevant, since racial/ethnic differences in the pathophysiology of hypertension are known and there is an urgent medical need for novel therapeutic approaches to hypertension among minorities.^{2,48–51} Patients with gout and concurrent hypertension and cardiovascular (CV) morbidities might require a ULT threshold goal lower than the one accepted for uncomplicated gout that is entirely defined in terms of monosodium urate solubility. This hyperuricemia threshold of 6.8 mg/dL might not be relevant for serum urate effects on the endothelium and their association with CV disease. Expected challenges of the study relate to recruitment of the targeted patient population of young adults, given the stringent enrollment criteria as well as participant retention and adherence related to the complex study procedures. SURPHER will uniquely contribute to our scientific understanding of the effect of serum urate in CV health and will open avenues to approach targeted treatments for hypertension and serum urate-related conditions.

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Abbreviations

AAs	African Americans
ABPM	ambulatory blood pressure monitoring
AEs	adverse events
AHS	allopurinol hypersensitivity syndrome
BP	blood pressure
CoRT	Center of Research Translation in Gout and Hyperuricemia
CRP	C-reactive protein
CV	cardiovascular

DBP	diastolic blood pressure
DSMB	Data Safety and Monitoring Board
FMD	flow-mediated dilation
GCP	Good Clinical Practice
GRF	glomerular filtration rate
hsCRP	high sensitivity C-reactive protein
IRB	Institutional Review Board
NIAMS	National Institute of Arthritis and Musculoskeletal and skin Diseases
PI	Principal Investigator
RAAS	renin-angiotensin-aldosterone system
REDCap	Research Electronic Data Capture
SAEs	serious adverse events
SBP	systolic blood pressure
SURPHER	Serum Urate Reduction to Prevent Hypertension
UAB	University of Alabama at Birmingham
ULT	urate lowering therapy
URAT-1	urate anion transporter-1

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Figure 1. Study Design Diagram

Participants are screened for eligibility and then begin a 2 week run-in period. Upon completion of run-in, participants are randomized in Phase 1 to allopurinol, 300 mg or placebo for 4 weeks. This is followed by a 4-week washout period and then crossover to Phase 2 (i.e. participants who previously received allopurinol receive placebo, and vice versa) for 4 weeks

Table 1**Enrollment Criteria**

Inclusion criteria	
Pre-hypertension or stage I hypertension, defined as the following after the mean of two clinic measurements:	
<ul style="list-style-type: none"> - Systolic blood pressure (SBP) 120 and <160 or; - Diastolic blood pressure (DBP) 80 and < 100 	
Serum urate	5.0 mg/dL for men or 4.0 mg/dL for women
Age 18–40	
Exclusion criteria	
Any current pharmacological treatment for hypertension excluding the Calcium channel Blockers	
Estimated glomerular filtration rate (GFR) < 60 mL/min/1.73m ²	
Individuals who are lactose intolerant	
Consuming > 2 alcoholic drinks per day	
Current or past use of any urate-lowering therapy or statins	
Prior diagnosis of gout or diabetes	
Pregnancy, recent delivery, or last trimester pregnancy loss more recent than 3 months	
Active smokers	
Immune-suppressed individuals including transplant recipients or current use of azathioprine.	
Leucopenia with absolute white cell count < 3000 /mL, anemia with hemoglobin < 12 g/dL, or thrombocytopenia with platelet count < 150,000/mL	
Abnormal liver function tests (Aspartate Aminotransferase (AST) > 40 Units/L, Alanine Aminotransferase (ALT) > 58 Units/L, Alkaline Phosphatase > 117 Units/L)	
Han Chinese or Thai descent with Human leukocyte Antigen phenotype B5801 (HLAB5801)	
Serious medical condition that in the investigator's judgment precludes utilization of a fixed dose of allopurinol	

Table 2

All Study Procedures

			Treatment Phase										
Visit description / Study Procedures	Screening	Run-in period	Phase 1	Washout period		Phase 2 (crossover)							
	Weeks												
	0	1–2	3	4	5	6	7–10	11	12	13	14		
	Visit 1		Visit 2			Visit 3		Visit 4			Visit 5 (final)		
Informed consent	X												
Pill count			X	X	X	X		X	X	X	X		
Adverse events reporting			X	X	X	X		X	X	X			
PE	X		X**			X**		X**			X**		
BP screening	X		X			X		X			X		
Focused medical history			X										
Updated medical history			X			X		X			X		
Randomization			X										
24-hr ABPM			X			X		X			X		
FMD			X			X		X			X		
Blood sample	X		X			X		X			X		
CBC	X		X			X		X			X		
Liver tests*	X		X			X		X			X		
Pregnancy test [†]	X		X			X		X			X		
Serum creatinine	X		X			X		X			X		
Serum urate	X		X			X		X			X		
hsCRP			X			X		X			X		
Serum / urine banking [§]			X			X		X			X		

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PE-physical examination, BP-blood pressure, 24- hr ABPM- 24 hours ambulatory blood pressure monitoring, FMD- flow mediated dilation of the brachial artery, hsCRP-highly sensitive C-reactive protein, CBC - complete blood count

* Liver tests are alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase

** Physical exams will be conducted following screening visit only in cases where rash has been reported

[†] Only in women

[§] Sera, whole blood (for DNA extraction), and urine will be stored.

