Project 2: The Effects of Urate Lowering Therapy on Inflammation, Endothelial Function, and Blood Pressure (NIAMS: CORT)

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Summary of Changes from Previous Version:

Protocol Version	Affected Section(s)	Summary of Revisions Made	Rationale
9	Appendix 1	Informed Consent Approval Updated with Annual Review (April 2018)	Informed consent was updated with Annual Review
8	1aE, G	Plasma oxypurinol and allopurinol levels measurement and increased compensation for ambulatory blood pressure monitoring (September, 2017)	Determine participant compliance and promote recruitment/retention
7	1aF	Urine urate and urine creatinine quantification with banked samples (May, 2017)	Measure fractional excretion of urate in urine samples
6	1aF	Reduction of Washout between phases from 4 to 2 weeks (August, 2016)	Promote recruitment and increase retention
5	1aG, 7a/b	Removal of stable/low dose calcium channel blockers as exclusion criteria (October, 2015)	Expand inclusion criteria and promote recruitment
4	1D, 1aI4,7, 5, 7a	Age of recruitment lower limit decreased from 19 to 18 (July, 2015)	Expand inclusion criteria and promote recruitment
3	1D, 5	Title of the study changed to Serum Uric acid Reduction to Prevent HypERtension Study (SURPHER) (April, 2014)	Promote the recruitment of younger study subjects with a shorter, more recognizable title.
2	1D, 1aI4,7, 5, 7a	Age of recruitment upper limit increased from 35 to 40 (September 2014)	Expand inclusion criteria and promote recruitment

Contents

1. Study Overview

1a. Study Protocol

A. Background

The association between elevated serum urate and increased blood pressure is well established from epidemiologic data.[1-13] Providing further support for the causal nature of this association, rat models describe new onset hypertension soon after serum urate increases following the administration of the uricase inhibitor oxonic acid.[14] Reduction of nitric oxide levels in endothelial cells and the concurrent activation of the renin-angiotensin system are mechanisms postulated to mediate this effect.[15-17] In humans, endothelial dysfunction and increased plasma renin activity have been associated with greater serum urate.[16-18] Despite these compelling data from physiologic and animal studies, there is a paucity of studies that evaluated the blood pressure lowering effects of urate-lowering therapy (ULT) in humans.[19] In one early study, significant reductions in blood pressure were demonstrated in adolescents with hyperuricemia and hypertension after treatment with allopurinol. Similar blood pressure reductions after ULT with the uricosuric drug probenecid favors the hypothesis that urate reduction leads to blood pressure lowering independent of xanthine-oxidase inhibition.[20] The trials, however, were small (n=30 and n=60),[19] did not investigate the mechanisms underlying the blood pressure reduction, and did not evaluate differential responses to ULT by race/ethnicity. This latter point is particularly relevant, since racial/ethnic differences in the pathophysiology of hypertension are known.[21-24] Translational research studies to define the mechanisms through which urate lowering might contribute to hypertension control in humans are scarce. Given the high burden of hyperuricemia and hypertension among US adults, particularly among those with gout, understanding the blood pressure lowering effects of ULT and its underlying mechanisms has important public health implications.

A.1. Hypertension is a common disorder that impacts patients with hyperuricemia and gout

Persons with gout and hyperuricemia suffer from hypertension and other cardiovascular outcomes at disproportionately greater rates compared with the general population and independently of other demographic considerations and traditional cardiovascular risk factors.[25-29] Since hyperuricemia is a necessary precondition for the development of gout, the demonstration that lowering serum urate confers the additional benefit of lowering blood pressure (BP) has special relevance for patients with gout. Hypertension affects about 37% of US adults.[30] Its health care impact is considerable and is one of the most important risk factors for chronic kidney disease, stroke, and myocardial infarction.[31-33] In 2003, direct costs associated with hypertension exceeded $37 billion.[34] Several mechanisms contribute to the etiology of hypertension, including the renin-angiotensin-aldosterone hormonal system (RAAS), the sympathetic nervous system, and the endothelin-nitric oxide system.[35] Approaches to hypertension management are based on pharmacological therapy to target these systems: angiotensin-converting enzyme blockers and blockers of the angiotensin receptor or the mineralocorticoid receptor for the RAAS, alpha and beta adrenergic blockade for the sympathetic nervous system, calcium channel blockers for calcium stimulated peripheral vasoconstriction, and diuretics for volume expansion.[36] Even after combining these approaches, a large proportion of treated

hypertensive patients who are adherent with therapy fail to achieve BP goals.[37] There is a clear need to develop novel approaches to hypertension control, particularly among persons with gout, and to better understand the pathophysiology of hypertension in the setting of hyperuricemia.

A.2. Elevated serum urate is independently linked to hypertension

The association between levels of serum urate and BP has been described for many decades.[1,2,4,6-10,25,26,38-48] This association has been reported even with serum urate levels within the "normal" range. [49] Animal models provided additional support for a causal hypothesis by demonstrating that serum urate increases, induced artificially in rats through administration of oxonic acid, lead to glomerular hypertension.[14,50-52] These changes were fully reversible after the administration of ULT.[53-55] Administration of 200 mg of twice daily allopurinol as ULT for one month resulted in a significant reduction in casual and ambulatory BP among 30 adolescents with hypertension and hyperuricemia when compared with placebo in a crossover trial.[19] Although this study had a very strong design and good medication compliance, it suffered from small sample size, lack of translational outcomes related to the mechanisms mediating the BP lowering, generalizability issues (large proportion of obese patients), and inability to explore outcomes by race and ethnicity. Another study evaluated the effect of urate lowering with 300 mg of allopurinol daily for 3 months on BP among 48 hyperuricemic adult patients in Turkey compared to 21 normouricemic age and sex-matched controls that received no treatment. Decreases in systolic BP (SBP), diastolic BP (DBP), C-reactive protein (CRP) levels, and serum urate were noted in the hyperuricemic patients but not in the control group.[56] Significant study limitations include the lack of an adequate placebo-controlled group, BP outcome based on casual measurements, and a limited sample-size. An additional study demonstrated that the uricosuric drug probenecid is also effective in reducing BP in children and adolescents, supporting the notion that BP reduction is associated with urate-lowering by mechanisms other than xanthine-oxidase inhibition.[20] We will make further progress in addressing these questions by including a larger sample size of mixed race/ethnicity, mechanistic and translational studies of the effect of ULT on BP, a careful methodology with a crossover design, and a placebo-control group.

A.3. Serum urate lowering is an innovative approach to BP lowering

A.3.1. Serum urate effects on inflammatory markers and hypertension

Serum urate concentrations have been associated with levels of inflammatory markers in individuals without gout.[57,58] Improvements in hypertension control have been associated with a decrease in levels of CRP and high sensitivity C-reactive protein (hsCRP).[59,60] Given their importance in the pathophysiology of endothelial dysfunction and cardiovascular disease, changes in inflammatory markers are important candidate mechanisms to better understand the action of urate-lowering on endothelial function and BP. A study done in a small number (n=30) of hyperuricemic adults (mean age 54 years) showed a reduction in hsCRP after treatment with allopurinol but no correlation with BP change.[61] The association between levels of inflammatory markers, such as hsCRP, and BP could be tested more directly in a younger population with less chronic vascular remodeling.[62,63]

A.3.2. Serum urate effects on endothelial function and hypertension

Endothelial dysfunction is an independent risk factor for cardiovascular events and a well-described predecessor and comorbid factor with hypertension, thus increasing cardiovascular risk.[64-66] Rat models have shown that hyperuricemia can induce endothelial dysfunction,[67] and human studies have described improvement in endothelial function after treatment of hyperuricemia with allopurinol.[16,18,61] Although prior studies suggested that improvements in endothelial function achieved with allopurinol were secondary to xanthine oxidase inhibition and not to urate lowering [68], data from our group have found that urate lowering by mechanisms other than xanthine-oxidase inhibition was effective at reducing BP in adolescents. [20] A study done in a small number of hyperuricemic older adults (n=30) reported that ULT with allopurinol improved endothelial function.[61] Estimation of endothelial function through ultrasound FMD is the most widely used method in children and adults, and is reflective of the integrity of the nitric oxide (NO)-mediated vasodilation mechanisms. This tool will allow our group to test whether there is an association between serum urate lowering, NO-dependent endothelial function, and BP lowering in young adults.

A.3.3. Decreases in serum urate reduce blood pressure

This hypothesis has been tested only in small groups of patients.[19,56,61] In all cases, reducing urate levels led to statistically significant decreases in BP. Given that hypertension is associated with anatomical changes leading to an increase in the wall/lumen ratio of the resistance arteries[62,63], we expect that our innovative approach will show its maximum effect in a population of young adults in whom chronic vascular remodeling induced by hypertension is less likely to have occurred. In addition, we expect our proposed translational study to be beneficial even in individuals that do not meet the formal biochemical definition of hyperuricemia (greater or equal than 6.8 mg/dL).[69] This is supported in part by epidemiological data reporting an increase in the risk of developing cardiovascular events associated with increases in serum urate even at serum urate concentrations below the threshold of hyperuricemia. [25,26] Our own findings showed an increased risk for incident hypertension over 20 years of follow-up for women with serum urate concentrations greater or equal to 4.0 mg/dL and men with serum urate concentrations greater or equal to 5.0 mg/dL. [49] The results of the proposed study could lead to more ambitious goals for ULT in individuals with gout and cardiovascular comorbidities.

A.4. Serum urate levels increase more rapidly over time and the prevalence of hypertension is higher among African Americans

Prior studies examining the BP response to ULT have not examined the question of racial/ethnic differences. It is well established that hypertension disproportionately affects African Americans[70] and that African Americans have differential responses to hypertension therapies.[21-24] Our group has reported that young African Americans have lower serum urate concentrations than Caucasians after adjustment for clinical and demographic factors. [71] However, the risk of developing hyperuricemia over 20 years of follow-up increased more rapidly in African American women than in men. Other investigators have reported higher concentrations of serum urate in African Americans later in life.[72] We hypothesize that BP in African Americans

is more sensitive to serum urate and that this may be a mechanism to partially explain the higher prevalence of hypertension among African Americans. Given these reported differences, we will investigate differences in hsCRP, FMD, and BP in response to ULT.

B. SPECIFIC AIMS

In the context of a prospective, randomized study with crossover, **our specific aims are to:**

1. Determine in young adults with pre- or stage I hypertension whether ULT with 300 mg of allopurinol once daily over 4 weeks will:

1a. Lower blood pressure

Hypothesis 1: Four weeks of ULT therapy will induce a greater reduction in ambulatory blood pressure levels when compared to four weeks on placebo

Hypothesis 2: ULT will induce a greater reduction in ambulatory blood pressure levels in African Americans when compared with other races/ethnicities.

1b. Induce a change in endothelial function

Hypothesis 3: Four weeks of ULT will induce an improvement in endothelial function as indexed by flow-mediated arterial vasodilation (FMD) compared to placebo.

Hypothesis 4: ULT will induce a greater increase in FMD in African Americans when compared with other races/ethnicities.

1c. Induce a change in serum levels of high sensitivity C-reactive protein

Hypothesis 5: Four weeks of ULT will induce a greater reduction in serum levels of high sensitivity C-reactive protein (hsCRP) than placebo.

Hypothesis 6: ULT will induce a greater reduction in hsCRP in African Americans when compared with other races/ethnicities.

2. Determine if the impact of ULT on hsCRP, FMD, and blood pressure is associated with the change in serum urate achieved

Hypothesis 7: Changes in hsCRP, FMD, and blood pressure will be associated with the serum urate change observed after four weeks of ULT with allopurinol compared to placebo.

We initially aimed to recruit 112 young adults (ages 18-40 years) with pre-hypertension or JNC 7 stage I hypertension[37] not taking antihypertensive medication. Recruitment includes men, women, African Americans, and other races/ethnicities in equal numbers. To increase generalizability and add novelty to the research question, participants will not be required to be hyperuricemic, but to have serum urate levels $\geq$ 5.0

mg/dL for men and 4.0 mg/dL for women. Participants will be randomized to receive allopurinol or placebo in a four-week study with crossover. This novel translational study will generate knowledge about the mechanism by which ULT lowers blood pressure and provide evidence that can be translated into clinical practice for patients with gout or high-normal urate levels and other cardiovascular risk factors, including hypertension.

C. RESEARCH STRATEGY

We propose a novel intervention for reducing BP that could have a preferential impact in patients with hyperuricemia and gout. There is a great need for new anti-hypertensives, particularly among those with gout. The proposed study is novel in its plans to investigate the physiologic mechanisms through which urate contributes to vascular disease and by which ULT may contribute to BP reduction. Also innovative, we will: 1) determine to what extent the described benefit of lowering serum urate extends beyond the adolescent population previously studied into young adults, 2) test whether a urate-lowering approach will benefit individuals that do not yet meet the current definition of hyperuricemia and do not have gout, and 3) begin to explore potential mechanisms for the higher prevalence of hypertension among African Americans. If successful, this work could translate to the standard of clinical care and to health care recommendations for the population as a whole.

D. Subject Selection

Similar to past successful recruitment for hypertension studies at our institution, announcements will be placed in the web page www.researchmatch.org , Birmingham metro area local newspapers, UAB publications (e.g. UAB reporter, distributed to all UAB campus), websites, and flyers that will be posted on UAB campus. Given the inclusion age range with this trial, participants will be also be recruited utilizing social media ads (e.g., Spotify, Facebook, and Instagram). The flyers and ads will request adults, ages 18-40, that are interested in participating in a clinical study for hypertension, to call the recruitment coordinating center. Additionally, participants will also be recruited directly from the UAB 700 physician outpatient care facility, The Kirklin Clinic, through brochures distributed to physicians and from the Hypertension clinic. UAB is a national leader in recruitment of African American study participants for clinical trials and large cohort studies.

We expect a screen fail rate of ≥66%, thus we anticipate screening roughly 3 or more persons by BP measurements and focused history to find one eligible participant for laboratory screening. From our previous epidemiological work with a population of similar age, we estimate that 30% of adults, 18-40 years of age, will have hyperuricemia as defined by a level of $\geq$ 6.8 mg/dL.[73] Since this information was obtained in 1986, and the trends for obesity and other conditions known to elevate serum urate have increased, it is likely that over 50% of the population of men and women will have serum urate levels $\geq$ 5.0 and $\geq$ 4.0 mg/dL, respectively. Also, based on NHANES 1999-2006 data, 53% of men and women age 20 to 35 years with pre-hypertension or stage 1 hypertension have serum urate ≥ 5.0 mg/dL (≥4.0 mg/dL for women). When considering this and the past experience of the UAB Hypertension group in screening, we anticipate that about 50% of the laboratory-

screened subjects will be ineligible. In conclusion, we will need to perform screening laboratory assessments in 236 individuals. We have carefully accounted for these recruitment challenges in our study design and budget and anticipate enrolling 30 individuals per year.

Table 1. Enrollment criteria.
Inclusion criteria
Pre-hypertension or stage I hypertension, defined as the following after the mean of two clinic measurements: -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, including diuretics**
Estimated glomerular filtration rate < 60 mL/min/1.73m2
Current use of any urate-lowering therapy or statins
Prior diagnosis of gout or past use of urate-lowering therapy for gout
Prior diagnosis of diabetes
Pregnancy, or 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 (AST > 40 Units/L, ALT > 58 Units/L, Alkaline Phosphatase > 117 Units/L)
Individuals of Han Chinese or Thai descent with HLAB5801 genetic phenotype
Serious medical condition that at investigator's judgment precludes utilization of a fixed dose of allopurinol

*Study was amended on 10/2014 and 07/2015 to allow inclusion of individuals ages 36-40 and 18, respectively.

**Study was amended on 10/2015 to allow of inclusion of individuals on stable doses of calcium channel blockers.

E. Participant Retention

Retention of participants is central to the internal validity of the proposed study and will be an extraordinarily high priority. To increase retention, we hire engaging, attentive, and responsive staff and provide participants with as much flexibility as we can to complete their study visits. Other features used to promote high retention rates will include tracking systems and collection of information (i.e., names and telephone numbers) for two friends/family members who do not live with the study participant but would know where to find them. Once enrolled, a proactive plan for retention will be implemented that includes in person visits

every 4 weeks, and phone calls in between each visit. The following major principles and commonly used strategies to maximize retention and minimize loss to follow-up will be employed during the trial:

- Stressing the idea that participants have an active role in the research and are part of the research team
- Enhancing participant's understanding of the study's mission and the protocol
- Stressing that retention efforts begin with recruitment and are an ongoing process
- Building patient relations and patient satisfaction, with the study staff taking a central role on this effort
- Emphasizing the importance of congeniality, respectfulness and friendliness in interactions with participants
- Actively discuss with patients any questions and concerns pertaining to their condition
- Identifying potential problems and key retention factors and developing intervention strategies regarding retention
- Assessing each patient's drop out potential and intervening as needed to keep patients interested in continuing to participate
- Subjects will be paid $25 for each completed clinic visit and flow mediated dilation procedure. In addition, subjects will receive $100 for each complete 24-hour ambulatory blood pressure recording session. Payments will be made at the completion of each study visit's procedures. Should subjects withdraw from the study, they will be paid for the completed visits up to the date of withdrawal.

F. Study Procedures

Recruitment is planned for commencement in month 9 of the first grant year and continue through the middle of year 5 of the funding period pending a no cost extension which would augment the proposed timeline. Participants will be randomized following confirmation of eligibility. Enrollment and participant follow-up will continue until all participants were enrolled. Data entry and quality control will occur concurrently with participant enrollment and follow-up.

Table 2. Projected Timeline of Research Tasks

	Year 1		Year 2		Year 3		Year 4		Year 5	
Tasks (start/stop dates)	**7/12 12/12**	**1/13 6/13**	**7/13 12/13**	**1/14 6/14**	**7/14 12/14**	**1/15 6/15**	**7/15 12/15**	**1/16 6/16**	**7/16 12/16**	**1/17 6/17**
Obtain IRB approvals	■	■	■							
Develop patient screening tools	■	■								
Develop data collection tools	■	■								
Testing, screening and intervention			■	■	■	■	■	■	■	
Pilot testing of instruments			■	■						
Screening and intervention			■	■	■	■	■	■	■	
Data management	■	■	■	■	■	■	■	■	■	■
Database construction	■	■	■							
Data entry			■	■	■	■	■	■	■	■
Data analysis						■	■	■	■	■
Report preparation/ dissemination								■	■	■

All study visits and procedures will be performed at the University of Alabama at Birmingham in facilities dedicated to clinical research studies.

Screening visit (Visit 1)

The first visit will take about 1-2 hours to complete. Men and women within the first 7 days of the menstrual cycle will be re-evaluated for a qualifying BP value, and if within the study range, invited to proceed with informed consent and randomization on the same day or as soon as convenient for them. During the first visit, the following procedures will be performed, and information will be obtained to determine eligibility to continue in this research study:

- Review inclusion/exclusion criteria
- Informed consent
- Focused medical history
- Medication review
- Screening Visit Laboratory
    - CBC
    - Hemoglobin
    - Liver tests*
    - Pregnancy test†
    - Cotinine test
    - Serum creatinine
    - Serum urate

- Administration of single-blind run-in placebo – 2-4 weeks duration (2 week minimum, but longer duration for pre-menopausal women).

Screening visits and study entry criteria assessment. At the screening visit, the study objectives will be explained to potential participants and once interest in participating is confirmed, they will be offered an initial informed consent for the screening evaluation. Three BP measurements will be performed following the recommendations published in the report of the Joint National Committee on Hypertension [37] participants will sit quietly for at least five minutes with feet on the floor and arms supported at heart level, appropriate cuff sizes will be used, restrictions on alcohol and caffeine consumption for a specified time period prior to obtaining BP measurements will be imposed, and appropriate maintenance of all equipment will be performed. We will use automated BP devices for measurement of clinic BP (Omron, Bannockburn, IL, Model HEM-705CP). If the average of the second and third systolic and diastolic BP measurements taken 3 minutes apart is found to be within the pre-hypertension or stage I hypertension range (SBP ≥ 120 and <160 mmHg or DBP ≥ 80 and < 100 mmHg) the participant will be allowed to continue with the full screening procedures to assess for study entry criteria (Table 3). Before randomization, participants will participate in a single blind run-in period lasting 2-4 weeks during which they will receive a 21-45 day supply of the daily dose of placebo.

Focused history and physical examination: Information collected will include date of birth, self-reported race/ethnicity (defined as in previous studies investigating its role in rheumatic diseases)[74], medical conditions that might preclude study participation including current or recent infections and gout, medication history (including use of aspirin, gout medications, losartan, fenofibrate), duration of hypertension, weight, and height. Gynecological history will include last menstrual date. This information will help assess the balanced allocation of participants during randomization, estimate glomerular filtration rate, establish phase of menstrual cycle for women, and identify exclusion criteria.

Laboratory: Serum urate, transaminases, alkaline phosphatase, serum creatinine, complete blood count, bilirubin, cotinine test, and pregnancy test for women. Blood samples will be analyzed at UAB lab facilities in the Kirklin clinic. Pregnancy and cotinine tests will be performed by trained personnel at the time of visit using urine samples. All lab samples will be discarded if the participant is deemed not eligible for the study.

Informed Consent

The process of informed consent will be carried out by one of the study coordinators or research assistants at the beginning of the screening in-person study visit. During the screening visit, the consent form will be read by the study participant and then each section will be explained by the research study coordinator obtaining consent. The participant will be given as much time as they need to read and ask questions about the consent form. The individual will be informed that he/she is not obligated to participate in the study and that it is strictly voluntary. The informed consent process will ensure that there is no penalty for not participating in a clinical trial and that treatment will not be compromised if individuals do not participate or if they cease participation at any time.

By signing the consent form, the participant authorizes the use of their personal health information, that they understand the study and its benefits and risks, and agree to all other aspects of the study outlined in the form. It allows the participant the opportunity to decide whether they want to participate in a study. During this process, individuals will be informed of all aspects of the study so that they can make an informed decision. Participants will then confirm their willingness to participate in the research study by signing the Informed Consent form.

After the participant has signed the consent form, the study coordinator/research assistant conducting the visit must countersign and date the Informed Consent Document, along with a third party witness to the consent. A signed version of the consent form will be kept by the study staff in the study binder and an additional copy of the consent form will also be given to the participant to keep.

The informed consent document contains the following:

- Disclosure of relevant information to prospective participants about the research;
- The participant's comprehension of the information;
- The participant's voluntary agreement to participate in a research study without coercion or undue influence.
- An optional participation in future research by allowing samples to be biobanked.
- Complete disclosure of any appropriate alternative procedures and their risks and benefits
- Disclosure of the extent of confidentiality that will be maintained
- Statement of compensation and/or medical treatment available if injury occurs
- Name, address, and telephone number of the Principal Investigator

If there is a change in any of the study procedures that may affect the participant, the informed consent document will be revised and approved by the IRB. Any participants active within the study prior to a change in procedures will sign the amended consent form. Per NIH policy, the signed consent forms will be kept as part of the study record for at least 7 years after completion of the study. Participants can withdraw their consent at any time by informing the study coordinator. Additionally, all participants will be provided with descriptions of allopurinol hypersensitivity induced rashes and specific instructions to contact the study staff immediately should a rash appear.

The UAB study staff will:

- Provide participants with adequate information concerning the study procedures and scope
- Provide adequate opportunity for the participant to consider all available options
- Respond to the participant's questions and concerns
- Ensure that each participant understands all information provided
- Confirm acceptable birth control is being used
- Obtain the participant's written voluntary consent to participate

- Sign the consent form as the person obtaining consent and functioning as a third-party witnesses
- Provide participants with a copy of the consent form
- Keep the signed form in the participant's binder
- Attempt to schedule an early end of study assessment in the case of study drug discontinuation

Table 3. Schedule of visits and evaluations

			Treatment Phase								
Visit description / Study Procedures	Screening	Run-in period	Phase 1				Washout period	Phase 2 (crossover)			
	Weeks										
Male participant (original)	0	1-2	3	4	5	6	7-10	11	12	13	14
Male participant (revised)††	*0*	*1-2*	*3*	*4*	*5*	*6*	*7-8*	*9*	*10*	*11*	*12*
Female participant (original)	0	1-4	5	6	7	8	9-12	13	14	15	16
Female participant (revised)††	*0*	*1-4*	*5*	*6*	*7*	*8*	*9-10*	*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	
Physical exam	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
Cotinine	X		X			X		X			X
Serum banking§			X			X		X			X
Contact check in				X	X		2X		X	X	

PE-physical examination, BP-blood pressure, ABPM-ambulatory blood pressure monitoring, FMD-flow mediated dilation of the brachial artery, hsCRP-highly sensitive C-reactive protein
*Liver tests are alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. At screening only, a bilirubin test was performed.
** Physical exams will be conducted following screening visit only in cases where rash has been reported
†Only in women
††Revised timeline corresponded to reduction in washout phase from 4 to 2 weeks for male participants and female participants depending on menstrual cycle.
§Sera and whole blood (for DNA extraction) will be stored.

Visit 1:

- Informed Consent
- Blood pressure
- Physical Exam
- Focused medical history
- Blood sample
- Urine sample
- CBC
- Liver tests-Liver tests are alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase
- Serum creatinine
- Serum urate
- Pregnancy test†
- Cotinine test
- Dispense placebo run-in if all qualifications are met

Visit 2 will occur 2 weeks following the screening procedures, signing of the consent form, and 2 week long (±3 days) placebo run-in period in the UAB Hypertension Clinic. For pre-menopausal women, run-in was timed so the end of the run-in will coincide with the onset of their menses and no later than day 7 of the next cycle for standardized measurements of FMD, thus run-in was typically of a 28-day duration. [75] Compliance with placebo run-in will be assessed through pill count. Before and after each intervention phase, the markers for

the study primary (serum urate, hsCRP, endothelial function as measured by FMD) and secondary outcomes (ambulatory BP monitoring) will be collected. Other data will be collected as needed for safety monitoring.

