# NSAIDs vs. Coxibs in the Presence of Aspirin: Effects on Platelet Function, Endothelial Function, and Biomarkers of Inflammation in Subjects with Rheumatoid Arthritis and Increased Cardiovascular Risk or Cardiovascular Disease

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## ABSTRACT

**Title**: NSAIDs vs. Coxibs in the Presence of Aspirin: Effects on Platelet Function, Endothelial Function, and Biomarkers of Inflammation in Subjects with Rheumatoid Arthritis and Increased Cardiovascular Risk or Cardiovascular Disease

**Short Title**: Aspirin, NSAIDS and Coxibs

**Rationale:** The relative cardiovascular safety of NSAIDs, particularly among patients with cardiovascular disease (CVD) or at higher CVD risk, has generated considerable concern among both patients and physicians because of knowledge gaps in the evidence relative to comparative safety and pharmacodynamic interactions between aspirin and NSAIDs. In the recently reported PRECISION trial, a moderate dose of celecoxib was found to be noninferior to ibuprofen or naproxen with respect to cardiovascular safety in patients with arthritis at increased CVD risk. At this time, no comparative prior data are available analyzing the effects of NSAIDs vs. Coxibs in the presence of aspirin on platelet function, biomarkers of inflammation and endothelial function.

**Objectives:** To study the pharmacodynamic interactions between aspirin, NSAIDs and Coxibs with respect to platelet function, biomarkers of inflammation and endothelial function.

**Study Type:** This is a randomized, crossover study.

**Study Design:** Thirty patients with rheumatoid arthritis who are at high cardiovascular (CV) risk or with established CV disease will be enrolled in the study. Patients taking anticoagulant therapy or any other antiplatelet agent other than aspirin will be excluded. The study will be conducted at LifeBridge Health facilities, Sinai Hospital of Baltimore and Greenspring Valley Cardiovascular Associates.

**Study Methodology:** Patients will be treated with immediate release 81mg aspirin for 4 weeks in the run-in period followed by randomization to celecoxib (200 mg bid) vs. naproxen sodium (550 mg bid) for 4 weeks and then cross over to the other drug for another 4 weeks. Blood and urine samples will be collected at baseline before the aspirin run in period, 24±4 hr after the last dose of aspirin in the run in period, 24±4 hr after the last dose of the first period study drug and 24±4 hr after the last dose of the second period study drug. Assays for platelet function, biomarkers of inflammation and endothelial function will be performed at these time points.

**Primary Outcomes**
Measurement of collagen, epinephrine, arachidonic acid (AA)-, and adenosine diphosphate (ADP)-induced platelet aggregation by light transmittance aggregometry in platelet rich plasma.

**Secondary outcomes**
Measurement of:
a) Serum $TxB_2$ levels
b) Urine 11-dh-$TxB_2$ and 8-iso-prostaglandin $F2_\alpha$.
c) Blood pressure.
d) Endothelial function by EndoPAT (Endothelial Peripheral Arterial Tone)

e) Soluble markers of endothelial dysfunction- VCAM, ICAM, oxLDL, hs CRP, and RCN2 (Reticulocalbin 2).

**Exploratory**
U466199 (thromboxane [Tx] A2 agonist)-induced platelet aggregation by light transmittance aggregometry in platelet rich plasma.

**Statistical Methodology:** Categorical variables will be compared using $\chi^2$ test or the Fisher exact test whereas continuous variables will be assessed with independent-samples t-test or the Mann-Whitney U test in case of non-parametric distribution of data; normality of data will be checked with the Kolmogorov Smirnov test. This is an exploratory study with no available historical data to determine sample size. No comparative prior data are available analyzing the effects of NSAIDs vs. coxibs in the presence of aspirin on platelet function, biomarkers of inflammation and endothelial function. Based on our previous experience with pharmacodynamic studies, we assume that 30 patients using a randomized cross over design is adequate to determine statistical difference between two groups. Analyses will be performed with SPSS software (SPSS, Inc., Chicago, IL) and $p<0.05$ will be considered significant.

# 1. INTRODUCTION

## 1.1.Specific Aims

Measurement of

a) Collagen-, epinephrine-, AA-, ADP-, and U466199-induced platelet aggregation by light transmittance aggregometry in platelet rich plasma.
b) Serum TxB2
c) Urine 11-dh-TxB2 and 8-iso-prostaglandin F2$\alpha$
d) Blood pressure
e) Endothelial function by EndoPAT (Endothelial Peripheral Arterial Tone)
f) Soluble markers of endothelial dysfunction- VCAM, ICAM, oxLDL, hsCRP, and RCN2.

