Impact of steady state Cobicistat and Darunavir/Cobicistat on the Pharmacokinetics and Pharmacodynamics of an Oral Anticoagulant (Rivaroxaban) in Healthy Volunteers (CLOTRx)

NIH Protocol Number: 19-CC-0063

Sponsored by:
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Version 4.0

29 July 2021

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List of Abbreviations

AE	adverse event
AF	atrial fibrillation
aPTT	activated partial thromboplastin time
ALT	alanine transaminase
APR	Antiretroviral Pregnancy Registry
ART	antiretroviral therapy
ARV	antiretroviral
AST	aspartate transaminase
AUC	area under the concentration vs time curve
$AUC_{0\text{-}24hr}$	area under the concentration vs time curve from 0 to 24 hours postdose
$AUC_{0\text{-}12hr}$	area under the concentration vs time curve from time 0 to 12 hours postdose
$AUC_{0\text{-}\infty}$	area under the concentration vs time curve from 0 to infinity; total drug exposure
$AUC_{0\text{-}tau}$	area under the curve from 0 to the dosing interval
$AUEC_{0\text{-}24}$	area under the effect curve from 0 to 24 hours
BCRP	breast cancer resistance protein
BID	twice daily
CBC/diff	complete blood count with differential
CC	Clinical Center
CL/F	apparent oral clearance
C_{max}	maximum total plasma concentration
C_{min}	minimum total plasma concentration
COBI	cobicistat
CPRU	Clinical Pharmacokinetics Research Unit
CrCl	creatinine clearance
CRIMSON	Clinical Research Information Management System of the NIAID
CYP	cytochrome P450 isozyme
DAIDS	Division of AIDS
DLM	Department of Laboratory Medicine
DOAC	direct oral anticoagulant
DOT	directly observed therapy
DRV	darunavir
DVT	deep vein thrombosis
eGFR	estimate glomerular filtration rate
ER_{max}	maximum effect ratio over baseline
FDA	Food and Drug Administration
FXa	factor Xa
GI	gastrointestinal
GMR	geometric mean ratio
Hgb	hemoglobin
HIV	human immunodeficiency virus
HRPP	Human Research Protections Program
INR	international normalized ratio
IRB	institutional review board
IV	intravenous
LFTs	liver function tests

LMWH	low molecular weight heparin
MAI	medical advisory investigator
MM	medical monitor
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OATP	organic anion transporting polypeptides
OHSRP	Office for Human Subjects Research Protections
OTC	over-the-counter
P-gp	permeability glycoprotein
PD	pharmacodynamics
PE	pulmonary embolism
PI	principal investigator
PK	pharmacokinetic(s)
PLT	platelet
PO	oral(ly)
PT	prothrombin time
QD	once daily
RECORD	Prevention of Venous Thromboembolic Events in Patients Undergoing Major Orthopedic Surgery of the lower Limbs: Clinical Trial
ROCKET-AF	Prevention of Stroke and Systemic Embolism in Patients with non-valvular Atrial Fibrillation: Clinical Trial
RTV	ritonavir
SAE	serious adverse event
SCr	serum creatinine
SRCP	safety review and communication plan
$t_{½}$	terminal elimination half-life
t_{max}	time to maximum plasma concentration
ULN	upper limit of normal
UP	unanticipated problem
V/F	apparent oral volume of distribution
VKAs	vitamin K antagonists
VTE	venous thromboembolism
WNL	within normal limits

Protocol Summary

Full Title:	Impact of steady state Cobicistat and Darunavir/Cobicistat on the Pharmacokinetics and Pharmacodynamics of an Oral Anticoagulant (Rivaroxaban) in Healthy Volunteers
Short Title:	CLOTRx
Sample Size:	N = 12
Accrual Ceiling:	N = 40
Study Population:	Healthy volunteers ages 18-65 years
Accrual Period:	3 years
Study duration:	**Start date: 01/01/2019** **End date: 12/31/2022** **Total length of individual participation: 17 to 23 days**
Study Design:	This is an open-label, fixed sequence, intrasubject drug-drug interaction study designed to evaluate the effect of CYP3A4/P-gp inhibition by cobicistat and darunavir/cobicistat on the pharmacokinetics (PK) and pharmacodynamics (PD) of rivaroxaban. The study will include 12 healthy adult volunteers. Subjects will receive study drugs sequentially as follows: **Phase 1:** a single dose of rivaroxaban on day 1 followed by serial PK/PD blood sampling **Phase 2:** cobicistat once daily (days 2 to 7), followed by single dose of rivaroxaban and serial PK/PD blood sampling on day 7 **Phase 3:** darunavir/cobicistat once daily (days 8 to 13) followed by single dose of rivaroxaban and serial PK/PD blood sampling on day 13.
Study Agent/ Intervention Description:	Participants will receive the following: - Rivaroxaban 10 mg by mouth (3 doses on days 1, 7, and 13) - Cobicistat 150 mg by mouth x 6 days (days 2 to 7) - Darunavir/Cobicistat 800/150 mg by mouth x 6 days (days 8 to 13) - PK/PD blood sampling will be performed on days 1, 7 and 13
Primary Objective:	To characterize the single dose PK of rivaroxaban alone and in combination with steady-state cobicistat or darunavir/cobicistat in healthy volunteers.

