Study Title: A Mechanistic Study with Non-Dialysis Chronic Kidney Disease to Investigate Altered Platelet Response to Antiplatelet Therapy

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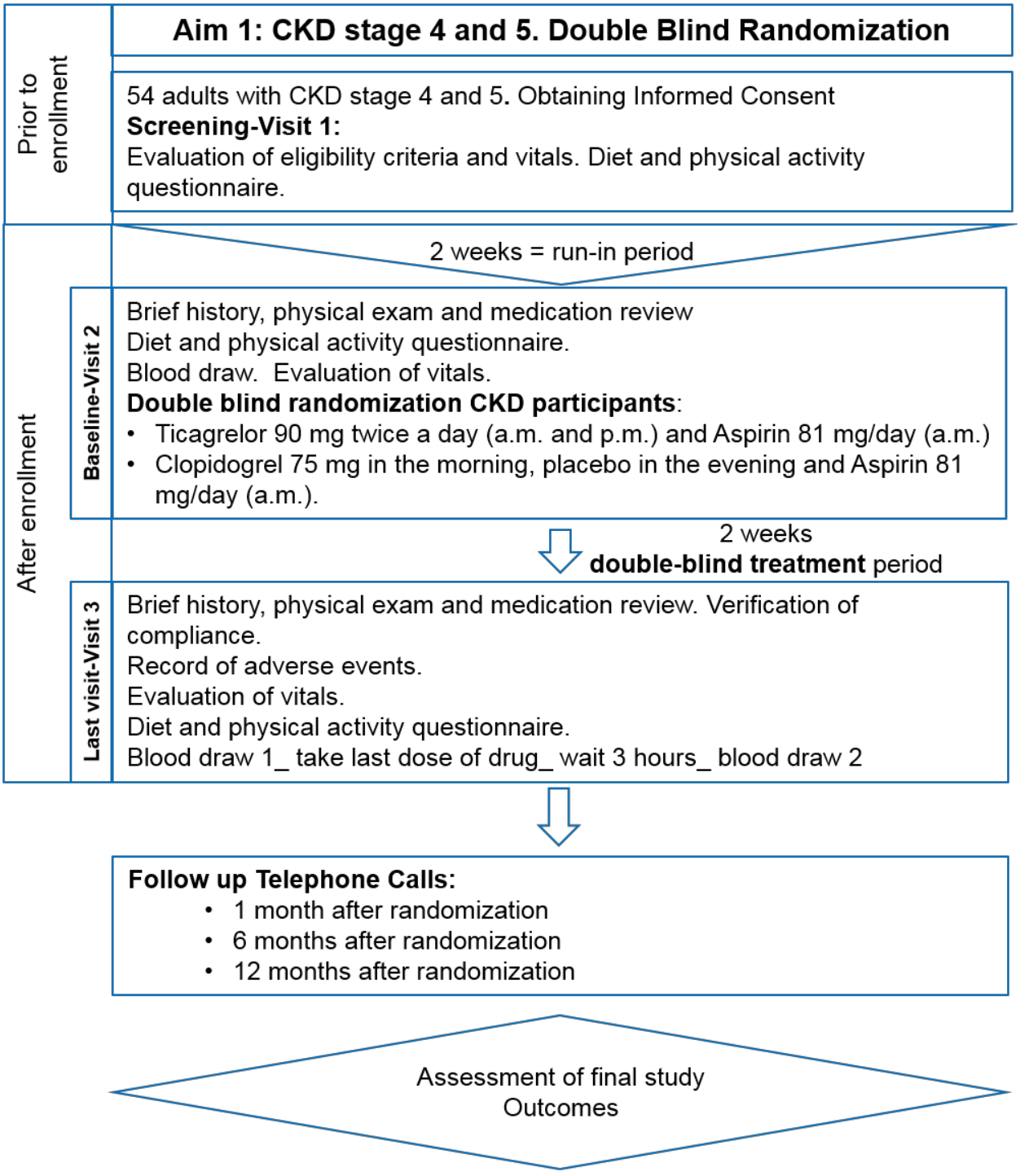
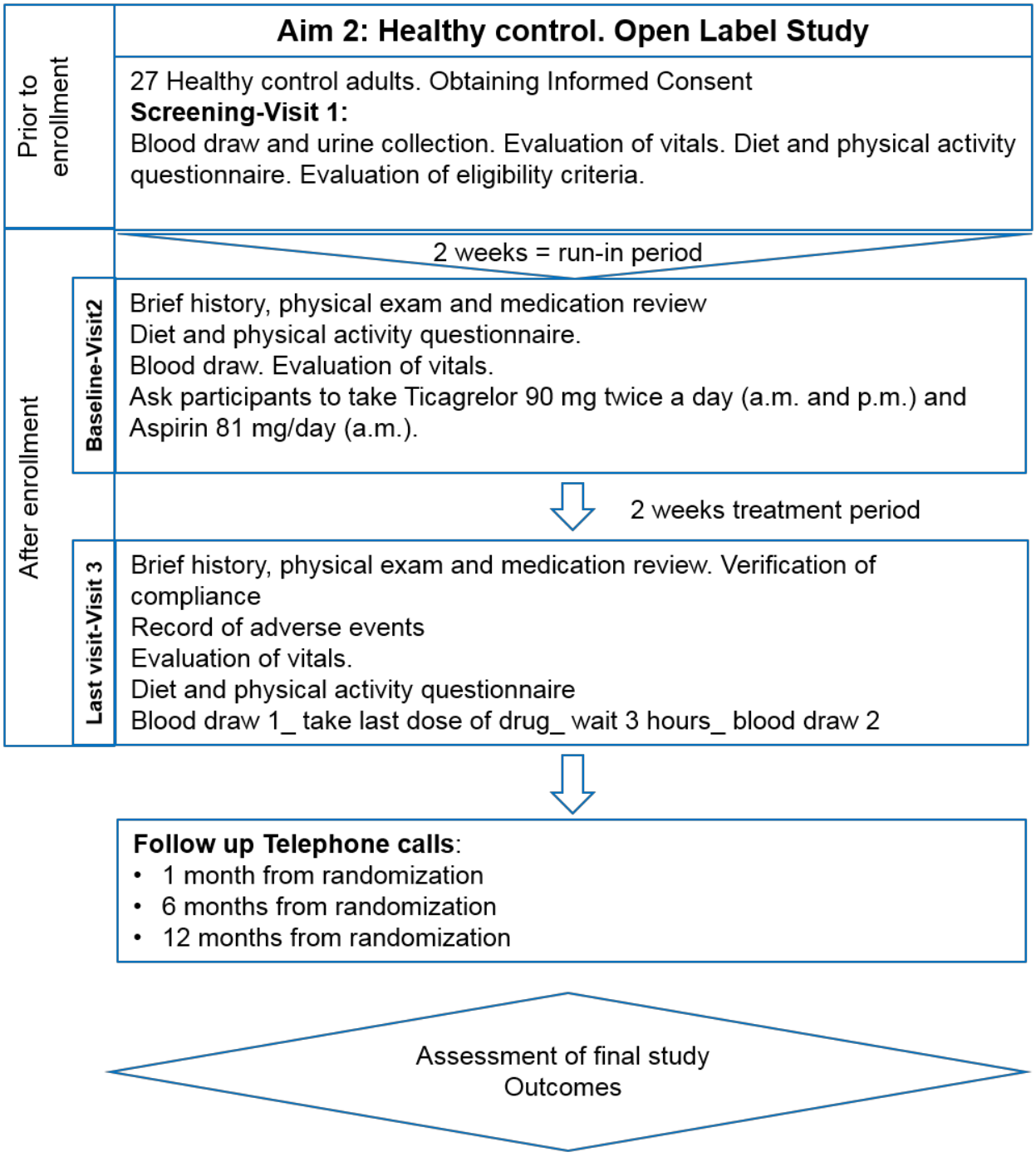
ABBREVIATIONS

ACS - Acute coronary syndrome
ADP - Adenosine-5 diphosphate
AE - Adverse Event
ANOVA - Analysis of Variance
ANCOVA - Analysis of Covariance
APA - Anti platelet agent
BMI - Body Mass Index
CV - Cardiovascular
CKD - Chronic Kidney Disease
CKD-ND - Non-dialysis dependent CKD
CKD-EPI - Chronic Kidney Disease Epidemiology Collaboration
GFR - Glomerular filtration rate
IL - Interleukin
IPA - Inhibition of platelet aggregation
IRB - Institutional Review Board
NKF - National Kidney Foundation
NSAIDs - Non-steroidal anti-inflammatory drugs
PCI - Percutaneous coronary interventions
PPIs - Protein pump inhibitors
PE - Physical examination
PI - Principal Investigator
RCT - Randomized Clinical Trial
RPA - Residual platelet aggregability
SNPs - Single Nucleotide Polymorphisms
SD - Standard Deviation
TNF - Tumor necrosis factor
TRI - Translational Research Institute
UAMS - University of Arkansas for Medical Sciences
UPIRTSO - Unanticipated Problems Involving Risk to Subjects or Others
WBPA - Whole blood platelet aggregation

1.0 STUDY SCHEMA



Study Flow Aim 1: describes the different steps of the study in chronological order for Aim1



Study Flow Aim 2: describes the different steps of the study in chronological order for Aim 2

2.0 STUDY SUMMARY

Title	A Mechanistic Study with Non-Dialysis Chronic Kidney Disease to Investigate Altered Platelet Response to Antiplatelet Therapy
Short Title	CKD Platelet Study
Protocol Number	227997
Phase	Phase III
Methodology	The study will be conducted in two phases. During the first phase of the study, CKD patients will be enrolled and will be randomized in two treatment groups (ticagrelor or clopidogrel) in a double blind manner. Healthy controls will be enrolled in the second phase of the study; they will receive open label study drugs. We want to recruit 20 positive controls from the UAMS hospital.
Study Duration	2 years and 3 months for subjects' recruitment. 2 years for data analysis. Total of 4 years and 3 months
Study Center(s)	CKD patients will be enrolled at the University of Arkansas for Medical Sciences (UAMS) renal clinic. Healthy controls will be recruited using the data from the UAMS Translational Research Institute (TRI) Research Participant Registry. We are also planning to recruit CKD and controls from Little Rock VA as a second site. Positive controls will be recruited from the UAMS hospital.
Objectives	**Aim 1**: Determine the mean changes from baseline in ADP-induced whole-blood platelet aggregation (WBPA) values and markers of platelet activation and inflammation in CKD patients on aspirin who are randomly assigned to ticagrelor or clopidogrel in double-blind fashion. Double blind randomization. **Aim 2**: Determine the mean changes from baseline in ADP-induced WBPA values and markers of platelet activation and inflammation after treatment with ticagrelor plus aspirin in chronic kidney disease (CKD) patients compared with non-CKD controls. Open label treatment. Aim 3: Recruit 20 active controls that are in the hospital who have had a heart attack or stroke. The data from the active controls group will be used to exhibit relevance of findings that represent similarities with already known abnormalities in positive controls and absence of them in negative controls.
Number of Subjects	Accrual goal is 81 participants (54 CKD patients and 27 healthy controls) and 20 positive controls
Diagnosis and Main Inclusion Criteria	*CKD 4 and 5 subjects*: Presence of CKD with an estimated GFR of <30 mL/min/1.73 m^2 for a period of ≥3 months. Not on dialysis Healthy Controls: These subjects will have an estimated GFR ≥60 mL/min/1.73 m^2. Positive controls will be patients with acute heart attack or acute stroke admitted to UAMS. They will not go through the intervention. They will only provide blood sample once.