## 1.2. Hypothesis

In patients with rheumatoid arthritis who are at high risk for or with established cardiovascular disease, in the presence of aspirin administration, celecoxib but not naproxen administration will be associated with elevated inflammation and deleterious effect on endothelial function.

## 1.3. Background and Significance

Aspirin is the bedrock of antiplatelet treatment strategies in patients at high risk for CVD (1). More than 50 million people in the United States alone i.e., 36% of the adult population, take about 10-20 billion aspirin tablets regularly for primary and secondary prevention of CVD (2). Patients with rheumatoid arthritis may require therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) for pain relief. However, patients with rheumatoid arthritis who are at CV risk will be treated with both NSAIDs and aspirin. In contrast to aspirin, which inhibits platelet COX-1 for the lifetime of the platelet, non-selective NSAIDs such as naproxen sodium bind reversibly to COX-1 and provide a transient antiplatelet effect. A pharmacodynamic interaction between naproxen sodium and low-dose aspirin when both agents are dosed concurrently has been suggested. Cyclooxygenase-2 inhibition reduces endothelial production of prostacyclin, a vasodilatory eicosanoid that blocks platelet aggregation *in vitro*. Celecoxib is COX-2 selective and do not substantially inhibit COX-1 at recommended dosages. The relative extent of COX-1 versus COX-2 inhibition has potential implications for development of both thrombotic CV events and adverse gastrointestinal (GI) effects in patients treated with NSAIDs. Both naproxen and celecoxib have been associated with an increase in CV risk. In the recently reported PRECISION trial, moderate doses of celecoxib was found to be noninferior to ibuprofen or naproxen with respect to CV safety in patients with arthritis with established CVD or increased CVD risk (3). NSAID treatment has been associated with effects on endothelial function, a biomarker that has been linked to cardiovascular risk (4,5). At this time, there have been no randomized comparative studies examining the pharmacodynamic interactions between aspirin, NSAIDs and coxibs with respect to platelet function, and biomarkers of inflammation and endothelial function.

# 2. STUDY DESIGN AND SUBJECT SELECTION

## 2.1. Study Type

This is a randomized, crossover study.

## 2.2.Setting/Location

The study will be conducted at the LifeBridge Health facilities, Sinai Hospital of Baltimore and Greenspring Valley Cardiovascular Associates.

**2.3.Duration of Study**
The study duration for each patient is approximately 12 weeks from the screening visit until the end of study visit.

**2.4.Number of Subjects**
Thirty patients will be randomized in this trial.

**2.5.Study Population**
Patients between 18 and 75 years of age with rheumatoid arthritis and increased cardiovascular risk or cardiovascular disease may be enrolled. For patients who do not regularly use NSAIDs, the upper age limit is 65 years of age.

2.5.1. **Subject Demographics**
Subjects of all genders, racial/ethnic background, able to give consent, with all inclusion criteria and none of the exclusionary criteria can participate in this trial. Subjects from the vulnerable population will not be approached to participate in this study.

**2.6.Recruitment**
Recruitment will continue until 30 qualified subjects have been enrolled and have completed all visits. The length of the recruitment period is 12 months. If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within the reasonable time frame as agreed upon, the recruitment period may be extended to reach the desired sample size.

**2.7.Study Design**
Qualified patients will be treated with immediate release 81mg aspirin for 4 weeks in the run-in period followed by randomization to celecoxib (200 mg bid) vs. naproxen sodium (550 mg bid) for 4 weeks and then cross over to the other drug for another 4 weeks. Blood and urine samples will be collected before the aspirin run-in period (baseline), 24±4 hrs after the last dose of aspirin in the run-in period, 24±4 hrs after the last dose of the study drug in the first period and 24±4 hrs after the last dose of the study drug in the second period. Assays for platelet function, biomarkers of inflammation and endothelial function will be performed at these time points (Table 2).

**2.8.Qualifying Criteria-** The inclusion and exclusion criteria is based on the PRECISION trial (6).

2.8.1. Inclusion Criteria (Qualified patients should have all 4 main criteria)

- Age 18-75 years of age for patients who regularly use NSAIDs or
  Age 18-65 years of age for patients who do not regularly use NSAIDs.
- Able to give informed consent.
- Clinical diagnosis of rheumatoid arthritis, as determined by individual patient and physician.
- Subjects with CVD or increased CV risk. Please see definitions for each criteria below:
  - Increased CV risk (Subjects should have at least 3 of the following)
    - > 55 years of age