Secondary Objectives:	(1) To characterize the PD (anti–factor Xa [FXa], activated partial thromboplastin time [aPTT], prothrombin time [PT]/international normalized ratio [INR]) of rivaroxaban alone and in combination with cobicistat or darunavir/cobicistat in healthy volunteers. (2) To evaluate the safety and tolerability of coadministration of rivaroxaban alone and in combination with cobicistat or darunavir/cobicistat in healthy volunteers.
Exploratory Objective:	To assess the role, if any, of gene polymorphisms (for enzymes involved in transportation and hepatic metabolism of study drugs) on the interaction between rivaroxaban and CYP3A4/P-gp inhibitors, cobicistat or darunavir/cobicistat.
Primary Endpoint:	Plasma area under the concentration vs time curve from 0 to 24 hours and 0 to infinity ($AUC_{0\text{-}24hr}$, $AUC_{0\text{-}\infty}$), maximum total plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), terminal elimination half-life ($t_{½}$), apparent oral clearance (CL/F), apparent oral volume of distribution (V/F), and minimum total plasma concentration (C_{min}) for rivaroxaban.
Secondary Endpoints:	(1) Area under the effect curve for factor Xa, aPTT, and PT/INR from 0 to 24 hours ($AUEC_{0\text{-}24}$), and the maximum effect ratio over baseline (ER_{max}). (2) AEs and abnormal laboratory values.

Precis

Rivaroxaban is a direct oral anticoagulant (DOAC) used for the prevention and treatment of various thromboembolic disorders. Predictable pharmacokinetic (PK) and pharmacodynamic (PD) properties, coupled with a few drug-drug and food-drug interactions, distinguishes DOACs from a traditionally used anticoagulant, warfarin, allowing fixed dosing without routine coagulation monitoring. Patients with human immunodeficiency virus (HIV) are living as long as their HIV-negative counterparts due to safe and efficacious antiretroviral therapy (ART). Persons with HIV are at higher risk for thromboembolic events and DOACs are a feasible option for anticoagulation in this population. However, there is a lack of drug interaction and safety data currently on the co-administration cobicistat (COBI)-boosted antiretroviral (ARV) regimens with rivaroxaban. Rivaroxaban is metabolized by cytochrome P450 isozyme (CYP) 3A4 and its absorption is modulated by permeability glycoprotein (P-gp), both of which are inhibited by the PK booster COBI. It is therefore possible that plasma concentrations of rivaroxaban may be significantly increased when co-administered together with COBI. This is of clinical concern as increased anticoagulant exposure may result in bleeding without the security of routine clinical monitoring. The purpose of this study is to determine the effects of steady state concentrations of COBI and darunavir (DRV)/COBI on the PK and PD of single oral doses of rivaroxaban.

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background

It is estimated that more than 50% of individuals infected with human immunodeficiency virus (HIV) are over the age of 50 years.[1] These patients have a near-normal life expectancy and as a result, they are experiencing aging-related comorbidities (e.g., atrial fibrillation [AF], stroke, etc.) and polypharmacy.[2,3] In addition, HIV infection itself is characterized as a hypercoagulable condition, with estimated prevalence of thromboembolic events up to 10 times higher than in the general population.[4–6] Thus, even the younger individuals living with HIV still remain at a high risk for developing thromboembolic events.