Study Product(s), Dose, Route, Regimen	CKD participants will be double-blind randomized in these two arms: Arm 1 - **Ticagrelor,** 90 mg twice daily (one pill in the morning and one pill in the evening) + **Aspirin** 81 mg/day. Ticagrelor is the test treatment. Arm 2 - **Clopidogrel**, 75 mg/day in the morning and a **matching placebo** in the evening + **Aspirin** 81 mg/day. Clopidogrel is the reference treatment. Participants (Arm1/ Arm2) are required to take the **oral** treatment for a total of **two weeks** plus one half day. Healthy control participants will be asked to take Ticagrelor, 90 mg twice daily (one pill in the morning and one pill in the evening) and aspirin 81 mg/day. Open label treatment. Participants are required to take the oral treatment for a total of two weeks plus one half day. Positive controls will not be part of the study intervention.
Duration of administration	Participants are required to take the daily oral treatment for a total of two weeks plus one half day (Exclude positive controls).
Statistical Methodology	**Aim 1.** We will use summary statistics to describe the distribution of the data. Post-treatment ADP-induced WBPA value in ohms (Ω) will be the primary outcome variable. We will use an analysis of covariance (ANCOVA) model to compare the treatment effects of ticagrelor vs. clopidogrel in CKD patients because this approach has higher statistical power than other methods to analyze drug effects. The primary outcome variable will be modeled as the dependent variable. The secondary outcome variables, including percentage of inhibition in platelet aggregation (IPA), residual platelet aggregability (RPA), adverse events at 2-weeks, 1, 6 and 12 months will be compared using Chi-square test. For the exploratory analyses, we will 1) compare proportions of metabolic phenotypes of CYP polymorphisms between groups with Fisher's exact test, 2) correlate WBPA values with cytokines, and drug/metabolite levels, and 3) measure two-way interactions between the exploratory markers and the study drug on the primary outcome variable. **Aim 2**. Summary statistics will be used to describe data distribution. Two-sample *t*-test or Mann-Whitney test will be used to compare markers between groups. *For the primary outcome*, a mixed effects linear model will be used to compare **mean change in WBPA to therapy** between groups. The model will have group factor, treatment factor (repeated, pre vs. post), and interaction between group and treatment. The pair effect and subject effect will be random factors to control for the clustering within matched pairs and within each subject. Other confounders except sex and DM will be adjusted in the analyses. All available data will be included in the analyses; the mixed model can accommodate cases with missing data. *RPA and adverse events* will be compared as Aim 1 as part of the secondary outcomes analyses.

3.0 BACKGROUND AND RATIONALE

3.1 Disease Background

Patients with chronic kidney disease (CKD) are at four-times higher risk of experiencing thrombotic cardiovascular (CV) events such as acute coronary syndrome (ACS) than the general population, and this risk increases with worsening kidney disease.[1-4] As a result, one in five of the millions of CKD patients in the U.S. is prescribed aspirin and an oral P2Y12 inhibitor (e.g., clopidogrel and ticagrelor) to reduce future events.[5,6] Despite treatment with clopidogrel, the most commonly used oral P2Y12 inhibitor, patients with stages 1–3 and stages 4–5 CKD are at, respectively, two- and four-times higher risk of thrombotic CV events or death than individuals without CKD.[7,8] Residual platelet aggregability (RPA) while on clopidogrel treatment, as determined by mean changes in adenosine diphosphate (ADP) induced whole blood platelet aggregation (WBPA) from baseline, is a novel modifiable risk factor associated with increased risk for thrombotic CV events or death.[9,10] Clopidogrel is a prodrug that requires metabolism by cytochrome P450 (CYP) enzyme to become active.[9,10] We reported that a higher proportion of patients with stages 4–5 non–dialysis dependent CKD (CKD-ND) demonstrated residual platelet aggregability on-clopidogrel treatment than patients without CKD, (56% vs. 8.3%, p=0.01), independent of metabolic phenotypes of CYP polymorphisms.[11] Our work generated the hypothesis that a baseline defect in WBPA may result from advanced kidney disease and may not be completely inhibited with a P2Y12 inhibitor. Ticagrelor, a newer oral P2Y12 inhibitor, is an active drug.[12] Recent clinical trials in the general population translated this favorable pharmacokinetic difference into greater clinical benefit from ticagrelor than clopidogrel by reporting a 15% reduced risk of future thrombotic CV events.[12] However, use of ticagrelor over clopidogrel in CKD-ND patients raises efficacy concerns despite its more favorable pharmacokinetic profile because CKD can alter drug metabolism and its interaction with the target receptor.[13-15] Previous studies that explored antiplatelet effects of ticagrelor in CKD were limited to dialysis patients and suffered from lack of randomization or blinding.[16-18] Surprisingly, scarce data exist to establish the antiplatelet efficacy of ticagrelor and its potential benefit when compared with clopidogrel in CKD-ND patients, precisely those at highest risk for thrombotic CV events.[19] Clinical practice guidelines remain ambiguous about identifying the preferred oral anti platelet agents (APAs) for CKD patients but endorse ticagrelor use in “high risk” ACS patients.[20,21] As a result, ticagrelor use is growing exponentially. It is therefore imperative to establish if ticagrelor is able to 1) demonstrate greater antiplatelet effects than clopidogrel in CKD-ND patients and 2) inhibit platelet function in CKD-ND as effectively as in non-CKD individuals; it is also important to 3) explore mechanisms to explain the poor response to APAs in CKD patients. We hypothesize that CKD-ND patients treated with ticagrelor will experience 1) greater mean changes in platelet function (i.e., greater platelet inhibition) than when treated with clopidogrel, but 2) lesser mean changes in platelet function (lesser platelet inhibition) than non-CKD controls treated with ticagrelor.

Advanced kidney disease is a special clinical condition with multiple defects in the hemostatic pathway that raise serious concerns about the efficacy of ticagrelor in CKD-ND patients.[14,22] First, CKD may cause alterations in platelet surface receptors and impair their ability to undergo conformational changes upon activation.[15] Because ticagrelor binds reversibly to a site on the P2Y12 receptor remote from the ADP-binding site and subsequently blocks ADP binding to the receptor via allosteric modulation, binding of the ligand (ticagrelor) to its receptor (P2Y12) and the resultant allosteric modulation of the receptor may be altered in the milieu of kidney disease. Second, ADP-induced platelet aggregation may be higher in CKD and may not be completely inhibited by a P2Y12 inhibitor that acts on this pathway.[11] Finally, CKD may alter CYP enzyme function and result in lower levels of a drug or its active metabolite in blood.[13] For these reasons, the differences between ticagrelor and clopidogrel may not translate into clinical benefits in

CKD-ND patients comparable to those reported in the general population. The current proposal will fill this knowledge gap by comparing the antiplatelet effects of ticagrelor and clopidogrel in a double-blind, parallel-design randomized controlled trial (RCT) involving CKD-ND patients (Aim 1). We will also investigate if presence of kidney disease alters antiplatelet responses to ticagrelor in CKD-ND patients vs. non-CKD controls (Aim 2 and sub-aims).

3.2 Product Background

Clopidogrel is a FDA approved drug. Clopidogrel is a prodrug, requiring metabolism by cytochrome P450 (especially CYP2C19) to become active (5-thiol metabolite) before irreversibly blocking ADP binding to the platelet P2Y12 receptor.[23] The requirement for multiple steps for a drug to become active may be disadvantageous in CKD because 1) genetic polymorphisms in CYP2C19 result in high inter- and intra-individual variability in antiplatelet action and 2) CKD may alter the CYP450 system and affect conversion of a prodrug to its active metabolite.[23,24] Observational studies reported that CKD vs. non-CKD patients had residual platelet aggregability, as defined by higher post-treatment platelet aggregability, when treated with aspirin plus clopidogrel.[11,25-28] Our preliminary studies extended these findings and demonstrated in a prospective trial that a higher proportion of non–dialysis dependent stages 4–5 CKD (CKD-ND) than non-CKD patients had residual platelet aggregability, when treated with aspirin plus clopidogrel (56% vs. 8.3%, p=0.01), independent of CYP2C19 polymorphisms. Our work generated the hypothesis that there may be a baseline defect in the ADP pathway in advanced kidney disease to potentially explain the poor efficacy of P2Y12 inhibitors in CKD-ND patients.

Ticagrelor is a FDA approved drug. Ticagrelor has the most desirable pharmacological properties among the oral P2Y12 inhibitors. Ticagrelor and its metabolite AR-C124910XX (primarily using the CYP3A4) are active.[29,30] The drug reversibly blocks ADP binding to the P2Y12 receptor.[31] In the general population, this pharmacokinetic difference translates into greater antiplatelet effects for ticagrelor than clopidogrel, with reduced inter- and intra-individual variability.[32,33] A recent RCT reported higher efficacy for ticagrelor than clopidogrel, each taken together with aspirin, in reducing CV events and mortality.[34] However, these trials excluded CKD-ND patients, precisely those at the highest risk of thrombotic CV events. In the subgroup with early-stage CKD (defined as creatinine clearance <60 mL/min), use of ticagrelor was associated with a 23% reduction in mortality and future CV events.[12] This subgroup analysis suggests that ticagrelor may be superior to clopidogrel in CKD patients. Recent studies exploring the antiplatelet effects of ticagrelor in CKD were limited by lack of controls, high dropout rates, or failure to enroll patients with advanced CKD-ND (estimated GFR <30 mL/min/1.73m2). There are no controlled studies to compare ticagrelor and clopidogrel in CKD-ND patients.

Clinical practice guidelines emphasize that providers should be aware of treatment strategies to choose newer APAs other than clopidogrel when appropriate. However, the guidelines remain ambiguous about the preferred APA in CKD-ND patients. Furthermore, guidelines endorse the use of the newer P2Y12 inhibitors for percutaneous coronary interventions (PCIs) that are “high-risk” procedures. Because PCIs in CKD-ND patients are considered high risk, there has been an exponential rise in ticagrelor prescriptions since its approval by FDA in 2011. Therefore, the proposed studies are critical to advancing this understudied field. A well-designed mechanistic study will be the first step to investigate antiplatelet efficacy of ticagrelor in these high-risk patients.