        - History of Hypertension
        - History of Dyslipidemia or subjects currently receiving lipid lowering therapy as standard of care (i.e. statin drugs, fibrates, prescription ω 3-acid ethyl esters, or prescription niacin [≥1,000 mg/dL]).
        - Family history of premature CV disease (MI, angina pectoris, heart failure, cardiac death or coronary revascularization, stroke, carotid endarterectomy, or other arterial surgery or angioplasty for atherosclerotic vascular disease in a parent, grandparent, or sibling with symptom onset or diagnosis before age 55 y for males and 65 y for females)
        - Current smoker (defined as any cigarette smoking within the past 30 days)
        - Left ventricular hypertrophy
        - Documented ankle brachial index of <0.9
        - History of microalbuminuria, urine protein-creatinine ratio of >2
        - Obesity (defined as BMI ≥ 30kg/$m^2$)
    - CV disease (defined as one of the following):
        - Calcium score of > 0
        - ≥ 50% occlusion of a coronary artery by angiography
        - ≥ 50% occlusion of a carotid artery by angiography or ultrasound
        - History of stable angina
        - Symptomatic peripheral arterial disease
        - Prior MI, unstable angina, percutaneous coronary intervention, CABG, TIA, ischemic stroke, carotid endarterectomy, or other arterial surgery or angioplasty, which have occurred > 3 months prior to screening visit
        - Diabetes Mellitus type 1 or 2 (considered a CV disease equivalent)

2.8.2. Exclusion Criteria

Subjects with any of the following criteria will be excluded from this study.

- Planned coronary, cerebrovascular, or peripheral revascularization
- Undergone major surgery within 3 months prior to screening visit or has planned major surgery during the study period.
- Uncontrolled hypertension (SBP >190, DBP >100 mm Hg) during screening visit (may be repeated after 15 minutes and exclusion will be based on the last measurement).
- Uncontrolled arrhythmia < 3 months from screening visit.
- NYHA class III-IV heart failure or if available, ejection fraction ≤ 35%.
- Current anticoagulation therapy within 14 days of screening visit.
- Current antiplatelet therapy except for aspirin within 14 days of screening visit.
- GI ulceration < 60 days before screening visit.
- GI bleeding, perforation, obstruction < 6 months of screening visit.

- Inflammatory bowel disease, diverticulitis active < 6 months of screening visit.
- AST, ALT, or BUN >2x the upper limit normal (within 30 days prior to screening visit).
- Creatinine level > 1.7 mg/dL in men, 1.5 mg dL in women (within 30 days prior to screening visit).
- On fluconazole, or lithium therapy.
- Malignancy < 5 years before screening visit.
- Other known, active, significant GI, hepatic, renal, or coagulation disorders
- Allergy, allergic-type reactions or hypersensitivity (e.g. asthma, urticaria, etc.) to any of the study medications and its components (i.e. sulfonamides).
- History of any disease or condition that, in the opinion of the investigator would place the subject at an unacceptable risk to participate in this study.
- Any clinically relevant abnormal findings in physical examination, vital signs, or previous laboratory works that, in the opinion of the investigator, may compromise the safety of the subject to participate.
- Subjects who are legally institutionalized. Subjects who are pregnant or lactating.
- Female subjects who are unwilling to use at least 1 effective birth control method, unless the subject is sterilized or postmenopausal.

## 2.9 Study Treatment

### 2.9.1. Aspirin

Subjects who have signed informed consent that qualify for this trial will complete the run-in period for 4 weeks. For the run-in period, 81mg per day of immediate release aspirin will be started and continued during the duration of the trial.

- Subjects already taking 81mg of immediate release aspirin daily prior to screening visit will be instructed to continue as prescribed; however, the medication will be provided to the subjects by the study site for compliance accounting purposes.
- For eligible subjects not currently taking aspirin daily, 81mg of immediate release aspirin daily will be started during the run-in period.
- For eligible subjects taking >81mg of aspirin prior to screening visit, their aspirin dose will be decreased to 81mg daily during the study duration.

### 2.9.2. Celecoxib and Naproxen

After a successful 4 week run-in period, subjects will be randomized to receive treatment sequence: celecoxib → naproxen or treatment sequence: naproxen → celecoxib concurrently taken with 81mg immediate release aspirin. Subjects randomized to the celecoxib→ naproxen sequence receive celecoxib in the first period (randomization thru cross-over visit) and naproxen in the second period (cross-over visit) thru end of study; whereas subjects randomized to the naproxen→ celecoxib treatment receive naproxen in the first period (randomization thru crossover visit) and celecoxib in the second period (cross-over visit) thru end of study.

Celecoxib dose and frequency for this trial is 200 mg twice daily, while naproxen sodium dose and frequency is 550 mg twice daily.

2.9.2.1. Aspirin

Aspirin will be supplied in a pill form for oral administration containing 81 mg of acetylsalicylic acid. Current FDA approved indications include relief of pain, fever, and inflammation from a variety of conditions and for its platelet aggregation inhibitory properties.

- Instruct patient to take aspirin once daily after food intake, with plenty of liquid.