Before the availability of direct oral anticoagulants (DOACs), warfarin, a vitamin K antagonist (VKA), has been the mainstay therapy in the prevention and treatment of thrombosis in individuals without cancer, including those living with HIV. For individuals with cancer, the American College of Chest Physicians recommends the use of low molecular weight heparin (LMWH) over warfarin.[7] Cancer, just like HIV, is also a known hypercoagulable state, leading to an increased risk of venous thromboembolism (VTE), with 4- to 7-fold increased risk of VTE.[8,9] VTE frequency is estimated to be 4% to 20% depending on the cancer type.[10] HIV infection has been associated with certain forms of cancer such as Non-Hodgkin's and Hodgkin's lymphoma.[11] Thus, individuals living with HIV and cancer are at a far greater risk of developing thrombosis than those with HIV alone. As such, effective anticoagulant therapy is necessary, with minimal drug-drug interaction, ease of dosing, and administration.

Warfarin, despite its long history of use, presents with many clinical challenges. It is associated with a risk of bleeding and requires close monitoring for signs and symptoms of bleeding—including monitoring for international normalized ratio (INR) for dose adjustment.[12,13] In addition, it is subject to clinically significant drug interactions with food and drugs, including commonly used antiretroviral (ARV) medications.[14] On the other hand, LMWH requires daily self-injection that can limit adherence, especially in the elderly. Thus, challenges in the use of both warfarin and LMWH have elicited large interest in the utilization of DOACs, such as rivaroxaban, that have more predictable pharmacokinetics and can be effectively administered as a fixed dose without routine pharmacological (e.g., plasma drug levels) or laboratory monitoring (e.g., blood clotting time). Multiple studies have demonstrated that the DOACs are non-inferior to warfarin or LMWH in preventing thrombotic events.[15–19] However, DOACs also carry a potential for increased risk of bleeding with increased drug exposure and it is therefore important to identify factors that could increase their exposure, especially concomitant medications that can inhibit enzymes and transporters involved in their metabolism and elimination respectively. In the ROCKET-AF study (Prevention of Stroke and Systemic Embolism in Patients with non-valvular Atrial Fibrillation) comparing the efficacy of rivaroxaban (n=7111) vs. warfarin (n=7125), the rate of major bleeds and non-major clinically relevant bleeds were 3.6%/year vs. 3.5%/year (p=0.58), and 11.8%/year vs. 11.4%/year (p=0.35) respectively. A factor Xa reversal agent, andexanet alfa (AndexXa®) was recently approved in May 2018 by the Food & Drug Administration (FDA) for patients treated with rivaroxaban or other DOACs when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding.[20,21] As such, the use of rivaroxaban is expected to further increase as clinicians may feel more comfortable at prescribing this DOAC with an approved antidote.

While new developments of efficacious anticoagulants exist, concerns for drug-drug interaction in patients taking pharmacokinetic (PK)-boosted HIV protease inhibitors, such as darunavir/cobicistat (DRV/COBI), still remain. Rivaroxaban is a substrate of CYP3A4, CYP2J2, permeability glycoprotein (P-gp), and breast cancer resistance protein (BCRP). Prescribing information for rivaroxaban recommends a 50% and avoidance[22] when taken concomitantly with strong dual CYP3A4 and P-gp inhibitors. These recommendations are based on drug interaction data using index inhibitors ketoconazole and ritonavir (RTV). COBI is a strong dual CYP3A4 and P-gp inhibitor and is expected to significantly increase the plasma concentrations of rivaroxaban when co-administered together. COBI and RTV inhibit CYP3A4 with similar magnitude, however, they differ in their potential to inhibit P-gp, with COBI being the more potent inhibitor.[23]

Available drug-drug interaction data between rivaroxaban and RTV describe a 2.5-fold increase in rivaroxaban exposure.[24] However, it should be noted that RTV was dosed at 600 mg twice daily (BID) in these healthy volunteers, a dose that is higher than the recommended booster dose (100 mg) for protease inhibitor–containing regimens. RTV has been described to exhibit dose- and exposure-dependent inhibition with a combination of reversible and time-dependent mechanisms[25]. Through this inhibition mechanism, a lower dose of 100 mg is expected to yield a potential difference in rivaroxaban PK compared to the 600-mg BID dose. Additionally, RTV inhibits both P-gp and BCRP efflux transporters, and it is unclear whether or not its inhibition is also dose-dependent. Of note, there are no studies that have examined the PK interaction between rivaroxaban and COBI.