Women will need to be evaluated between days 1-7 of their menstrual cycles for study visits. This will be done to make FMD results comparable among women, and between women and men, since hormone levels have been shown to affect FMD results.[75] Participants will be entered into the trial and will be randomized to receive allopurinol or placebo. Neither the participant nor the Principal Investigator will know the drug assignment. This will begin Phase 1 (i.e., the first four weeks of the intervention). Randomization lists, stratified by race and gender, will be generated using a random number generator prior to the initiation of the study (i.e., in the first six months of study funding). Randomization will occur in blocks of size 2, 4, or 8 and will be stratified on sex and race/ethnicity to ensure order of treatment administration is balanced by sex and race. Randomization will occur using a random number generator. The sequence of treatment assignment will be determined by the random number generator and held by the pharmacist creating treatment arm study pill over-encapsulation. The pharmacist will maintain the master list and securely store the randomization files.

Visit 2:

- Blood pressure
- Updated medical history
- Blood sample
- Urine sample
- 24-hr ABPM
- FMD
- CBC
- Liver tests-Liver tests are alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase
- Serum creatinine
- Serum urate
- hsCRP
- Serum banking
- Pregnancy test†
- Cotinine test
- Pill count from run-in
- Potential adverse events
    - Assess any gastrointestinal symptoms, rash, or any other untoward effect
    - If potential untoward effect, study investigator will follow-up with subject

- Dispense 4 week course of allopurinol or placebo based on treatment assignment following return of the ABPM monitor

Interim visit check-in:

Between study visits, there will be four contact check-ins to encourage study compliance. These will be made by the study coordinator or research assistant. Additionally the study coordinator and/or research associate will assess the following during the call.

- Pill count
- Potential adverse events
    - Assess any gastrointestinal symptoms, rash, or any other untoward effect
    - If potential untoward effect, study investigator will follow-up with subject
    - If no untoward effects, subject will be encouraged to continue medication

Visit 3:

- Blood pressure
- Physical exam (head/neck/heart/abdomen)
- Updated medical history
- 24-hr ABPM
- FMD
- Blood sample
- Urine sample
- CBC
- Liver tests-Liver tests are alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase
- Serum creatinine
- Serum urate
- hsCRP
- Serum banking
- Pregnancy test†
- Cotinine test
- Pill count
- Potential adverse events
    - Assess any gastrointestinal symptoms, rash, or any other untoward effect
    - If potential untoward effect, study investigator will follow-up with subject

Following Visit 3, participants will begin a “wash-out period” before crossing over to the alternate treatment assignment. The wash-out period will last one month. Allopurinol has a half-life of 1-2 hours before being converted to oxypurinol, which has a half-life of 18 to 30 hours. [2] Thus, thirty days from the last allopurinol

dose is an adequate wash-out period and no persistent effects from the drug should be expected at this point. In fact, a subsequent amendment to the protocol (approved by IRB on August 2016) allowed the duration of washout for male participants and some female participants without a regular menstrual cycle (e.g., hysterectomy) to be shortened to 2 weeks. This approach is supported by interventional studies by our co-Investigator [redacted] and others investigating the effect of allopurinol on cardiovascular disease. Additionally during the washout period we will continue our phone check-ins with participants. These phone calls will be made by the study coordinator or research assistant, will take about 10-15 minutes each, and will assess any potential adverse events the participants may be experiencing.

Visit 4

- Blood pressure
- Physical exam (head/neck/heart/abdomen)
- Updated medical history
- Blood sample
- Urine sample
- 24-hr ABPM
- FMD
- CBC
- Liver tests-Liver tests are alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase
- Serum creatinine
- Serum urate
- hsCRP
- Serum banking
- Pregnancy test†
- Cotinine test
- Potential adverse events
    - Assess any gastrointestinal symptoms, rash, or any other untoward effect
    - If potential untoward effect, study investigator will follow-up with subject
- Dispense 4 week course of allopurinol or placebo based on treatment assignment following return of the ABPM monitor

Interim visit phone call-in :

Between study visits, there will be four follow-up phone calls to encourage study compliance. These phone calls will be made by the study coordinator or research assistant and will take about 10-15 minutes each. Additionally the study coordinator and/or research associate will assess the following during the call.

- Pill count

- Potential adverse events
    - Assess any gastrointestinal symptoms, rash, or any other untoward effect
    - If potential untoward effect, study investigator will follow-up with subject
    - If no untoward effects, subject will be encouraged to continue medication

Visit 5:

- Blood pressure
- Updated medical history
- Blood sample
- Urine sample
- 24-hr ABPM
- FMD
- CBC
- Liver tests-Liver tests are alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase
- Serum creatinine
- Serum urate
- hsCRP
- Serum banking
- Pregnancy test†
- Cotinine test
- Pill count
- Potential adverse events
    - Assess any gastrointestinal symptoms, rash, or any other untoward effect
    - If potential untoward effect, study investigator will follow-up with subject

24-hour Ambulatory Blood Pressure Measurements (ABPM)

All patients had 24-hour ABPM evaluations with an automated, noninvasive, oscillometric device (Spacelabs Healthcare, Inc., 35301 SE Center Street, Snoqualmie, WA 98065, U.S.A). An appropriate sized cuff is used with a cuff bladder encircling at least 80% of the arm, according to guidelines.[37] Participants will be receive careful instructions at each visit about the appropriate use of the device, including a handout that contains information about error codes that may occur and possible ways to resolve them. They will be asked to wear the device as much as possible within the 24 hour recording period. Participants will be encouraged to contact study staff immediately should they not any abnormalities in cuff or monitor function (e.g., cuff failure to inflate fully, repeat error codes). The first measurement will be obtained at the end of the clinic visit to ensure proper fit and function. ABPM monitors will be outfitted with new AA batteries for each participant and will

be programmed to obtain blood pressure recordings every 20 minutes for the daytime (awake) and every 30 minutes for the nighttime (asleep) for a 24-hour period. For the purposes of this study, daytime will be defined as 06:00-21:59 and nighttime will be defined as 22:00-05:59. Compliant 24-hour ABPM will be defined as a minimum of 14 successful daytime recordings and 5 nighttime readings. Those with less than these minimal thresholds will be asked to repeat the test. Should a non-compliant ABPM be unattainable, missing data will be handled with multiple imputation analysis. 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. Average ABPM will be weighted for the proportion of daytime and nighttime blood pressure readings. At the time of data analysis, manual readings will be discarded when there is a concurrent automated reading within the same 5-minute period. In instances where the ABPM was recorded outside of the appropriate time (e.g., the participant did not follow instructions as to the timing of the test), each ABPM will be evaluated by two data analysts blinded to condition to determine

Flow-Mediated Dilation analyses (FMD)

At visits 2, 3, 4, and 5, vascular endothelial function is measured by flow-mediated dilation (FMD) of the brachial artery performed by a study investigator who is blinded to treatment assignment. In women participants, FMD is performed within the first 7 days after onset of menstrual cycle to avoid known cyclic variations in FMD. [76,77] 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 were asked to rest for 10 minutes in the supine position in a quiet room. Ultrasound of the brachial artery was taken at approximately 5 cm proximal from the elbow in longitudinal section on the right arm. The probe was maintained in a fixed position at a fixed angle, and baseline artery diameter is recorded for 1 minute. Blood flow was estimated by pulsed Doppler velocity, and baseline artery diameter was assessed with simultaneous ECG recording for baseline assessment at every study visit. Reactive hyperemia was induced by inflating a BP cuff around the forearm to 50 mmHg above resting systolic BP. The cuff remained inflated for a five-minute period, and then was rapidly deflated. The longitudinal image of the brachial artery was recorded continuously from 30 seconds before to 3 minutes after cuff deflation. A mid-artery pulsed Doppler signal was obtained at an < 60° angle upon immediate cuff release and no later than 15 seconds after cuff deflation to assess hyperemic velocity parameters. Ultrasound images were recorded 30 seconds before cuff deflation, and 3 minutes after cuff deflation. The five largest diameters after deflation were averaged, and FMD was expressed as the percentage increase in diameter from the baseline average measurement to the peak average dilation. Brachial arterial flow-mediated dilation related parameters were 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 was 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 were calculated at the beginning and end of allopurinol and placebo phases for FMD.

G. Study Drug

The subjects will be randomized to receive allopurinol as ULT, at a daily dose of 300 mg once daily by mouth. Because of the crossover design of this study, all subjects who complete the protocol will be exposed to the active compound at some point in the study. Allopurinol will be purchased in bulk quantity through the UAB Investigational Drugs Pharmacy, which will also oversee the process of over-encapsulation with cellulose of the active medication and placebos, handle medication storage, distribution, and assignment of randomization sequence. The centralized management of the medication will allow maintenance of a double blind trial. A research nurse will contact participants approximately a week after the initiation of both intervention phases to ask if the participant has any medication concerns and to encourage compliance. Adherence to the medication will be recorded by pill counts at the follow-up study visits and consumption of at least 80% will be required to consider the participant compliant. A non-compliant participant will continue in the study and enter analyses as mandated by the intention-to-treat study design. At the end of the study, biobanked plasma samples (1.0-1.5 mL) will be evaluated for levels of allopurinol and the metabolite, oxypurinol, to determine an objective measure of study drug adherence. An amendment approved by the UAB IRB in September of 2017 allowed for testing oxypurinol and allopurinol prospectively on all participants enrolled (previously restricted to biobanked samples).

Individuals with transaminases or alkaline phosphatase values more than double the upper limit of normal will be excluded. The initial plan was to exclude subjects taking hypertension medications (including diuretics, that some participants do not regard as hypertension treatments), as this will have an unpredictable effect on the study outcomes. An amendment approved in 10/2015 allowed later for inclusion of individuals in stable doses of calcium channel blockers.

As allopurinol is classified as FDA class C during pregnancy pregnant women will not be allowed in the study. Women with recent deliveries or third trimester pregnancy losses in the previous 3 months will be excluded because of unpredictable effects on FMD. A special question about the use of azathioprine will be included, since this can have a severe interaction with allopurinol.[78] Participants with serious medical conditions that in the investigator's judgment pose an unacceptable risk for exposure to a fixed dose of allopurinol (e.g. advanced heart failure, advanced liver disease or cirrhosis) will also be excluded.

H. Data collection and Statistical Analyses (see also Data Analysis plan)

Measures of central tendency (sample mean, sample media) and dispersion (sample variance, sample standard deviation) will be calculated for all outcome variables (e.g., hsCRP, endothelial function, and BP on ABPM) and covariates (e.g. age, race, etc.). For outcome variables, distributional properties will be evaluated using histograms and normal probability plots. When the assumption of normality is questionable, outcome

variable tranformations will be examined to determine if the transformed variables approximate a normal distribution. will be transformed to achieve normality. Of the main study measures, we anticipate the distribution of hsCRP to be highly skewed. Consistent with prior studies, we will log transform hsCRP values for analysis. Possible outlying or inconsistent values will be detected and explored

Aim 1a: ULT and change in BP. The change in the average weighted 24-hour mean SBP and DBP will be compared among participants during the active treatment and placebo phases by paired t-test analysis.

Aim 1b: ULT and change in FMD. The change in the FMD will be compared among participants during the active treatment and placebo phases by paired t-test analysis.

Aim 1c: ULT and change in hsCRP. For each participant, we will first calculate the change in log transformed hsCRP from the beginning to the end of each crossover phase. The lack of an effect for the treatment sequence on the relative treatment responses will be confirmed using a treatment by sequence interaction term. Next, the within-participant change in hsCRP will be compared during the allopurinol treatment and placebo phases by paired t-test analysis.

Assessing differential treatment effects by race/ethnicity

Comparison in the mean change achieved in the allopurinol treatment minus placebo phase between African Americans and other races/ethnicities will be performed initially using a t-test for independent samples. If differences are identified in the distribution of covariates, then the analysis will be completed with analysis of variance.

Assessing change in serum urate on change in hsCRP, FMD, and blood pressure

For this aim, the independent variable is change in serum urate and the outcome is change in hsCRP, FMD, and BP. First, participant factors associated with a significant change in serum urate will be identified using unadjusted age, race/ethnicity, and gender adjusted linear regression models. The correlation between change in serum urate with change in hsCRP, FMD, and SBP and DBP will be calculated using Spearman correlation coefficients. Using linear regression models, the association between changes in serum urate with changes in levels of each outcome, separately, will be determined. Models will include initial adjustment for age, gender, and race/ethnicity with subsequent models adjusted for other potential confounders (e.g. estimated glomerular filtration rate). In analyzing the association of change in serum urate on change in BP, we will additionally adjust for hsCRP and FMD, individually and together. The reduction in the association (i.e. beta coefficient) between change in serum urate and change in BP from before to after adjustment for these variables will provide insight into the degree that these mechanisms mediate the relation between ULT and BP reduction.

With 56 African Americans and 56 other races/ethnicities, we will 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.

The primary analyses will be on an intent-to-treat basis. All statistical analysis will be performed using SAS statistical software (SAS, Cary, NC) by analysts masked to randomization assignment. All study related information will be entered into a study database. This database will be maintained on a secure computer with access limited to study personnel only.

Research information that identifies subjects will be shared with the UAB Institutional Review Board (IRB) and others who are responsible for ensuring compliance with laws and regulations related to research, including the U.S. Food and Drug Administration (FDA); and the Office for Humans Research Protections (OHRP). The results of this study may be published in scientific journals or presented at medical meetings, but study participants identity will remain confidential.

Calculation of sample size.

Power and sample size for aim 1c

In a prior study, a standard deviation of 8.5 mmHg for mean SBP by 24-hour ABPM in young and normotensive patients studied over 4 months has been reported.[81] Using this standard deviation and similar assumptions as outlined above (alpha of 0.05 and a correlation of 10% between pairs of SBP measurements for each subject), we will have 90% statistical power to detect a 3.7 mmHg difference in the change in SBP with allopurinol treatment versus placebo. An intra-individual variation (standard deviation) of the difference between two values on a same patient in DBP by ABPM of 6.3 mmHg has been reported previously by an international consensus conference.[82] Using this standard deviation and 90% statistical power, we will be able to detect a change in DBP of 2.0 mmHg for treatment with allopurinol versus placebo. For comparing African Americans versus participants of other race/ethnicities (assuming 46 participants in each group), we will have 90% statistical power to detect a difference in SBP and DBP of 5.8 mmHg and 4.3 mmHg, respectively.

Power and sample size for aim 1b.

The standard deviation of the difference between two values obtained in the same patient has been reported to be 2.5%. [80] With an alpha of 0.05, statistical power of 0.90, assuming a 10% within-period correlation of change in FMD and the sample size of 93 individuals calculated for hsCRP, we will be able to detect a change in FMD of 1.15% between allopurinol treatment and placebo. With 46 African American s and 46 other races/ethnicities, we will 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.

Power and sample size for aim 1a.

As mentioned above, it is anticipated that the distribution for hsCRP will be skewed, and thus, we will log transform hsCRP. The sample size for the study is based on having 90% statistical power, assuming a two-tailed alpha error of 5% and a within person correlation of log transformed hsCRP between measurements of 10%. The intra-person standard deviation for log transformed hsCRP has been reported to be 0.52 log mg/L on serial measurements extending into a one year period.[79] To detect a difference in log transformed hsCRP of 0.25 (log mg/L) will require a sample size of 93 participants with complete hsCRP measurements (i.e., from baseline and closeout visits for each of the study phases). This is equivalent to a net reduction of hsCRP of 25% (e.g., from 2 mg/L to 1.5 mg/L or from 1 mg/L to 0.75 mg/L). This difference was chosen as it represents approximately half of one standard deviation in log-transformed hsCRP and is a meaningful decline to be observed over a four week period. Although we expect minimal loss to follow-up, we have taken the conservative step of increasing the sample size to account for 20% of participants not having complete data. Thus, we will recruit and randomize 112 participants for the proposed trial. For comparing differences in the net reduction in hsCRP for African Americans in comparison to other races/ethnicities, with 46 African Americans and 46 other races/ethnicities, we will 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).

Table 4. Statistical Power for Aim 2			
Proportion of Outcome Variance Explained by Control Variables	**Incremental increases in R^2 due to the addition of change in serum urate in the presence of the control variables**		
	.05	.075	.10
.10	.64	.82	.92
.20	.69	.87	.95
.30	.75	.91	.97

Power and sample size for aim 2

Table 4 shows the statistical power to detect various incremental increases in R^2 due to the addition of change in serum urate in various regression models. For example, 92 individuals will provide 87% power using a Type I error rate of 0.05 to detect an increase of 7.5% in R^2 by adding change in serum urate to a regression model that already explains 20% of the variability in the change in BP with 5 control variables (age, sex, race/ethnicity, body mass-index, glomerular filtration rate). Due to the nature of multivariable linear regression, identical results hold for changes in hsCRP and FMD.

I. Risks and discomforts

1.Risks associated with phlebotomy

Subjects may experience pain from the needle used during phlebotomy, as well as possible bruising and soreness at the phlebotomy site.

2.Twenty-four hour ambulatory blood pressure monitoring

The procedure may cause some mild discomfort during cuff inflation, but otherwise has no associated risk.

3.Ultrasonographic measurement of flow-mediated dilation (FMD) of the brachial artery

The procedure requires the subject to be supine for approximately 20 minutes. The inflation of the blood pressure cuff for 5 minutes may cause arm discomfort.

4.Risks associated with allopurinol

Allopurinol is a competitive xanthine oxidase inhibitor used as a urate-lowering agent in patients with gout and for secondary hyperuricemia associated with chemotherapy. The risks of therapy with allopurinol include cutaneous reactions, onset of gout, diarrhea, nausea, and elevation of alkaline phosphatase or transaminases. Most of these side effects are mild and reversible, but severe cutaneous reaction of the Stevens-Johnson type and bone marrow suppression, events that are reported in less than 1% of allopurinol recipients, may not be reversible.

Allopurinol hypersensitivity syndrome (AHS) is a very rare (occurs in less than 1 in 1000 patients), life threatening idiosyncratic reaction that is commonly preceded by rash. published prior report mentions a crude incidence rate of .69 per 1000 person years for severe AHS cutaneous reactions. [83] It should be noted that during the course of this study we will expose to allopurinol a total 112 (<30 participants/year) ambulatory young adults aged 18-40, with normal kidney function. The rate of AHS in younger ambulatory patients with normal kidney function has not been established but is possibly lower than in individuals with gout and other medical comorbidities. Allopurinol hypersensitivity syndrome has principally been observed in individuals that are HLAB5801 positive (primarily individuals of Han Chinese or Thai descent). As recommended in the recently released American College of Rheumatology guidelines, we will test for HLAB5801 during initial screening in individuals of Han Chinese or Thai descent. [84]

5.Hypertension

Participants might be exposed to elevated blood pressure during a short period of the study. Given that adverse outcomes associated with hypertension tend to occur over time-periods measured in years and our study extends over a period of only 3-4 months, the risk of short exposure to elevated blood pressure leading to an adverse outcome is small.

To maximize the safety of the participants we have added a safety monitoring rule. When patients are seen at visits 2-5, if present with high blood pressure >160/110, first it will be rechecked 24 hrs later, if blood pressure

is still elevated, they will excluded from the study, received consultation by PI and/or Co-PI, and referred to their primary care provider.

6.Hyperuricemia

Participants will not be treated for asymptomatic hyperuricemia outside the study protocol, given that they are understood not to have been diagnosed with gout for enrollment in the study.

7.Pregnancy

This study will enroll women between the ages of 18-40 years, at which age we expect a greater majority to be fertile. If pregnancy occurs during the active treatment study period, there is a theoretical risk for the unborn as allopurinol is considered FDA class C during pregnancy. Untreated hypertension during the early pregnancy can also carry a small risk. Pregnant women will not be allowed in the study because allopurinol is classified as FDA class C during pregnancy, additionally prisoners, and institutionalized individuals will be excluded. Proper birth control methods for male and female participants will be confirmed and discussed during the screening visit.

8. Psychosocial risks

There are no obvious psychosocial risks

J. Potential Benefits

Currently, cardiovascular events caused by hypertension are the leading cause of morbidity and mortality in patients with gout and in the general population. If the addition of urate-lowering therapy to the standard therapy for hypertension reduces cardiovascular morbidity through improvement in endothelial function and inflammation in patients with hyperuricemia and those in the higher percentiles of normal serum urate concentration, this knowledge might have a significant impact on the quality of life of a large proportion of the general population.

K. Withdrawal of Participant Consent and Discontinuation of Study Drug

Participants can withdraw their consent at any time by informing the study coordinator.
This may include but is not limited to the following

a. The subject deciding to withdraw consent for study
b. An intolerable adverse event as judged by study investigator and/or participant
c. The subject discontinuing acceptable birth control methods or becoming pregnant
d. The subject enrolling in a conflicting investigational drug trial
e. The participant BP>160/110 between visit 2-5

If the study drug is discontinued, unless the subject withdraws consent, the subject will be followed for the full treatment period and all data will be collected as scheduled. Attempt will be made to schedule an early end of study assessment in the case of study drug discontinuation

As stated above as the risks to pregnant women or an unborn baby when taking allopurinol are not fully known, women must have a pregnancy test before the study starts and again throughout the study. Women must not become pregnant during this study. Acceptable methods of birth control include hormonal control methods, inter-uterine device, a double-barrier method (diaphragm with spermicide, condom with spermicide) or abstinence.

If participants suspect that they may have become pregnant during the study, the study coordinator must contact the study PI immediately and instruct the participant to stop taking their study medication. If it is confirmed that the participant is pregnant, they will be withdrawn from the study. The study PI may want to schedule a follow up visit and may choose to follow the outcome of the pregnancy. Additionally, taking allopurinol may involve unknown risks to a nursing infant. If it is discovered that participants are breastfeeding, they are not eligible to participate in the study and their participation will be discontinued immediately.

The rate of AHS is estimated at 1/1000, and may be a particular concern for those with reduced renal function or caring the HLA B5801 allele. We will expose 112 young adults with normal renal function to a dose of 300 mg per day of allopurinol as part of the study protocol, which include checking for the HLAB5801 allele in individuals of Han Chinese or Thai descent before exposure to allopurinol. Based on these rates of AHS and the procedures we have in place, we believe it is very unlikely, but possible that we could witness one severe cutaneous adverse reaction including AHS. Our stopping rule for safety re-evaluation would occur if we register more than one of such cutaneous adverse reaction (toxic epidermal necrolysis, Steven-Johnson's syndrome, AHS). We would then stop the study to comprehensively review safety and allopurinol dosing protocols. Other severe adverse reaction or deaths may or may not be related to the study drug and stopping or discontinuation of the study drug will be considered on an individual basis.

L. QUALITY and Safety ASSURANCE

1. Study team monitoring of data

Goal enrollment is 112 subjects. The history, physical examination and review of laboratory data will be conducted by the principal investigator or co-investigator, all of whom are experienced in clinical trials. Case report forms will be cross-checked with source documents by study personnel. The research team will meet biweekly to review recruitment, enrollment, laboratory tests, source documents, and case report forms.

2. Safety Monitoring

As this is proposed to be an NIAMS-funded study, NIAMS selected a Safety Officer, [redacted], to work under a subcontract with KAI Research INC to oversee the project. [redacted] will serve

as the independent Safety Officer for this project. [redacted] will provide independent oversight and act in an advisory capacity to the NIAMS to monitor research participant safety and data quality and to alert the Institute to potential issues in either of these areas. We will work with [redacted] and provide regular reports about blinded clinical outcomes, adverse events, and all laboratory information. In addition, all blinded laboratory data will be reviewed by the PI or co-investigator and case report forms. In addition to [redacted], we will submit monthly reports to the CORT Project Advisory Committee detailing the study progress and participant status, any adverse events, and any protocol deviations. The safety of the study participants is the highest priority for this project. The ability to make appropriate, sound scientific decisions regarding the outcome of each participant as early as possible is the top priority and as such, a process for the ongoing monitoring of results by independent scientists is important to maintain throughout the duration of the project.

In addition to [redacted], we will convene a Data Safety and Monitoring Board (DSMB) for additional monitoring of study procedures. The safety of the study participants is the highest priority for this project. The ability to make appropriate, sound scientific decisions regarding the outcome of each patient as early as possible is the top priority and as such, a process for the ongoing monitoring of results by independent scientists is important to maintain throughout the duration of the project.

The DSMB will be comprised of scientists who are independent of the study, the study investigators, and of UAB for this clinical trial. [redacted] will chair the DSMB. [redacted] is an internationally known rheumatologist at [redacted]. Additional members include [redacted], a biostatistician with cardiovascular expertise and [redacted], a nephrologist with gout expertise.

DSMB Responsibilities

The DSMB responsibilities are to:

- review the research protocol, informed consent documents and plans for data safety and monitoring;
- advise on the readiness of the study staff to initiate recruitment;
- evaluate the progress of the trial, including periodic assessments of data quality and timeliness, recruitment, accrual and retention, participant risk versus benefit, and other factors that can affect study outcome;
- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- review study performance, make recommendations and assist in the resolution of problems reported by the Principal Investigator;
- protect the safety of the study participants;
- report on the safety and progress of the trial;

- make recommendations to the Principal Investigator, and, if required, to the Food and Drug Administration (FDA) concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
- the Principal Investigator will report findings of the DSMB to the FDA in accordance to reporting guidelines;
- if appropriate, review interim analyses in accordance with stopping rules, which are clearly defined in advance of data analysis and have the approval of the DSMB;
- ensure the confidentiality of the study data and the results of monitoring; and,
- assist in the commenting on any problems with study conduct, enrollment, sample size and/or data collection.

The DSMB will discharge itself from its duties when the last participant completes the study.

Membership

The DSMB will include experts in or representatives of the fields of:

- rheumatology,
- cardiovascular disease
- biostatistics.

The Chair is the contact person for the DSMB. The *University of Alabama at Birmingham* shall provide the logistical management and support of the DSMB.

Board Process

At the first meeting the DSMB will discuss the protocol, suggest modifications, and establish guidelines to study monitoring to be reported. The DSMB Chairperson [redacted] in consultation with the Principal Investigator and the study team will prepare the agenda to address the review of study materials, modifications to the study protocol and informed consent document, initiation of the trial, reporting of adverse events, statistical analysis plan including interim analysis, etc.