2.9.2.2. Celecoxib

Celecoxib will be supplied in a capsule form for oral administration containing 200 mg of celecoxib. It is currently indicated for relief of the signs and symptoms of RA with a recommended dosage of 100 to 200 mg twice daily.

- Instruct patient to take celecoxib 200 mg capsules twice daily (once in the morning and once in the evening). It should be swallowed whole, with or without food.

2.9.2.3. Naproxen

Naproxen will be supplied in a pill form for oral administration containing 550 mg of naproxen sodium (naproxen 500 mg with 50 mg sodium). It is currently indicated for the relief of the signs and symptoms of RA with a recommended dosage of 550 mg twice daily.

- Instruct patient to take this medication by mouth twice daily (once in the morning and once in the evening) with a full glass of water (8 ounces) and can be taken with food.

2.9.2.4. Storage and Handling

Store all study drugs at room temperature, 20° to 25° C (68° to 77° F), excursions permitted 15° to 30° C (59° to 86° F). Dispense in a child proof, well-closed container.

2.9.3. Permissible/Prohibited Concurrent Medications

- Inhaled or systemic corticosteroids are permissible, provided dose has been stable for 30 days prior to baseline and is continued throughout the duration of the trial. Corticosteroids administered for treatment of flare-ups is prohibited. Permissible treatment options for flare-ups include acetaminophen or opioids.
- Gastroprotective agents such as proton pump inhibitors or histimine-2 blockers may be taken prophylactically or in response to GI symptoms.

Subjects taking NSAIDS prior to Visit 1 may continue to take NSAIDS up until Visit 2 - Randomization.

# 3. STUDY METHODS AND PROCEDURES

## 3.1 Endpoints/Outcomes Measurements

### 3.1.1 **Primary Outcomes**

The primary outcome measures are a change in measurements of collagen-, epinephrine-, AA-, ADP-, and U466199-induced platelet aggregation by light transmittance aggregometry in platelet rich plasma.

### 3.1.2 **Secondary Outcomes**

Secondary outcomes measurements are changes in:

a) Collagen-, epinephrine, AA-, ADP, and U466199-induced platelet aggregation by Multiplate analyzer in whole blood.
b) Serum TxB2
c) Urine 11-dh-TxB2 and 8-iso-prostaglandin $F2_{\alpha}$
d) Blood pressure
e) Endothelial function by EndoPAT (Endothelial Peripheral Arterial Tone)
f) Soluble markers of endothelial dysfunction-VCAM, ICAM, oxLDL, hs CRP, and RCN2 (Reticulocalbin 2).

3.1.3 **Exploratory Measures**
a) U466199 (thromboxane (Tx) A2 agonist)-induced platelet aggregation by light transmittance aggregometry in platelet rich plasma.

3.1.4. **Leftover Blood Sampling**
Leftover serum and plasma samples will be stored to no more than 2 years post collection. Leftover samples from participants who do not consent for leftover sampling storage will be discarded.

## 3.2 Consent/Assent

Eligible subjects may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC/REB-approved informed consent, if the subject is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document or a separate assent form. Informed consent must be obtained before conducting any study-specific procedures (i.e., all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the subject's source documents.

## 3.3 Schedule of Visits and Procedures

The study is comprised of 3 periods: screening visit/run-in, randomization, and cross-over. All subjects will be taking immediate release aspirin 81 mg daily during the study duration. During randomization, subjects are assigned to a sequence of 2 additional treatments in combination with aspirin wherein subjects cross over from one treatment to another. Subjects randomized to the celecoxib-naproxen sequence receive celecoxib in the first period and naproxen in the second period, whereas subjects randomized to the naproxen-celecoxib treatment receive naproxen in the first period and celecoxib in the second period (Table 1).

**Table 1**

| | **Period 1 (n=15)** | **Period 2 (n=15)** |
|---|---|---|
| Treatment sequence: celecoxib→naproxen | Immediate release aspirin 81 mg daily + celecoxib 200 mg bid | Immediate release aspirin 81 mg daily + naproxen sodium 550 mg bid |
| Treatment sequence: naproxen→celecoxib | Immediate release aspirin 81 mg daily + naproxen sodium 550 mg bid | Immediate release aspirin 81 mg daily + celecoxib 200 mg bid |

Blood sample for safety labs, and blood and urine samples for pharmacodynamics measurements will be collected. An Endo-PAT test will be completed 24±4 hours after last dose of run-in aspirin, and 24±4 hours after the last dose of each study period. Subjects will get a reminder, prior to randomization, cross-over, and end of study visits, from their study

coordinator to confirm visit appointment and to review patient requirements for each visit. A tabulated overview (Table 2) of the procedures conducted in each of these periods is provided where in procedures and their timing are described in more detail.