We chose to study rivaroxaban not only because it is one of the most frequently prescribed as of 2016,[20] but also due to the general lack of PK data on the co-administration of this DOAC with COBI and COBI boosted DRV. In addition, rivaroxaban will be going generic in 2021, potentially making it a more attractive DOAC option for prescribers. In this study, rivaroxaban 10-mg will be studied, as this is the clinical dose used. Additionally, rivaroxaban 10 mg is the commonly used dose in healthy volunteer PK studies. Higher doses of rivaroxaban have been used in healthy volunteer PK studies, and have been tolerable with mild to moderate AEs.

The aims of this study include:

1) To characterize the PK of rivaroxaban alone and in combination with a) COBI and b) DRV/COBI;
2) To characterize the PD of rivaroxaban alone and in combination with a) COBI and b) DRV/COBI;
3) To explore the potential influence if any, of gene expression on the interaction between DOACs (rivaroxaban) and strong dual CYP3A4/P-gp inhibitors (COBI and DRV/COBI). Genes of interest are those associated with enzymes and transporters involved in the metabolism and elimination of study drugs.

1.2 Direct Oral Anticoagulants

In the coagulation cascade, factor Xa directly converts prothrombin to thrombin through the prothrombinase complex, leading to the formation of fibrin clot formation and activation of platelets (PLTs) by thrombin.

1.2.1 Rivaroxaban (Xarelto®)

Rivaroxaban is a potent and highly selective oral direct factor Xa inhibitor, indicated for the treatment and prevention of thrombosis. Rivaroxaban is FDA approved for (1) reducing the risk of stroke and systemic embolism in patients with nonvalvular AF, (2) for the treatment of deep venous thrombosis (DVT) and pulmonary embolism (PE), (3) for reducing the risk of recurrence of DVT and PE, and (4) for prophylaxis of DVT, which may lead to PE in patients undergoing knee or hip replacement surgery. For AF, the recommended dosage is 20 mg once daily, or 15 mg for patients with creatinine clearance (CrCl) of 15-50 mL/min. For treatment of DVT/PE, the recommended dose is 15 mg BID with food for the first 21 days, followed by 20 mg once daily with food for the remainder of the treatment period. For long-term reduction in the risk of recurrence of DVT and PE, the recommended dose is 10 mg once daily. The bioavailability of rivaroxaban is dose dependent, with the 10-mg dose having a bioavailability of 80%-100%, with or without food. The solubility of rivaroxaban is rate limiting beyond 15 mg and therefore it is recommended that doses beyond 15 mg be administered with food. In fasted conditions, the bioavailability of 20-mg dose is 66%. Rivaroxaban area under the concentration vs time curve (AUC) and maximum total plasma concentration (C_{max}) were dose proportional, independent of food up to 10 mg. Under fasting conditions, AUC and C_{max} for the 15-mg and 20-mg doses increased with dose, but were less than dose proportional.[26] However, AUC and C_{max} were dose proportional for rivaroxaban doses of 10 mg, 15 mg, and 20 mg in the fed state. In addition, there was no relevant accumulation observed in a multiple dose escalation study,[27] indicating that rivaroxaban concentrations after a single dose can be considered representative for steady-state concentrations. The peak plasma concentration is achieved approximately 2-4 hours following oral intake, with a terminal elimination half-life ($t_{½}$)of 5-9 hours in healthy subjects ages 20-45 years old, and 11-13 hours in the elderly (ages 60-76). Rivaroxaban is eliminated both by renal and non-renal pathways, with 36% of unchanged drug recovered in the urine, and 7% in the feces. Non-renal metabolism includes CYP3A4/5 (18%), 2J2 (14%), and hydrolysis (14%), with no active circulating metabolites. It is a substrate of the efflux transporters P-gp and BCRP. The product label recommends that concomitant use of rivaroxaban with strong dual P-gp and CYP3A4 inhibitors should be avoided.[22] Rivaroxaban does not inhibit or induce CYP450 enzymes or the drug transporters.