Product safety data in healthy volunteers and CKD patients. In a randomized, open-label, cross-over, trial conducted by Teng *et al*. among healthy volunteers comparing pharmacokinetic and pharmacodynamics effects of ticagrelor to clopidogrel. Ticagrelor and

clopidogrel with aspirin was well tolerated overall by healthy volunteers. There were no serious adverse events or death reported during the study (total duration 34 days, two 10-day intervention periods interspersed by a washout period of 14 days). There were no changes in complete blood count, vital signs or other lab parameters in healthy volunteers except increased bleeding time. In addition, following adverse events were reported:

Study drug	n	Adverse events	Outcome
Ticagrelor	3	Moderate epistaxis, hematoma, petechaie, gingivitis or urticaria	Discontinued study drug
Ticagrelor	4	Headache, petechaie	Continued study drug
Clopidogrel	2	Nausea and flatulence	Continued study drug

Similar tolerability and no serious adverse events were noted on ticagrelor in healthy volunteers by Husted *et al*. (**Br J Clin Pharmacol** 70: 65–77, 2010). The adverse events reported in this study by healthy volunteers were as follows:

Study drug	n	Adverse events	Outcome
Ticagrelor	4	Myalgias or gingival bleeding	Continued study drug
Clopidogrel	2	Gingival bleeding	Continued study drug

Most of the side effects reported in the two studies may be due to:

1) ***Higher doses of ticagrelor*** (200 mg twice daily or 300 mg twice daily);
2) ***Prolonged period of therapy*** (>14 days);
3) ***Cross-over design with repeated drug exposure*** (treat with ticagrelor for 10 days, washout period of 14 days and then treat again for 10 days).

Other large RCTs have reported rare side effects for the products as follows:

- **Aspirin** may cause some, all or none of the side-effects listed below:
  Common side effects (<10%) of aspirin are ulcers and bleeding of the stomach and intestines, which could happen if you are taking aspirin for long time. This study requires only 2 weeks' treatment with aspirin which limits your risk.
  Rare side effects (<1%) are ringing in the ears, breathing problems, swelling and allergic problems. Reye's syndrome (fever, rash, vomiting, headaches and lethargy) is also reported, which however, occurs more commonly in children and those with a known allergy to aspirin.

- **Clopidogrel** may cause some, all or none of the side-effects listed below:
  Common side effects (<10%) of clopidogrel are bleeding that occurs in 3.5-6.1% of the patients. Less common side effects are bleeding from stomach in approximately 2% of the patients and 2.7% of the patients when combined with aspirin. Any bleeding requiring hospitalization and blood transfusion occurs in 0.8% of patients on clopidogrel.
  Rare side effects of clopidogrel (<1%) are low blood counts (low white cell count, low red cell count, low platelets); damage to liver (hepatitis, liver failure), bleeding in brain; bleeding in the eyes (0.05%); breathing problems such as pneumonia.

- **Aspirin and clopidogrel** have been used together safely in some patients in previous studies to reduce risk of heart attack. The risk of bleeding does increase minimally as compared to using either drug alone. However, the risk low do to the short duration of the treatment (only 2 weeks).

- **Ticagrelor** may cause some, all or none of the side effects listed below:
  Common side effects of ticagrelor are bleeding from any site that can occur in up to 4% of patients. Other reported side effects seen >10% of the time is dyspnea (14%). Approximately 1–10% of patients may develop ECG abnormality, dizziness, nausea, and increases in serum creatinine or serum uric acid levels.
  Rare side effects of ticagrelor (<1%) are angioedema, atrioventricular block, bradycardia, gout, hypersensitivity, and skin rash. As ticagrelor has an FDA-required boxed warning regarding risk factors for bleeding, we are excluding all those patients meeting exclusion criteria. In addition, ticagrelor and clopidogrel are metabolized by cytochrome P450 liver enzymes, especially CYP2C19 and CYP3A4. Therefore, we are excluding any patients with liver disease to minimize risks of bleeding. Neither clopidogrel nor ticagrelor requires renal dosing for low GFR.

Potential major drug-drug interactions of aspirin, clopidogrel or ticagrelor with the following drugs:

- Antithrombotic drugs, like Ranolazine, Aggrenox and Cilostazol. Taking these medicines with the study drugs could potentially increase risk of bleeding.
- Non-steroidal anti-inflammatory drugs (NSAIDs). These medicines can reduce the function of platelets and cause bleeding complications. Subjects will be asked to do not take any NSAIDs during the study.
- Medicines for acid reflux called Proton pump inhibitors (PPIs), fish oil, Vitamin E and herbal supplements.

Anticipated safety of controls/CKD in our study: Given above reasons, our protocol will use safest possible strategy - lower dose for 14 days without cross over. We anticipate lower number of adverse events in the healthy volunteers and CKD.

3.3 Study Rationale

Of the nearly 13 million patients with chronic kidney disease (CKD) in the U.S., nearly one-third experience thrombotic cardiovascular (CV) events, such as acute coronary syndrome (ACS).[4,35-38] Risk of such events rises with advancing kidney failure. Furthermore, the CKD population is the fastest growing among chronic diseases, and the number of CKD patients undergoing percutaneous coronary interventions (PCIs) has increased by 50% in the last decade.[4,38] Patients experiencing ACS and/or undergoing PCIs are subsequently prescribed the combination of aspirin, 81 mg/day, and an oral P2Y12 inhibitor (e.g., clopidogrel, ticagrelor) to reduce future CV events. Currently, at least one in five CKD patients is prescribed these drugs. Yet CKD patients continue to have thrombotic CV events, such as coronary in-stent thrombosis, despite treatment.

Scientific Premise. Use of clopidogrel plus aspirin became standard of care for patients with ACS after the publication of two large randomized, controlled trials (RCTs), which excluded patients with advanced CKD and enrolled very few with early-stage CKD. Despite treatment with clopidogrel, patients with stages 1–3 and 4–5 CKD are at, respectively, two- and four-times higher risk of thrombotic CV events than those without CKD. [7,8,10,39] The landmark trials of oral P2Y12 inhibitors systematically excluded CKD-ND patients, precisely those who are at a higher risk for thrombotic CV events. Our previous work showed that the baseline ADP pathway of platelet aggregation in CKD-ND patients may be abnormal. Further, the ADP pathway may not be completely inhibited by clopidogrel, possibly due to altered platelet aggregation in advanced CKD. These findings raise questions regarding the antiplatelet efficacy of ticagrelor in CKD-ND patients, despite its more favorable pharmacokinetic profile than that of clopidogrel, because it targets the

same pathway that may be abnormal in advanced CKD. The current proposal addresses these knowledge gaps by comparing the antiplatelet effects of ticagrelor and clopidogrel, the most commonly used oral P2Y12 inhibitor, in a double blind, parallel-design RCT involving CKD-ND patients (Aim 1). In addition, we will investigate if kidney disease alters the antiplatelet response to ticagrelor in CKD-ND patients vs. controls without CKD (Aim 2). We will also measure the metabolic phenotypes of CYP2C19/CYP3A4 and drug and active metabolite levels, using mass spectrometry, to explore mechanisms responsible for the poor efficacy of APAs in CKD-ND patients (exploratory aim).

Impact of the proposed study: In summary, the results of this study will be informative and move the field forward regardless of whether the hypothesis is proven to be true. Completion of the proposed trials will advance the understudied field of platelet dysfunction in CKD while elucidating novel mechanisms underlying the poor efficacy of APAs in CKD patients. Most important, it will provide urgently needed data to power future, larger trials to determine if newer P2Y12 inhibitors are efficacious and will improve outcomes in CKD-ND patients, a group at disproportionately higher risk than the general population for CV events, where traditional risk factor modification interventions have failed to do so.

4.0 STUDY OBJECTIVES

4.1 Aim 1: Determine the mean changes from baseline in ADP-induced whole-blood platelet aggregation (WBPA) values and markers of platelet activation and inflammation in CKD-ND patients on aspirin who are randomly assigned to ticagrelor or clopidogrel in double-blind fashion.

4.2 Aim 2: Determine the mean changes from baseline in ADP-induced WBPA values and markers of platelet activation and inflammation after treatment with ticagrelor plus aspirin in CKD-ND patients compared with non-CKD controls.

4.3 Primary Objectives:

Aim 1: To determine mean changes from baseline in ADP-induced whole blood platelet aggregation (WBPA) values and markers of platelet activation in non-dialysis CKD patients on aspirin who are randomly assigned to ticagrelor or clopidogrel in a double-blind fashion. We will randomly assign 54 CKD patients (GFR <30 mL/min/1.73m^2), stratified by diabetes mellitus presence and treated with aspirin, 81 mg/day, to receive either ticagrelor, 90 mg twice daily, or clopidogrel, 75 mg/day, and placebo, for 2 weeks. WBPA values and P-selectin expression on platelet surface will be measured before and after treatment and compared between groups. The *post-treatment WBPA* will be the primary outcome variable.

Aim 2: To determine mean changes from baseline in WBPA values and markers of platelet activation and inflammation after treatment with ticagrelor plus aspirin in non-dialysis CKD patients compared with non-CKD controls. We will recruit 27 non-CKD controls (GFR ≥60 mL/min/1.73 m^2 and no other evidence of kidney damage). The *post-treatment WBPA* will be the primary outcome variable.

4.4 Secondary Objectives:

Aim 1: The secondary outcomes will include the proportion with IPA, RPA and adverse events (i.e. bleeding, CV events, hospitalizations, and deaths etc.) at 2 weeks, 30 days, 6 months and 12 months between groups.

Sub-aim 1: To investigate mechanisms for WBPA differences between treatment groups, we will measure a) the metabolic phenotypes of CYP to analyze interaction effects with treatment on changes in WBPA; and b) the drug and metabolite levels and the levels of circulating inflammatory cytokines to correlate them with changes in WBPA.

Aim 2: The secondary outcomes will include the proportion with IPA, RPA and adverse events (i.e., bleeding, CV events, hospitalizations, deaths etc.) at 2 weeks, 30 days, 6 months and 12 months between groups.

Sub-aim 2: To investigate mechanisms for WBPA differences between groups, we will measure a) the metabolic phenotypes of CYP to analyze interaction effects with treatment on changes in WBPA; b) the drug and metabolite levels and the levels of circulating inflammatory cytokines to correlate them with changes in WBPA; and c) uremic toxins.

Aim3: Recruit 20 active controls that are in the hospital who have had a heart attack or stroke. To compare baseline platelet flow cytometry data from CKD with non-CKD controls and positive controls.

5.0 STUDY POPULATION

Subjects must meet all of the inclusion and exclusion criteria to be enrolled in the study. Study treatment may not begin until a subject is enrolled.

5.1 Inclusion Criteria for CKD subjects

- Males and females, age-18 years and older
- Non-dialysis CKD patients: Presence of CKD with an estimated GFR of <30 mL/min/1.73 m2 for a period of ≥3 months, as defined by the National Kidney Foundation (NKF) and determined with the CKD-EPI creatinine-based formula
- Ability to understand and sign informed consent after the nature of the study has been fully explained

Inclusion Criteria for Healthy Controls

- Males and females, age-18 years and older
- These subjects will have an estimated GFR ≥60 mL/min/1.73 m^2 as determined by the CKD-EPI creatinine-based formula and a urine albumin-to-creatinine ratio <30 mg/g as defined by the National Kidney Foundation
- Ability to understand and sign informed consent after the nature of the study has been fully explained.