3.3.1 Tabulated Overview

**Table 2**

| | V1 | | V2 | V3 | V4/EOS |
|---|---|---|---|---|---|
| | **Screening** | **Run-In[2] (0 to 7 days from screening visit; -28+3 days from V2)** | **Randomization[3]** | **Cross-Over[3,4] (28±3 days from randomization)** | **End of study[3,5] (28±3 days from Cross-Over)** |
| Informed Consent | X | | | | |
| Inclusion/Exclusion Criteria | X | | | | |
| Medical History | X | X | X | X | X |
| Review Prior/Concomitant Medications | X | X | X | X | X |
| Blood for Safety Labs (CMP) | X[1] | | | | X |
| Physical Examination[8] and vital signs (Blood pressure, HR) | X | | | | |
| Nursing Assessment (Blood pressure, HR) | | X | X | X | X |
| Height and weight measurement | X | | | | |
| Endo-PAT2000 Test[6] | | X | X | X | X |
| Blood and urine samples for PD measurements[7] | | X | X | X | X |
| Dispense Study Drug | | X | X | X | |
| Administer/continue 81mg of immediate-release aspirin | | X | X | X | |
| Administer Treatment 1 | | | X | | |
| Cross-over to treatment 2 | | | | X | |
| Drug accountability | | | X | X | X |
| Adverse Events | X | X | X | X | X |

[1] Blood safety labs collection *only* for subjects with no lab results on records that are ≤ 3 months from screening visit.
[2] Subjects need to have CMP reviewed prior to initiation of study-aspirin therapy. If CMP results are not readily available for review, subjects may complete 2 separate visits for Visit 1(allowing time for CMP collection and review) to complete all study procedures. Screening visit and run-in visit can occur at the same day if the subject's medical records and safety laboratory results are readily available for review prior to first-dose of run-in aspirin.
[3]Instruct subject prior to visit to hold run-in aspirin 24 +/- 4 hours prior to randomization visit
[4]Instruct subject to hold treatment 1 (naproxen or celecoxib) 24 +/- 4 hours prior to cross-over visit
[5]Instruct subject to hold treatment 2 (celecoxib or naproxen) 24 +/- 4 hours prior to end of study visit
[6]EndoPAT test is completed prior to first dose of run-in aspirin, treatment 1, and treatment 2
[7]Obtain blood and urine samples for PD measurements at V1, V2 and V3 prior to study drug administration and at V4.
[8]Screening visit/Run-In physical to be completed by a study provider (NP, PA, DO or MD).

## 4. STATISTICAL CONSIDERATIONS/DATA ANALYSIS

### 4.1. Sample Size

This is an observational study, and we assume that a sample size of 30 qualified subjects is sufficient to assess the pharmacodynamics interactions between aspirin, NSAIDs, and coxibs.

### 4.2. Statistical Calculations

Categorical variables will be compared using $\chi 2$ test or the Fisher exact test whereas continuous variables will be assessed with independent-samples t-test or the Mann-Whitney U test in case of non-parametric distribution of data; normality of data will be checked with the Kolmogorov Smirnov test. Analyses will be performed with SPSS software (SPSS, Inc., Chicago, IL) and $p<0.05$ will be considered significant**.**

### 4.3 Data Storage and Management

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Privacy and confidentiality of all enrolled patients must be maintained. De-identification of participants from their personal health information (PHI) will be accomplished with a personal identification number (PIN). The PIN will not be derived from any PHI (in part or in the entirety), e.g., social security number or medical record. The "key" linking a PIN to a patient's PHI will be maintained separately from collected data. The "key" will be secured; e.g., locked cabinet or password protected data file on a secure internet server. Only members of the research team will be able to access this data on the secure server and the file password.

### 4.4 Confidentiality

Subject information will be kept confidential as according to HIPAA requirements. Subject data will be stored and managed as outlined in section 4.3. All data records will be stored on site until 3 years after the investigation is formally discontinued. Paper records will be shredded and recycled. Records stored on a computer hard drive will be erased using a commercial software application designed to remove all data from the storage device.

## 5 HUMAN SUBJECTS PROTECTION (RISKS, BENEFITS, AND ALTERNATIVES)

### 5.1. Risks

5.1.1. Potential Loss of Privacy

Protected health information (PHI) will be collected during the study. The risk for breach of confidentiality and privacy will be minimized by shielding the subjects unlinking his or her identity from his or her personal health information.

5.1.2. Potential Adverse Events

Lactating and women of childbearing potential are excluded from participating in this trial secondary to potential harmful effects to breastfed infants and developing fetus. All male participants and/or their female partner will be advised to use at least one effective form of contraceptive method* during the study duration and at least 30 days from End of Study visit.