Maximum inhibition of factor Xa (FXa) occurred 1-4 hours after administration of rivaroxaban 1.25-80 mg, with the half-life for the biological effect of 6-7 hours. After 24 hours, FXa activities had not returned to baseline for doses above 5 mg. Inhibition of FXa activity was found to be dose dependent over a dose range of 5-80 mg. Inhibition of factor Xa activity and prolongation of prothrombin time (PT) were also strongly correlated with plasma concentrations ($r=0.949$, $r=0.935$, respectively).[27] In addition, concentration-dependent changes in other PD measures such as PT and prothrombinase-induced clotting time were observed. PT measurements increased linearly with rivaroxaban concentrations, and this relationship was similar in both healthy subjects and patients with non-valvular AF. Increase in PT resulted in an increase in major bleeding events, while the probability of ischemic stroke was found to be independent of PT.[20]

Rivaroxaban exposure in elderly subjects is generally higher than that in younger subjects, without relevant changes in C_{max},[27,28] a finding also reported in a previous study.[29] Increase in AUC (1.5-fold higher) in the elderly was largely due to reduced renal function, however, dose adjustment is not required based solely on age.[22,29] Similar increases in PD markers were

observed (factor Xa activity and PT) as it is expected with the well-established correlation between rivaroxaban plasma concentrations and both factor Xa activity and PT prolongation. Extremes in body weight (<50 kg or >120 kg) had a less than 25% effect on the PK of rivaroxaban and did not require a dose adjustment.[22]

Refer to section 8.1.1 (Potential Risks of rivaroxaban) for more information on the side effects of rivaroxaban.

1.2.1.1 Select Drug Interaction Studies Between Rivaroxaban and Dual CYP3A4/P-gp Inhibitors

Table 1: Summary of Drug Interaction Studies with Rivaroxaban

Concomitant Drug	**Change in rivaroxaban PK**	**Change in rivaroxaban PD**
Ketoconazole 200 mg PO daily days 1-4. Rivaroxaban 10 mg PO day 4. N=12[24]	C_{max}: ↑53% AUC: ↑82% CL/F: ↓45% $t_{1/2}$: ↓ 22 % (5.7 hrs)	Not measured
Rivaroxaban 10 mg PO daily days 1-10. Ketoconazole 400 mg PO daily days 6-10. N=20[24]	C_{max}: ↑72% AUC_{τ}: ↑158% CL/F: ↓61% CL_{RS}: ↓44% $t_{1/2}$: ↑ 35% (6.5 hrs)	Not measured
Rivaroxaban 10 mg PO days 1 & 8. Ritonavir 600 mg PO BID days 3-8 N=18 (12 for PK analysis)[24]	C_{max}: ↑55% AUC: ↑153% CL/F: ↓60% CL_{RS}: ↓82% $t_{1/2}$: ↑ 21% (6.9 hrs)	Not measured

1.3 CYP3A4 and P-gp Inhibitors

1.3.1 Cobicistat (Tybost®)

COBI is a PK enhancer used in combination with certain ARV drugs to increase their exposure and peak plasma concentrations, and prolong half-life. COBI has no activity against HIV.[30] It is primarily metabolized by CYP3A4, with minor contribution by CYP2D6. The $t_{½}$ of COBI is 3 to 4 hours, with 86.2% excreted in feces and 8.2% in urine. It is a potent inhibitor of CYP3A4 and P-gp.[30] In addition, COBI inhibits BCRP, organic anion transporting polypeptides (OATP1B1, OATP1B3)[30] responsible for drug uptake, and MATE1, a transporter involved in the tubular secretion of creatinine.[31] Consequently, a modest increase in SCr (not more than 0.4 mg/dL) is observed when patients are started on a COBI-based therapy. However, this modest decline in estimated glomerular filtration rate (eGFR) does not affect actual renal glomerular function.[30] Its inhibition properties allow for less frequent dosing, lower pill burden, reduced variability of systemic drug exposure, and improved drug efficacy.[32] When co-administered with CYP3A4 and/or P-gp substrates (victim), COBI (perpetrator) is expected to increase the concentration of the victim drug. COBI has been shown to have limited effect on activating pregnane X receptor, which regulates expression of metabolizing enzymes, and thus induction of these enzymes is

unlikely,[33] a phenomenon observed with the other PK booster, ritonavir. Currently COBI, at a dose of 150 mg, is used to boost the plasma concentrations of the protease inhibitors darunavir, atazanavir, and the integrase inhibitor elvitegravir. It is available as a co-formulation with atazanavir (Evotaz®), darunavir (Prezcobix® and Symtuza® [in combination with other ARVs]), and elvitegravir (as Genvoya® and Stribild® in combination with other ARV agents). It is also available as a single agent (Tybost®). Refer to section 8.1.2 (Potential Risks of Cobicistat) for more information on the side effects of COBI.