Inclusion criteria for positive controls for AMI (Sarma et al. Circulation. 2002;105:2166-2171)

- Patients admitted with ischemic symptoms (for e.g., chest pain) with EKG changes and positive troponin within 1 day

Inclusion criteria for positive controls for acute ischemic stroke (Schmalbach et al. Cerebrovasc Dis 2015;39:176–180)

- Patients admitted with acute ischemic stroke to blood draw within 1 day

5.2 Exclusion Criteria for CKD patients and Healthy Control Subjects:

- Inability to sign consent and HIPAA form. Legal Authorized Representatives are not allowed to sign the consent form.

- Unwillingness or inability to participate in the protocol or comply with any of its components.
- Subjects unable or unwilling to stop taking:
  - Aspirin and other antithrombotic agents, like cilostazol, ranolazine, aggrenox, prasugrel, warfarin, xarelto, pradaxa, eliquis.
  - Glycoprotein IIb/IIIa antagonist (abciximab-ReoPro, eptifibatide-Integrilin, tirofiban-Aggrastal)
  - NSAIDs and PPIs
  - Fish oil, Vitamin E and herbal supplements
- Acute kidney injury superimposed on CKD
- Kidney transplant or any other solid organ transplant recipient
- End-stage kidney disease on maintenance dialysis (peritoneal or hemodialysis)
- Nephrotic syndrome defined as nephrotic range proteinuria, hypoalbuminemia, hyperlipidemia and generalized edema
- Recent hospitalization or surgery <3 months
- Acute coronary or cerebrovascular event in the last 12 months
- Blood dyscrasias, active bleeding, or bleeding diathesis
- Gastrointestinal bleeding in the last 6 months
- Recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist (Integrelin).
- Hematocrit <25%, white blood cell count >20,000/μL, or platelet count <50,000/μL
- Any active malignancy or liver disease.
- Pregnancy
- Positive urine pregnancy test in a woman of childbearing potential prior to study entry. A female of childbearing potential is any woman who meets the following criteria:
  - Has not undergone a hysterectomy or bilateral oophorectomy; or
  - Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).
- Patients must not be nursing due to the potential for congenital abnormalities and the potential of this regimen to harm nursing infants.

Exclusion criteria for positive controls of AMI (Sarma et al. Circulation. 2002;105:2166-2171)

- Exclude if CABG of PCI in the last 6 months
- Exclude if receiving any antiplatelet therapy other than aspirin

Exclusion criteria for positive controls of acute stroke (Schmalbach et al. Cerebrovasc Dis 2015;39:176–180)

- Exclude if intracranial hemorrhage
- Exclude if receiving any antiplatelet therapy other than aspirin

5.3 Accrual Goal

Roughly, one in 5 patients with CKD is already on antiplatelet agents; therefore, 4 out of every 5 patients will be potential candidates. To complete recruitment of 54 subjects in 13 months, it is necessary to randomize four subjects over each 4-week interval. If 75% of those who are enrolled and sign consent forms come for Visit 1, about 6 subjects need to be enrolled/consented every 4 weeks to qualify for randomization. Therefore, nine patients will need to be screened every 4 weeks to find six not taking an oral antiplatelet agent. If we assume a conservative 50% refusal rate, 18 patients will need to be screened every 4 weeks (4/week) for four to be randomized.

The recruitment goal will be 4–5 controls per month to be completed in 4-6 months. Potential healthy control subjects will be contacted using the TRI participant research registry (AR research). Less than 10% are expected to respond and come for screening visit and meet eligibility criteria for controls. We estimate that nearly 200 contacted potential subjects will be identified, of which nearly 10% (i.e., n=20) will be enrolled as controls in the study. We will also planning to recruit Veterans from the Little Rock VA as a second site.

Positive controls will be recruited from the UAMS hospital. We plan to recruit 20 consecutive patients who meet inclusion/exclusion criteria.

5.4 Recruitment Plan

Potential participants with CKD stages 4-5 will be enrolled in the study at the UAMS and Little Rock VA Renal Clinic targeting new and existing patients. We will speak with the possible subjects after their clinical visit with the kidney doctor and will go over the consent process and if possible, we will perform the screening visit. A flyer with a brief description of the study will be posted in the waiting area and visit room of the UAMS Renal clinic. Flyer has been included in the application.
Healthy control subjects will be recruited using the data from the UAMS TRI Research Participant Registry. People recruited through the AR Research website, can choose to be contacted by the researchers by e-mail or letter or telephone call. We will contact the possible participants in relation to their preference (e-mail, call or letter). The letter, e-mail and call scripts have been included in the application. We will include the consent form and HIPAA authorization with the recruitment e-mail or mail, in this way the possible participants have time to go over a more detailed description of the research study and make a better-informed decision during the consent process. Veterans will also be approached through Little Rock VA and the details of recruitment activities at Little Rock VA are written in VA protocol.
Study will be advertised on the Translational Research Institute Facebook's page and on UAMS social media accounts to increase enrollment.
The message and graphic that will be posted in social media is included in the submission.

We will recruit positive controls as quickly as possible depending on the admissions on a given weekday.

5.5 Participant Compensation

Visit 1 (Screening visit)

We will not conduct in-person screening visit for controls due to COVID-19 pandemic. The CKD subjects will be performed either after their clinical appointment with the renal doctors or on telephone to minimize contact. Both healthy control and CKD subjects will not receive a compensation for the screening visit.

Visit 2 (Baseline visit). For all participants:

At the end of this visit, the subjects will receive a Walmart gift card for a value of $25. We will also validate the UAMS parking ticket.

Visit 3 (Last visit). For all participants:

At the end of this visit, the participant will receive a Walmart gift card for a value of $100. Moreover, we will validate the UAMS parking ticket.

The participants that withdraw or are removed from the study will not receive gift cards for visits that they do not attend. We will give the Walmart gift card at the end of each visit and

if the subject does not come to the scheduled visit, we will not give the subject compensation for that specific visit. All information concerning payment (amount, schedule and type of payment) will be discussed in the informed consent document. Receipts of the payment will be stored with the consent process note.

Positive controls will have only one visit for blood draw. They will be given $50 Walmart gift card after completing blood draw.

6.0 INVESTIGATIONAL PRODUCT

6.1 Study Drugs

Clopidogrel is a FDA approved drug. Clopidogrel is a prodrug, requiring metabolism by cytochrome P450 (especially CYP2C19) to become active (5-thiol metabolite) before irreversibly blocking ADP binding to the platelet P2Y12 receptor.

Ticagrelor is a FDA approved drug. Ticagrelor has the most desirable pharmacological properties among the oral P2Y12 inhibitors. Ticagrelor and its metabolite AR-C124910XX (primarily using the CYP3A4) are active. The drug reversibly blocks ADP binding to the P2Y12 receptor. In the general population, this pharmacokinetic difference translates into greater antiplatelet effects for ticagrelor than clopidogrel, with reduced inter- and intra-individual variability.

6.1.1 Dispensing

For CKD subjects: Ticagrelor/clopidogrel/placebo will be dispensed by the UAMS investigational pharmacy in a double-blind manner using blister pack with morning (AM) and evening (PM) dosing labels. Each subject will receive open label aspirin in the same blister pack labeled AM. The blister pack will be labeled appropriately with subject ID, IRB number for the study protocol. UAMS research pharmacy will put the blister package in a specific second container reporting the administration instructions for the participant. The study drugs will be dispensed for 14 days plus one extra day, for a total of 15 days.

The research staff will pick up the study drug from the research pharmacy. Dr Jain or other research staff member with clinical background (doctors or nurses) will give the study drugs to the participant. At the end of the treatment, left over pills will be returned to the research pharmacy for destruction.

For Control subjects: Ticagrelor and aspirin will be dispensed by the UAMS investigational pharmacy in an open label fashion. The blister pack will be labeled appropriately with subject ID, IRB number for the study protocol and the instructions for the subjects. The study drugs will be dispensed for 14 days plus one extra day for a total of 15 days. The research personnel will pick up the study drugs from the research pharmacy and will return the left over pills after last visit to the research pharmacy for destruction.

Positive controls will not be part of the intervention study.

6.1.2 Treatment Compliance

Treatment compliance will be determined by asking the participants to bring the blister pack of the study drugs to the final visit. We will perform pill count to ensure adherence. Based on the remaining pills, we will calculate proportion of days the study drug was not taken by a subject and record it as non-adherence in percentage. Moreover, during the mid-treatment phone call we will ask the

participants the pill count consumed. Pill non-adherence is defined as the ingestion of <80% or >110% of study drug recorded during Visit 3. Reasons for noncompliance will be sought and recorded, and subjects who are noncompliant with pills or visits will be counseled and reeducated about the importance of compliance and will also receive additional telephone calls to remind them about compliance between visit 2 and visit 3.

6.2 Treatment Dosage and Administration

Intervention for CKD Participants. Randomization, Blinding and Intervention Aim 1.
Stratified randomization will be used to ensure the balance between two treatments in the strata defined by diabetic status. All eligible CKD patients who provide written informed consent will be classified into a particular stratum (diabetics or non-diabetics) and then randomized in double-blind fashion to receive either 2 weeks of ticagrelor (90 mg twice daily) or clopidogrel (75 mg/day in the morning, plus a matching placebo once daily at night) in a 1:1 ratio. To minimize imbalance in treatment allocation and to maximize power, a computerized random number generator will be used to create a blocked randomization list separately for diabetic and non-diabetic strata. Block size, variable for each stratum, will be determined by the statistician and revealed to the research pharmacist but not to the research personnel.

Matching placebo will be compacted by the research pharmacist to conceal frequency of dosing for clopidogrel. All study pills will be placed in larger identical capsules in blister pack (labeled AM and PM) to conceal allocation and frequency of dosing.

The research pharmacist will randomize all eligible CKD participants in a double-blind fashion way in two arms:

- Ticagrelor 90 mg twice daily + Aspirin 81 mg once daily
- Clopidogrel (75 mg once daily) + placebo once daily + Aspirin 81 mg once daily

Intervention for Healthy Control Participants. Open Label Intervention - Aim 2
Overall, healthy controls will be recruited to match 1:1 for age and sex with subjects in the Ticagrelor arm. No randomization will be performed for the healthy controls subjects. We want to improve the pace of enrollment in the study as it is a two-year grant and the funds expire in June 2021 after getting NCE. With this in mind, we want to start enrolling controls concurrent to CKD patients. We will keep track of average age of recruited CKD subjects and proportion of males enrolled in the CKD arm. This will allow us to recruit some controls with similar average age and sex. We feel confident that this strategy will be able to match subjects with CKD to controls without CKD for age and sex in the end when the trial concludes.
UAMS research pharmacist will provide ticagrelor and aspirin for the healthy control participants. We will enroll healthy controls in this open-label study together with the CKD's recruitment for Aim 1.

Healthy control participants will be asked to take ticagrelor, 90 mg twice daily (one pill in the morning and one pill in the evening) and aspirin 81 mg/day. Participants are required to take the oral treatment for a total of two weeks plus one half day.

All subjects will be asked to take the specific oral treatment daily for a total of 2 weeks plus one half day as instructed.