*Total Abstinence, absence of menstrual periods in women for more than one year after menopause, sterilization surgery, including tubal ligation (tubes tied) or hysterectomy (removal of the uterus or womb) in women or a vasectomy in men. Oral

contraceptives, intrauterine device (IUD), implantable or injectable contraceptives (Norplant or Depo-Provera), contraceptive patch, vaginal ring or use of condom with spermicide (these methods must be used exactly as directed).

5.1.2.1. Aspirin

5.1.2.1.1. Hypersensitivity. Aspirin intake may precipitate bronchospasm and induce asthma attacks. Subjects with previous allergic or hypersensitivity reactions to aspirin will be excluded from this trial.

5.1.2.1.2. Hematologic. Aspirin may be associated with an increased risk of bleeding due to its effect on platelet aggregation.

5.1.2.1.3. Pregnancy. Aspirin inhibits prostaglandin synthesis which may affect the pregnancy and/or the embryo/fetal development. Women planning on conceiving a child during the trial period or who becomes pregnant while taking study drug/s will be excluded/discontinued from the study.

5.1.2.1.4. Low uric acid excretion. At low doses, aspirin reduces excretion of uric acid. This can trigger gout in patients who already tend to have low uric acid excretion.

5.1.2.1.5. Other adverse reactions. Many adverse reactions due to aspirin ingestion may be dose-related. Some of the adverse reactions reported in the literature, from pre and post-marketing experience include: GI effects-nausea, vomiting, abdominal pain; bleeding; hematologic effects- anemia. More information about aspirin's adverse effects can be found on the drug's product label or package insert.

5.1.2.2. Naproxen and celecoxib. The use of NSAIDs, including naproxen and celecoxib is associated with a wide range of potential adverse effects. The risk of adverse events varies depending upon each individual, taking into consideration varying clinical context and concomitant medications. NSAIDs can interact with numerous drugs thus a careful review of each patient's concomitant medications is of utmost importance to reduce risk for renal impairment, additive hemorrhagic and hepatic toxicity, and other effects brought about by its effects in drug metabolism.

5.1.2.2.1. Cardiovascular effects

5.1.2.2.1.1. Clinical trials of COX-2 selective and nonselective NSAIDS have shown an increased risk of serious cardiovascular (CV) thrombotic events, myocardial infarction, and stroke, which can be fatal. All NSAIDS, both COX-2 selective and nonselective, may have a similar risk.

5.1.2.2.1.2. Hypertension. Celecoxib and naproxen can lead to onset of new hypertension or worsening of pre-existing hypertension. Throughout the course of this trial, blood pressure will be monitored, including careful review of concomitant medications to prevent drug-drug interactions that can cause impaired response to the study treatment (e.g.concurrent use of thiazide or loop diuretics, ACE inhibitors).

5.1.2.2.1.3. Congestive heart failure and edema. Fluid retention and edema have been observed in some patients taking NSAIDS. Patients with heart failure need to be monitored for new or worsening of heart failure signs and symptoms such as edema and weight gain of > 3 pounds per day.

5.1.2.2.2. Risk for gastrointestinal (GI) ulceration, bleeding, and perforation

NSAIDs can cause serious GI adverse events including inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or large intestine, which can be fatal. Upper GI ulcers, gross bleeding, or perforation caused by NSAIDs occur in approximately 1% of patients treated for 3-6 months, and in

about 2-4% of patients treated for one year. These trends continue with longer duration of use, increasing the likelihood of developing a serious GI event at some time during the course of therapy. However, even short-term therapy is not without risk. Since serious GI tract ulcerations and bleeding can occur without warning symptoms, investigators need to monitor for signs and symptoms of GI bleeding.

5.1.2.2.3. Renal effects. Renal toxicity, renal papillary necrosis and other renal injury can result from long-term administration of NSAIDS. Patients with impaired renal function (creatinine level > 1.7 mg/dL in men, 1.5 mg dL in women), heart failure, liver dysfunction, and those taking diuretics and ACE inhibitors are at greatest risk of this reaction.

5.1.2.2.4. Anaphylactoid reactions. Anaphylactoid reactions may occur in patients without known prior exposure to celecoxib and naproxen. Patients with aspirin triad, hypersensitivity, or previous allergic reaction to any of the study drug/s and its components will be excluded from participating in this trial.

5.1.2.2.5. Severe skin reactions. NSAIDS, including naproxen and celecoxib can cause serious skin adverse events such as exfoliative dermatitis, Steven-Johnson Syndrome, and toxic epidermal necrolysis, which can be fatal. Patient education about potential skin manifestations such as skin rash or any sign of hypersensitivity is very important for early study drug termination and AE treatment.