Meetings of the DSMB will be held at the call of the Chairperson. The study investigators or designee will attend most of the meetings. An emergency meeting of the DSMB may be called at any time by the Chair or by the Principal Investigator should participant safety questions or other unanticipated problems arise.

Meeting Format

DSMB meetings will consist of open and closed sessions. Discussion held in both sessions is confidential. The Principal Investigator and key members of the study team attend the **open sessions.** Open session discussion will focus on the conduct and progress of the study, including participant accrual, protocol compliance, and problems encountered. Unblinded data are not presented in the open session.

The **closed session** will be attended by the DSMB members and the study representative(s). The study statistician may be present, at the request of the DSMB. Any data by blinded study group and, as necessary, unblinded data, are presented during the closed session.

Each meeting must include a recommendation to continue or to terminate the study and whether the DSMB has any concerns about participant safety made by a formal DSMB majority or unanimous vote. Should the DSMB decide to issue a termination recommendation, the full vote of the DSMB is required. In the event of a split vote, majority vote will rule and a minority report should be appended. The DSMB Chair provides the tiebreaking vote in the event of a 50-50 split vote.

A recommendation to terminate the study may be made by the DSMB at any time by majority vote. The Chair should provide such a recommendation to the Principal Investigator immediately by telephone and email.

Meeting Materials

DSMB interim report templates will be prepared by the study staff, typically the statistician, to be reviewed by the DSMB members at the first meeting. Interim data reports generally consist of two parts:

- Part 1 - Open Session Report and
- Part 2 - Closed Session Report

Format and content of the reports for both the open and closed sessions and plans for interim analyses should be finalized and approved at the initial DSMB meeting, although changes throughout the trial may be requested by the Board.

The reports will list and summarize safety data and describe the status of the study. All meeting materials should be sent to Principal Investigator who will forward the materials to the DSMB at least 7 to 14 days prior to the meeting. The reports are numbered and provided in sealed envelopes within an express mailing package or by secure email as the DSMB prefers. In select cases, Principal Investigator may approve direct mailing of the reports from the study to the DSMB.

1. **Part 1 - Open Session Reports:** Open session reports generally include administrative reports by site that describe participants screened, enrolled, completed, and discontinued, as well as baseline characteristics of the study population. Other general information on study status may also be presented. Listings of adverse events and serious adverse events as well as any other information requested by the DSMB may also be in the open session report, but none of the data should be presented in an unblinded manner. The DSMB may direct additions and other modifications to the reports on a one-time or continuing basis.

2. **Part 2 – Closed Session Report:** Closed session reports generally present the same information as presented in the open session but by blinded treatment group (e.g., A/B, etc.). The reports may also contain data on study outcomes, including safety data, and depending on the study, perhaps efficacy data. The Closed Session reports should be destroyed at the conclusion of the meeting. If the meetings are held by telephone, printed copies of the closed reports should be destroyed immediately following the meeting. If a study has an interim analysis, it is also discussed in the closed session.

Additional Reports

1. Mailings to the DSMB: On a scheduled basis (as agreed upon by the DSMB) blinded safety data could be communicated to all DSMB members and to the designated Safety Officer (VIA KAI). The study may provide these data directly to the DSMB, if such communication procedures are approved by Principal Investigator.

Reports from the DSMB

A formal report containing the recommendations for continuation or modifications of the study will be prepared by the DSMB Chairperson, Principal Investigator or designee. The draft report will be sent to the DSMB members for review and approval. It is the responsibility of the Principal Investigator to distribute the DSMB recommendation to all co-investigators and to ensure that necessary reports are submitted to the UAB IRB.

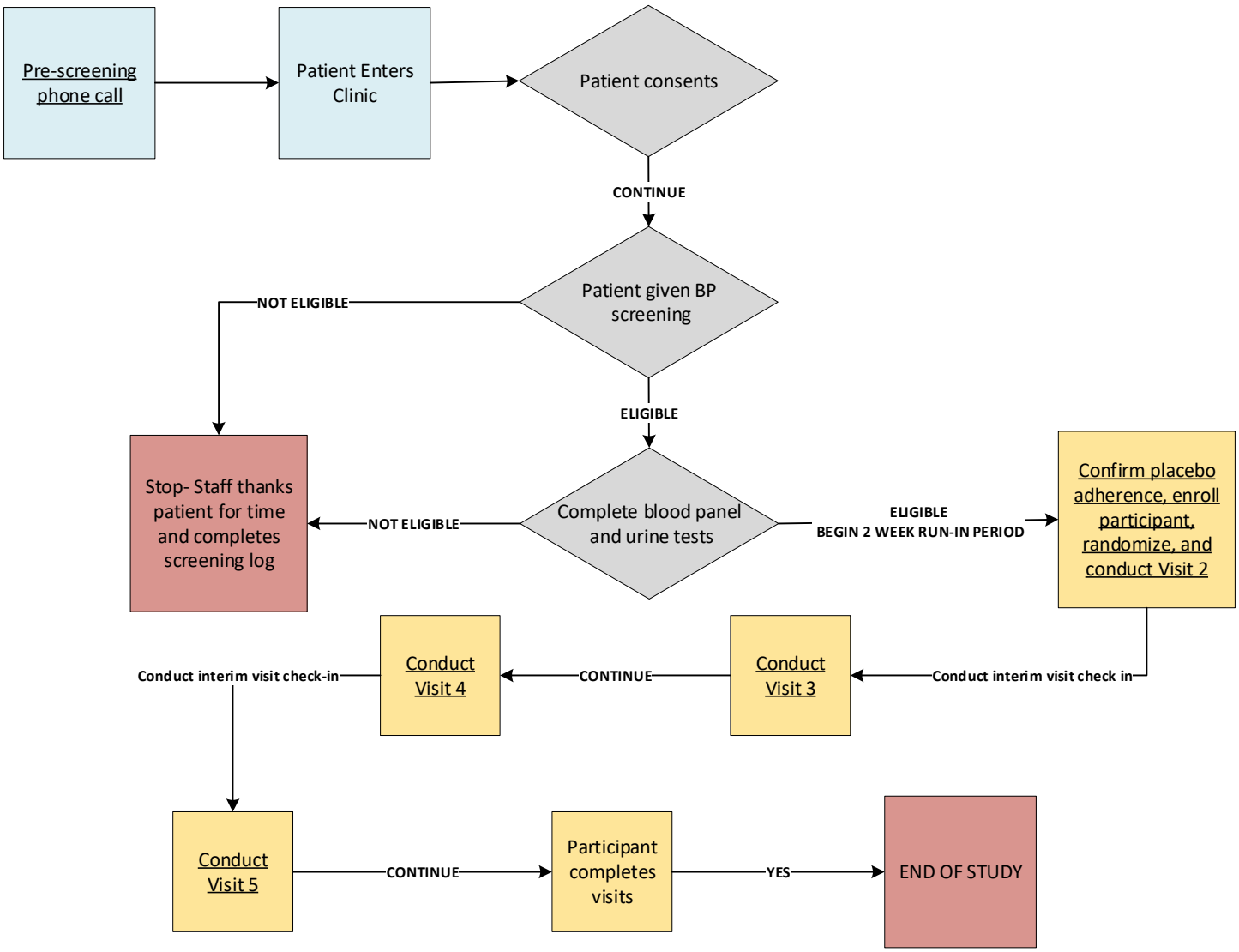
As previously stated, the formal DSMB report must include a recommendation to continue or to terminate the study. This recommendation should be made by formal majority vote. A termination recommendation may be made by the DSMB at any time by majority vote. In the event of a split vote in favor of continuation, a minority report should be contained within the regular DSMB report. The report should not include unblinded data or discussion of the unblinded data.

Confidentiality

All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.

1b. Schedule of visits and evaluations (see Protocol, Table 3. Schedule of visits and evaluations, page 13)

1c. Figure 1-Study Flow



2. Study Organization and Responsibilities

2a: Table 5 Study Roster

REDACTED

2b. Study Center

This is a single site study, and the staff listed above will be responsible for all aspects of the trial. This includes but is not limited to the following:

1. Development of the study protocol
2. Development of the manual of procedures and its maintenance
3. Participant randomization
4. Development and implementation of the data flow and data tracking
5. Development of procedures for data entry, error identification, and error correction
6. Adverse event monitoring and reporting
7. Quality control procedures
8. Submitting for IRB review and approval
9. Creating reports - enrollment, adverse events, participant status (e.g., withdrawals)
10. Preparing and sending required reports to the Safety Officer and the IRB
11. Submitting all required reports to KAI for distribution to the study appointed Safety Officer
12. Distribution of all changes, updates and policies of above mentioned reports and documents to KAI, NIAMS, and the study appointed Safety Officer.
13. Maintaining the study binder (regulatory and clinical documents)
14. Preparation of all study materials- data tables, recruitment materials, official reports
15. Identifying, recruiting, screening and enrolling participants
16. Obtaining informed consent from each participant
17. Protecting participants' rights
18. Collecting study data and following participants through study completion
19. Compliance and accountability of administration of study intervention, and
20. Communicating questions, concerns, and/or observations to the Principal Investigators

All of the above activities will be carried out by the study's project coordinator, project managers, and research assistants on a weekly basis (or more frequently as needed) and monitored by the principal and co-investigators. All instructions, forms, and documents necessary for these tasks are contained in the PROTOCOL and/or study binder.

2c. UAB CORT Executive Committee

As this project is a part of the UAB CORT of Gout and Hyperuricemia this project is subject to review by the UAB CORT Executive Committee. This committee is made up of project principal investigators, co-

investigators, and senior UAB researchers who meet periodically to discuss all facets of the CORT and its projects. Any concerns voiced by study staff members will be brought to the attention of this committee.

2d. UAB Pharmacy

The UAB Investigational Drug Service (IDS) is responsible for the storage and preparation of both the study drug and placebo for this study. Placebo run-in medication will be picked up for dispensement after a participant is deemed eligible. The labeled study drug is picked up by a research assistant to dispense to study participants (depending on assigned group) at visits 2 and 4. A drug log will be used to track the study drug from pharmacy to study participant. Further questions about pharmacy activities can be directed to: [redacted]. IDS Pharmacy, University of Alabama at Birmingham Hospital, [redacted].

3. Training Plan

Each study staff member will be trained in the protocol by the investigators named above. The investigators and all staff involved in the study will have completed their required IRB / human participants training. New study staff members will be trained on the protocol and spend a visit shadowing another trained staff member before carrying out visits independently. If necessary, any non-MD study personnel will be trained to take a blood pressure reading, height, and weight; those with additional phlebotomy training will be permitted to draw blood labs. Any study personnel tasked to enter participant information/data will be certified to enter data utilizing the using the REDCap (Research Electronic Data Capture) system. Prior to study start up, the project manager and Data Work Group Leader will certify study personnel to enter in study data and they have completed REDCap training.

Prior to conducting visits, the investigator will be asked to sign off that the staff member has been appropriately trained in the study protocol.

If a staff performance problem arises, several steps will be taken to resolve the problem. First, the project manager will communicate the problem to the PIs. At this stage, the PIs will work with study personnel to identify causes of the problem and offer solutions. If the problem continues, additional training will be considered. In cases of problems of sufficient severity or intractable problems that are not resolved by the procedures described above, the under-performing team member may be removed from active participation in the study.

4. Communications plan

As this protocol is currently only being carried out at one site (the University of Alabama at Birmingham-UAB), meetings will be held between the entire study staff on a monthly basis to communicate the on-going progress of the study.

Topics of discussion at each meeting will include:

- Recruitment progress
- Adverse events
- Issues or concerns with the study and possible solutions
- Any data available at that time
- Safety reporting
- Other study updates

Additionally, study personnel will keep investigators informed of the weekly progress of the study with weekly study work group meetings and email updates that include the following information:

- Number of patients screened that week
- Number of patient visits overall
- Prospective study participants to speak with the following week
- A recap of any adverse events (which, if classifies as a severe adverse event or requiring additional review, would have already been reported immediately following the event)
- Other study updates
- Any recent data collection that is useful for viewing or necessary to see for safety monitoring

Finally, routine progress and safety reports will be provided to NIAMS-KAI within the intervals requested, but no less than quarterly.

5. Recruitment and Retention Plan

5a. Recruitment Plan

The target population to be recruited is patients with Pre-hypertension or stage I hypertension. Similar to past successful recruitment for hypertension studies by [redacted], announcements will be placed in the web page www.researchmatch.org , Birmingham metro area local newspapers, UAB publications (e.g. UAB reporter, distributed to all UAB campus), and flyers that will be posted on UAB campus requesting adults, ages 18-40, that are interested in participating in a clinical study for hypertension, to call the recruitment coordinating center. Given the inclusion age range with this trial, participants will be also be recruited utilizing social media print and radio ads (e.g., Spotify, Facebook, and Instagram). Patients will also be recruited directly from the UAB 700 physician outpatient care facility, The Kirklin Clinic, through brochures distributed to physicians and from the Hypertension clinic. The inclusion and exclusion criteria will be reviewed during a telephone interview (pre screening), and if the participant wishes to proceed, a screening visit will be scheduled (section 7). Informed consent will be obtained by the principal investigator, one of the co-investigators, or a trained member of the study staff. Study procedures will not begin until signed informed consent has been obtained (section 8).

Informed consent will be obtained by the principal investigator, one of the co-investigators, or a trained member of the study staff (ex. Study coordinator). Study procedures will not begin until signed informed consent has been obtained (section 8).

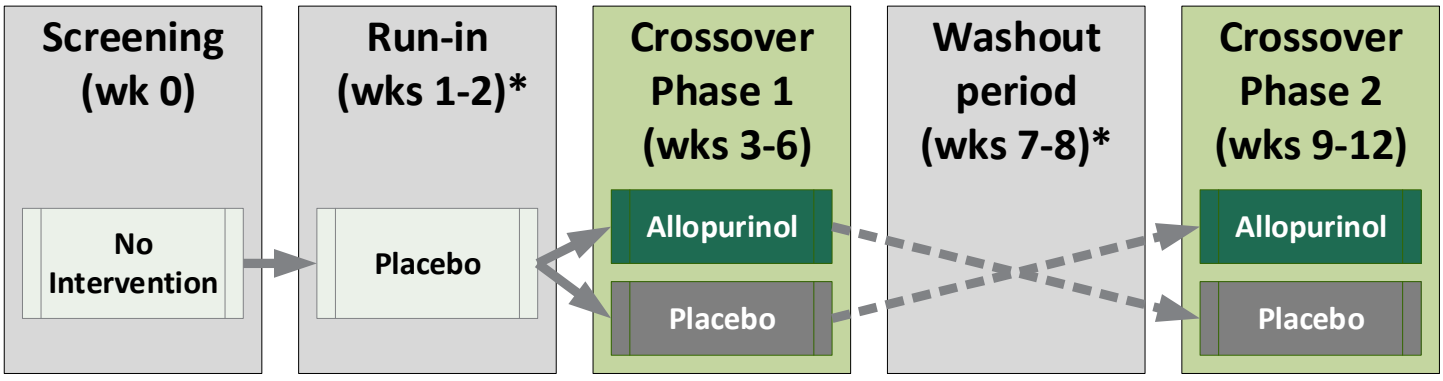
Figure 2. Example patient recruitment flyer

5b. Participant Retention

Retention of participants is central to the internal validity of the proposed study and will be an extraordinarily high priority. We will aim to have an 80% retention rate. During the screening and enrollment process, contact will be made with participants in a variety of ways (letters, staff contact by phone and in person) for optimal retention. To increase retention, we hire engaging, attentive, and responsive staff and provide participants with a flexible time to complete their study visits. Other features used to promote high retention rates will include tracking systems and collection of information (i.e., names and telephone numbers) for two friends/family members who do not live with the study participant but would know where to find them. Once enrolled, a proactive plan for retention will be implemented that includes in person visits every 4 weeks, phone calls between each visit, and transportation vouchers to encourage participants to come to their visits. The following major principals and commonly used strategies to maximize retention and minimize loss to follow-up will be employed during the trial:

- Stressing the idea that participants have an active role in the research and are part of the research team
- Enhancing participant's understanding of the study's mission and the protocol
- Stressing that retention efforts begin with recruitment and are an ongoing process
- Building participant relations and participant satisfaction, with the study staff taking a central role on this effort
- Emphasizing the importance of congeniality, respectfulness and friendliness in interactions with participants
- Actively discuss with participants any questions and concerns pertaining to their condition
- Identifying potential problems and key retention factors and developing intervention strategies regarding retention
- Assessing each participant's drop out potential and intervening as needed to keep participant interested in continuing to participate

Figure 3. Study Diagram



*Timing within these phases were up to 4 weeks for pre-menopausal women

7. Screening and eligibility criteria

During the screening phase, potential participants will be determined as described in the Recruitment Plan in section 5A. Study personnel following instructions outlined in the study binder will carry out screenings. The set of screening questions will be asked prior to starting any study procedures and immediately after obtaining consent. These questions will be based on eligibility and exclusion criteria described below (see Appendix 2 for REDCap screenshots). Screening logs will be kept in electronic format and saved in a password protected file folder in the REDCap system (See Appendix 2 for REDCap screenshots) and updated as needed during recruitment.

7. Criteria

7a. Eligibility criteria

A screening visit will occur to confirm study eligibility, explain procedures, and sign an informed consent for screening. For men and women in days 1-7 of the menstrual cycle eligible for the study, randomization and the baseline visit will occur 2 weeks after the screening procedures and signing of the consent form. Women after day 7 of the menstrual cycle will be invited to return after the onset of their menses and no later than day 7 of the next cycle for standardized measurements of FMD (4 week placebo run-in ±3 days) in the UAB Hypertension Clinic. [85,84] Participants will be randomized following confirmation of eligibility. We anticipate an additional 10% that initially fulfill inclusion criteria will fail to randomize because their BP will be outside the study range at visit 1. In conclusion, we project the need to perform screening laboratory assessments in 236 individuals. Enrollment and participant follow-up will continue until 112 participants are enrolled. We anticipate recruiting 30 persons per year based on the following inclusion criteria.

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 or;
    - -Diastolic blood pressure (DBP) ≥ 80 and < 100
2. Serum urate ≥ 5.0 mg/dL for men or ≥ 4.0 mg/dL for women
3. Age 18-40

7b. Exclusion criteria

Individuals below 18 years of age will be excluded given that although hyperuricemia may affect children, it is predominately a condition of adults and gout is almost exclusively a problem of adulthood. The science proposed and the rationale for our study of this disorder in adults only is clarified in our research strategy and significance section. While prior research of this type has examined adolescents, part of the novelty of our study is to focus only on adults and given the increased risk to benefit ratio of our proposed study in children, children will not be included in this research. Prisoners and institutionalized individuals will be excluded. In individuals of Thai or Han Chinese descent that test positive for HLAB5801 will be excluded due to the high risk of allopurinol hypersensitivity.

The risks to pregnant women or an unborn baby when taking allopurinol is not fully known. For this reason, women must have a pregnancy test before the study starts and again throughout the study. Women must not become pregnant during this study. All females of childbearing potential must use an effective form of birth control during this study. Acceptable methods of birth control include hormonal control methods, inter-uterine device, a double-barrier method (diaphragm with spermicide, condom with spermicide) or abstinence.

If participants suspect that they may have become pregnant during the study, the study coordinator must contact the study PI immediately and instruct the participant to stop taking their study medication. If it is confirmed that the participant is pregnant, they will be withdrawn from the study. The study PI may want to schedule a follow up visit and may choose to follow the outcome of the pregnancy. Additionally, taking allopurinol may involve unknown risks to a nursing infant. If potential participants are breastfeeding, they are not eligible to participate in the study.

If participants suffer from very high BP >160/110 ,first it will be rechecked 24hrs later, if blood pressure is still elevated, they will excluded from the study, received consultation by PI and/or Co-PI, and referred to their primary care provider.

8. Informed Consent and HIPAA

8a. Overview of the informed consent process

During the screening visit, the form will be read by the study participant and then each section will be explained by the research study coordinator obtaining consent. The participant will be given as much time as they need to read and ask questions about the consent form. After the participant has signed the consent form, the Principal Investigator, and the research assistant conducting the visit must each sign and date the Informed Consent Document. A signed version of the consent form will be kept by the study staff in the study binder and an additional copy of the consent form will also be given to the participant to keep.

The individual will be informed that he/she is not obligated to participate in the study and that it is strictly voluntary. The informed consent process will ensure that there is no penalty for not participating in a clinical trial and that treatment will not be compromised if individuals do not participate or if they cease participation at any time.

By signing the consent form, the participant authorizes the use of their personal health information, that they understand the study and its benefits and risks, and agree to all other aspects of the study outlined in the form. Participants can withdraw their consent at any time.

8b. Overview of the Informed consent document

The process of informed consent will be carried out by a study coordinator or the research assistant involved in the screening visit after the participant appears to meet the pre-screening criteria. It allows the participant the opportunity to decide whether they want to participate in a study. During this process, individuals will be informed of all aspects of the study so that they can make an informed decision. Participants will then confirm their willingness to participate in the research study by signing the Informed Consent form (see Appendix 1 for the complete informed consent). After the participant has signed the consent form, the research coordinator conducting the visit must sign and date the Informed Consent Document.

The informed consent document contains the following:

- Disclosure of relevant information to prospective participants about the research;
- The participant's comprehension of the information;
- The participant's voluntary agreement to participate in a research study without coercion or undue influence.
- An optional participation in future research by allowing samples to be biobanked.
- Complete disclosure of any appropriate alternative procedures and their risks and benefits
- Disclosure of the extent of confidentiality that will be maintained
- Statement of compensation and/or medical treatment available if injury occurs
- Name, address, and telephone number of the Principal Investigator

If there is a change in any of the study procedures that may affect the participant, the informed consent document will be revised and approved by the IRB. Any participants active in the study prior to a change in procedures will sign the amended consent form. Per NIH policy, the signed consent forms will be kept as part of the study record for at least 7 years after completion of the study.

Important Steps for the Study Staff:

- Provide participants with adequate information concerning the study procedures and scope
- Provide adequate opportunity for the participant to consider all available options
- Respond to the participant's questions and concerns
- Ensure that each participant understands all information provided
- Obtain the participant's written voluntary consent to participate
- Countersign the consent form
- Obtain a third-party witness signature
- Provide participants with a copy of the consent form
- Keep the signed form in the study binder

8c. HIPAA Authorization

The privacy requirements, as outlined by The Health Insurance Portability & Accountability Act (HIPAA) to protect the participant's confidentiality, are met by reading through the consent form with the potential study participant before obtaining a signature. Additionally, the following HIPAA Specific requirements are met in the last section of the consent form:

- The form contains language that satisfies the HIPAA requirements and outline the protection of health information utilized in the study
- That participants are authorizing investigators, IRBs, research administrators, and others to share and disclose their Protected Health Information (PHI) for research purposes.

9. UAB Pharmacy

The UAB Investigational Drug Service (IDS) is responsible for the storage and preparation of both the study drug and placebo for this study. The labeled study drug is then picked up by a research assistant to dispense to study participants (depending on assigned group) at visits 2 and 4. A drug log will be used to track the study drug from pharmacy to study participant.

The manual of operations and procedures provides instructions to site personnel on the requirements and processes for handling and management of the study drugs and other study medication-related supplies. Instructions provided in this document must be followed precisely.
Conduct of clinical studies and the associated activities outlined in this Manual are subject to various local, national, and international regulations. All policies and procedures must be adhered to.

STUDY SUPPLIES

Study drugs used in the study are:

1. Allopurinol 300 mg, oral administration
2. Placebo, oral administration

Placebo and allopurinol will be over encapsulated at UAB pharmacy by [redacted].

The UAB pharmacy will provide storage for non-encapsulated allopurinol and placebo. [redacted] will prepare in advance for up to 10 subjects. Encapsulated study supplies should be picked up on the day of study visit appointments or stored in a locked drug cabinet in the clinic space until the time of this visit. The study coordinator will be responsible for distribution of study supplies to participants. Upon distribution the study coordinator should log the distributed pill bottle.

Further questions about pharmacy activities can be directed to: [redacted].

10. Randomization

Randomization will occur using a random number generator. Specifically, permuted block randomization will be used allowing for balance between crossover arms throughout the investigation. Block sizes will be variable to prevent research staff from anticipating subsequent crossover arm allocations. The sequence of treatment assignment will be held by the pharmacist creating treatment arm study pill containers. The pharmacist will maintain the master list and securely store the randomization files. The study coordinator will initiate the randomization procedure after a subject has consented. The coordinator will let the pharmacist know that a study pill container is needed according to the randomization scheme. If any concerns arise during the randomization procedures, both the pharmacist and study coordinator will contact the study statistician.

11. Blinding and unblinding

All study investigators and participating coordinators will be blinded to treatment assignment. If there is a serious adverse event, then unblinding will be discussed and if deemed necessary to unblind the participant, then the ID of the unblinded participant, reasons for unblinding, name of the pharmacist and a list of whomever becomes unblinded will be kept in the study binder. The pharmacist will contact that participant's primary physician about the exposure. If necessary, the participant will be withdrawn from the study. The pharmacist will hold the randomization codes securely until the completion of the study.

12. Study Intervention

This study meets the criteria of a clinical trial but it is not a NIH-Defined Phase III Clinical Trial as defined by NIH. The UAB IDS pharmacy is responsible for the preparation and labeling of allopurinol and the placebo. Participants will be asked to take 4 weeks of allopurinol or placebo, then will crossover to the other drug (after 2-4 week washout period) and take either allopurinol or placebo for an additional 4 weeks. Allopurinol will be prescribed in 300mg tablets. At visit 2 and 4, participants will be given a 5-week (with an additional 7 days to account for variability in appt availability) supply of medication. During visits 3 and 5 study staff will count pills (any extra will be discarded) to determine compliance. Allopurinol and placebo prescriptions will be administered by the research study coordinator conducting the visit, daily doses will be administered by the participants themselves.