Regiment Description				
Agent	**Dose**	**Route**	**Schedule**	**Cycle Length**

Ticagrelor	90 mg	Oral	AM and PM	2 weeks (14 days)
Clopidogrel	75 mg	Oral	AM	
Placebo (to conceal frequency of clopidogrel dosing)	--	Oral	PM	
Aspirin	81 mg	Oral	AM	

6.3 Concomitant Medications/Treatments

As reported in the exclusion criteria, subjects will not be included in the study if:

- Concomitant use of antiplatelet agents other than aspirin or antithrombotic agents
- Recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist
- Unwilling to stop NSAIDs, PPIs, Aspirin (if they are taking it for primary CV prevention), Fish oil, Vitamin E and herbal supplements

6.4 Duration of Therapy

Treatment is to continue for two weeks plus one half day or until:

- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s). Any adverse event attributed to blinded study drug that in the opinion of the PIs would obviate the reinstitution of drug, such as bleeding requiring hospitalization or transfusion
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Pregnancy

Each stopping point will be documented and the participant notified to discontinue and return study drugs. The participant will be scheduled for a close-out visit to arrange follow-up with his or her personal physician as indicated. A stopping point will be reported to the IRBs not more than 5 working days after the study staff becomes aware of the problem. If the stopping point is a serious adverse event, it will be reported within 2 working days.

Data Safety and Monitoring Plan

Routine data reports will be provided by the PIs to the IRB every 6 months and will include laboratory data and clinical data on study drug side effects. Non-routine data reports will be provided as needed and include data on serious adverse events, stop points, unblinding episodes, pill non-adherence, and loss to follow-up. The PIs will report any serious adverse events to the IRBs not more than 2 working days after study staff become aware of the event, and unanticipated problems will be reported not more than 5 working days after study staff become aware of the problem. We have included a medical monitor, Dr. Karakala, as part of the protocol. He will be responsible for monitoring of serious adverse events and to identify safety concerns during the study.

6.5 Duration of Follow Up

Telephone follow up will occur at 30-days, 6-months and 12-months post randomization to obtain information about patients' health. Specifically, we will collect discharge summaries for any hospitalizations, procedure notes/operative notes for any procedures that may occur or death summary for any death that may occur during follow up. If there are any

unexplained adverse events, UAMS electronic medical records will be reviewed or records of non-UAMS care will be obtained to report adverse events by the research personnel. The medical records that will be reviewed and/or requested are related to hospitalization and/or procedure that can be associated to study-related adverse events. Data will be collected over the course of 12 months following enrollment.
The telephone script is included in the application.

Positive controls will not be followed with phone calls.

6.6 Removal of Patients from Protocol Therapy

Stopping Points. All potential stopping points will be reviewed by the PIs prior to designation. A subject can leave the trial or be withdrawn if any one of the following occurs:

1) Any adverse event attributed to blinded study drug that in the opinion of the PIs would obviate the reinstitution of drug, such as bleeding requiring hospitalization or transfusion.
2) Intolerable side effects and the subject decides to withdraw.
3) Pregnancy.
4) The subject is unwilling or unable to continue with study protocol and procedures.

Each stopping point will be documented and the participant notified to discontinue and return study drug. The participant will be scheduled for a close-out visit to arrange follow-up with his or her personal physician as indicated. A stopping point will be reported to the IRBs not more than 5 working days after the study staff becomes aware of the problem. If the stopping point is a serious adverse event, it will be reported not more than 2 working days afterward.

7.0 STUDY PROCEDURES

7.1 VISIT 1 (Consent Process and Screening Procedures)

7.1.1 Informed Consent (for all subjects)

CKD subjects. The medical records of patients will be reviewed to evaluate if they are eligible to be part of the study. For subjects enrolled in the study, assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the assessments were done before informed consent was obtained.

Research staff will go over the consent process with the potential CKD subject after their clinical appointment at the renal clinic. If the subject does not have enough time to go over the consent process, but he/she is interested in our study, we will provide the subject with a printed copy of the ICF and we will perform the consent process on the phone.

Healthy control subjects. To minimize contact with potential subjects (COVID-19 pandemic), the consent process and screening questionnaire will be performed on the phone. After the phone conversation, research staff will e-mail the consent form to the potential subject for review and inform the subjects that a printed copy of the consent form will be provided during the baseline visit for signature. Moreover, subject will be informed that during baseline we evaluate their kidney functionality to determine eligibility (blood draw and urine collection).

Phone consent process will be documented in the process note. Signed consent form will be obtained from each subjects enrolled in the study during the screening and/or baseline visit.

7.1.2 Eligibility Questionnaires

CKD subjects. We will ask permission to collect data related from UAMS medical records. This will avoid an extra blood draw. We will collect:

- Laboratory data: Comprehensive metabolic panel. Complete blood count, Serum phosphate levels, Urine albumin to creatinine ratio, serum uric acid level.
- Clinical data: Vital signs, body mass index.
- Demographic data: age; gender; race; ethnicity.
- Medical history:
  - Diabetes mellitus (defined as a fasting blood glucose level ≥126 mg/dL and/or use of hypoglycemic agents);
  - Hyperlipidemia (defined by use of lipid-lowering agents, or total cholesterol level ≥ 240 mg/dL or a serum LDL level ≥160 mg/dL);
  - Hypertension (defined as four office blood pressure readings averaging ≥140/90 mm Hg and/or use of antihypertensive drugs);
  - Current smoking (defined as any cigarette smoking in the past 30 days) status will be recorded as data. Smoking status is not an exclusion criterion.
  - Nephrotic syndrome (spot urine protein-to-creatinine ratio >3, serum albumin concentration <3 g/dl, serum total cholesterol >200 mg/dl and generalized edema).
- Medication in use (aspirin, clopidogrel, warfarin, prasugrel, beta-blockers, proton pump inhibitors, calcium antagonist, nitrates and statins).

Pregnancy test. Performed at screening visit for CKD subjects.
In a female of childbearing potential, perform urine pregnancy test. The test must be negative for subject to be eligible for inclusion. Research staff will perform the pregnancy test in the urine sample collected by the subject. Results of the pregnancy test will be recorded in the consent process note.
CKD and Healthy Control Subjects will be asked to complete the following questionnaires:

- Concomitant Medication Form (see questionnaire 1)
- Demographic Form (see questionnaire 2)
- Past Medical History Form (see questionnaire 3)
- COVID-19 questionnaire

Healthy control subjects will complete the questionnaires on the phone.

All the subjects will be asked to schedule an in person visit “Visit 2 –Baseline”.

Washout Period. Subjects will be asked to:

- Stop taking for two weeks the following drug/supplement:
  - Aspirin (if they are taking it) for primary CV prevention
  - Vitamin E and herbal supplements
  - Nonsteroidal anti-inflammatory drugs
  - Fish oil
  - Proton pump inhibitors
- Stop drinking alcohol 12 hours before each visit
- Do not eat or smoke for at least 6 hours before the blood test during Visit 2.

If the subject is taking aspirin, the baseline visit can be scheduled only after the washout period (plus 7 days). For the subjects that are not taking aspirin before the study, the baseline visit will be scheduled any time after the screening visit as specified also in the baseline section of the protocol.

We will send to all the subject an e-mail or mail with instruction for the baseline visit, date, time of the visit and the location of the Visit 2. E-mail and mail script included in this application.

Eligibility of healthy control will be determined only after baseline visit.

Positive controls will sign consent after meeting inclusion/exclusion criteria. The research staff will ask the patient questions about their medical history, social history, physical history, and the medications that they are currently taking. The Stanford 7-Day Physical Activity Recall questionnaire will be used to assess the patient's physical activity.

Visit 2 – Baseline Visit (for all subjects)

Baseline Visit 2 will be scheduled 2 weeks (+7 days) from the screening Visit 1. In case an eligible subject is not taking aspirin we will schedule visit 2 without the washout period. These participants do not need to wait for 2 weeks (+7 days) before the baseline visit 2. The Baseline Visit 2 can be scheduled any time after the screening visit for those subjects that are not taking aspirin before being in the research study.
If an eligible subject is taking the following:

- Vitamin E and herbal supplements
- Nonsteroidal anti-inflammatory drugs
- Fish oil
- Proton pump inhibitors

The baseline visit can be scheduled any time after the screening visit.

<u>If the participant is not eligible</u>, research staff will contact the subject by phone and notify that he/she is not eligible to be part of the study.

7.1.3 Medical History

- Review any medical problems since the screening visit.
  - We will also ask questions in order to perform COVID-19 screening if the last screening was done 2 weeks ago.

7.1.4 Review of medication in use

Record any concomitant medication. Confirm that the subject has not taken in the past 2 weeks any of the following drugs:

- Aspirin (if they are taking it) for primary CV prevention
- Vitamin E and herbal supplements
- Nonsteroidal anti-inflammatory drugs
- Fish oil
- Proton pump inhibitors

Confirm that the subject stopped eating and smoking at least 6 hours before the visit and stopped drinking alcohol 12 hours before the visit.

Record any symptoms occurred since the screening visit.

7.1.5 Diet and Physical Activity Questionnaire

An interviewer-administered assessment of diet and exercise with a modified 24-hour Dietary Recall Questionnaire and the Stanford 7-Day Physical Activity Recall Questionnaire will be performed to ensure dietary consistency which may affect platelet aggregability. Questionnaire forms included in the application.

7.1.6 Blood draw and urine collection

For all subjects_ seven blood tubes will be drawn from trained staff members (total of 40 ml of blood). Blood will be used to evaluate:

- Complete blood count (CBC). UAMS Clinical Lab.
- Uric Acid, creatinine and lipid panel. UAMS Clinical Lab
- Comprehensive Metabolic panel. UAMS Clinical Lab.
- Complete Blood Count. UAMS Clinical Lab.
- Serum phosphate. UAMS Clinical Lab.
- Serum uric acid level. UAMS Clinical Lab.
- HbA1c. UAMS Clinical Lab.
- Levels of circulating cytokines including, but not limited to interleukin-1beta, IL-6, TNF-alpha
- WBPA
- Circulating drug/metabolites
- DNA extraction and analyze of polymorphisms in relevant SNPs.

:

Moreover, subjects will be provided with a sterile urine collection cup by the research staff. Urine collected will be send to UAMS lab for evaluation of kidney functionality. Part of the same urine sample will be used for pregnancy test in woman of childbearing age (as described below).

For all subjects, blood draw need to be done in fasting condition. Participants will be instructed by research staff to not eat and smoke for at least 6 hours and to avoid alcoholic beverage 12 hours before the blood test.

7.1.7 Pregnancy test. Performed at baseline visit for healthy control and CKD patients if necessary..

In a female of childbearing potential, perform urine pregnancy test. The test must be negative for subject to be eligible for inclusion. Research staff will perform the pregnancy test in the urine sample collected by the subject. Results of the pregnancy test will be recorded in the consent process note.