5.1.2.2.6. Pregnancy. All lactating or females of childbearing potential are excluded from study participation since celebrex and naproxen may cause premature closure of the ductus arteriosus.

5.1.2.2.7. Hepatic effects. Borderline elevations of one or more liver tests may occur in up to 15% of patients taking NSAIDS, including naproxen and celecoxib. Notable elevations of AST or ALT have been reported in approximately 1% of patients in clinical trials with NSAIDs. Rare cases of severe hepatic reactions, some with fatal outcomes, have been reported.

5.1.2.2.8. Hematological effects. Anemia is sometimes seen in patients receiving NSAIDs, including celecoxib and naproxen. This can be due to fluid retention, occult or gross blood loss. Patients exhibiting any signs or symptoms of anemia should have their hemoglobin and hematocrit checked. Naproxen may decrease platelet aggregation and prolong bleeding time. Celecoxib does not generally affect platelet counts, prothrombin time (PT), or partial thromboplastin time (PTT), and does not inhibit platelet aggregation at the dosage indicated for this trial. Unlike aspirin, their effect on platelet function is less severe, of shorter duration, and reversible. Patients with coagulation disorders or patients receiving anticoagulants will be excluded from this trial.

5.1.2.2.9. Pre-existing asthma. Patient with aspirin-sensitive asthma will be excluded from this trial. The use of aspirin in patients with aspirin-sensitive asthma has been associated with severe bronchospasm, which can be fatal. Since cross reactivity, between aspirin and other nonsteroidal anti-inflammatory drugs has been reported in such aspirin-sensitive patients, naproxen and celecoxib should not be administered to patients with this form of aspirin sensitivity and should be used with caution in patients with preexisting asthma.

5.1.2.2.10. Other adverse reactions. For more information on adverse events documented pre and post marketing experience for each study drug, please see drug databases at the FDA U.S. food and drug administration website.

5.1.3. Economic Risk

Subjects in the study may lose time at work or home and spend more time in the research site more than usual. Visit schedules will be made flexible for subjects (as allowed by protocol).

## 5.2. Safety

5.2.1. Study Treatment Titration

For the purpose of this trial, study treatment dosage adjustment is not permitted. If a subject requires dosage adjustment of study drug (e.g. for better pain control), the subject needs to be exited from the study.

5.2.2. Study Treatment Discontinuation

Study treatment will be stopped and subject removed from study participation if any of the following criteria listed below are met:

- Withdrawal of informed consent
- Death from any cause
- Any serious adverse event (as defined in section 7.2)
- Major adverse cardiovascular events
- Development of renal insufficiency or renal failure
- Hospitalization for hypertension
- Hospitalization for congestive heart failure
- Serious gastrointestinal events
  - Clinically significant GI events
  - Iron-deficiency anemia of GI origin
- Allergic or hypersensitivity reaction to any of the study treatment
- Pregnancy
- Subject requires an invasive or surgical procedure within the study period
- Non-compliance
- Subject develops a need for anticoagulant therapy
- Subject develops a need for antiplatelet therapy not originally assigned in his/her respective group
- Subject develops a need for any medication in the exclusion list
- Subject necessitating increased dose of study drug/s for symptom alleviation (e.g. naproxen dose increase for pain control)
- Any other reason that the investigator feels would pose a significant hazard to the subject if investigational therapy were continued

## 5.3 Benefits and Alternatives

There are no direct benefits to the patient. Participation in the study is entirely voluntary. The alternative is not to participate in the trial.

## 5.4. Protocol Deviations

The principal investigator will not deviate from the protocol without obtaining approval from the IRB or Ethics Committee and the sponsor.

## 6. SUBJECT COMPENSATION

### 6.1. Costs

The subject or their insurance company will not be billed for this study. All study related tests that is not considered as standard of care will be paid for by the research site. Protocol-related study drugs will be provided by the study.

### 6.2. Payment

A total of $400 financial compensation will be provided for study participation with the following breakdown each visit (Visit 1: Screening visit-$50, run-in $50, Visit 2: $100, Visit 3:$100, Visit 4: $100) to cover transportation, parking, and meal expenses. Compensation will only be paid for completed visits. Payment will be received by subjects after completion of each visit.

## 7. ADVERSE EVENT REPORTING

### 7.1.Adverse Event (AE) Definitions

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product which does not necessarily have to have a causal relationship with this treatment. An adverse event can be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality (i.e., whether or not it is considered to be drug-related). This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of the study treatment/intervention.

A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. An adverse event or suspected adverse reaction is considered "unexpected" if it is not specifically mentioned as occurring with the particular drug under investigation. Information about common side effects already known about the study drug/s drug can be found in the study drug/s package inserts. This information will be included in the subject's informed consent and should be discussed with the subject during the study as needed.