1.3.2 Darunavir (Prezista®)

DRV (Prezista®) is an HIV protease inhibitor approved by the FDA in 2006. It is also available as a fixed dose combination with COBI (DRV/COBI 800/150 mg), with the brand name Prezcobix®, approved in 2015, and DRV/COBI/tenofovir alafenamide/emtricitabine (Symtuza®, 2018). In combination with other ARVs, it is indicated for the treatment of HIV-1 infection in adults.[34] DRV is chiefly metabolized by CYP3A4, with minor contribution by CYP2D6. Following oral administration, maximum plasma concentration is achieved within approximately 4 to 4.5 hours for DRV and 4 to 5 hours for COBI, under fed conditions.[35] The $t_{½}$ of DRV and COBI are 7 and 4 hours, respectively when administered together. Both DRV and COBI are bound to plasma protein, approximately 95% and 97% to 98%, respectively. Both drugs are eliminated via renal and non-renal pathways. Renal elimination accounts for 13.9% and 8.2% for DRV and COBI respectively. Non-renal elimination, mainly fecal, accounts for 79.5% and 86.2% for DRV and COBI respectively. DRV is both a substrate for and a modulator of P-gp. Some studies have demonstrated that DRV is an inhibitor, with P-gp inhibition similar to that of verapamil, a known moderate dual CYP3A4/P-gp inhibitor.[36,37] Other studies have also shown that it is capable of inducing P-gp.[38,39] COBI is strong dual inhibitor of CYP3A4 and P-gp. Subsequently, co-administration of DRV/COBI with substrates of CYP3A4 and P-gp may be of significant clinical concern.

Prior to the approval of Prezcobix®, DRV was coadministered with RTV (DRV/RTV) and COBI separately. In clinical trials and post-marketing surveillance of DRV/RTV, the most frequent AEs occurring in ≥5% of individuals included diarrhea, nausea, rash, headache, abdominal pain, and vomiting.[35] Long-term studies with co-formulated DRV/COBI have shown similar AEs to those observed with DRV/RTV and COBI alone.[35,40] In short-term PK studies, DRV/COBI has been well tolerated, with similar AEs to those reported in long-term clinical studies.[41,42] Mild-to-moderate rash occurred within the first four weeks of starting therapy, but resolved with continued administration.[35] Warnings with the use of DRV/COBI include drug-induced hepatitis (0.5%), liver failure (notably in patients with pre-existing liver dysfunction), severe skin reactions accompanied by fever and/or transaminase elevation (0.4%), Stevens-Johnson Syndrome (<0.1%), and elevation of SCr due to inhibition of tubular secretion by COBI.[35] This increase in SCr is not associated with an actual decrease in renal function. However, patients who experience an increase over 0.4 mg/dL from baseline should be monitored.

In the 3rd phase of this study, the combination product, DRV/COBI will be used.

Refer to section 8.1.3 (Potential Risks of Darunavir/Cobicistat) for more information on the side effects of DRV/COBI.

1.4 Scientific Rationale

COBI is a strong dual inhibitor of CYP3A4 and P-gp and is expected to increase the plasma concentrations of rivaroxaban. COBI is used to boost the plasma concentrations of protease inhibitors DRV and atazanavir, and integrase strand transfer inhibitor elvitegravir. To date, the impact of COBI, alone or in combination with other ARVs, on the PK of rivaroxaban has not been studied. Hence, the purpose of this study is to determine the magnitude of change in exposure of rivaroxaban when given alone and in combination with COBI and DRV/COBI in healthy volunteers, and to further determine if this change is clinically relevant to require a dose adjustment. In addition, the PK and PD of rivaroxaban will be characterized. DRV was chosen to be studied as it the preferred protease inhibitor per the DHHS ARV guidelines.[34] Elvitegravir is not an inhibitor of CYP3A4 nor P-gp, and is therefore not expected to interact with rivaroxaban, and thus will not be studied.

2 STUDY OBJECTIVES

2.1 Primary Objective

To characterize the PK of single-dose rivaroxaban when administered alone and in combination with COBI or DRV/COBI in healthy volunteers.

2.2 Secondary Objective

1. To characterize the PD of rivaroxaban alone and in combination with COBI or DRV/COBI in healthy volunteers.
2. To assess safety and tolerability of rivaroxaban in combination with COBI or DRV/COBI in healthy volunteers.