7.1.8 Double blind randomization for CKD

CKD participants will be randomized by a research pharmacist in a double-blind fashion way in two arms:

- Ticagrelor 90 mg twice daily (AM and PM) + Aspirin 81 mg once daily (AM)
- Clopidogrel 75 mg once daily (AM) + placebo once daily (PM) + Aspirin 81 mg once daily (AM)

7.1.9 Open label for healthy control:

Recruitment of healthy controls will start together with the recruitment for CKD subjects. Healthy control participants will receive:

- Ticagrelor 90 mg twice daily (AM and PM) + Aspirin 81 mg once daily (AM)

Intervention for healthy controls will be open-label. Control subjects will be recruited matching 1:1 for age and sex the CKD subjects in the ticagrelor arm.

At the end of the baseline-Visit 2, clinical research staff will provide the participant with the appropriate drug to cover the 2 weeks treatment period.

In summary for both CKD and healthy controls, each subject will take the study drugs for 14 days at home and they will come for the last visit on the 15th day in the morning. During the last visit, we will ask the subject to take one more morning dose of the study drugs (15th day) as shown in the table below:

Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		
am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	
Day 8		Day 9		Day 10		Day 11		Day 12		Day 13		Day 14		Day 15
am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am

7.1.10 Healthy control subjects eligibility

If the subject is eligible, research staff will contact the subject to inform them about their eligibility and to start taking the study drugs as instructed during the baseline visit.

If the subject is not eligible, research staff will contact the subject by phone and notify that he/she is not eligible to be part of the study. If the participant's lab test results show abnormal values, the subject will be provided with the lab test results and he/she will be asked to discuss about the results with his/her primary physician. We will instruct the not-eligible subject to return the study drug to the research staff.

7.1.11 Instruction to subjects about last visit

Instruct subjects that they need to take the last dose of prescribed research drug during the last visit-Visit 3. Describe procedures that are going to happen during the last visit. Remind the subjects to collect the empty pill pocket and bring them back to the last visit as a proof of the total number of pills they had.

7.2 Mid-Treatment Telephone Call (7-10 days after Baseline-Visit 2):

- Record when subject started taking the study treatment;
- Record any adverse event;
- Make sure that the participant did not take any of the following:
  - Vitamin E and herbal supplements
  - Nonsteroidal anti-inflammatory drugs
  - Fish oil
  - Proton pump inhibitors
- Schedule the date for the last visit-Visit 3 in relation to when they started taking the study drugs. We will send to the subject an e-mail or mail regarding date, time of the visit and the location of the last visit-Visit 3. E-mail and mail script are included in this application.
- Remind the subject to:
  - Stop eating and smoking at least 6 hours before the last visit-Visit 3;
  - Stop drinking alcoholic beverages 12 hours before the last visit-Visit 3;
  - Bring back the pill pocket (empty/used/not used) at the last visit-Visit 3;
- Remind the participant that the last pill must be taken during the last visit-Visit 3.
- Record the number of pills taken by participant since the start of the treatment.

7.3 Visit 3 (Last visit)

7.3.1 Physical exam will be performed only on the last visit

Vital signs (temperature and blood pressure), height and weight

7.3.2 Review of medication in use

Record any concomitant medication. Confirm that the subject has not taken the following drugs:

- Vitamin E and herbal supplements
- Nonsteroidal anti-inflammatory drugs
- Fish oil
- Proton pump inhibitors

Confirm that the subject stopped eating and smoking 6 hours before the visit and stopped drinking any alcoholic beverage 12 hours before the visit.

7.3.3 Record any adverse events that occurred

7.3.4 Pill count to verify compliance with the study. In order to verify compliance, participants are requested to bring back the used study drug containers at the last visit

7.3.5 Blood draw

- Time point 0: Draw seven tubes of blood (about 42 ml) by peripheral venipuncture.
- Time point 1: Subjects will be asked to take the last dose of prescribed drug
- Time point 2: After 3 hours from taking the last dose of prescribed research drug, we will draw two tubes of blood (about 12 ml) by peripheral venipuncture

Blood collected at time point 0 will be used to evaluate:

- Complete blood count (CBC), serum creatinine and serum uric acid. UAMS lab will evaluate them
- Levels of circulating cytokines including, but not limited to interleukin-1beta, IL-6, TNF-alpha
- WBPA
- Drug/metabolite.

Blood collected at time point 2 will be used to evaluate drug/metabolite.

7.3.6 Diet and Physical Activity Questionnaire will not be collected on this visit

7.4 Follow Up Calls

Follow up calls will be done 4 weeks (±3 days), 26 weeks (±3 days) and 52 weeks (±3 days) after the baseline visit (Visit 2). At each phone call we will ask the participant about any hospitalization, any procedure related to heart and blood vessels a stroke. If so, we will ask date and location of hospitalization and/or procedures. The medical records that will be reviewed and/or requested are related to hospitalization and/or procedure that can be associated to study-related adverse events. Data will be collected over the course of 12 months following enrollment.

7.5 Schedule of Events

The following table reports steps during different visits and follow up calls:

	Screening	Treatment	Follow up

Weeks	**0**	**2**	**3**	**4**	**6**	**28**	**54**
Visit	Visit 1	Baseline V2	Mid-Treatment phone call	Last Visit 3	Phone call 1	Phone call 2	Phone call 3
General Assessments							
Informed Consent	X						
Eligibility criteria	X						
Demographics	X						
Urine Pregnancy Test		X					
Vital Sign				X			
History, medication review	X	X	X	X			
Diet and Physical Activity Questionnaire		X					
Adverse Events			X	X	X	X	X
Blood draw		X		X			
Urine collection		X					
Randomization		X					
Pill Count				X			

Schedule of events table: Follow up calls will be done 4 weeks (±3 days), 26 weeks (±3 days) and 52 weeks (±3 days) after the baseline visit (Visit 2).

7.6 Removal of Subjects from the Study

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

- Patient voluntarily withdraws from treatment (follow-up permitted);
- Patient withdraws consent (termination of treatment and follow-up);
- Patient is unable to comply with protocol requirements;
- Patient experiences toxicity that makes continuation in the protocol unsafe;
- Treating physician judges that continuation on the study would not be in the patient's best interest;
- Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);

8.0 ADVERSE EVENTS

8.2 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of subject's safety and care.

8.3 Definitions

8.3.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a subject receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

In this study, we will monitor adverse events from the changes in blood laboratory parameters as recorded in the lab test from the final lab draw or other patient reported events. We will call each subject after 7-10 days from the baseline visit in order to verify any adverse event recorded during the first part of the treatment.

8.3.2 Severity of Adverse Events

The severity of adverse events will be graded as follows

Mild: the event causes discomfort without disruption of normal daily activities.

Moderate: the event causes discomfort that affects normal daily activities.

Severe: the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.

8.3.3 Serious Adverse Events

A “serious” adverse event is defined in regulatory terminology as any untoward medical occurrence that meets one or more of the following criteria:

8.3.3.1 Results in death.

If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

8.3.3.2 Is life-threatening (the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).

8.3.3.3 Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.

8.3.3.4 Results in persistent or significant disability or incapacity

8.3.3.5 Is a congenital anomaly/birth defect

8.3.3.6 Is an important medical event

8.3.3.7 Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event“.

For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

8.4 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the adverse event.

Step 2: Determine whether the adverse event is related to the protocol therapy.

Attribution categories are as follows:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

Step 3: Determine the prior experience of the adverse event.

Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in the current known adverse events.

Step 4: Determine whether the adverse event is a Serious Adverse Event

8.5 Reporting Requirements for Adverse Events

8.5.1 Expedited Reporting

The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study.

The IRB must be notified within 10 business days of any **U**nanticipated Problems Involving Risk to Subjects or Others" (UPIRTSO). A UPIRTSO is defined as any problem, event or new information that is:

1) Unanticipated or unexpected;
2) Related to the research; and
3) Involves new or increased risks to subjects or others.

8.5.2 Routine Reporting

All other adverse events - such as those that are expected, or are unlikely or definitely not related to the study participation - are to be reported annually as part of regular data submission.

8.6 Unblinding Procedures

While the safety of the subject always comes first, it is still important to seriously consider if unblinding the study therapy is necessary to ensure a subject's safety. In most cases, the unblinding will be part of managing an SAE, and will be reported with the SAE, however, in cases where unblinding was not associated with an SAE, such actions should be reported in a timely manner. While there is no regulation governing this timeline, it is suggested to use the same timeline requirements for investigator reporting of SAEs.

8.7 Sample Collection Guidelines

Samples from participants will be handled, processed by research staff members. The blood tubes and the urine containers will be labeled with the unique study number corresponding to the subject and they will be stored in a double container with ice until the end of the collection period.

Blood and urine samples from participants will be transported by authorized and trained staff members to the UAMS Biomedical Building II (Bio-Med II) in Dr. Arthur's laboratory (room #638-2) where they will be processed for long-term storage. The samples will be transported to the Bio-Med II building using a double container system that will be labeled as Biohazard container. Bio-Med II building can be accessed only by authorized personal using an access card. The freezer is locked and the key will be stored in a separate locked room.

Blood and urine samples from participants will be centrifuged, and will be aliquoted in cryovials for long-term storage at -80°C. Each cryovial will be labeled only with the unique study number. De-identified DNA extracted from blood sample will be frozen banked in Dr. Arthur's laboratory (room #638-2) and analyzed at the Pharmacogenomics Lab VA Little Rock (G-128). VA sub-award with scope of work is included in this amendment.

Only authorized staff members will be able to access the protected file that associates the assigned unique study number to the identifiers of the participant.

8.8 Assay Methodology

WBPA. Ten mL of whole blood will be collected in 3.2% sodium citrate (9:1 ratio) for platelet aggregation test by *ex vivo* whole blood impedance (WB) platelet aggregometry via a Chrono-log aggregometer. This instrument uses electrical impedance in whole blood. Blood from all subjects will be tested within 3 hours of collection at Dr. Jerry Ware Platelet laboratory at UAMS BioMed II building, 2nd floor. Whole blood platelet aggregation induced by 2 µg/ml collagen, 10 and 20 µM ADP, 0.5 and 0.25 mM arachidonic acid, thrombin and ristocetin will be tested.

Rationale for using whole blood aggregometry: This is a method developed 20 years after the first aggregation test used in laboratory practice to assess platelet function involving platelet rich plasma. This method is superior to optical method: a) it is more sensitive and faster; b) evaluates platelets in physiological milieu in the presence of red blood cells and white blood cells known to affect platelet function and; c) does not require centrifugation which results in some platelet injury. The specimen is placed in a plastic cuvette containing magnetic stir bar. It is incubated at 37 degree Celsius for 5 minutes in wells prior to testing. An AC voltage in millivolt range is applied to the circuit, which creates stable resistance value during equilibrium with the formation of monolayer of platelets covering wires. After introducing agonists, platelets aggregate, which increases resistance to electrical flow, measured in ohms. ATP secretion is measured using firefly luciferin-luciferase reaction. Whole blood platelet aggregometry method allows larger platelets to be present and can potentially use thrombocytopenic samples and icteric/lipemic/hemolyzed samples. Previous studies have used optical aggregometry or multi-plate analyzers and Platelet Function Analyzer (PFA)-100 which are sub-optimal for testing and the results may not be reproducible.