13. Participant Evaluations- Study timeline, visits, and procedures (See Protocol, Section F, for details on the study timeline, visit schedules, and procedures)

For each visit, the study coordinator and staff conducting the visit will systematically enter all participant information electronically to the Research Electronic Data Capture system (REDCap). Our team has extensive experience using the REDCap system. All data will be collected in REDCap. Additionally electronic records are kept by visit. The study coordinator and project manager will review all data collected on a regular basis. This will help ensure all electronic forms are being completed correctly, are intact, and have been entered into system correctly. Lab forms provided by the research study coordinator describing the blood work to be done should be filled out at all visits (See *Appendix 3. Standard Operating Procedures* for instruction of preparation and storage of biobanked samples). If any issues arise (including, but not limited to adverse events) during the visits that prevent the participant from continuing, these will be documented and reported to the principal investigator immediately. The laboratories and visits are outlined in the study Protocol, section F.

14. Data Collection, Study Forms, and Logs

All data for this study will be collected by study staff in accordance with 21 CFR Part 11 rules and will meet all regulatory requirements. Due to the fact that data is almost always recorded directly on a CRF, we developed electronic case report forms for all data entry using REDCap. Our team has extensive experience using the REDCap system. All forms will be completed electronically in REDCap by the study coordinator(s). All REDCap forms filled out by study personnel will be considered source documents and kept electronically. All electronic forms and logs in REDCap are encrypted and password protected and hard copies can be printed if required. A detailed listing of all current study forms is below.

14a. Table 6. Study Participant Forms- Data will be collected by the study staff at each visit on study forms as described and presented below:

Form	Description	Visit administered	Instructions	Location
Informed consent	Informed consent	Screening visit	Participant reads through consent and initials each page in the box. Participant, doctor obtaining consent and study staff should all sign, date and time stamp	Appendix 1 Study Binder
Participant information/contact	Detailed contact sheet for	Screening visit	Participant is asked for	Appendix 2

	participant and two other listed contacts		contact information for his/herself and 2 other persons	
Screening form	Ensures eligibility by cross referencing participants labs in one form, and determining that all inclusion criteria are met.	Screening visit	Completed by study staff following prescreen and initial exam. Certified by study coordinator.	Appendix 2
Exclusions form	Ensures eligibility by cross referencing participants labs in one form, and determining that no exclusion criteria are met.	Screening visit	Completed by study staff following prescreen and initial exam. Certified by study coordinator.	Appendix 2
Initial participant questionnaire including focused medical history and health	Initial data collected on participants medical history, health status, anthropometric data, status, blood pressure, heart rate, and medication use.	Screening visit	Study coordinator queries participant on his/her medical history. Entered in REDCap	Appendix 2
Interval medical history	Updates medical history of participant between visits,	Visits 2-5	Study coordinator queries participant on	Appendix 2

	blood pressure, heart rate, and medication use.		updates to medical history. Entered in REDCap	
Concomitant medication	Record of all prescribed and other the counter medication participant is currently using	Screening visit and visits 2-5	Completed by study coordinator and entered into REDCap	Appendix 2
Lab results	All study lab data entered.	Screening visit and visits 2-5	Data Obtained from UAB labs and entered by study staff into REDCap system.	Appendix 2
FMD results	Data collected during FMD procedure	Visits 2-5	Completed by [redacted] and entered in to REDCap system.	Appendix 2
24-hour ABPM	Data collected by ambulatory blood pressure monitor	Visits 2-5	Data downloaded from device.	Raw data files housed on secure server
Adverse event	Adverse event data entered on this form	Visits-2-5, and during follow-up phone calls	Completed by study coordinator. PIs notified within 24 hours of any adverse event	Appendix 2

14b. Table 7. Study Specific Administrative Forms, Logs, and Data Tables

Form	Description	Location
Study roster	Complete study personnel roster and signature of all members of the study team. It is the responsibility of the Principal Investigator and/or Clinical	Study Binder REDCap System

	Research Coordinator to: designate individuals approved to make form entries and changes, and note the date when any study team member is removed from the team for any reason.	
Timeline	Detailed study timeline	Study Binder Protocol Protocol REDCap System
Schedule of Visits and Evaluations	Listing of all schedule study visits and evaluations	Study Binder Protocol Protocol -Appendix 3 REDCap System
Screening log	Log of individuals screened and enrolled	EXCEL document
Participant contact log	Record of all calls/contact between study staff and participants. Study coordinator(s) complete log showing interval visit calls. Assess medication compliance and adverse events.	Appendix 2
Serious adverse event report	Serious adverse event data entered on this form.	Appendix 2
Study drug accountability record	Will be maintained in the Pharmacy by the research pharmacist and must not be shared with other members of the study team.	Available by request at the IDS Pharmacy
Record of Destruction of Clinical Product	This log is used to document the destruction of any unused study drug. The date of incineration as well as how many vials were incinerated must be recorded. This record should be attached to the Study Drug Accountability Record.	Available by request at the IDS Pharmacy

Enrollment tables	Data Tables will be exported from REDCap database and entered into the NIAMS provided EXCEL template.	Data Tables will be generated from REDCap database and downloaded into the NIAMS provided EXCEL template.
Demographic characteristics	Demographic characteristics of the study population (ex. Sex, Race, Ethnicity, Age)	Data Tables will be generated from REDCap database and entered into the NIAMS provided EXCEL template.
Randomization/Enrollment log	Record of randomized and enrolled participants	EXCEL screening log and REDCap system
Study Completion form	Record of participant study completion/withdrawal	Appendix 2
Adverse Events Summary	Summary of all adverse events reported by participant during study period.	Data Tables will be generated from REDCap database and entered into the NIAMS provided EXCEL template.
Adverse Events Summary(by severity)	Summary of all adverse events by body system and severity reported by participant during study period.	Data Tables will be generated from REDCap database and entered into the NIAMS provided EXCEL template.
Adverse Events-Details	Summary of adverse events by specific participants upon entering study with details related to event.	Data Tables will be generated from REDCap database and entered into the NIAMS provided EXCEL template
Serious Adverse Events	Summary of serious adverse events by participants upon entering study.	Data Tables will be generated from REDCap database and entered into the NIAMS provided EXCEL template.

Death	Descriptive summary of all participants who die during study by cause of death.	Data Tables will be generated from REDCap database and entered into the NIAMS provided EXCEL template.

All study personnel will agree to use of electronic signature for REDCap, defined by a private username and password, with the same legal significance as their traditional handwritten signature. We will define user roles (clinical investigators, clinical coordinators, and system administrators) with secured log-in. We will track current user, database accessed, date, and time and the application will be stopped automatically after 10 minutes of user inactivity requiring a fresh log-in. All data will be collected on the eCRFs in REDCap and copies can be printed and included in the study binder after each visit or phone check in if necessary. The study coordinator and project manager will review all data collected weekly. This will help ensure all electronic forms are being completed correctly, are intact, and have been entered into system correctly. No data will leave the site, as this is a single site study.

14c. General Instructions for Completing Forms in REDCap

All data recorded in REDCap will be maintained according to FDA and ICH Good Clinical Practice (GCP) guidelines. Instructions for completing eCRFs to ensure quality and consistency in data collection are below.

Sample instructions: When completing electronic study forms participants should only be identified by name on the screening document. Identification (ID) numbers will be used to reference the participant in all other eCRFs.

Header: Complete the header information in every field, including fields for which no study data are recorded. From the drop down box choose Not Applicable, Unknown, or UNK for fields where no data can be provided.

Participant ID: The participant ID will be prepopulated in the header of each page.

Time: Use a 24 hour clock (e.g., 14:00 to indicate 2:00 p.m.) unless otherwise specified.

Dates: All dates will be verified by the study coordinator. Historical dates are sometimes not known (e.g., date of first symptom); in this case, chose Not Applicable, UNKNOWN or UNK to indicate missing data.

Abbreviations: Do not use of abbreviations not specifically noted in the instructions for completing the forms. Comments should only be written where text fields to do so are provided.

Correcting errors: Errors can be corrected within the REDCap system where a timestamp and date are associated with each entry and revision.

Completion of forms:
All forms must be completed and saved in REDCap. The study team member responsible for the entry of data into the REDCap system must ensure that all fields have been entered and that the form has been saved and marked as complete. If a form cannot be completed or data cannot be verified the form should be marked incomplete or unverified. Forms should only be marked unverified if PI or investigator review is required. This may include questions surrounding lab or FMD values recorded in REDCap.

14d. Missing or Incomplete data
Data may not be available to complete the eform for various reasons. Electronic forms that are missing or not complete should be noted, in the REDCap system as INCOMPLETE and saved. Forms with missing data in fields or sections should be marked as INCOMPLETE in the REDCap form. Indicate the reason for the missing data in the text field when available. If the evaluation was not done, type NOT DONE or ND and provide a reason in the available text field or from the dropdown.

Incomplete or Illegible forms:
If any section or an entire page of the forms cannot be completed (e.g., no parts have any responses), and it is unlikely that it will be completed, choose or type NOT DONE/ND, NOT AVAILABLE/NA or NOT APPLICABLE, as appropriate in the text field. Do not leave forms incomplete or unused without explanation.

All form header information must be completed even though no data are recorded on the electronic form. If an electronic form can only be partially completed at the time of visit, but will be completed when the information becomes available, note this in the MISSING FORMS / INCOMPLETE FORMS field and follow the direction of the study coordinator and principal investigator.

Every effort should be made to obtain and ensure that the electronic forms are being completed correctly.

14e. Retention of Study Documentation
Investigators will adhere to rigorous requirements and should retain forms and all other study documents for a period of no less than 7 years.

15. Data Management
Data management will be the responsibility of the research assistants, study coordinator, project manager and study statistician. All data will be entered into electronic case report study forms using the REDCap (Research Electronic Data Capture) system. REDCap is a secure web application for building and managing online surveys and databases. REDCap provides audit trails for tracking data manipulation and user activity, as well as automated export procedures for seamless data downloads to Excel, PDF, and common statistical packages (SPSS, SAS, Stata, R). From REDCap all data will be exported into SAS files and stored on a secure and

encrypted server or hard drive. The study statistician will oversee the data management Data entry, editing and updating will be the responsibility of the research assistants, while tracking and corrections will be the responsibility of the entire staff. The study statistician and coordinator will train the research assistants on proper data entry in the REDCap system. All efforts will be made to ensure the data is in compliance with the UAB Institutional Review Board (IRB) policies, Good Clinical Practice (GCP) guidelines, and Federal regulations. Since this is a single site study, no data will be transmitted between sites.

15a. External Data

The UAB Arthritis Clinical Intervention Program (ACIP) and Hypertension Clinic will handle all laboratory data collection. No external data will be collected. All Serum and DNA will be processed and stored in the lab of [redacted]-the UAB CORT co-director. Serum and DNA processing and storage will be done by so in accordance with the standard operating procedures (see Appendix 3). The study coordinator will be responsible for the labeling and transport of all blood samples. The study coordinator will collect the lab results and record in the eCRFs for each study participant.

15b. Data Entry

A database will be designed for the current study in the REDCap system. These computerized templates, are accessible through a secure web interface. Data entered into the system will be downloaded weekly and the database will have range and validity checks built in. All abnormal values will be flagged for further investigation. Research staff performing data entry will be trained in the use of the REDCap system and the study database. The study statistician will develop validity checks for outlier values, consistency, and completeness and will notify the principal investigator if issues are identified. Discrepancies will be adjudicated by consultation with the principal investigators. Once data entry is complete, the study data will be transferred to SAS statistical software and reviewed by the statistician.

16. Safety Monitoring and Reporting

The following defines the types of adverse events that study staff should inquire about at each visit. The type and possible causes of the adverse event will be discussed with the principal investigator, an examination will be conducted if deemed necessary, and it will be reported to the required parties. It is the responsibility of each and every study staff member to determine the appropriate line of action, to take the action agreed upon with the participant, and to subsequently report the event.

- Adverse Event (AE) - An AE is any unfavorable and unintended diagnosis, sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the study intervention, which may or may not be related to the intervention. AEs include any new events not present during the pre-intervention period or events that were present during the pre-intervention period which have increased in severity.

- Serious Adverse Event (SAE) - An SAE is any untoward medical occurrence that results is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, in death, or, in the opinion of the investigators, represents other significant hazards or potentially serious harm to research participants or others. All SAEs will be reported to the Safety officer (via KAI), Data Safety and Monitoring Board (DSMB), and IRB within 48 hours of the Principal Investigator becoming aware of the event.
- *Unexpected adverse event: Any adverse event, the specificity, frequency or severity of which is not consistent with either:*
    - *the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, the current IRB-approved informed consent document, and other relevant sources of information, such as product labeling and package inserts; or*
    - *the expected natural progression of any underlying disease or condition of the participant(s) experiencing the adverse event*.

All AEs should be collected, analyzed, and monitored by using the serious adverse event form using the Adverse Event Reporting Form as well as the AE Tracking Log in the Research Electronic Data Capture system (REDCap). All laboratory abnormalities identified in the protocol as critical to participant safety must be reported. All AEs experienced by the participant from the time study drug administration through the end of the study are to be reported, as outlined in the protocol. The following delineates specific responsibilities of staff members:

- The research coordinator will complete the Adverse Event Form and AE Tracking Log in REDCap; assist the PI to notify the IRB, and Safety Officer of all the SAEs, and assist them to prepare SAE reports to IRB, and Safety Officer.
- The Principal Investigator will confirm that all AEs are correctly entered into the AE log with the study coordinator; be available to answer any questions that the coordinators may have concerning AEs; notify the IRB, FDA of all SAEs and AEs as appropriate.

The study coordinator will confirm that the AEs are correctly entered into the Adverse Event form and log in REDCap. They will confirm that the adverse events are reported to the appropriate regulatory bodies, as required. Additionally the project Safety Officer will receive safety tables every 12 weeks during the study period that include AE and SAE reports.

The proposed research meets the criteria of a clinical trial but it is not an NIH-Defined Phase III Clinical Trial since we propose to evaluate the mechanisms by which urate-lowering might contribute to blood pressure

control and to compare its effects to placebo in 112 participants with prehypertension and stage I hypertension. [redacted] will be responsible for data safety and monitoring. As PIs they will complete and submit to the Project Advisory Committee monthly reports detailing the study progress and participant status, any adverse events, and any protocol deviations. Continuous, close monitoring of individual participant by the PIs will be assured throughout the study period. Events determined by the PIs to be serious, unanticipated problems involving risks, those not described in ***Table 8. Adverse Events***, to the participants will be reported by the PIs to the IRB, DSMB, and the Safety Officer (via KAI) within 48 hours.

Table 8. Adverse Events – This document will be updated as needed.

Activities of Daily Living-ADL
Body Surface Area- BSA

Body System			
Cardiovascular	**Mild**	**Moderate**	**Serious**
Myocardial Infarction	[redacted]	[redacted]	All reported myocardial infarction will be classified as serious
Increased Blood Pressure	Systolic BP 140 - 159 mm Hg or diastolic BP 90 - 99 mm Hg; medical intervention indicated	Systolic BP >=160 mm Hg or diastolic BP >=100 mm Hg; medical intervention indicated	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis)
Hematology	**Mild**	**Moderate**	**Serious**
Anemia	13-10 Hb g/dl)	10-8 Hb g/dl	<8 Hb g/dl
Thrombocytopenia	150 000- 75 000/mm3	<75,000 – 50,000/mm3	<50,000 – 25,000/mm3
Neutropenia	<LLN – 1500/mm3	<1500-1000/mm3	<1000-500/mm3
Gastrointestinal	**Mild**	**Moderate**	**Serious**
Vomiting	1 - 2 episodes (separated by 5 minutes) in 24 hrs	3 - 5 episodes (separated by 5 minutes) in 24 hrs	>=6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN 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**	**Serious**
Joint pain	Mild pain	Moderate pain; limiting instrumental ADL	Serious pain; limiting self-care

Nervous	**Mild**	**Moderate**	**Serious**
Any patient reported event	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Moderate symptoms; limiting instrumental ADL	Serious symptoms; limiting self care ADL or death
Skin and subcutaneous tissue disorders	**Mild**	**Moderate**	**Serious**
Rash- characterized by a circumscribed and elevated skin lesion	Papules and/or pustules covering <10% BSA	Papules and/or pustules covering 10-30% BSA	Papules and/or pustules covering any % BSA, with life threatening consequences or death
Stevens Johnson Syndrome		Skin sloughing covering <10% BSA with associated signs (e.g., erythema, purpura, epidermal detachment and mucous membrane detachment)	Skin sloughing covering 10 - 30% BSA with associated signs (e.g., erythema, purpura, epidermal detachment and mucous membrane detachment) or death
Itch	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Minimal, local or noninvasive intervention indicated	Medically significant or immediately life threatening; hospitalization or urgent intervention indicated

Hepatobiliary	Mild	Moderate	Serious
Abnormal liver enzymes	Above the upper limit to 2x ULN	2x ULN to 4X -	Greater than 4x ULN

Values and definitions derived from:
Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0
Published: May 28, 2009 (v4.03: June 14, 2010) U.S.DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health National Cancer Institute

All adverse events will be reported to the UAB IRB, our study DSMB and the Safety Officer (via KAI) at the time of continuing review. All serious adverse events (SAEs) will be reported to KAI and the DSMB within 48 hours of the Principal Investigator becoming aware of the event. All study staff members will be informed by the PIs about any unanticipated problems involving risks to the participant. 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 the research participant. In such case the IRB will be promptly informed of the change following implementation (within 1 week).

17. Study compliance

All deviations will be reported to the appropriate parties, including the Principal Investigator and the Safety Officer (via KAI), within 48 hours of occurrence or as soon as they are discovered. In addition, if monitors discover any of these deviations during a monitoring visit, they should notify Safety Officer (via KAI) of the occurrence in writing. The study staff and research assistants will maintain the log of all protocol

Protocol deviations include, but are not limited to, the following:

- Randomization of an ineligible participant
- Failure to obtain Informed Consent
- Entering a participant into another study
- Failure to keep IRB approval up to date
- Wrong treatment administered to participant

18. Quality Control and Standard Operating Procedures

All study procedures are to take place at the University of Alabama at Birmingham and will do so in accordance with the PROTOCOL.

18a. Standard Operating Procedures

Standard operating procedures for this project can be found in Appendix 3.

18b. Data and Form Control

All data collection and operating procedures are to take place at UAB and will be done in accordance with the PROTOCOL. Study files will be monitored by research assistants on a weekly basis, and will also be monitored

by other study staff members (study coordinator, manager, statistician, and PIs) on a monthly basis to ensure quality control. The appropriate regulatory and IRB documentations will be kept on file and up to date by the research assistants and administrative staff.

IRB documents

- All IRB Correspondence is electronically archived
- The study staff are IRB approved prior to performing any study procedures
- Adverse events and deviations are reported to IRB per current guidelines
- All versions of the IRB protocols and informed consent forms are electronically archived
- Reporting all protocol deviations (exceptions and violations) are reported to IRB as required.

Informed Consent

- Ensure that participant identification is on all pages of the ICF
- There is documentation that the participant is given a copy of the consent form
- The participant and study representative signed and dated the consent form for him/herself.
- The participant initialed and dated all appropriate pages on the informed consent form.
- Note to file made for any informed consent deviations.
- Ensure a valid (current version date) copy of the consent form was used
- Contact information collected on study participant and two additional study contacts.

Protocol:

- Confirm that the study staff is conducting the study in compliance with the protocol approved by IRB and if applicable, FDA.
- The protocol deviations (exceptions and violations) are documented appropriately and reported to IRB as required.

Electronic Case Report Forms:

- Review participant files to ensure that accuracy, and completeness of the data
- Any correction made to the eCRFs is automatically dated in REDCap. The original entry is archived and hard copies can be printed.
- Note files made for missing or incomplete data and to explain any discrepancies or additional comments in appropriate text fields.

Other documents:

- CVs for all study staff are on file and updated every 2 years
- Medical licenses for the PIs and Co-Investigators are on file and updated prior to expiration
- Yearly financial disclosures for PIs and consultants

18c. Clinical Monitoring

No clinical monitoring will take place in this study as it is a single site trial.

18d. Reports

Reports as outlined in the communications plan above will be prepared to keep the study staff up to date and engaged, as well as help to check quality control. These reports will take the form of:

- Weekly status emails to the PI by the research assistants and study coordinator
- Data and safety monitoring reports prepared by the study investigator and other staff to be reported to the Safety Officer, DSMB, and the UAB IRB as requested.
- Monthly reports that will be compiled by the research assistant and staff statistician in presentation form for study meetings will include
  - Target and actual enrollment,
  - Individuals screened with reasons for screen failure,
  - Enrollment status (enrolled, active, completed, discontinued treatment, and lost to follow-up).
  - Electronic forms completed and entered
  - Missing or erroneous data.
  - AEs and SAEs

19. Study Closeout

At the conclusion of the study the following procedures will be done to verify all study related obligations have been met. Verification that all study procedures have been completed including, but not limited to the following procedures include:

- All data has been collected
- All data queries have been completed.
- All electronic source materials have been properly documented and archived
- Any study related supplies and unused medication is returned for destruction.
- Assurance that correspondence and study files are accessible for audits.
- Reminder to investigators of their ongoing responsibility to maintain study records and to report any relevant study information to the KAI.
- IRB notification of the study completion and stored copy of the notification.
- Preparation of a report summarizing the study's conduct and results.
- Participant notification of the study completion.

20. Policies

All staff will be instructed in their study specific responsibilities regarding data safety and confidentiality. Additionally all staff will be cautioned against the release of data to any unauthorized individuals and no data will be released to any individual without first obtaining approval from NIAMS and/or the UAB IRB.

This section of the PROTOCOL will discuss the safeguards which have been put in place by the PIs to ensure participant confidentiality and data security.

The following is a list of study participant confidentiality safeguards:

- Data flow procedures – As this is a single site study and therefore no data identifying participants will be transmitted between sites. The study statistician and coordinator will provide thorough data entry and confidentiality training to all staff involved in data collection and data entry. All eCRFs and copies will be included in participant electronic study binder on day of visit or phone check in. The study statistician, project manager, and coordinator will review all data collected weekly to ensure all forms are being completed correctly in REDCap and are intact. No data will leave the site, as this is a single site study, so no other data flow will occur.
- Electronic files – All data identifying participants will be stored electronically will be maintained by the study statistician and will be maintained behind the UAB firewall.
- Data listings – Data or information containing the participant name, study ID, medical information, record number, Social Security Number, or any other unique identifier will under no circumstances be included in any published data listing.
- Data distribution - data listings that contain participant name, name code, or other identifiers easily associated with a specific participant will not be distributed.
- Data disposal - computer listings that contain participant-identifying information should be disposed of in an appropriate manner.
- Access - participant records stored in the data center should not be accessible to persons outside the center without the express written consent of the participant.
- Storage – All data collected during this will be stored on UAB Department of Medicine Servers. No data will leave the site, as this is a single site study, so no other data flow will occur. Any printed study forms and related documents created both during and after study completion will be stored in a secure location in UAB Faculty Office Tower.
- Passwords – Each study related file will be password protected. Additionally, limitations will be placed on general access to the study's REDCap system and to the functions that individuals can use. All passwords will be changed on a regular basis.
- User Training - Study staff with access to clinical computer systems will be trained in their use with an emphasis on, and the importance of, system security.
- System Testing - Prior to the use of the Redcap system, and subsequent to any modifications, the system will be tested to verify that it performs as expected. Testing will verify that the password-activated access system performs as intended.
- System Backups - Backup copies of electronic data will be made by the study coordinator or study statistician.

21. Publications

Upon completion of the study we will publish our findings in peered reviewed journals.

Authors must participate in the writing of the paper in accordance with the International Committee of Medical Journal Editors guidelines (N Engl J Med 1991;324:424-8). First authors are expected to delete names from the final list of authors if those individuals have not satisfied criteria for authorship in accordance with those guidelines. The first author on proposed or active writing projects will be decided on by the project's Publication Working Group. The project's Publication Working Group will develop a listing of first (lead), second and senior (last) author for all potential reports. In general, the researcher who first conceived of the project and submitted a plan for the manuscript should have the option of serving as first author. Conflicts in first authorship should be resolved by all members of the Publication Working Group. In the event that the Publication Working Group is unable to resolve a conflict, the UAB CORT Executive Committee will adjudicate and may assign the first authorship.

All project manuscripts must be approved by the Publication Work Group prior to submission to journal or presentation. The UAB CORT Executive Committee will assist in the review of all manuscripts or scientific presentations with the goal to ensure their content and face validity.

UAB CORT project investigators are required to inform and submit copies to the UAB CORT Executive Committee of all abstracts, posters, presentations, slides and manuscripts. It is the responsibility of the first (lead) author to ensure that appropriate journal submission procedure is adhered to. Any substantial changes to an approved manuscript prior to publication whether required by the journal or not, must be discussed with the co-authors. The co-authors should receive the proposed re-submissions at least 1 week prior to the journal re-submission.

22. Protocol Maintenance

The Protocol will be maintained and updated throughout the study by the study staff as necessary. The most current copies of the Protocol will be kept on a shared project drive, and a hard copy, in loose leaf form, will be kept in staff issued binders along with other study files for staff for their reference. Each page of the Protocol is numbered, dated, and contains the version number to eliminate any confusion. As a living document the Protocol will be continuously reviewed by study staff to ensure the operating procedures described are accurate. If any procedures are changed or modified, the protocol will be updated and the new version will be emailed and printed for study binders for all study staff members.

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Informed Consent

TITLE: Project 2: Serum Uric acid Reduction to Prevent HypERtension Study (NIAMS: CORT)

IRB PROTOCOL NUMBER: IRB130408004

PRINCIPAL INVESTIGATOR: Kenneth Saag, MD and David Calhoun, MD

HOSPITAL/INSTITUTION: University of Alabama at Birmingham
LOCATION: 115 Community Health Services Building
Birmingham, AL 35294
[redacted]

SPONSOR: National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

INTRODUCTION

You are being asked to participate in a clinical research study, being conducted by Dr. Kenneth Saag and Dr. David A. Calhoun. Drs. Saag and Calhoun have determined that you meet the initial requirements to be considered for participation in this study. The information in this form is provided to tell you about the research study and your participation in it.

PURPOSE OF THE STUDY

The purpose of this study is to study the safety and effectiveness of 300 mg of allopurinol in pre- or stage I hypertensive patients between the ages of 18-40 years. We aim to determine if allopurinol will be effective in reducing blood pressure. Additionally, we would like to evaluate how allopurinol leads to a decrease in blood pressure. Allopurinol has been approved by the US Food and Drug Administration (FDA) for the treatment of gout and hyperuricemia (high uric acid).