Table 3

Ambulatory Blood Pressure Variables

Blood pressure variables
24 –hour average (Systolic, diastolic, mean)
Awake average (systolic, diastolic mean)
Asleep average (systolic, diastolic mean)
Blood pressure load *
24-hour average BP load
Awake BP load
Asleep BP load
Pulse pressure **
Dipping patterns ***
Blood pressure variability
Heart rate variability

* Awake BP Load: Systolic: % of awake systolic BP's were greater than 135 mmHg, Diastolic: % of awake diastolic BP's was greater than 85 mmHg; Asleep BP Load: Systolic: % of asleep systolic BP's were greater than 120 mmHg, Diastolic: % of asleep diastolic BP's was greater than 70 mmHg;

** Psystolic - Pdiastolic = 120mmHg - 80mmHg = 40mmHg;

$$***\text{Dip} = \left(1 - \frac{\text{SBP}_{\text{Sleeping}}}{\text{SBP}_{\text{Waking}}}\right) \times 100\%$$

Table 4

Adverse Events (AEs)

Body System			
Cardiovascular	Mild	Moderate	Severe
Myocardial Infarction			All reported myocardial infarction will be classified as severe
Increased Blood Pressure	Systolic: BP 140 – 159 mm Hg or diastolic BP 90 – 99 mm Hg; medical intervention indicated	Systolic Blood pressure: BP $\geq$ 160 mm Hg or diastolic BP $\geq$ 100 mm Hg; medical intervention indicated	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis)
Hematology	Mild	Moderate	Severe
Anemia	Hemoglobin between 13–10 g/dl	Hemoglobin between 10–8 g/dl	Hemoglobin <8 g/dl
Thrombocytopenia	Platelet between – 75-150,000/mm ³	Platelet <50,000–75,000 /mm ³	Platelet <25,000–50,000 /mm ³
Neutropenia	Neutrophil < 1500/mm ³	Neutrophil <1000-1500/mm ³	Neutrophil <500-1000/mm ³
Gastrointestinal	Mild	Moderate	Severe
Vomiting	1 – 2 episodes (separated by 5 minutes) in 24 hours	3 –5 episodes (separated by 5 minutes) in 24 hours	6 episodes (separated by 5 minutes) in 24 hours; tube feeding, Total parental nutrition or hospitalization indicated
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to normal	Increase of 4 –6 stools per day over baseline; moderate increase in ostomy output compared to normal	Increase of 7 stools per day over baseline; incontinence; hospitalization indicated; serious increase in ostomy output compared to normal or death
Other	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL	Medically significant or immediately life threatening; hospitalization or urgent intervention indicated
Musculoskeletal	Mild	Moderate	Severe
Joint pain	Mild pain	Moderate pain; limiting instrumental activities of daily living	Serious pain; limiting self-care
Nervous	Mild	Moderate	Severe
Any patient reported event	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Moderate symptoms; limiting instrumental activities of daily living	Severe symptoms; limiting self-care activities of daily living or death
Skin and subcutaneous tissue disorders	Mild	Moderate	Severe
Rash- characterized by a circumscribed and elevated skin lesion	Papules and/or pustules covering <10% body surface area (BSA)	Papules and/or pustules covering 10–30% body surface area (BSA)	Papules and/or pustules covering any % body surface area (BSA), with life threatening consequences or death
Stevens Johnson Syndrome		Skin sloughing covering <10% body surface area	Skin sloughing covering 10 –30% body surface

Body System			
		(BSA) with associated signs(e.g., erythema, purpura, epidermal detachment and mucous membrane detachment)	area (BSA) with associated signs (e.g., erythema, purpura, epidermal detachment and mucous membrane detachment) or death
Hepatobiliary	Mild	Moderate	Severe
Abnormal liver enzymes	Above the upper limit to 2x (ULN)	2x to 4x (ULN)	Greater than 4x (ULN)

BP- Blood pressure, ULN - Upper Limit of Normal, BSA - Body Surface area