### 7.2 Serious Adverse Event (SAE) Definition

An SAE is defined as an event that:

- is fatal or life-threatening;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly/birth defect;
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
-elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
-treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
-social reasons and respite care in the absence of any deterioration in the subject's general condition

- is medically significant, i.e., defined as an event that jeopardizes the subject or may require medical or surgical intervention to prevent one of the outcomes listed above

**7.3 AE Grading Scale**

The descriptions and grading scales found in NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be used for AE reporting. Each AE term is associated with a 5-point severity scale.

**7.4 Reportable Adverse Events**

Aspirin, naproxen, and celecoxib have been extensively studied and each drug's overall adverse event profile has been well described. For the purpose of this trial, All SAEs, AEs which are not serious but which lead to permanent discontinuation of study medication, and AEs necessitating subject withdrawal will be captured in the CRF. Non-serious AEs which do not lead to discontinuation of study medication or subject withdrawal will not be collected. AE collection will commence once study medication is started. A detected SAE or an AE deemed related to study drug that led to permanent study drug discontinuation should be followed until its resolution or until the subject completes the study, whichever occurs first.

**7.5 Procedures for Recording and Reporting of Adverse Events**

7.5.1. All AEs will be reported to the principal investigator for evaluation. For both serious and non-serious AEs, the investigator has the primary responsibility for AE identification, documentation, grading, and assignment of attribution to the study treatment/intervention.

7.5.2. AEs occurring from the start of study medication administration through the last day of study participation must be recorded on the AE CRF with the following information:

·The intensity grade (grade 1, 2, 3, 4, 5; see CTCAE v5.0 grading)
·The relationship to the study drug(s)
·Attribution: An assessment of the relationship between the AE and the medical intervention (i.e., study drug administration). After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

| RELATIONSHIP | ATTRIBUTION | DESCRIPTION |
|---|---|---|
| Unrelated to study treatment/intervention | Unrelated | The AE *is clearly NOT related* to the study treatment/intervention |
| | Unlikely | The AE *Is doubtfully related* to the study treatment/intervention |

| | | |
|---|---|---|
| Related to study treatment/intervention | Possible | The AE *may be related* to the study treatment/intervention |
| | Probable | The AE *is likely related* to the study treatment/intervention |
| | Definite | The AE *is clearly related* to the study treatment/intervention |

·The duration (start and end dates or if continuing at final exam)
·Occurrence (known risks for study drug/s, underlying illness or population)
- Expected
- Unexpected

·Other contributing causes
·Action taken with study drug
·Any other actions in response to event
·Outcome
- Death related to AE
- Recovered/resolved with sequelae
- Not recovered/resolved
- Recovered/resolved without sequelae
- Recovering/resolving
- Intervention for AE continues
- Unknown

·Whether it constitutes a serious adverse event (SAE)

7.5.3. AE Collection

The occurrence of adverse events should be sought by non-directive questioning of the subject at each visit during the study. AEs may also be detected when these are volunteered by the subject during or between visits or through physical examination, laboratory test, or other assessments. Medical conditions/diseases present before starting the study drug are considered AEs only if they worsen after starting the study drug. Abnormal laboratory values or test results constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy. Abnormal values that constitute a SAE or lead to discontinuation of administration of study drug must be reported and recorded as an AE. Adverse event collection will commence when the subject starts taking study medication.

7.5.4. Adverse Event Treatment

All AEs should be treated appropriately and managed as according to standard of care, at the discretion of the investigator. The action taken to treat the AE should be recorded on the AE CRF. A detected AE should be followed until its resolution or until the subject completes the study. Assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, relationship to the study drug, the interventions required to treat it, and the outcome.

## 8. FUNDING

This is a clinical study initiated, developed, designed and managed by Platelet and Thrombosis Research, LLC. Funds to conduct this study will be provided by Bayer HealthCare, LLC.

## 9. CONFLICTS OF INTEREST.

Paul Gurbel, the principal investigator for this study, has received consulting fees and grant funds from the funding sponsor, Bayer HealthCare, LLC in the past.

## 10. FACILITIES AND EQUIPMENT

The research site is equipped with its own laboratory equipment, which includes state of the art technologies for platelet assays and PD measurements, centrifuges, refrigerators, and freezers for investigational specimen processing and storage. Subjects will be seen in the site's outpatient clinic room equipped with an Endo-PAT2000 machine, blood pressure equipment, weight scale, and phlebotomy supplies necessary for subject assessment.

## 11. OUTSIDE CONSULTANTS/COLLABORATORS

There are no outside consultants/collaborators participating.

## 12. CONTRACTURAL AGREEMENTS

There are no outside consultants/collaborators participating.

## 13. REFERENCES


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