DESCRIPTION OF THE STUDY AND PROCEDURES

The research will be conducted at the following location(s): UAB Arthritis Clinical Intervention Program (ACIP) and UAB Hypertension Clinic. The study will include five visits (including screening visit), five blood collections, five urine collections, four flow mediated dilation procedures (FMD), four 24-hour ambulatory blood pressure monitoring sessions (ABPM), and four surveys. These clinic visits will last 45-60 minutes. These procedures will be described in detail later in the consent form.

Your participation in this study will be 14 weeks and will include five study visits. The first visit will be for screening to determine your initial eligibility. You will be one of approximately 112 subjects to be asked to participate in this study. Before you can start the study, the study doctor or study staff will talk to you about the study.

At this initial screening visit, participants will undergo a physical examination, a blood sample, blood pressure screening, and urine collection. If you are unable to complete all screening tests, or did not fast before the screening visit for at least 8 hours, you will be scheduled to return to the study center within two days of the

screening visit to complete any outstanding screening tests. If you continue to qualify to participate in this study by meeting all study requirements, you will be scheduled to return to the study center two weeks after the screening visit.

It is also possible that you may not meet certain study requirements at some study visits and the study doctor may discontinue your participation. If you are unable to continue in the study, you will stop receiving the study medication and your study doctor may advise you to start on other treatments to control your blood pressure. At that point, your participation in the study will end.

If you qualify, you will be randomly assigned, like the flip of a coin, and have a 50-50 chance of receiving either placebo or a dose (300mg) of allopurinol. You will not know whether you are receiving allopurinol or the inactive placebo. All participants will receive placebo at some point in the study. During the screening visit, you will be given a two-week supply of study medication. You will take one pill once every morning between 8 and 10 am.

Blood Tests: Blood will be drawn five times during this study. Needle sticks are painful for short period of time and sometimes will bruise at the spot for a couple of days. You might feel dizzy or faint during a blood draw but this generally passes within a few minutes. Each time we draw blood, we will take approximately about 1½ tablespoons of blood. We will do several tests including ones to make sure it is safe to use the study medicines, to look for any side effects of the study medicine and to measure several chemicals in the blood that control blood vessels and blood pressure.

Urine Test and Collection: Women capable of becoming pregnant will have a urine pregnancy test to exclude pregnancy at the beginning of the study before enrollment. The study doctor or study staff will tell you if the pregnancy test results are positive. The results of the pregnancy test must be negative in order for you to be in the study. At visits 1-5, urine will be collected from men and women using a collection cup. Will do several tests including ones to make sure it is safe to use the study medicines, to look for any side effects of the study medicine and to measure several chemicals in the urine that affect blood pressure.

Blood Pressure Tests: We will do two types of blood pressure tests during this study. The first will use a common blood pressure cuff just like in any doctor's office. At each visit we will measure blood pressure twice on each arm. The second blood pressure test is called ambulatory blood pressure monitoring. You will wear a blood pressure cuff on one arm for 24 hours and an automatic monitor will measure and record blood pressure 2-3 times an hour while you are at home, work, or school.

Flow Mediated Dilation (FMD): At visits 2-5 a FMD will be done to measure the function of your blood vessels. You will wear a cuff, similar to a blood pressure cuff, on your upper and lower arm while an image of your blood vessel is recorded.

Survey: At visits 2-5, you will complete a survey that asks questions about your feelings and behavior. The survey will take approximately 5 minutes to complete.

<u>Screening Visit (Visit 1)</u>

Before the study starts, you will be asked to sign this consent form and give your health history. The study doctor will perform procedures and tests to find out if you can be in the study. These tests include:

- Review inclusion and exclusion criteria to assess your eligibility for the study.
- Recording of your personal information, such as your name, age, race, etc.
- Recording of your medical and surgical history including documentation of blood pressure.
- Recording of all prior and current prescription medications, over the counter medications, vitamins and herbs. The study doctor may instruct you not to take certain medications within 48 hours of the next visit.
- Measurement of your pulse rate, respiratory rate (your breathing rate), temperature, height, and weight.
- A complete physical exam. You should ask the study doctor about what will happen during this exam.
- Collection of urine and blood samples (about 1½ tablespoons of blood) for clinical laboratory analysis and pregnancy screening for women of childbearing potential. The study doctor or study staff will tell you if the pregnancy test results are positive. The results of the pregnancy test must be negative in order for you to be in the study

Visits 2, 3, 4, 5

You will return to the UAB Hypertension Clinic every 2-4 weeks (weeks 3, 6, 9, and 12) for scheduled clinic visits. These clinic visits will last about 60 minutes (1 hour). During these visits you will have your blood drawn, urine collected, blood pressure measured and a flow mediated dilation will be performed to evaluate endothelial function. You will be given a 24-hour blood pressure monitoring device and will have the opportunity to report any problems or concerns you have. Additionally, you will be given a survey that will take approximately 5 minutes to complete.

Starting at Visit 2 (week 3) you will be given a 4-week supply of allopurinol or placebo. You will take one pill once every morning between 8 and 10 am. Neither the physician nor you will know if you are taking allopurinol or placebo. At visit 4 you will receive a new 4-week supply of supply of allopurinol or placebo. All participants will receive placebo at some point in the study.

The following procedures will be performed at Visits 2, 3, 4, 5:

- Confirm you did not exercise, smoke, drink any caffeinated beverages, or eat or drink grapefruit for at least two hours prior to this visit
- Review of your current medications and any medical complaints or improvements you may have had since your last visit.
- Measurement of your blood pressure and heart rate between 6:30 am and 10 am.
- Collection of fasting blood samples (about 1.5 tablespoons of blood) for clinical laboratory analysis. If you are not fasting at least 8 hours at the time of the visit, the visit will be re-scheduled within 1-2 days. Fasting means you are to have no food or drink except for water for at least 8 hours before the study visit.
- A flow mediated dilation will be performed.
- You will be assigned a 24-hour blood pressure monitor and provided materials to return it to the UAB Hypertension Clinic in the provided stamped addressed box.
- You will complete a brief survey.
- At Visit 2, dispense study medication to last 4 weeks until the next study visit. You will be instructed to take one pill each morning between 8 am and 10 am
- At Visit 3, collection of all used/unused study medication. No medication will be dispensed at this visit.
- At Visit 4, dispense study medication to last 4 weeks until the next study visit. You will be instructed to take one pill each morning between 8 am and 10 am
- At Visit 5, you will return all unused study medication.

RESEARCH PARTICIPANT RESPONSIBILITIES

As a participant in this research study, it is asked that you carefully follow the instructions of the study doctor and study staff. You will be asked to :

- Follow the instructions you are given.
- Read this Information and Consent Form and ask as many questions as needed.
- Provide the study doctor with a complete history of illnesses (and medications) you have had in the past.
- Tell the study doctor or study staff about any changes in your health or the way you feel.
- Tell the study doctor or study staff if you want to stop being in the study at any time.
- Provide the study doctor with the name, address and phone number of your current regular doctor, and whether or not the study doctor can contact this doctor about your being in this study or to request your medical records.
- Schedule and attend visits with the study center in the morning to ensure that blood pressure measurements are taken between 6:30 am and 10 am.
- Keep to the schedule of visits and if there is a problem, call the study staff to reschedule.
- Take your study medication as directed. Do not abruptly stop taking study drug.
- Complete all tests and procedures to the best of your ability.
- Inform the study doctor or study staff of any medications you may have taken between visits.
- Ask questions during the study about anything you may be concerned about.
- Refrain from drinking excessive amounts of alcohol (no more than 2 drinks per day) and refrain from taking any illegal substances during this study.
- Refrain from exercise, smoking, drinking any caffeinated beverages, and eating or drinking grapefruit for at least two hours prior to each visit.
- Refrain from taking medications that the study doctor has asked you not to take while participating in this study.
- Fast overnight and the morning before coming to certain study visits if instructed to do so. Fasting means you are to have no food or drink except water for at least 8 hours before your study visit.
- Keep the study medication at room temperature and out of the reach of children or other adults. The study medication must be taken only by you, the person participating in the study.
- Do not donate blood while you are participating in the study and for up to one month after you have completed the study.
- You cannot participate in this study if you are pregnant.

POSSIBLE HAZARDS, RISKS, AND DISCOMFORTS OF PARTICIPATING IN THE STUDY

Participation in this study may involve some inconvenience, risks, or discomforts. The most common side effect for allopurinol is stomach upset. Allopurinol can also rarely irritate the liver or cause blood counts to fall and changes to blood, skin, and liver are not always reversible.

Another uncommon side effect of allopurinol is skin rash. Very rarely (less than one in one-thousand people) this skin rash may be followed by a severe reaction known as allopurinol hypersensitivity syndrome (AHS), a condition that may become life-threatening. You should not take part in the study if you have ever had a serious reaction to allopurinol. You should tell the study doctor immediately if you have a rash or another allergic reaction during the study.

Other side effects of allopurinol include:

- diarrhea
- nausea
- Drowsiness (sleepiness)

Ask the study doctor if you have questions about these or other risks of taking allopurinol.

Venous blood sampling will rarely cause bruising or a superficial skin infection.

The 24-hour ambulatory blood pressure monitoring can cause some mild discomfort during cuff inflation. The 24 hour ambulatory blood pressure monitors must be worn overnight and may interrupt sleep. The blood pressure may remain elevated during the study period, which does increase risk of having a stroke, heart attack, heart failure, or kidney damage.

The flow mediated dilation procedure can cause some mild discomfort during cuff inflation.

The survey may cause some mild unease when answering questions about your feelings and behavior.

Information for Women of Childbearing Potential

The risks to pregnant women or an unborn baby when taking allopurinol is not fully known. For this reason, women must have a pregnancy test before the study starts and again throughout the study. You must not become pregnant during this study. If you are a woman of childbearing potential, you must use an effective non-hormonal form of birth control during this study and for 30 days after completion of the study. Acceptable methods of birth control include a double-barrier method (diaphragm with spermicide, condom with spermicide) or abstinence.

If you suspect that you may have become pregnant during the study, you must contact the study doctor immediately and stop taking your study medication. If it is confirmed that you are pregnant, you will be withdrawn from the study. Your study doctor may want to follow you and the outcome of your pregnancy. Your pregnant partner may be asked for permission for the study doctor to follow the pregnancy to its outcome.

Taking allopurinol may involve unknown risks to a nursing infant. If you are breastfeeding, you cannot participate in the study.

ALTERNATIVE

The alternative is to not participate in this study and receive standard care for high blood pressure.

SIGNIFICANT NEW FINDINGS

All new findings discovered during this research study that may reasonably influence your willingness to continue participation in this study will be provided to you when they become available.

BENEFITS OF THE STUDY

There may be no direct benefit to you. If successful, the study results may allow for safer treatment of hypertension.

COSTS

There is no cost for participating in this study. There will be no charge for the study visits or study procedures. The cost of any routine medical care needed during the study period will be billed to your insurance company in the usual manner. The study medication will be provided to you at no charge.

Payment for Participation

You will be paid $25 for each completed clinic visit and flow mediated dilation procedure. In addition, you will receive $100 for each complete 24-hour ambulatory blood pressure recording session. Should you withdraw from the study, you will be paid for the completed visits up to the date of withdrawal. If you complete all study visits and procedures you will receive up to a total of $525.00. Ask the study staff about the method of payment that will be used for this study (e.g., check, cash, gift card, direct deposit).

CONFIDENTIALITY

Your personal information will be entered into a study database. Access to this database will be limited to study personnel. Research information that identifies you may be shared with the UAB Institutional Review Board (IRB) and others who are responsible for ensuring compliance with laws and regulations related to research, including the U.S. Food and Drug Administration (FDA), the Office for Humans Research Protections (OHRP); and the study sponsor the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS). Because of the need to release information to these parties, absolute confidentiality cannot be guaranteed. The information gathered during this study will be kept confidential to the extent permitted by law. The results of this study may be published in scientific journals or presented at medical meetings, but your identity will remain confidential.

Information relating to this study, including your name, medical record number, and date of birth may be shared with the billing offices of UAB and UAB Health System-affiliated entities so that claims may be appropriately submitted to the study sponsor or to the participant's insurance company for clinical services and procedures provided to participant during the course of this study.

COMPENSATION FOR INJURY

In the event that you experience an injury during the course of this study, you should immediately contact Drs. [redacted]

The University of Alabama at Birmingham (UAB) and the study sponsor the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) have not provided for any payment if you are harmed as a result of taking part of this study. If such harm occurs, treatment will be provided. However, this treatment will not be provided free of charge.

STUDY RIGHTS AND STUDY WITHDRAWAL

Participation in this study is entirely voluntary. Your treatment and the attitude of your doctor toward you will not be affected should you decide not to take part in this study. Refusal to participate will not affect any benefits to which you are otherwise entitled. If you agree to participate, you may withdraw from the study at any time.

If you are a UAB student or employee, taking part in this research is not a part of your UAB class work or duties. You can refuse to enroll or withdraw after enrolling at any time before the study is over with no effect on your class standing, grades, or job at UAB. You will not be offered or receive any special consideration if you take part in this research.

You may refuse to participate or even withdraw once the study has started. In either case, you will not be penalized or lose any benefits to which you are otherwise entitled.

QUESTIONS

While you are participating in this study, if you have any questions or concerns, you should contact Drs. Saag, Calhoun, or a member of their staff at [redacted] After regular business hours (after 4pm and before 7am), please call the UAB operator at [redacted] and ask for Dr. Kenneth G. Saag or Dr. David Calhoun to be paged.

You will be given a copy of this informed consent document and may ask for additional information at any time during the study from Drs. Saag or Calhoun or a member of their staff at [redacted]
If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the Institutional Review Board for Human Use (OIRB) a [redacted] egular hours for the Office of the IRB are 8:00 a.m. to 5:00 p.m. CT, Monday through Friday. You may also call this number in the event the research staff cannot be reached or you wish to talk to someone else.

A description of this clinical trial will be available on http://www.clinicaltrials.gov as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

LEGAL RIGHTS

You are not waiving any of your legal rights by signing this consent form.

STORAGE OF SPECIMENS FOR FUTURE USE

As part of this study, we would like to store some of the blood and urine specimens collected from you for future research in hypertension and rheumatology. The future research may be conducted by Dr. Kenneth Saag or Dr. David Calhoun or by other researchers that obtain IRB approval for their research. The specimens will be labeled with a code that only Dr. Kenneth Saag and Dr. David Calhoun can link back to you. Results of any future research will not be given to you or your doctor. The specimens obtained from you in this research may help in the development of a future commercial product. There are no plans to provide financial compensation to you should this occur.

You do not have to agree to allow your blood and urine specimens to be stored in order to be part of this study.

You may request at any time that your research samples be removed from storage and not be used for future research. If you decide you want your samples removed, you may contact Dr. Kenneth Saag or Dr. David Calhoun at the University of Alabama at Birmingham at 205-996-6086. Once the request is received, and if your samples have not already been used for other research, they will be destroyed. If you do not make such a request, your specimens will be stored indefinitely or until used.

Initial your choice below:

___ I agree to allow my samples to be kept and used for future research on hypertension.

___ I do not agree to allow my samples to be kept and used for future research .

SIGNATURES

Your signature below indicates that you agree to participate in this study. You will receive a copy of this signed informed consent.

_____

Printed Name of Participant

_____	_____
Signature of Participant	Date
_____	_____
Signature of Witness	Date
_____	_____
Signature of Person Obtaining Consent	Date

University of Alabama at Birmingham
AUTHORIZATION FOR USE/DISCLOSURE OF
PROTECTED HEALTH INFORMATION (PHI) FOR RESEARCH

Participant Name: _____
Research Protocol: Project 2: Serum Uric acid Reduction to Prevent HypERtension Study

UAB IRB Protocol Number: F130408004
Principal Investigator: Kenneth Saag, MD and David Calhoun, MD
Sponsor: National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

What is the purpose of this form? You are being asked to sign this form so that UAB may use and release your protected health information for research. Participation in research is voluntary. If you choose to participate in the research, you must sign this form so that your protected health information may be used for the research.

Why do the researchers want my protected health information? The researchers want to use your protected health information as part of the research protocol listed above and as described to you in the informed consent.

What protected health information do the researchers want to use? All medical information, including but not limited to information and/or records of any diagnosis or treatment of disease or condition, which may include sexually transmitted diseases (e.g., HIV, etc.) or communicable diseases, drug/alcohol dependency, etc.; all personal identifiers, including but not limited to your name, social security number, medical record number, date of birth, dates of service, etc.; any past, present, and future history, examinations, laboratory results, imaging studies and reports and treatments of whatever kind, including but not limited to drug/alcohol treatment, psychiatric/psychological treatment; financial/billing information, including but not limited to copies of your medical bills, and any other information related to or collected for use in the research protocol, regardless of whether the information was collected for research or non-research (e.g., treatment) purposes.

Who will disclose, use and/or receive my protected health information? All Individuals/entities listed in the informed consent documents, including but not limited to, the physicians, nurses and staff and others performing services related to the research (whether at UAB or elsewhere); other operating units of UAB, HSF, UAB Highlands, Children's of Alabama, Eye Foundation Hospital, and the Jefferson County Department of Health, as necessary for their operations; the IRB and its staff; the sponsor of the research and its employees and agents, including any CRO; and any outside regulatory agencies, such as the Food and Drug Administration, providing oversight or performing other legal and/or regulatory functions for which access to participant information is required.

How will my protected health information be protected once it is given to others? Your protected health information that is given to the study sponsor will remain private to the extent possible, even though the study sponsor is not required to follow the federal privacy laws. However, once your information is given to other organizations that are not required to follow federal privacy laws, we cannot assure that the information will remain protected.

How long will this Authorization last? Your authorization for the uses and disclosures described in this Authorization does not have an expiration date.

Can I cancel this Authorization? You may cancel this Authorization at any time by notifying the Principal Investigator, in writing, referencing the research protocol and IRB Protocol Number. If you cancel this Authorization, the study doctor and staff will not use any new health information for research. However, researchers may continue to use the protected health information that was provided before you cancelled your authorization.

Can I see my protected health information? You have a right to request to see your protected health information. However, to ensure the scientific integrity of the research, you will not be able to review the research information until after the research protocol has been completed.

Signature of participant: _____ Date: _____

or participant's legally authorized representative: _____ Date: _____

Printed Name of participant's representative: _____

Relationship to the participant: _____

Appendix 2- Study Forms and Screenshots from the REDCap System

Screen shots from the REDCap System

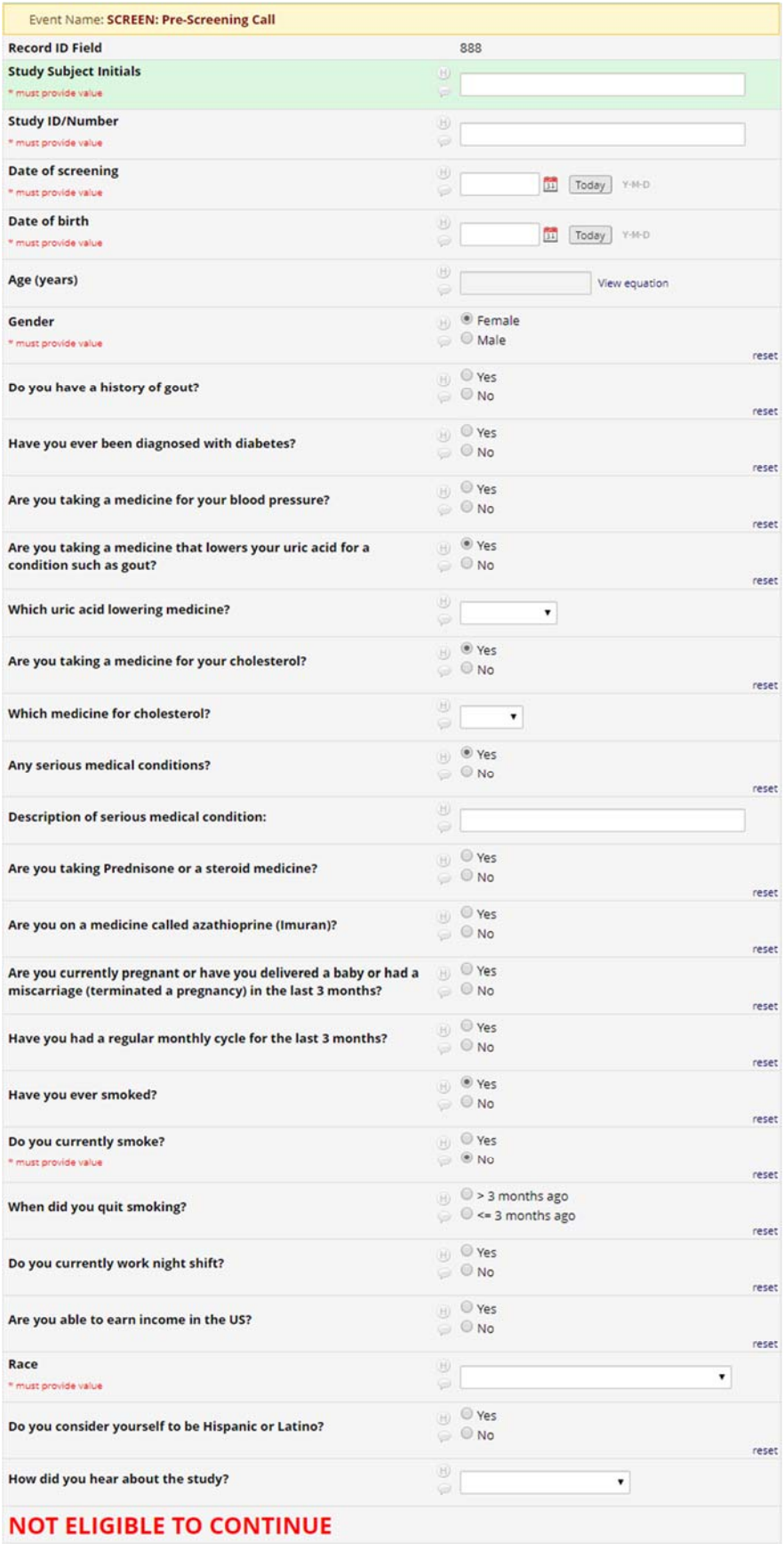
Project 2: The Effects of Urate Lowering Therapy on Inflammation, Endothelial Function, and Blood Pressure (NIAMS: CORT)

1. Prescreening call #1 – Eligibility criteria
2. Prescreening call #2 – Contact information
3. Screening visit #1 – Blood pressure measurement
4. Screening visit #2 – Medical history, sample collection and pill distribution
5. Screening visit #3 – Laboratory results
6. Phone Check-in #1
7. Phone Check-in #2
8. Visits 2, 3, 5 #1 – Pill count, adverse event check, and physical exam
9. Visit 4 #1 – Adverse event check and physical exam
10. Visit 2-5 #2 – Current medical conditions and medications, blood pressure measurement
11. Visit 2-5 #3 – Sample collection and laboratory results
12. Visit 2-5 #4 – Flow-mediated dilation
13. Between visits phone check-in
14. Adverse event report
15. Study completion

Following the screening visit (visit 1) the screen shots for study visits 2-5 repeat in the system.

Appendix 2

Screenshot 1. Prescreening call #1 – Eligibility criteria



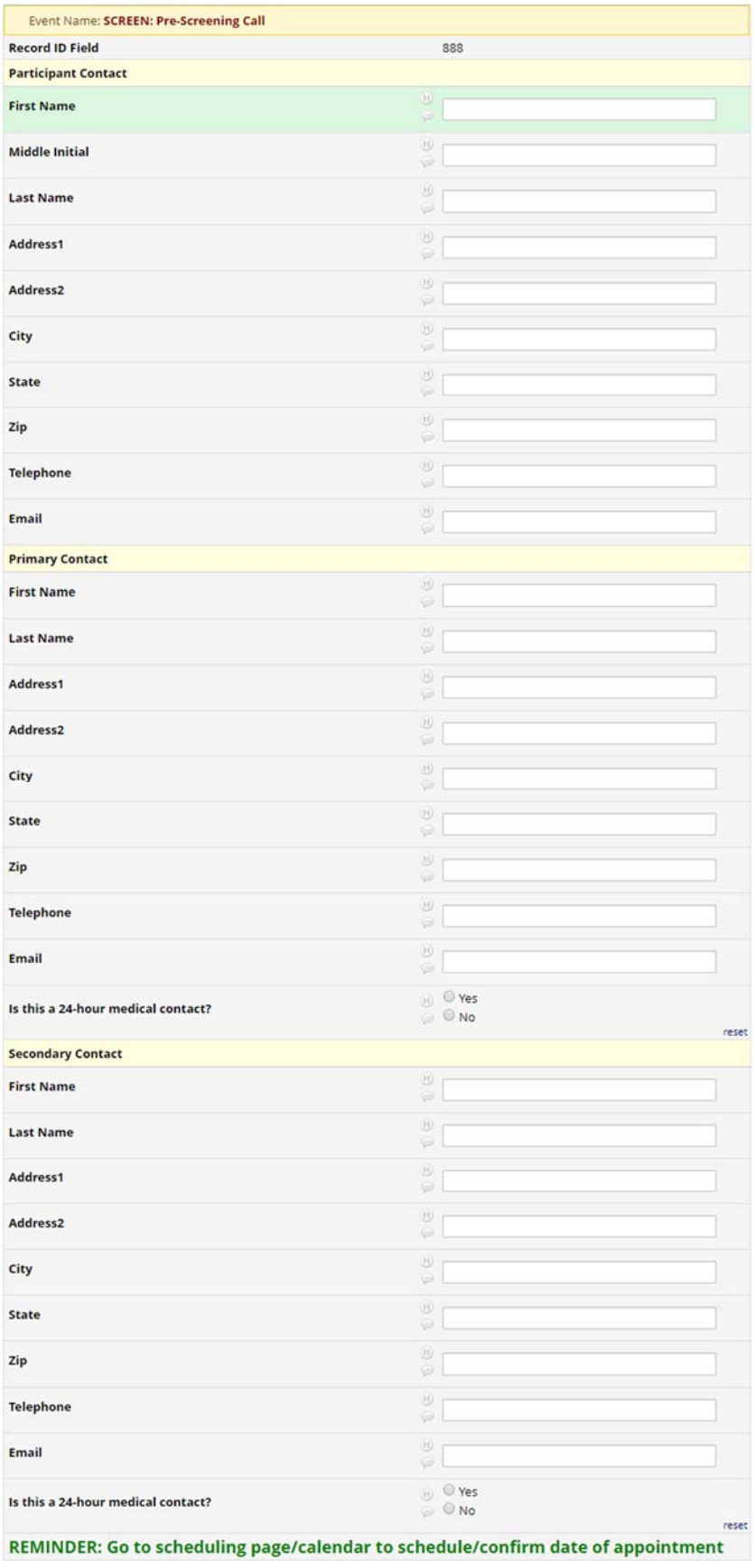
Event Name: **SCREEN: Pre-Screening Call**

Record ID Field	888
Study Subject Initials * must provide value	
Study ID/Number * must provide value	
Date of screening * must provide value	Today Y-M-D
Date of birth * must provide value	Today Y-M-D
Age (years)	View equation
Gender * must provide value	Female / Male reset
Do you have a history of gout?	Yes / No reset
Have you ever been diagnosed with diabetes?	Yes / No reset
Are you taking a medicine for your blood pressure?	Yes / No reset
Are you taking a medicine that lowers your uric acid for a condition such as gout?	Yes / No reset
Which uric acid lowering medicine?	
Are you taking a medicine for your cholesterol?	Yes / No reset
Which medicine for cholesterol?	
Any serious medical conditions?	Yes / No reset
Description of serious medical condition:	
Are you taking Prednisone or a steroid medicine?	Yes / No reset
Are you on a medicine called azathioprine (Imuran)?	Yes / No reset
Are you currently pregnant or have you delivered a baby or had a miscarriage (terminated a pregnancy) in the last 3 months?	Yes / No reset
Have you had a regular monthly cycle for the last 3 months?	Yes / No reset
Have you ever smoked?	Yes / No reset
Do you currently smoke? * must provide value	Yes / No reset
When did you quit smoking?	> 3 months ago / <= 3 months ago reset
Do you currently work night shift?	Yes / No reset
Are you able to earn income in the US?	Yes / No reset
Race * must provide value	
Do you consider yourself to be Hispanic or Latino?	Yes / No reset
How did you hear about the study?	