P-selectin by Flow Cytometry: Whole citrate blood will be fixed and mixed with antibodies (BD Biosciences) to measure P-selectin expression on the platelet surface by flow cytometry (UAMS Flow Cytometry Core Facility). P-selectin (CD62P) is a membrane glycoprotein stored in platelet granules. Upon platelet activation, granules are released,

and P-selectin is expressed on the platelet surface. P-selectin by flow cytometry is a classic and specific marker of platelet activation.

Cytokines analyses: Plasma will be frozen at –80 °C. Levels of circulating cytokines will be measured on batched frozen samples by Proteome Profiler Human Cytokine Array kit (R & D Systems, Catalog # ARY005B). Since studies investigating mechanisms of platelet aggregability in CKD patients are limited, and to not limit our inquiry to just a few cytokines, we are using a commercial cytokine array kit that detects 36 human circulating cytokines, chemokines and acute phase reactants simultaneously. It includes, but is not limited to, plasma interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α.

Rationale for Using Multiplex Cytokine Array: Platelet activation is linked to inflammation. Typical measures of inflammation are plasma IL-1β, IL-6 and TNF-α levels. Although P2Ys reduce levels of circulating cytokines in the general population,[40] these changes have not been evaluated in the CKD population. Furthermore, some of the circulating cytokines, especially, IL-6 and TNF-α were implicated in decreasing metabolism of certain drugs in CKD patients.[41] Finally, IL-6 and TNF-α were reported to be predictors of CV events in non-dialysis CKD patients[42,43] but it remains unclear if these cytokines and other unknown cytokines explain the poor efficacy of P2Ys in this high risk patient population. Therefore we decided to use the multiplex kit.

Drug and metabolite levels: We will measure the trough- and peak- drug/metabolite levels by multiple reaction monitoring. Due to the highly reactive nature of the active thiol metabolite of clopidogrel, it cannot be reliably measured. We will instead measure both the intact drug and the inactive carboxylic acid metabolite (Figure 1). A mixed mode solid phase extraction (SPE) plate will be used for processing the plasma samples. Briefly, the plasma samples will be diluted in an aqueous buffer and loaded onto the SPE well-plate and washed with a water methanol solution, and finally eluted with 5% NH_4OH in 60:40 IPA:ACN. The intact clopidogrel will be measured by monitoring the 322 to 212 transition and its carboxylic acid metabolite will use the 308 to 198 transition, both in the positive ion mode.[60] These will be measured using a Thermo Scientific Quantiva triple quadrupole mass spectrometer that is coupled to a Waters nanoAcquity UPLC. The system will operate at a flow rate of 50 ul/min using a 1mm x 10cm BEH C18 column with 1.7 um particles. Measuring ticagrelor will be performed in the negative ion mode with the 521 to 361 and the 477 to 361 transitions for drug and metabolite, respectively. The concentration of each drug will be determined by comparing the areas of the internal standards to the unlabeled drug in the plasma and the relationship to each standard curve and validated using GLP standards.

Rationale for Measurement of Levels of Drugs and Their Metabolites by Mass Spectrometry: To investigate if the favorable pharmacokinetic profile of ticagrelor over clopidogrel is seen in non-dialysis CKD patients as in the general population, we will measure levels of drugs (clopidogrel and ticagrelor) and metabolites (carboxylic acid metabolite for clopidogrel and AR-C124910XX for ticagrelor) using mass spectrometry. Phase I and II studies of the two drugs reported achievement of steady state concentrations of drug and metabolite concentrations within 2 weeks regardless of loading dose.[23,44] So, we should be able to determine levels of drugs and metabolites in 2 weeks.

Rationale for Measuring Trough and Peak Drug and Metabolite Levels: Trough drug and metabolite levels at 2 weeks of antiplatelet therapy are stable and accurate compared to their peak levels.[30,45,46] Therefore, we chose to measure the trough levels. In addition, we will measure peak levels 3 hours after the administration of the drug at Visit 2. This will ensure unbiased results and minimize variability.

Measurement of uremic toxins: On Visit 1, the plasma sample will be de-proteinized by acetonitrile precipitation at physiological pH,[47] followed by concentrating it down to a small volume and dissolving it in the liquid chromatography mobile phase. A sensitive and selective MRM method was reported for quantifying p-cresol sulfate in human plasma by using m/z 187->80 and 187->107 transition.[48] Similarly, an MRM method was reported for quantification of indoxyl sulfate.[49] Both molecules will be detected in the same sample at physiological pH in negative ion mode. In addition, similar MRM methods exist for trimethylammonium N-oxide,[50] albeit in a positive ion mode. Stable isotopic dilution will be used to validate the method in a few patients prior to applying it to the whole group. Stable isotopic dilution is the gold standard of quantification of low molecular weight metabolites and achieves the highest accuracy. Also, the TSQ Quantum mass spectrometer allows for 20 ms polarity switching without any loss of the signal. Therefore, it is expected that implementation of these MRM methods will occur in a way that would result in quantification of the three toxins in the same run for the same 0.5 mL plasma sample.

Rationale for indoxyl sulfate, guanidine succinic acid and p-cresol sulfate measurements. Widely known uremic toxins are indoxyl sulfate and p-cresol sulfate. Although animal studies have reported these uremic toxins to be pro-thrombotic,[51] there are no controlled human studies to investigate their association with changes in platelet function and levels of drug and metabolite. This will be novel to the proposed study.

CYP Polymorphims Measurement by blood sample collection, storage, DNA Extraction, Quantification, and Quality Assessment:

Sample collection: 8.5 mL of blood will be collected into PAXgene Blood DNA Tubes, which contain a proprietary blend of reagents that both prevents blood coagulation and stabilizes white blood cells. These tubes will be stored at -70degrees C refrigerator at Arthur's lab. All DNA samples will be sent to PAL, G128, Research Service, Little Rock VA in a batch where DNA will be isolated from the tubes using appropriate buffers supplied in the PAXgene Blood DNA Kit.

For DNA isolation, the blood is transferred to processing tubes (supplied already filled with cell lysis buffer), and the solution is mixed to lyse red and white blood cells. Cell nuclei and mitochondria are pelleted by centrifugation, washed, and resuspended in digestion buffer. Protein contaminants are removed by incubation with a protease. DNA is precipitated in isopropanol, washed in 70% ethanol, dried, and resuspended in resuspension buffer. DNA will be extracted from whole-blood using the PAXgene Blood DNA System (Qiagen, Valencia, CA). Highly pure genomic DNA will be obtained. DNA quantity and quality will be ascertained by RNAseP Quantitation™ (Applied Biosystems, Life Technologies, Carlsbad, CA) with fluorescence detection using an ABI7900HT Real-Time Quantitation System (Applied Biosystems, Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. DNA samples are normalized to 50ng/µl and 200ng per sample is required for analysis by microarray.

Performing Pharmacogenetic analyses by microarray: Each DNA sample will undergo whole-genome amplification, enzymatic end-point fragmentation, precipitation, resuspension and hybridization to microarrays for 20 hours at 48°C according to the manufacturer's procedures (Illumina, Inc., San Diego, CA). Microarrays used for Pharmacogenetics analyses are the Infinium® Human Gloval Screening Arrays V2 (Illumina, Inc., San Diego, CA) which feature more than 17,220 PGx polymorphisms covering all genes listed on the PharGKB and CPIC-17 websites plus 800,000 additional markers per sample., After single-base extension and staining, beadchips are scanned using the Illumina iScan system and Autoloader2. **Analysis of Pharmacogenetic data**: Preliminary data analyses to assess internal quality control will be performed with

GenomeStudio V2 software using Genotyping Module 2.0.2 (Illumina, Inc.). Reproducibility between intentional replicates and gender calls will be determined. Samples with call rates below 0.99 will be repeated and SNPs with GenTrain scores below 0.4 will be clustered manually. GenomeStudio software can export a custom report with only PGx data.

8.9 Specimen Banking

Blood and urine samples of the participants will be stored indefinitely for possible future use, only if participants consented for future use of the samples. The samples and health information stored for future research will be labeled only with study ID number and they will not have any identifiable information. The genetic material collected from participants will not be store for future research. All genetic material will be used only for this study and any left over at the end of the study will be discharged. Banking these samples and health information for future use is fundamental for understanding the correlation between the kidney functionality and several other diseases. We can use these samples to correlate kidney function/dysfunction with a broad spectrum of diseases in the future. The plasma from the blood and the supernatant collected from the urine samples of the participants will be stored in a -80°C freezer in Dr. Arthur's laboratory in the Biomedical Building II UAMS on the 6th floor. The Bio-Med II building can be accessed only by authorized personal using an access card. The freezer is locked and the key will be stored in a separate locked room. Future use of these samples which is not specifically described here for the current study will require additional approval from the IRB or designation by the IRB as non-human subjects research. We are planning to use these samples for research purposes until they are completely consumed. ***Genetic tests:*** The DNA sample will be frozen for analyses of polymorphisms affecting metabolism of the study drugs.

Specimens will be stored indefinitely or until they are used up. If future use is denied or withdrawn by the patient, best efforts will be made to stop any additional studies and to destroy the specimens.

9.0 STATISTICAL CONSIDERATIONS

9.1 Study Design/Study Endpoints

Statistical Considerations Summary statistics will be used to describe the data distribution. Median (interquartile range) and mean (SD) will be reported for markers as in Aim 1 for both groups (CKD vs. non-CKD) pre- and post-therapy. Two-sample t-test or Mann-Whitney test will be used to compare markers between groups at baseline. A mixed effects model will be used to compare WBPA (change) to ticagrelor and aspirin therapy between groups. The model will have group factor, ticagrelor treatment factor (repeated, pre vs. post), and interaction between group and treatment. The pair effect and subject effect will be random factors to control for the clustering within matched pairs and within each subject. Potential confounders (age and BMI) will be adjusted in the models. Differential response to ticagrelor treatment will be evaluated by testing the null hypothesis that there is no significant interaction term by using F-test statistics. In an exploratory analysis, levels of ticagrelor and its active metabolite will be compared between groups. Finally, two-way interactions between the metabolic phenotypes of CYP3A4 and CKD group on WBPA will be investigated.

9.2 Sample Size and Accrual

Sample-size Calculation and Power Considerations. We used baseline measurements of WBPA from our previous work. For CKD-ND and non-CKD patients at baseline, we reported mean (SD) WBPA of 11.32 (5.14) Ω and 7.67 (3.52) Ω, respectively. We

calculated post-treatment WBPA values in CKD-ND and non-CKD patients (at 75% IPA) to be 2.88 Ω and 2.77 Ω. We used the linear mixed effects model to compare between-group (CKD vs. non-CKD) differences in ticagrelor treatment effect, where matched pair will be a random effect. With a fixed sample size of 27 pairs at a 2-sided α of 0.05, the power for detecting a difference of at least 4 Ω in mean WBPA changes between CKD and controls with an F test will be 91.3%.