NOT ELIGIBLE TO CONTINUE

Appendix 2

Screenshot 2. Prescreening call #2 – Contact information



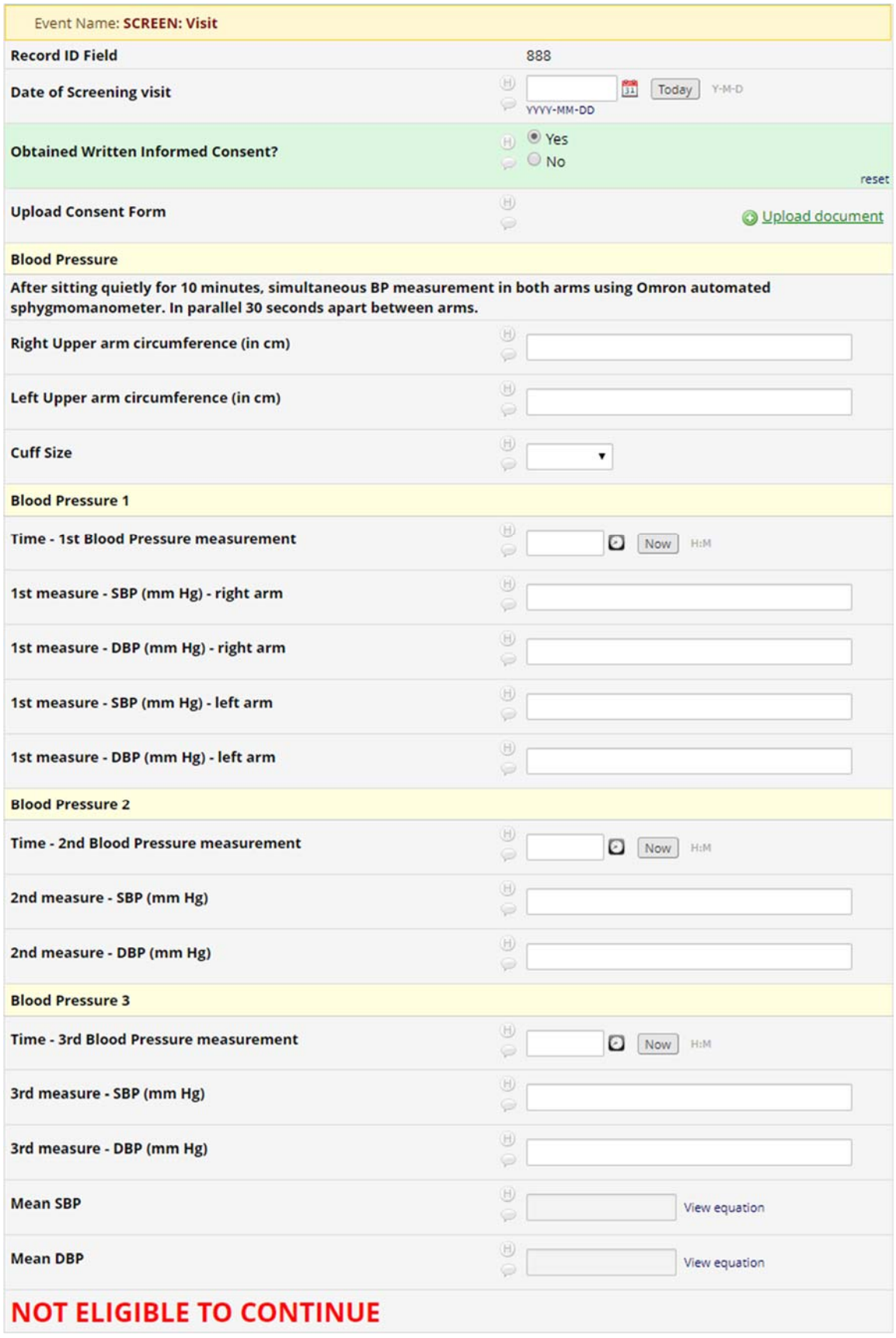
Event Name: **SCREEN: Pre-Screening Call**

Record ID Field	888
Participant Contact	
First Name	
Middle Initial	
Last Name	
Address1	
Address2	
City	
State	
Zip	
Telephone	
Email	
Primary Contact	
First Name	
Last Name	
Address1	
Address2	
City	
State	
Zip	
Telephone	
Email	
Is this a 24-hour medical contact?	Yes / No — reset
Secondary Contact	
First Name	
Last Name	
Address1	
Address2	
City	
State	
Zip	
Telephone	
Email	
Is this a 24-hour medical contact?	Yes / No — reset

REMINDER: Go to scheduling page/calendar to schedule/confirm date of appointment

Appendix 2

Screenshot 3. Screening visit #1 – Blood pressure measurement



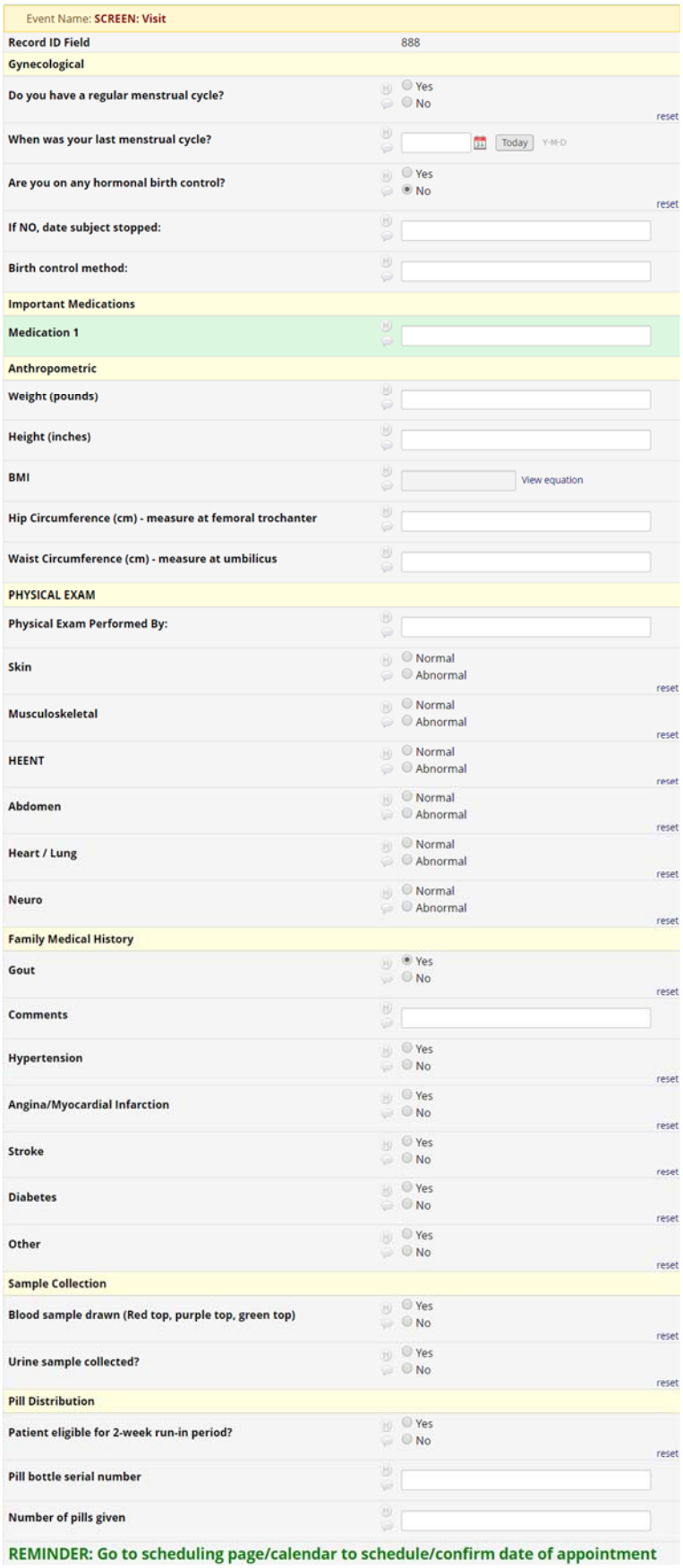
Event Name: **SCREEN: Visit**

Record ID Field	888
Date of Screening visit	Today Y-M-D YYYY-MM-DD
Obtained Written Informed Consent?	Yes / No reset
Upload Consent Form	Upload document
Blood Pressure	
After sitting quietly for 10 minutes, simultaneous BP measurement in both arms using Omron automated sphygmomanometer. In parallel 30 seconds apart between arms.	
Right Upper arm circumference (in cm)	
Left Upper arm circumference (in cm)	
Cuff Size	
Blood Pressure 1	
Time - 1st Blood Pressure measurement	Now H:M
1st measure - SBP (mm Hg) - right arm	
1st measure - DBP (mm Hg) - right arm	
1st measure - SBP (mm Hg) - left arm	
1st measure - DBP (mm Hg) - left arm	
Blood Pressure 2	
Time - 2nd Blood Pressure measurement	Now H:M
2nd measure - SBP (mm Hg)	
2nd measure - DBP (mm Hg)	
Blood Pressure 3	
Time - 3rd Blood Pressure measurement	Now H:M
3rd measure - SBP (mm Hg)	
3rd measure - DBP (mm Hg)	
Mean SBP	View equation
Mean DBP	View equation

NOT ELIGIBLE TO CONTINUE

Appendix 2

Screenshot 4. Screening visit #2 – Medical history, sample collection and pill distribution

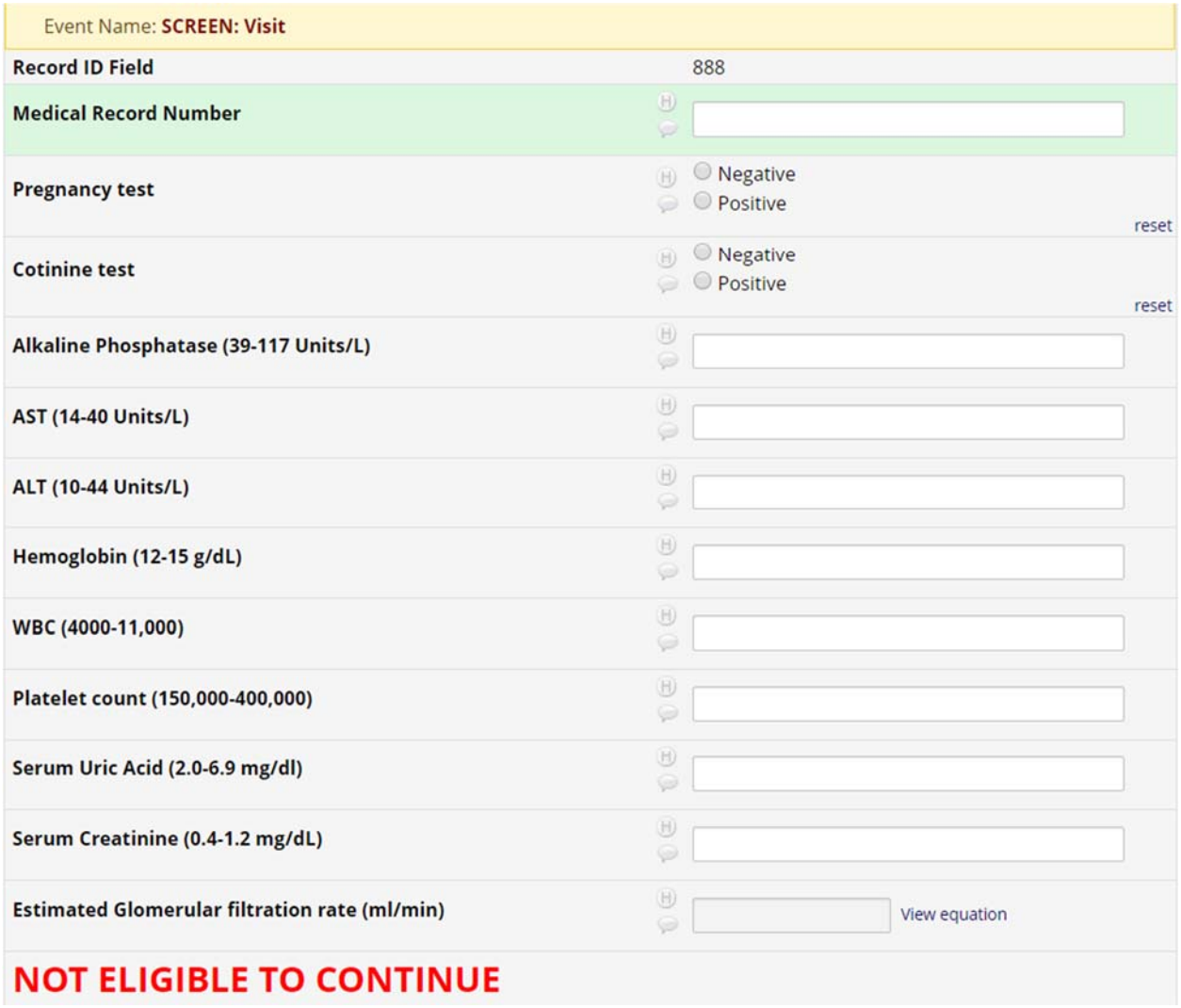


Event Name: **SCREEN: Visit**

Record ID Field	888
Gynecological	
Do you have a regular menstrual cycle?	○ Yes ○ No reset
When was your last menstrual cycle?	Today Y-M-D
Are you on any hormonal birth control?	○ Yes ◉ No reset
If NO, date subject stopped:	
Birth control method:	
Important Medications	
Medication 1	
Anthropometric	
Weight (pounds)	
Height (inches)	
BMI	View equation
Hip Circumference (cm) - measure at femoral trochanter	
Waist Circumference (cm) - measure at umbilicus	
PHYSICAL EXAM	
Physical Exam Performed By:	
Skin	○ Normal ○ Abnormal reset
Musculoskeletal	○ Normal ○ Abnormal reset
HEENT	○ Normal ○ Abnormal reset
Abdomen	○ Normal ○ Abnormal reset
Heart / Lung	○ Normal ○ Abnormal reset
Neuro	○ Normal ○ Abnormal reset
Family Medical History	
Gout	◉ Yes ○ No reset
Comments	
Hypertension	○ Yes ○ No reset
Angina/Myocardial Infarction	○ Yes ○ No reset
Stroke	○ Yes ○ No reset
Diabetes	○ Yes ○ No reset
Other	○ Yes ○ No reset
Sample Collection	
Blood sample drawn (Red top, purple top, green top)	○ Yes ○ No reset
Urine sample collected?	○ Yes ○ No reset
Pill Distribution	
Patient eligible for 2-week run-in period?	○ Yes ○ No reset
Pill bottle serial number	
Number of pills given	

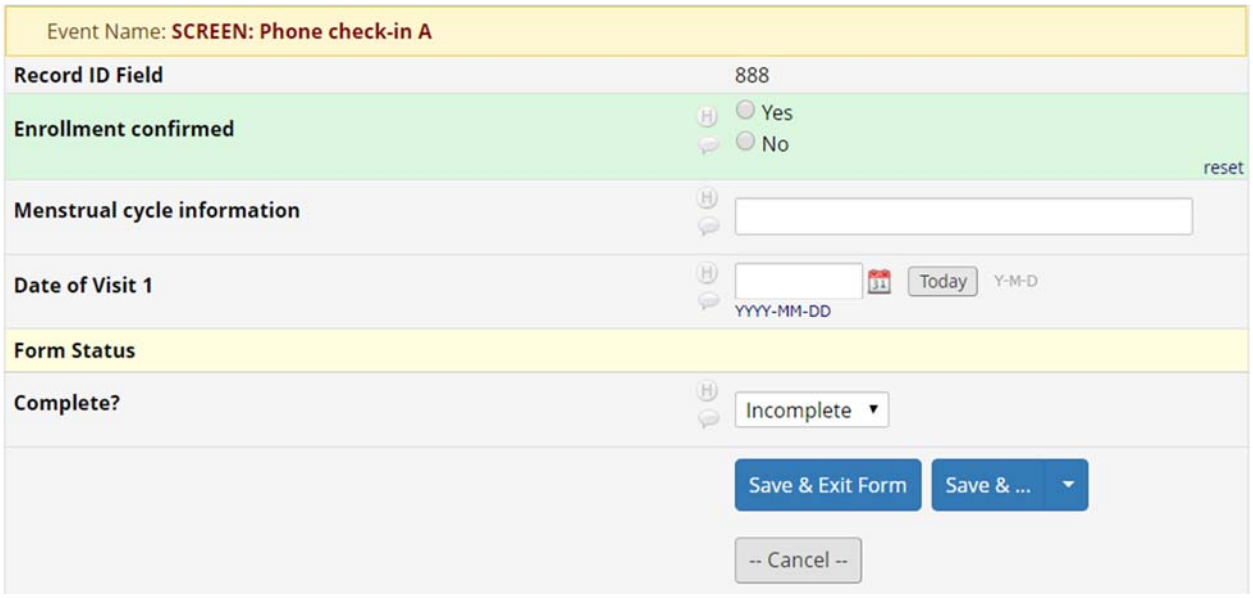
REMINDER: Go to scheduling page/calendar to schedule/confirm date of appointment

Screenshot 5. Screening visit #3 – Laboratory results

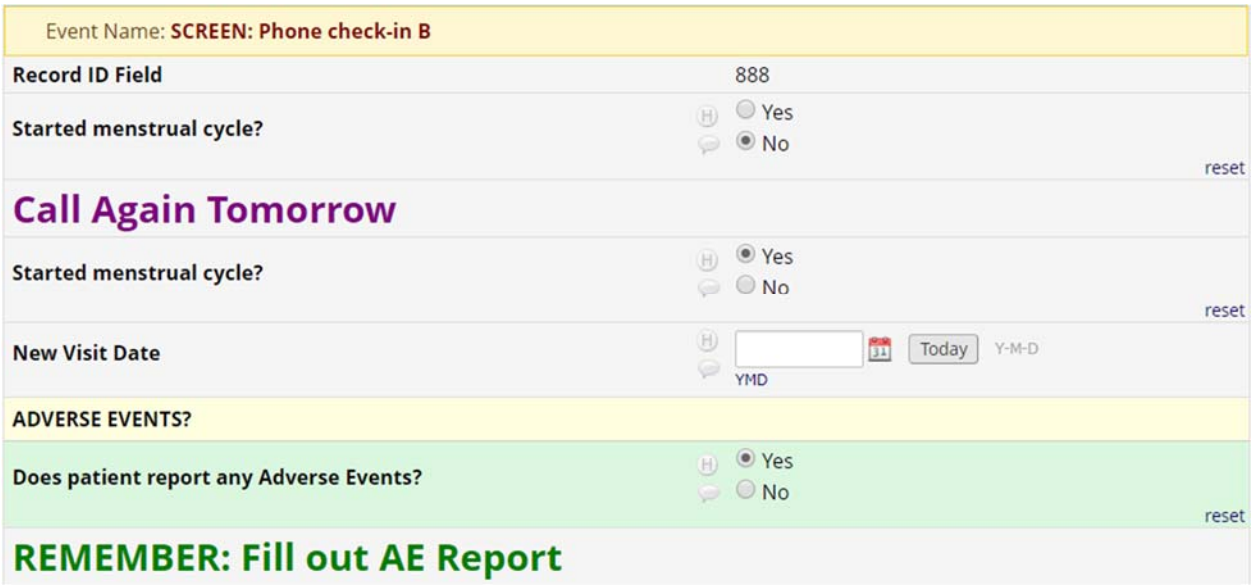


Appendix 2

Screenshot 6. Phone Check-in #1

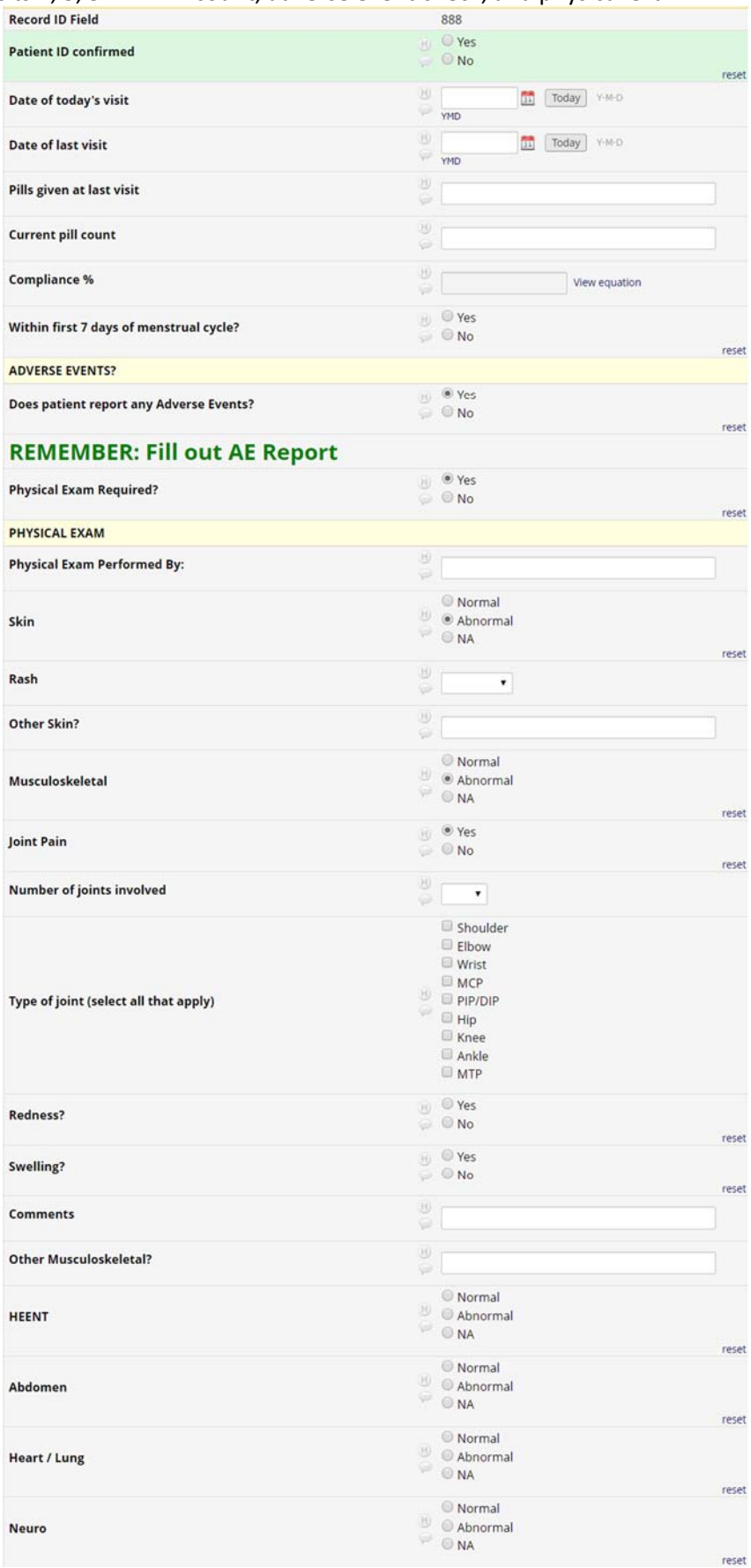


Screenshot 7. Phone Check-in #2



Appendix 2

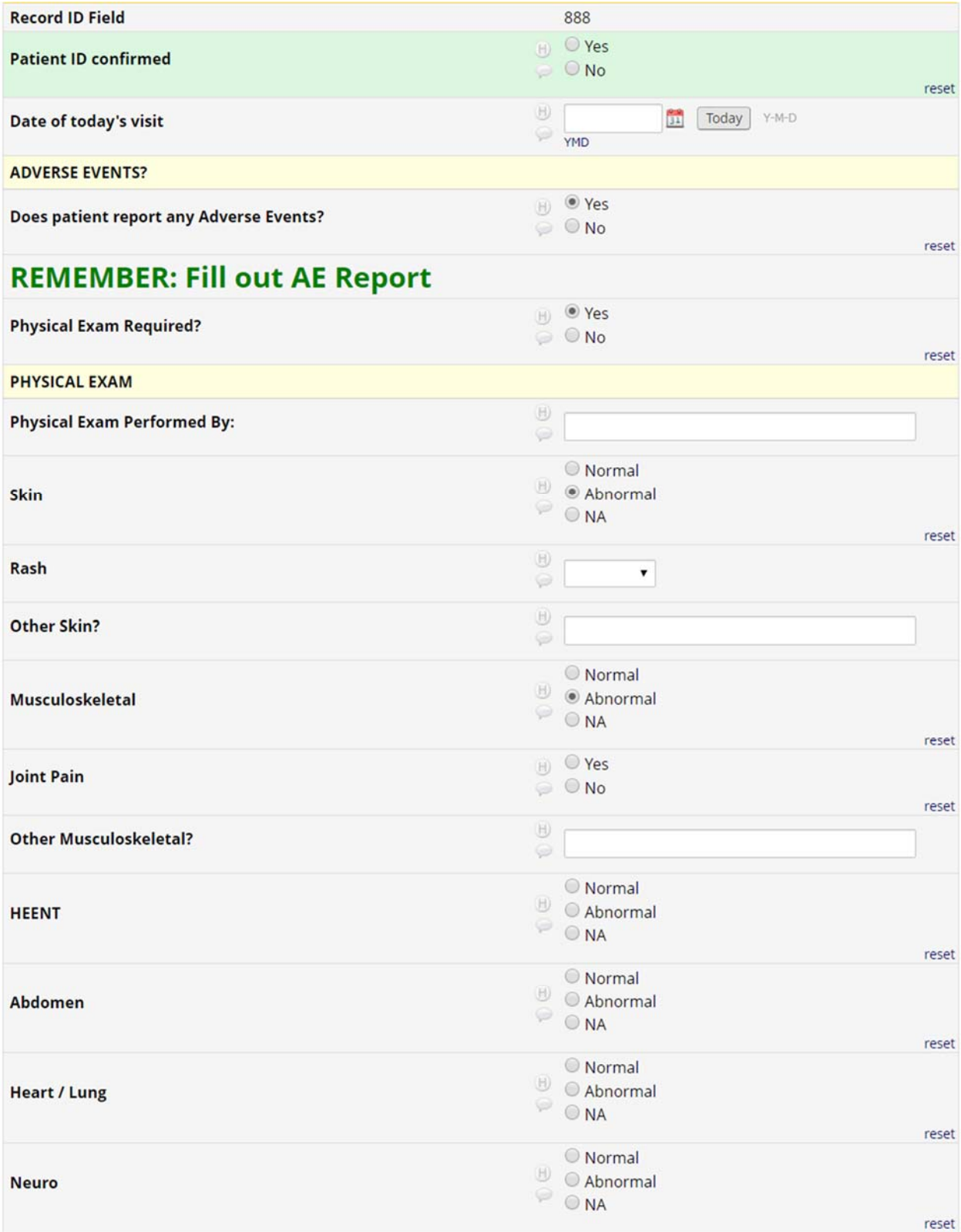
Screenshot 8. Visits 2, 3, 5 #1 – Pill count, adverse event check, and physical exam



Record ID Field	888
Patient ID confirmed	○ Yes ○ No reset
Date of today's visit	Today Y-M-D YMD
Date of last visit	Today Y-M-D YMD
Pills given at last visit	
Current pill count	
Compliance %	View equation
Within first 7 days of menstrual cycle?	○ Yes ○ No reset
ADVERSE EVENTS?	
Does patient report any Adverse Events?	◉ Yes ○ No reset
REMEMBER: Fill out AE Report	
Physical Exam Required?	◉ Yes ○ No reset
PHYSICAL EXAM	
Physical Exam Performed By:	
Skin	○ Normal ◉ Abnormal ○ NA reset
Rash	
Other Skin?	
Musculoskeletal	○ Normal ◉ Abnormal ○ NA reset
Joint Pain	◉ Yes ○ No reset
Number of joints involved	
Type of joint (select all that apply)	☐ Shoulder ☐ Elbow ☐ Wrist ☐ MCP ☐ PIP/DIP ☐ Hip ☐ Knee ☐ Ankle ☐ MTP
Redness?	○ Yes ○ No reset
Swelling?	○ Yes ○ No reset
Comments	
Other Musculoskeletal?	
HEENT	○ Normal ○ Abnormal ○ NA reset
Abdomen	○ Normal ○ Abnormal ○ NA reset
Heart / Lung	○ Normal ○ Abnormal ○ NA reset
Neuro	○ Normal ○ Abnormal ○ NA reset

Appendix 2

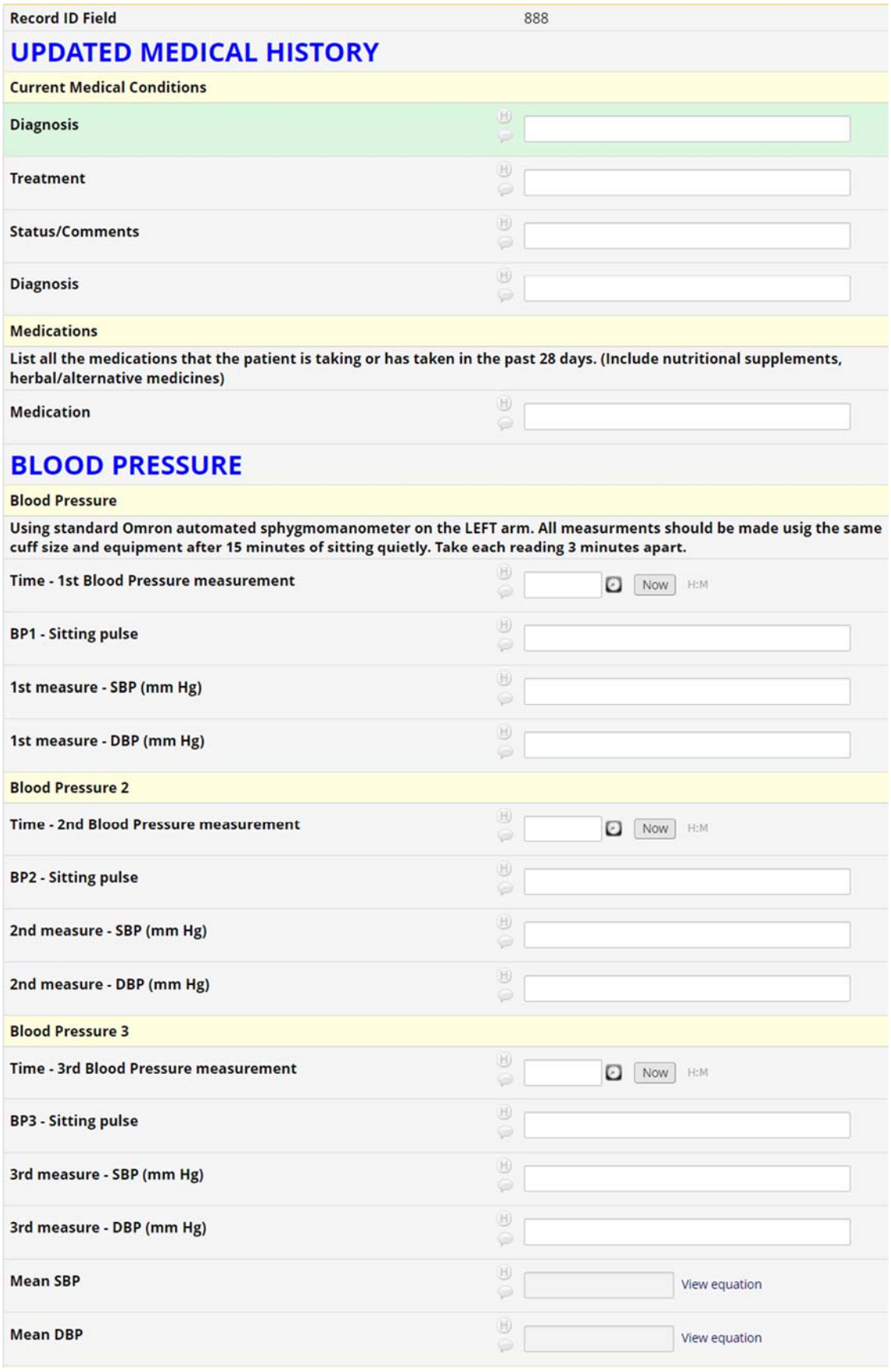
Screenshot 9. Visit 4 #1 – Adverse event check and physical exam



Record ID Field	888
Patient ID confirmed	○ Yes ○ No reset
Date of today's visit	Today Y-M-D YMD
ADVERSE EVENTS?	
Does patient report any Adverse Events?	◉ Yes ○ No reset
REMEMBER: Fill out AE Report	
Physical Exam Required?	◉ Yes ○ No reset
PHYSICAL EXAM	
Physical Exam Performed By:	
Skin	○ Normal ◉ Abnormal ○ NA reset
Rash	
Other Skin?	
Musculoskeletal	○ Normal ◉ Abnormal ○ NA reset
Joint Pain	○ Yes ○ No reset
Other Musculoskeletal?	
HEENT	○ Normal ○ Abnormal ○ NA reset
Abdomen	○ Normal ○ Abnormal ○ NA reset
Heart / Lung	○ Normal ○ Abnormal ○ NA reset
Neuro	○ Normal ○ Abnormal ○ NA reset

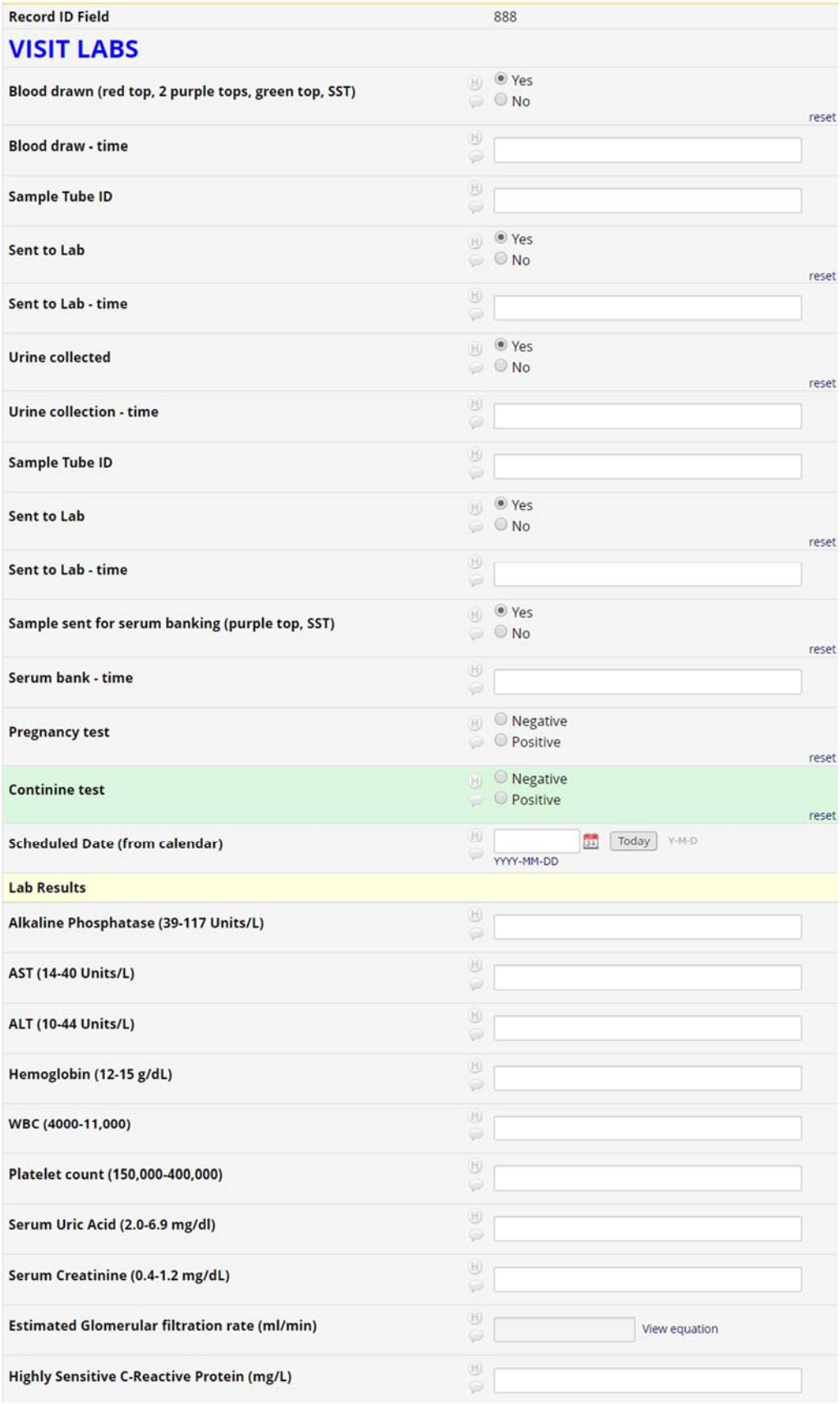
Appendix 2

Screenshot 10. Visit 2-5 #2 – Current medical conditions and medications, blood pressure measurement



Appendix 2

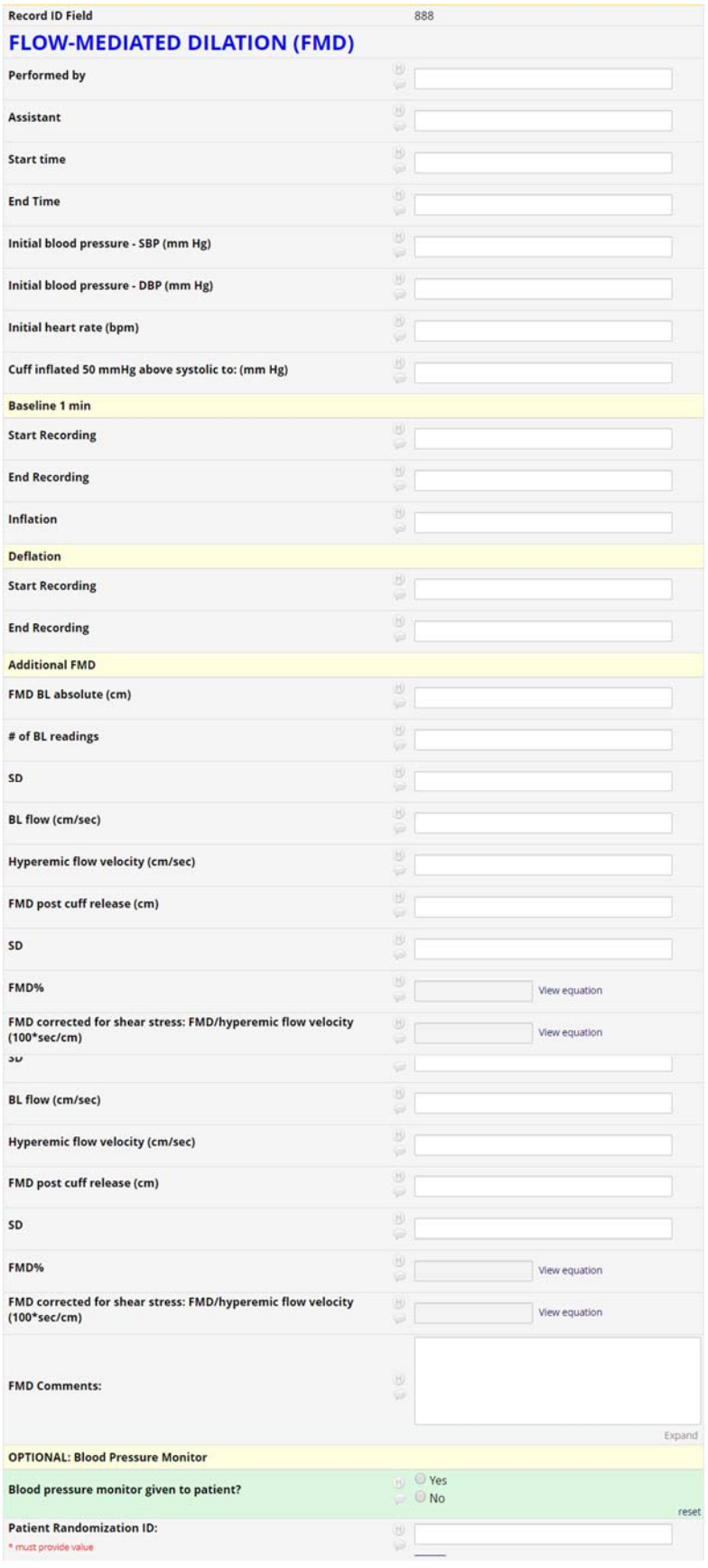
Screenshot 11. Visit 2-5 #3 – Sample collection and laboratory results



Record ID Field	888
VISIT LABS	
Blood drawn (red top, 2 purple tops, green top, SST)	Yes / No reset
Blood draw - time	
Sample Tube ID	
Sent to Lab	Yes / No reset
Sent to Lab - time	
Urine collected	Yes / No reset
Urine collection - time	
Sample Tube ID	
Sent to Lab	Yes / No reset
Sent to Lab - time	
Sample sent for serum banking (purple top, SST)	Yes / No reset
Serum bank - time	
Pregnancy test	Negative / Positive reset
Continine test	Negative / Positive reset
Scheduled Date (from calendar)	Today Y-M-D YYYY-MM-DD
Lab Results	
Alkaline Phosphatase (39-117 Units/L)	
AST (14-40 Units/L)	
ALT (10-44 Units/L)	
Hemoglobin (12-15 g/dL)	
WBC (4000-11,000)	
Platelet count (150,000-400,000)	
Serum Uric Acid (2.0-6.9 mg/dl)	
Serum Creatinine (0.4-1.2 mg/dL)	
Estimated Glomerular filtration rate (ml/min)	View equation
Highly Sensitive C-Reactive Protein (mg/L)	

Appendix 2

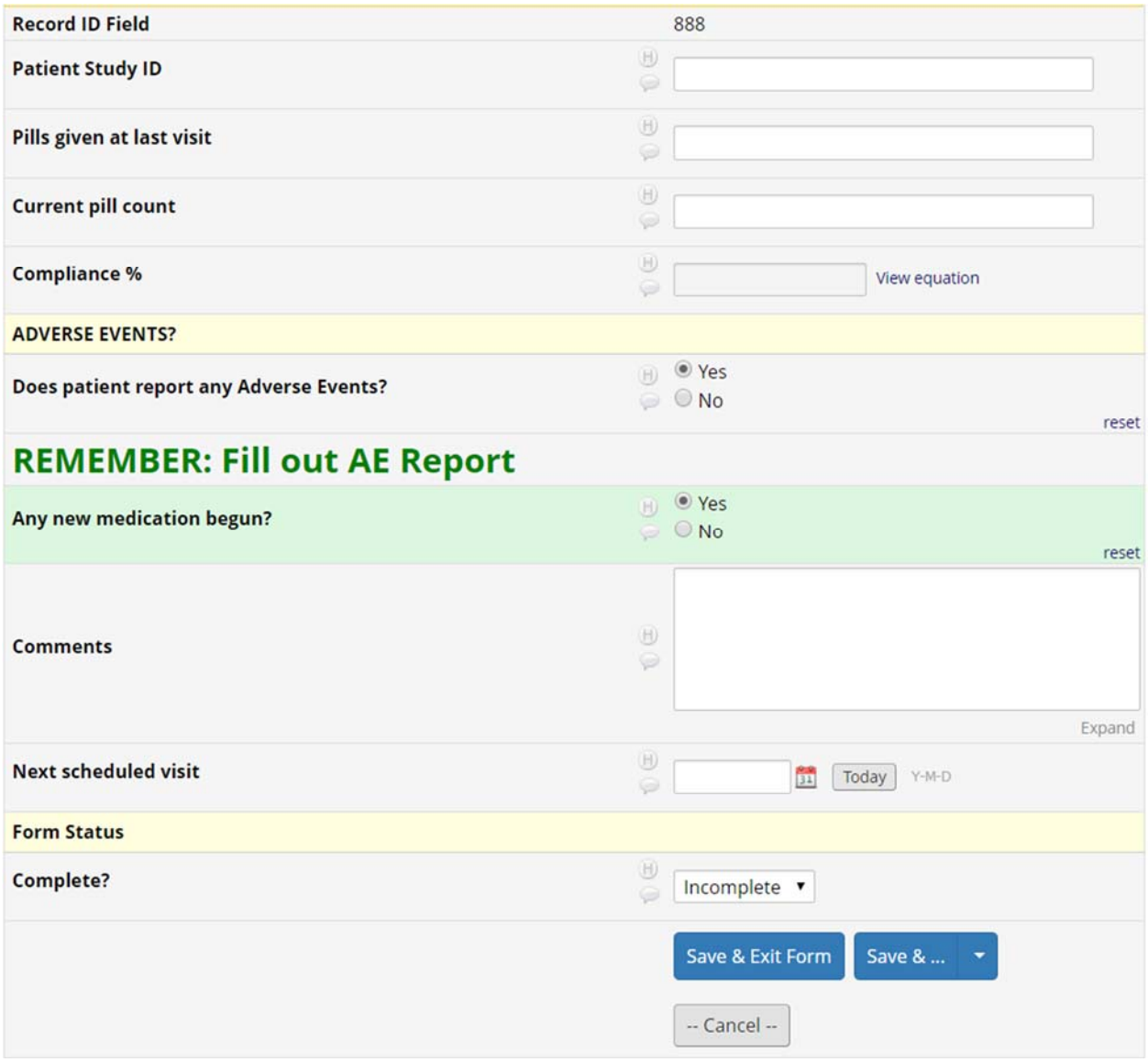
Screenshot 12. Visit 2-5 #4 – Flow-mediated dilation



Record ID Field	888
FLOW-MEDIATED DILATION (FMD)	
Performed by	
Assistant	
Start time	
End Time	
Initial blood pressure - SBP (mm Hg)	
Initial blood pressure - DBP (mm Hg)	
Initial heart rate (bpm)	
Cuff inflated 50 mmHg above systolic to: (mm Hg)	
Baseline 1 min	
Start Recording	
End Recording	
Inflation	
Deflation	
Start Recording	
End Recording	
Additional FMD	
FMD BL absolute (cm)	
# of BL readings	
SD	
BL flow (cm/sec)	
Hyperemic flow velocity (cm/sec)	
FMD post cuff release (cm)	
SD	
FMD%	View equation
FMD corrected for shear stress: FMD/hyperemic flow velocity (100*sec/cm)	View equation
SD	
BL flow (cm/sec)	
Hyperemic flow velocity (cm/sec)	
FMD post cuff release (cm)	
SD	
FMD%	View equation
FMD corrected for shear stress: FMD/hyperemic flow velocity (100*sec/cm)	View equation
FMD Comments:	Expand
OPTIONAL: Blood Pressure Monitor	
Blood pressure monitor given to patient?	Yes / No reset
Patient Randomization ID: * must provide value	

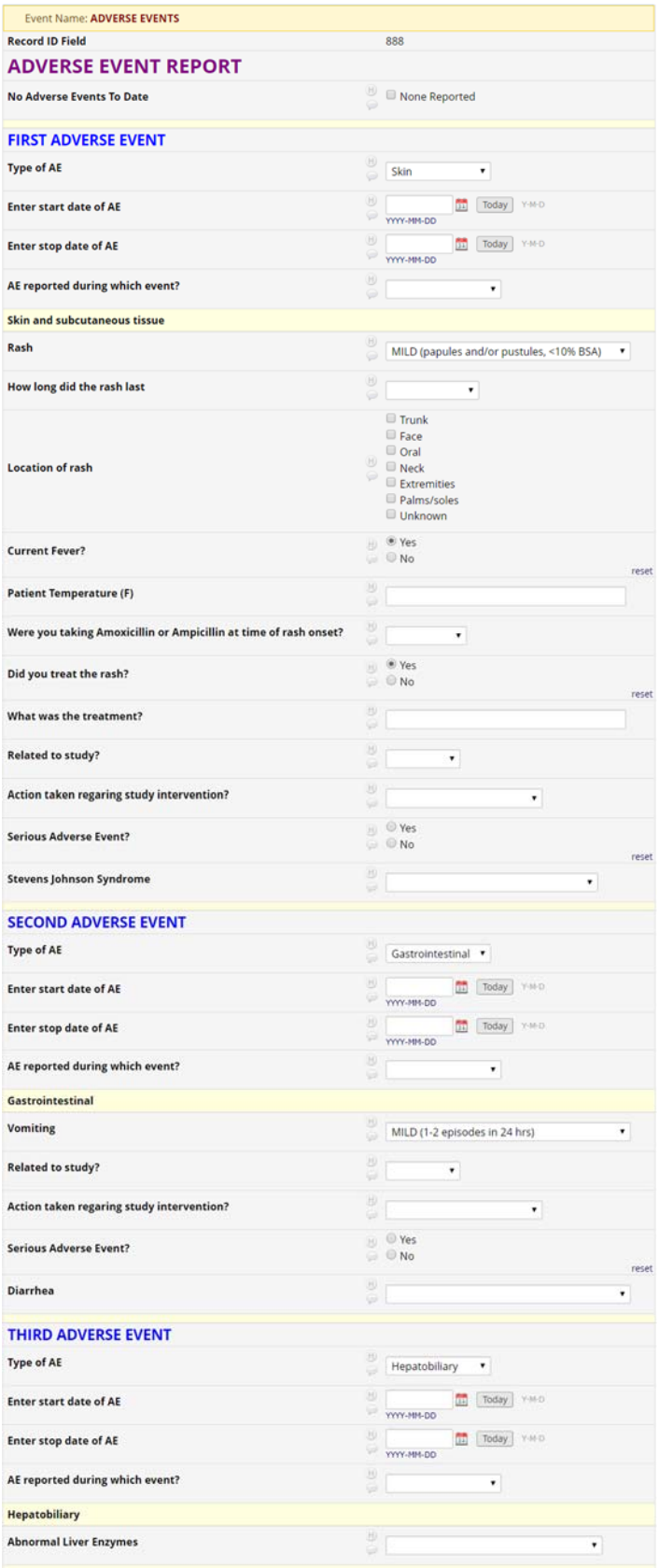
Appendix 2

Screenshot 13. Between visits phone check-in



Appendix 2

Screenshot 14. Adverse event report



Event Name: **ADVERSE EVENTS**

Record ID Field	888

ADVERSE EVENT REPORT

No Adverse Events To Date	☐ None Reported

FIRST ADVERSE EVENT

Type of AE	Skin
Enter start date of AE	Today Y-M-D YYYY-MM-DD
Enter stop date of AE	Today Y-M-D YYYY-MM-DD
AE reported during which event?	
Skin and subcutaneous tissue	
Rash	MILD (papules and/or pustules, <10% BSA)
How long did the rash last	
Location of rash	☐ Trunk ☐ Face ☐ Oral ☐ Neck ☐ Extremities ☐ Palms/soles ☐ Unknown
Current Fever?	◉ Yes ○ No reset
Patient Temperature (F)	
Were you taking Amoxicillin or Ampicillin at time of rash onset?	
Did you treat the rash?	◉ Yes ○ No reset
What was the treatment?	
Related to study?	
Action taken regaring study intervention?	
Serious Adverse Event?	○ Yes ○ No reset
Stevens Johnson Syndrome	