9.3 Data Analyses Plans

Data analyses plan for Aim 1

Aim1 Primary Objectives Analysis Plan. Summary statistics will be used to describe the distribution of the data. Median (interquartile range) and mean (plus standard deviation [SD]) will be reported for all variables. An analysis of covariance (ANCOVA) model will be used in the primary analysis to compare treatment effects of ticagrelor vs. clopidogrel in CKD patients because this approach has higher statistical power than other methods to analyze drug effects.[56] **Post-treatment ADP-induced WBPA values** in ohms (Ω) will be modeled as the dependent variable (the **primary outcome measure**). *Specifically, we will use the WBPA values drawn after 3 hours of the drug administration at Visit 2*. Baseline measurement of WBPA, diabetic status (1- diabetics; 0-non-diabetics) and a binary treatment variable (1-ticagrelor arm; 0-clopidogrel arm) will be included as independent variables. The coefficient associated with the treatment variable will be the parameter of interest. Under the assumption that there are no significant differences in baseline measurements between groups (due to randomization), this coefficient will reflect the mean difference in WBPA values (or difference in mean change from baseline) between groups. In the case of missing data, we will do a complete case analysis and compare estimates by using imputation of the means or the mixed-effects model approach. We will also calculate the following **secondary outcome measures between the groups** and compare between groups by Chi-square test:

1) **Percentage of inhibition in platelet aggregation (IPA)** is the ratio of the *difference in baseline and post-treatment values* divided by the *baseline value* of WBPA.
2) **Residual platelet aggregability (RPA)** is defined as those with IPA < 75% on treatment.
3) **Pre-specified adverse events at 2-weeks, at 30-days, at 6-months and at 12-months including** any bleeding (skin bruising, nose bleeds, major bleedings), CV events, hospitalizations or death

***Aim 1 Secondary Objectives Analysis Plan*.** We will report summary statistics of drug and metabolite levels. We will test correlations between changes in WBPA values in the treatment arms and levels of drug and metabolites. We will perform summary statistics of cytokine levels. We will correlate changes in levels of circulating cytokines before and after treatment with changes in WBPA values in the two treatment arms and drug/metabolite levels. In addition, we will use one-way ANOVA to analyze interaction of cytokines with treatment group to modify IPA. For statistical analysis, we will collapse CYP2C19 groups based on metabolic phenotypes based on previous published studies including our preliminary work. Proportions of metabolic phenotypes of CYP polymorphisms will be compared between groups with Chi Square or Fisher's exact test. Two-way interactions between the metabolic phenotypes and the study drug on the outcome measure will be investigated. Specifically, we will test interaction of poor (vs. wild) and rapid (vs. wild) metabolic phenotype of CYP2C19 with the study drug on IPA (Visit 2 from Visit 1). We will conduct analyses similar to CYP2C19 for CYP3A4/5 alleles after collapsing them as binary variables.

Data analyses plan for Aim 2

Aim 2 Primary objective Analysis Plan. Summary statistics will be used to describe the data distribution. Median (interquartile range) and mean (SD) will be reported for markers as in Aim 1 for both groups (CKD vs. non-CKD) pre- and post-therapy. Two-sample t-test or Mann-Whitney test will be used to compare markers between groups at baseline. For the primary outcome measure, a mixed effects linear model will be used to compare mean change in WBPA to ticagrelor and aspirin therapy between groups. The model will have group factor, ticagrelor treatment factor (repeated, pre vs. post), and interaction between group and treatment. The pair effect and subject effect will be random factors to control for the clustering within matched pairs and within each subject. Models will be adjusted for potential confounders (age and BMI) that were not used in matching. Differential response to ticagrelor treatment will be evaluated by testing the null hypothesis that there is no significant interaction term by using F-test statistics. All available data will be included in the analyses; the mixed model can accommodate cases with incomplete data. Data transformations will be employed if needed to meet analysis assumptions.

Aim 2 Secondary Objectives Analysis Plan. Secondary outcomes will be analyzed as Aim 1. Summary statistics will be performed. Student's t-test or Wilcoxon rank sum test will be used to compare continuous and Chi-Square test to compare categorical variables between groups. Any two-way interactions of CYP polymorphisms (present/absent) and CKD status on IPA after treatment will be investigated. Similar interactions will be analyzed between uremic toxins and CKD status on changes in WBPA values and levels of drug/metabolite.

Aim 3 Primary Objectives Analysis Plan. We will collect data on CBC, CMP, lipid panel, A1c, phosphorus, uric acid and urine microalbumin/creatinine ratio from positive controls medical chart as these are routinely done on admitted patients. We will also perform platelet function analysis along with flow cytometry to analyze platelet phenotype as already outlined in the protocol for the other two groups.

10.0 STUDY MANAGEMENT

10.1 Institutional Review Board (IRB) Approval and Consent

This study will be conducted in accordance with all applicable government regulations and University of Arkansas for Medical Sciences research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB).

The formal consent of each subject, using the IRB-approved consent form, will be obtained before the subject is submitted to any study procedure. All subjects for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The person obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and requirements of the study. The consent process will take place in a quiet and private room, and subjects may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. This consent form must be signed by the subject or legally acceptable surrogate, and the individual obtaining the consent. A copy of the signed consent will be given to the participant, and the informed consent process will be documented in each subject's research record.

10.2 Data Management and Monitoring/Auditing

All study subject material from participants will be assigned a unique identifying code or number. The key to the code (the instrument associating the data with subject identity) will be kept in a locked file in the principal investigator's office, if hardcopy, or on a password-protected UAMS server, both located behind locked doors in a restricted access area of the UAMS campus. Only those individuals listed as investigators for this protocol will have access to the code and information that identifies the subject in this study.

10.3 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol. Investigators may only implement a deviation from or a change to the protocol to eliminate an immediate hazard(s) to subjects without prior IRB approval.

10.4 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

10.5 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms). All study records will be retained in accordance with applicable institutional and applicable regulatory requirements.

10.6 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with applicable regulatory requirements. The Principal Investigator is responsible for personally overseeing the treatment of all study subjects. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all applicable regulations and guidelines regarding clinical trials both during and after study completion.

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Concomitant Medication Form (Questionnaire 1)

Subject ID #: _____ **Date of visit ___ ___ /___ ___ / ___ ___**

Ask the participant if they are taking:

- **Antithrombotic Agents (mark yes or no):**

Aspirin	Cilostazol	Ranolazine	Aggrenox	Prasugrel
Pradaxa	Eliquis	Warfarin	Xarelto	

- **Herbal supplements and antiplatelet agents (mark yes or no):**

Coumadin	Fish Oil	Vitamin E	Herbal supplements

- **Non-steroidal anti-inflammatory drugs (NSAIDS) (mark yes or no):**

Motrin	Advil	Aleve	Mobic	Naprosyn	Goody Powder
Lodine	Celebrex		Ibuprofen	BC Powder	Pamprin

- **Proton Pump Inhibitors (mark yes or no):**

Protonix (Pantoprazole)	Nexium (Esomeprazole)	Prevacid (Lansoprazole)	Prilosec (Omeprazole)	Aciphex (rabeprazole)

If participant is taking any of the above medications complete:

For screening visit use only (visit 1)			
Those already on **_____**	Dose of (current) ----------mg	How long on it? ---------- Primary prevention __ ___	**Stop date of _____between screening and baseline visit:**

		Secondary prevention (exclude) ___	___ ___ /___ ___ / ___ ___
Those already on _____	Dose of (current) ----------mg	How long on it? ---------- Primary prevention __ ___ Secondary prevention (exclude) ___	**Stop date of _____between screening and baseline visit:** ___ ___ /___ ___ / ___ ___
Those already on _____	Dose of (current) ----------mg	How long on it? ---------- Primary prevention __ ___ Secondary prevention (exclude) ___	**Stop date of _____between screening and baseline visit:** ___ ___ /___ ___ / ___ ___

If the participant is taking any of the above medications, ask the participant if he/she agree to stop taking them:

☐ **YES**, the participant can stop taking these medication	☐ **NO**, the participant can NOT stop taking these medications
Continue the study	Participant can NOT continue the study

Ask the participant about any other medication that is taking:

Medication name†	Total daily dose	Route	Med type	Type of change	Date of change

†Screening visit: list all medications patient is currently taking. All other visits: list any change during the period from the last visit

1. Beta blockers
2. ACEI & ARB`s
3. Calcium channel blockers
4. Diuretics
5. Other antihypertensives
6. Statins
7. TCA`s
8. SSRI
9. Antipsychotics
10. Hypnotic/sedatives
11. Hypoglycemics
12. NSAID`s
13. Narcotics
14. Antihistamines
15. Antibiotics
16. Steroids
17. HAART
18. PPI
19. Laxative/stool softner
20. Phosphate binders
21. Antiplatelets
22. Anticoagulants
23. Antiarhythmics
24. Others

Demographic Form (Questionnaire 2)
Subject ID #: _____ **IRB # 227997**
Date of visit ___ ___ /___ ___ / ___
___

DOB (date of birth)	__ __ /__ __ / __ __ __ __	Age _____
Gender	□M (1) □F (0)	
Race(check 1 or more)	□African-American / black (1) □American-Indian/Alaska native(2)	□Asian (3) □White (4) □Native Hawaiian/Pacific islander(5)
Ethnicity	□Hispanic (1)	□Non-hispanic (0)
Obesity	□ BMI ≥30 □ BMI ≥25 and <30 □ BMI <25	
Education	□ High school or below □ Undergraduate school □ Graduate school □ Postgraduate training	
Employment	□ Unemployed □ Self employed □ Employed	
Health insurance	□ Medicare □ Medicaid □ Private □ Unisured	

Past Medical History Form (Questionnaire 3)

Subject ID #: _____ **Date of visit** ___ ___ /___ ___ / ___ ___

PMH	Cause CKD	□DM (1) □HTN (2) □unknown (3) □multifactorial (4) □PKD (5) □RAS (6) □ GN (7) □ other (8)	
	DM	□yes (1)	□No (0)
	HTN	□yes (1)	□No (0)
	CVA in last 12 months	□yes (1)	□No (0)
	HIV	□yes (1)	□No (0)
	MI or CABG or PCI in last 12 months	□yes (1)	□No (0)
	MI or CABG or PCI > 12 months	□yes (1)	□No (0)
	CHF	□yes (1)	□No (0)
	CVA (remote)	□yes (1)	□No (0)
	CAD	□yes (1)	□No (0)
	LungDz	□yes (1)	□No (0)
	LiverDz	□yes (1)	□No (0)
	PVD	□yes (1)	□No (0)
	Cancer	□yes (1)	□No (0)
	DepressionHx	□yes (1)	□No (0)
	Surgery (within 3 month)	□yes (1)	□No (0)
	Recent bleeding episode	□yes (1)	□No (0)
	Bleeding disorder	□yes (1)	□No (0)
Social history			
	DrugAbuse	□yes (1)	□No (0)
	Alcohol	□yes (1)	□No (0)
	Tobacco	□yes (1)	□No (0)
	Married	□yes (1) □Divorced (2)	□No (0) □Widowed (3)
	Lives Alone	□yes (1)	□No (0)
	Employed	□yes (1)	□No (0)
Allergies	□yes (1) □No (0)	specify medication _____	
Any other Conditions?	□yes (1) □No (0)	please specify _____	