SECOND ADVERSE EVENT

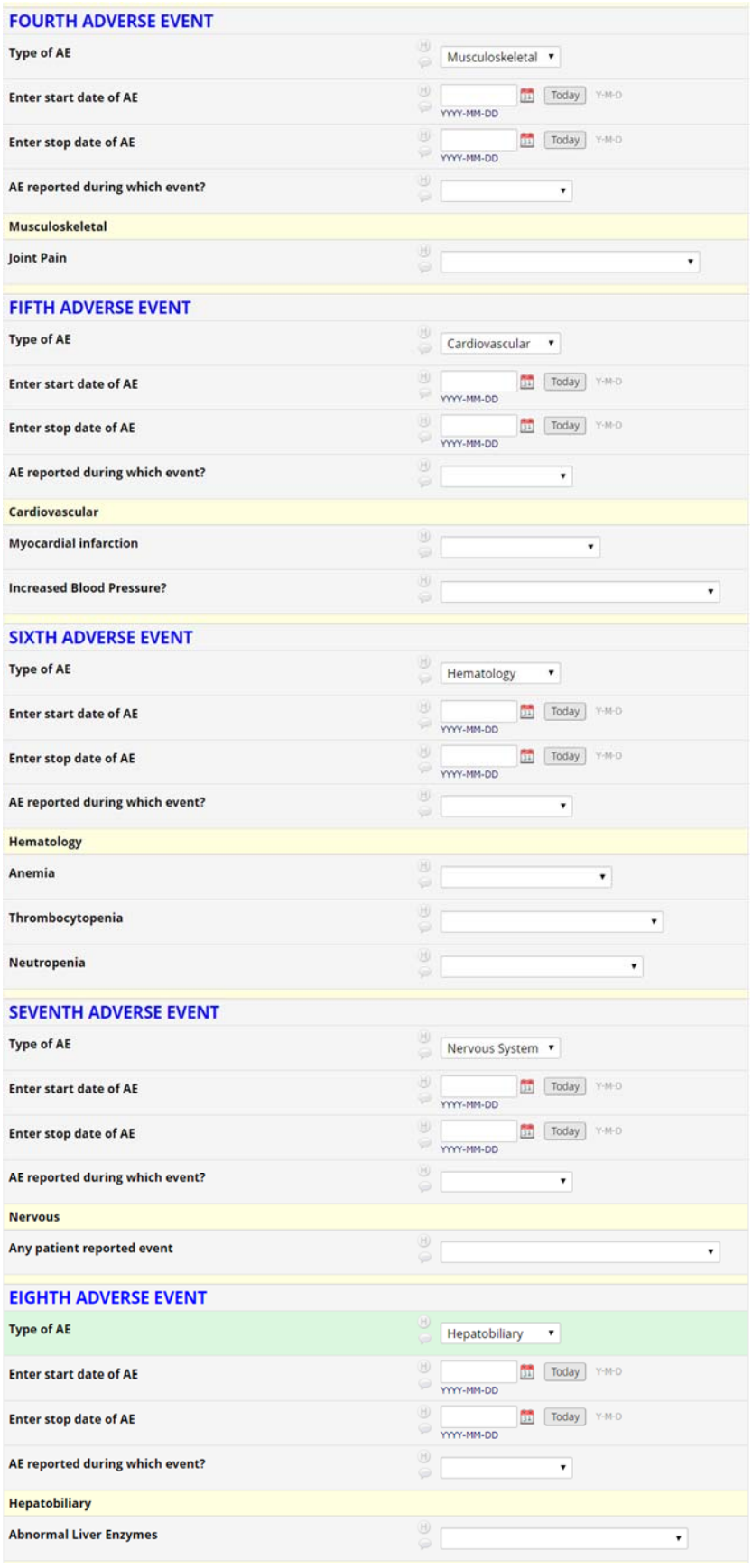
Type of AE	Gastrointestinal
Enter start date of AE	Today Y-M-D YYYY-MM-DD
Enter stop date of AE	Today Y-M-D YYYY-MM-DD
AE reported during which event?	
Gastrointestinal	
Vomiting	MILD (1-2 episodes in 24 hrs)
Related to study?	
Action taken regaring study intervention?	
Serious Adverse Event?	○ Yes ○ No reset
Diarrhea	

THIRD ADVERSE EVENT

Type of AE	Hepatobiliary
Enter start date of AE	Today Y-M-D YYYY-MM-DD
Enter stop date of AE	Today Y-M-D YYYY-MM-DD
AE reported during which event?	
Hepatobiliary	
Abnormal Liver Enzymes	

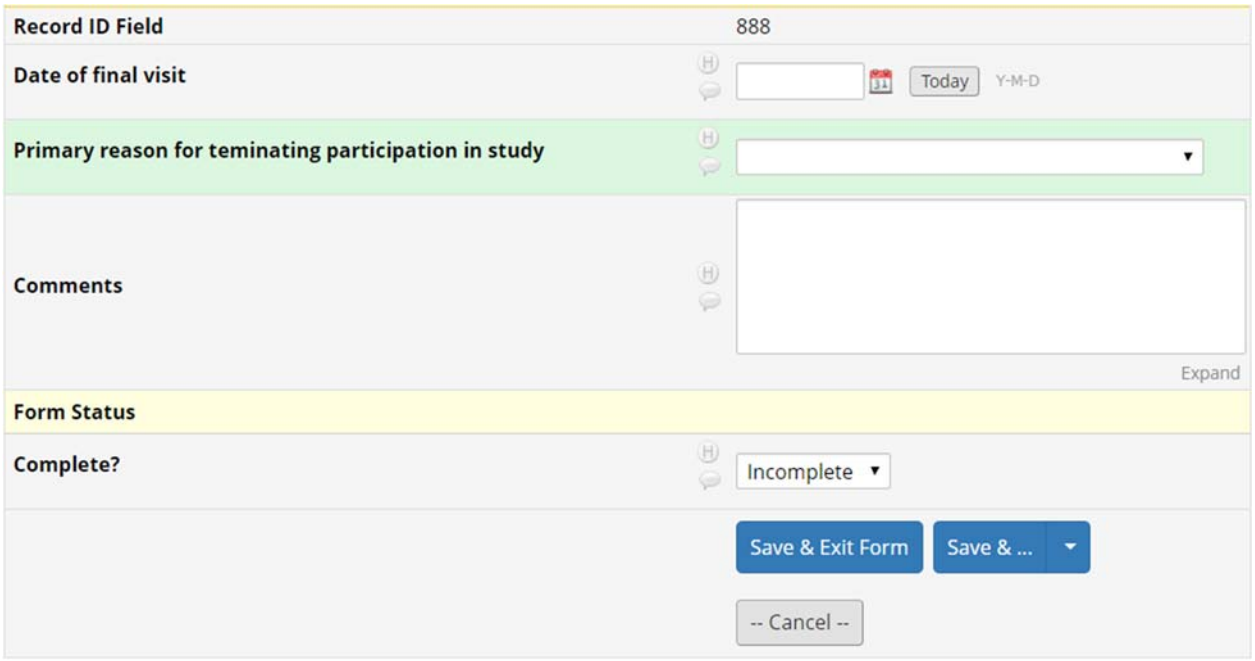
Appendix 2

Screenshot 14 (continued). Adverse event report



Appendix 2

Screenshot 15. Study completion



APPENDIX 3. Standard Operating Procedures

A. Standard Operating Procedure - Obtaining Consent

1. The informed consent will be obtained before any study procedures are performed (including washout from any medications).
2. Study coordinator will check the shared drive to ensure the correct and current IRB approved consent form is used for consenting.
3. Use the most recently approved consent form to give to the patient.
4. All patient will be given adequate time to read the informed consent in a quiet room.
5. Study coordinator will administer the informed consent and will provide an opportunity to discuss any questions the patient might have. [redacted] will also be available to answer any questions prior to or on the day of enrollment of the patient into a study.

   Review the following details with the patient: risks, benefits, alternative treatments, length of the study, interval of visits including time involvement, purpose of the study, any placebo use, FDA approval of medication, procedures to be performed along with risks, side effects of drug(s) used, need for compliance in the study, and benefits.

6. Any patient who states either they cannot see or cannot read will have the consent read to them
7. After the consenting process is completed the patient will sign and date each required page. Verify participant signatures and authorization to store lab samples for future use (not required for participation).
8. Review the consent for completeness of signatures and dates.
9. After the participant has signed the consent form, the Principle Investigator and/or the study coordinator must sign and date the Informed Consent document.
10. A signed version of the consent form will be kept by the study staff in the study binder.
11. An additional copy of the signed consent form will be given to the patient the same day along with the visit reminder brochure and instruction for ambulatory blood pressure monitor.

B. Standard Operating Procedure - Blood Pressure Measurement

1. Patient Preparation

   Have patient sitting comfortably for at least 5 minutes. The patient's legs should be uncrossed with his or her feet flat on the floor. The arm to be used for assessing blood pressure must be exposed and free of constrictive clothing and supported by a table or armrest. The patient should remain quiet during the examination process.

2. Sphygmomanometer Placement

   The sphygmomanometer should be at the eye level of the examiner. The cuff when placed on the patient's arm should be at his or her heart level.

3. Proper Cuff Size

   The arm circumference is measured at the midpoint of the upper arm, between the olecranon and the acromion processes. Arm circumferences of 22 cm-26.9 cm require use of a small adult/pediatric cuff; arm circumferences between 27cm-32.9 cm require a regular adult cuff; circumferences between 33 and 40.9 cm require use of a large adult cuff; arm circumferences between 41cm and 52cm require use of a thigh cuff.

4. Cuff Placement

   Locate the patient's brachial artery at the midpoint of the upper arm. Wrap the cuff smoothly and snugly around the arm with the bladder center directly over the palpated artery and lower edge of the cuff 2.5 cm above the antecubital fossa.

5. Procedure

   Press the START/STOP button. The cuff will inflate automatically. The patient should remain still and not move their arm prior to measurement completion. Inflation stops automatically and the measured is started. When complete, the arm cuff automatically deflates and SBP/DBP and HR are displayed. A minimum of 3 recorded measurements taken 5 min apart are necessary.

Special Techniques

6. Standing Blood Pressure

   Standing blood pressure is measured after making sitting assessments. Using the same arm used to obtain sitting values, have the patient stand fully upright. While supporting the arm in which the blood pressure is being measured, determine values as above. By convention, pressures are obtained 1 and 3 minutes after standing.

7. Korotkoff Sounds Poorly Heard

If the Korotkoff sounds are poorly heard, ensure proper placement of stethoscope directly over the brachial artery, attempt using the diaphragm of the stethoscope instead of the bell, and/or have the patient hold his or her arm upright for a few minutes before repeating procedures.

C. Standard Operating Procedure – Brachial Artery Ultrasound Protocol (Flow Mediated Dilation)

FMD are recorded on tapes, but simultaneously digitalized all recording can be electronically emailed and archived. This ensures that all investigators can easily exchange recordings, peer-review recordings, analyze data over the study period. Project Co-investigator [redacted] will administer and monitor all FMDs.

Ultrasound machine: Philips Agilent SONOS 5500, the ultrasound head is 15-6L.
Software system to be used: Vascular Research Tool 5, by Medical imaging Applications, LLC.
Steps:

1. Baseline
    - Acquire 15 baseline 2D images
    - Acquire 10 cycles of baseline Doppler

2. Inflation
   Inflate cuff pressure 50 mm/Hg>SBP (or until vessel occludes) for 5 minutes

3. Deflation
    - Acquire 10 cycles of hyperemic Doppler
    - Acquire 120 seconds of 2D images

D. Standard Operating Procedure- 24-hour Ambulatory Blood Pressure Monitoring

Twenty-four hour ABPM monitors (Spacelabs Healthcare, Snoqualmie, WA) are to be worn by the enrolled participants for the entire 24-hour period.
Software system: Reports of 24-hour ABPM are available as PDF and can be easily exported to an EXCEL database.
Monitor: 90207, 90217 from Spacelabs Healthcare

1. Determine Cuff Size
   Using incorrect cuff size could result in erroneous and misleading blood pressure measurements. Determine correct cuff size before beginning.
    - Wrap the cuff around the patients upper arm without sliding the arm through the sleeve
    - Use the color coded RANGE indicator on the inside of the cuff and the bold INDEX marker to check that the arm circumference falls within the cuff range
    - If the arm is within range, this cuff is the correct. If the measurement is outside the RANGE indicator, select a new cuff size as indicated by color.
2. Apply the Cuff
    - Simply slide the sleeve up the patients arm, ensuring the artery arrow points

down the arm. The cuff should be midway between the elbow and shoulder.

- Be sure the artery indicator is over the patient's brachial artery, between the biceps and triceps muscles (see illustration below)

- Wrap the cuff snugly around the patients upper arm
- Take the initial BP reading and ensure hook up is working properly.

3. Prepare the Patient

Preparing the patient is the most important step in obtaining accurate, reliable blood pressure measurement.

The following must be reviewed with all participants:

- Avoid excess movement during readings
- Relax the instrumented arm, slightly away from body
- Avoid hand movement
- Avoid flexing muscles during reading
- Do not remove the cuff between readings

E. Standard Operating Procedure- Adverse And Serious Adverse Event Reporting

1. Definition

The International Conference on Harmonization (ICH) Good Clinical Practice defines an adverse event as an "untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment." An Adverse Event can become a Serious Adverse Event. A Serious Adverse Event is defined by the ICH as "any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect

2. Observation

At each visit after screening during the study, a patient will be queried and observed in an objective manner for any changes in signs or symptoms or any new signs or symptoms that may have occurred since the preceding visit. If any adverse event has

occurred, the investigator or a designated member of his staff will determine the following in regards to the adverse event:

- the location of the sign or symptom
- the time of the start and end of the sign or symptom
- the clinical description and measurement of the sign or symptom
- how the sign or symptom came about in terms of setting, activity, or previous episodes
- possible causes of the sign or symptom
- the intensity of the sign or symptom
- the degree to which the sign or symptom may be related to the study drug.

3. Management

The investigator or a designated staff member will take action to assure that patient receives whatever treatment for the adverse event is required or appropriate, either by providing treatment or instructing the patient to seek the care of a physician of the patient's choice, such as the patient's primary care physician. The investigator or a designated staff member will continue to follow the patient with regard to the progress and/or resolution of the adverse event.

Whether the study medication should be interrupted or discontinued in response to an adverse event is a determination made at the investigator's discretion unless it is specified in the study protocol. If an acute emergency arises in the patient's condition, the randomization code may be broken, if this is required for the welfare of the patient.

4. Documentation

The description of the adverse event, including all of the above observations and management, will be recorded in Adverse Event Form, in REDCap and printed for the principal investigator to review and sign the documentation of the adverse event. This signed document will be archived electronically and in hard copy in the study binder.

5. Reporting

It is the responsibility of the investigator to report any adverse event to the sponsor and the institutional review board (IRB) of the study as soon as possible or "within 10 working days after the investigator learns of the adverse event." (ICH) If the Adverse Event is determined to be a Serious Adverse Event (SAE), it is the responsibility of the investigator to report the Serious Adverse Event to the sponsor immediately, and no later than 48 hours. Appropriate reports will be filed or faxed to the sponsor according to the study protocol.

F. General Instructions for Completing Forms in REDCap

All data recorded in REDCap will be maintained according to FDA and ICH Good Clinical Practice (GCP) guidelines. Instructions for completing eCRFs to ensure quality and consistency in data collection are below.

When completing electronic study forms participants must not be identified by name on any study document submitted with the forms (e.g. lab reports). Replace the participant name with the participant initials and study identification (ID) number.

Participant ID: The participant ID will be prepopulated in the header of each page.

Dates: All dates will be entered using the calendar feature in REDCap. For AEs historical dates are sometimes not known (e.g., date of first symptom); in this case, chose Not Applicable, UNKNOWN or UNK to indicate missing data.

Abbreviations: Do not use abbreviations not specifically noted in the instructions for completing the forms. Comments should only be written where text fields are provided.

Correcting errors: Errors can be corrected within the REDCap system where a timestamp and date are associated with each entry and revision.

Missing or Incomplete data
Data may not be available to complete the eform for various reasons. Electronic forms that are missing or not complete should be noted, in the field INCOMPLETE/UNVERIFIED field and brought to the attention of the investigator. Forms with missing data in fields or sections should be marked as such. Indicate the reason for the missing data in the field. If the evaluation was not done, type NOT DONE or ND and provide a reason in the available text field or from the dropdown.

Incomplete or Illegible forms:
If any section or an entire page of the forms cannot be completed (e.g., no parts have any responses), and it is unlikely that it will be completed, choose INCOMPLETE/UNVERIFIED, as appropriate. Do not leave forms incomplete or unused without explanation in the MISSING FORMS / INCOMPLETE FORMS field.

All form header information must be completed even though no data are recorded on the electronic form. If an electronic form can only be partially completed at the time of visit, but will be completed when the information becomes available, note this in the INCOMPLETE/UNVERIFIED field and follow the direction of the study coordinator and principle investigator.

Every effort should be made to obtain and ensure that the electronic forms are being completed correctly.

G. Standard Operating Procedures- Serum and DNA Processing And Storage

If specimen collection is scheduled the day before a holiday, check with [redacted] lab manger to see if lab personnel will be available.

Processing of Blood Samples

Blood Collection, Processing and Storage
Instructions and forms
Sample Processing Instructions
(Serum/DNA)

Laboratory Test		Procedures
Serum Sample		♦ Collect blood into **<u>one 10.0 cc red/black tiger top tube</u>** (SST tube) ♦ Invert the tube gently 8 times, then let the tube sit at room temperature for approximately 30 minutes or until the blood has completely clotted. ♦ Record draw date and time). ♦ **Process SST tube per protocol for separation of serum.**
DNA Testing: Purple EDTA Tube		♦ Collect blood into **<u>TWO purple top 10 ml (EDTA) tubes and one 4 cc EDTA for CBC and ESR.</u>** ♦ Invert the tube several times gently after the blood draw. (***Do NOT Centrifuge samples!)*** ♦ Place the aliquot tube in a 100 position box, record the locations in an excel file and place the box in a -80C freezer.

Processing in [redacted] Lab

Upon receipt of blood, [redacted] research assistant(s) will process the blood as follows: Document receipt of blood samples including number of each type of tube, and date, on the Specimen Processing Sheet.

1.) **Purple top tubes:**

a. Extract genomic DNA from one 500µl aliquot of whole blood using the Gentra PureGene Kit:

(Cell Lysis)

- Add 500ul whole blood to a 2.0ml microfuge tube containing 1500ul RBC Lysis Solution. Invert to mix and incubate 10 minutes at room temp; invert again once during incubation
- Centrifuge for **1 minute at 13K-16K x g**. Remove supernatant with a micropipette leaving behind visible white cell pellet and 5-10ul of residual liquid
- Vortex tube vigorously to resuspend white blood cells in the residual supernatant
- Add 300ul Cell Lysis Solution to tube and pipet up and down to lyse cells. If clumps are visible after mixing, incubate at **37ºC** or room temp until solution is homogenous. Samples are stable in Cell Lysis Solution at room temp for at least 18 months

(Rnase A Treatment)

- Add 1.5ul RNase A Solution to the cell lysate
- Mix the sample by inverting the tube 25 times and incubate at **37ºC for 15 minutes**

(Protein Precipitation)

- Cool sample to room temp
- Add 100ul Protein Precipitation Solution to the cell lysate
- Vortex vigorously at high speed for 20 seconds to mix
- Centrifuge at **13K-16K x g for 3 minutes**. The precipitated proteins will form a tight, dark brown pellet

(DNA Precipitation)

- Pour supernatant containing DNA (leaving behind the protein pellet) into a clean 1.5ml microfuge tube containing 300ul **100% Isopropanol**
- Mix sample by inverting gently 50 times until the white threads of DNA form a visible clump
- Centrifuge at **13K – 16K x g for 1 minute**; the DNA will be visible as a small white pellet
- Pour off supernatant and drain tube on clean absorbent paper. Add 300ul **70% Ethanol**. Invert tube several times to wash DNA pellet
- Centrifuge at **13K – 16K x g for 1 minute**. Carefully pour off the ethanol. Pellet may be loose so pour slowly and watch pellet.

- Drain tube on clean absorbent paper and allow to air dry at room temp for 15 minutes

(DNA Hydration)

- Add 150ul **DNA Hydration Solution**
- Allow DNA to rehydrate by heating at **65ºC for 1 hour**
- Determine [DNA] with spectrophotometer and store at –70ºC

b. Let the tube stand in a rack for 30 minutes at room temperature.
c. Spin in Hamilton tabletop centrifuge for 15 minutes. The three portions are plasma, buffy coat at interface, and red blood cells on the bottom.
d. Transfer with a pipet 2 ml aliquots of plasma into 2 – 4 cryovials.
e. Label vials with bar-coded FreezerWorks labels. Discard any volume of plasma more than 7.2 ml (enough to fill 4 cryovials).
f. Isolate the buffy coat layer from the remaining blood and aliquot into two cryovials. Label all vials with bar-coded FreezerWorks labels.
g. Isolate 1 ml of Red blood Cells from the bottom of the purple top tube and aliquot into one cyrovial. Add 500ul of saline to the tube and spin at 1500 rpm for 1 min. Discard supernatant. Label tube with packed RBCs or pRBCs and store in -70° C.
h. Store the plasma, genomic DNA, and buffy coat (for future DNA extraction) at -70º C.
i. Enter the location of all tubes in the Freezer Works database.

2.) Red/gray SST tube:

a. Centrifuge sample for 15 minutes at room temperature. Transfer with a pipet 2 ml aliquots of serum into 2 – 4 cryovials with preprinted, bar-coded WrapAround labels. Discard any volume of serum more than 7.2 ml (enough to fill 4 cryovials).
b. Store the serum samples at –80C.
c. Record the location of all tubes in the FreezerWorks database.

3.) File the completed Specimen Processing Sheet in 3 ring binder that is properly labeled in the laboratory.

Specimen Processing Sheet

[redacted] Lab

Patient ID Number _____
Date of Processing _____
Visit No. _____
Number of blood tubes received:
Purple _____
SST/Tiger _____
Green _____
Aliquots of plasma from purple top tube:
Number of cryovials _____
Barcode Freezer location
Aliquots of buffy coat from purple top tube:
Number of cryovials _____
Barcode Freezer location
Genomic DNA extracted from 500 microliters whole blood from purple top tube
Yield of DNA _____
Barcodes Freezer location
Aliquots of serum from red/gray top tubes:
Number of cryovials _____
Barcode Freezer location

Blood Collection Information

The table shows the quantity of each sample type along with volume and other processing information. All aliquots will be processed and stored in 1.8 ml Nunc tubes.

Sample Type	BD Collection Tube or Collection Container	Final Tube Type	Final Number of Aliquots	Volume (ML)	Date drawn	Storage Temperature	Date Processed
DNA/Plasma	EDTA 10 cc (1)	EDTA				Sent directly to lab for processing	
Serum	SST 10cc (1)	1.8	5			-80	

H. High Performance Liquid Chromatography (HPLC) Assay for Allopurinol and Oxypurinol in Plasma

Allopurinol and oxypurinol in plasma (extraction protocol above) were analysed by HPLC with ultraviolet (UV) detection. Plasma (0.1 ml) was mixed with 0.1 ml of water, vortexed briefly and 0.1 ml of 1 M perchloric acid added to precipitate the proteins. After centrifugation, 20 μl of supernatant was injected into the HPLC system. Chromatographic separation was performed on a C8 column (Agilent Zorbax Eclipse XDB-C8 5 μm, 150 x 4.6 mm i.d.). The mobile phase was 0.01 M sodium phosphate buffer pH 3.5 containing 0.8% acetonitrile at a flow rate of 1.0 ml/min. Eluting peaks were detected at 254 nm. Under these conditions, allopurinol and oxypurinol eluted at 3.9 and 4.8 min respectively. The chromatogram was free of interference from endogenous substances present in blank plasma. Allopurinol and oxypurinol standard curves were linear (r^2>0.99) up to 294 μmol/L. Absolute recoveries from plasma were >90% for both allopurinol (at 1.2, 37 and 294 μmol/L) and oxypurinol (at 1.0, 33 and 263 μmol/L). Intra- and inter-day coefficients of variation at the above concentrations were <9%, and the limit of quantification was 0.2 μmol/L, for both allopurinol and oxypurinol. All the plasma samples were analysed in duplicate and the mean values used in subsequent analyses.