2.3 Exploratory Objective

To explore the potential influence, if any, of gene expression (genes associated with CYP450 enzymes and drug transporters) on the interaction between rivaroxaban and strong dual CYP3A4/P-gp inhibitors (COBI, DRV/COBI)

3 STUDY DESIGN

3.1 Description of the Study Design

This is an open-label, non-randomized, three-period, fixed sequence, drug-drug interaction PK study in healthy volunteers ≥18 years of age. The study will evaluate the impact of steady state concentrations of COBI and DRV/COBI on the PK and PD of rivaroxaban. A schematic of the study design is presented in Figure 1. This study will involve administering these drugs to 12 volunteers participating in 3 phases as follows.

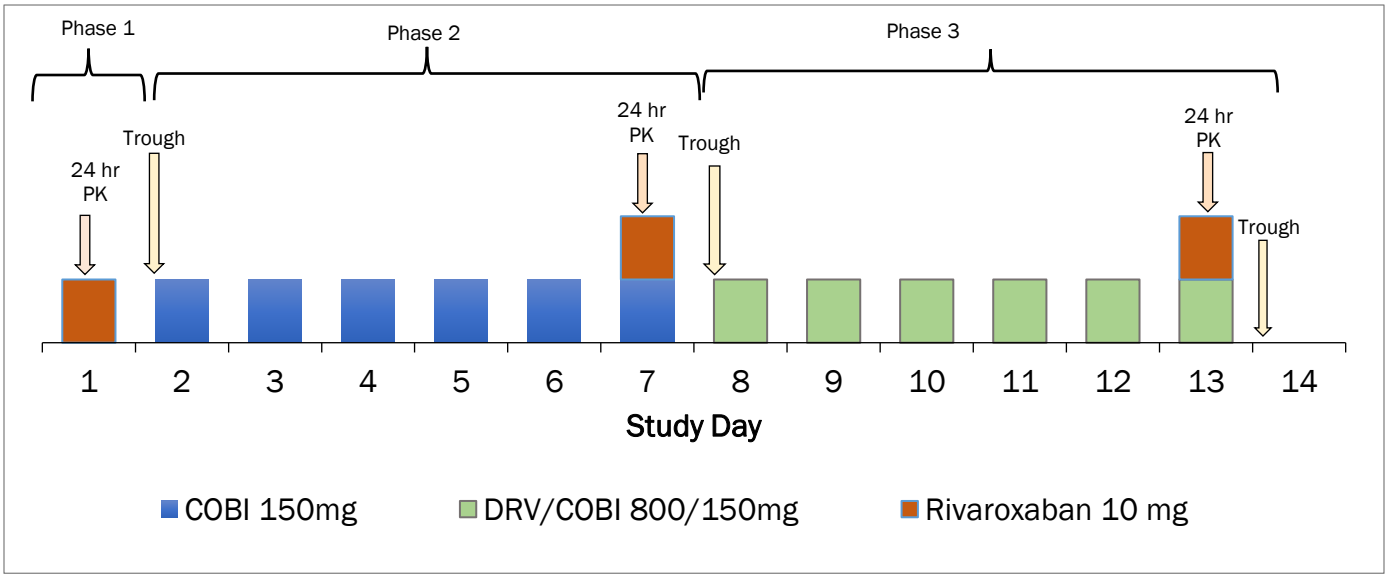
Rivaroxaban 10 mg PO, single dose (days 1, 7, 13), COBI 150 mg PO daily (days 2 to 7), DRV/COBI 800/150 mg PO daily (days 8 to 13).

Phase 1 consists of administration of rivaroxaban alone on day 1, with intense PK/PD sampling. Phase 2 consists of administration of COBI alone for a period of 5 days, then followed by co-administration of rivaroxaban and COBI on the 7th day in addition to intense PK/PD sampling. Administration of COBI alone for 5 days prior to co-administration with rivaroxaban allows for achievement of steady-state COBI plasma concentrations, maximum enzyme inhibition, and sufficient time for rivaroxaban to wash off. Enzyme inhibition is regarded as an almost instantaneous effect, with maximum enzyme inhibition observed after 2 days of dosing.[43]

Immediately following phase 2, subjects will be administered DRV/COBI in phase 3 for 5 days, then in combination with rivaroxaban on day 13 with intense PK/PD sampling. Transition from phase 2 to phase 3 does not require a wash-out period. Adequate time spacing between the single doses of rivaroxaban is necessary to ensure that the anticoagulant are sufficiently eliminated. The $t_{½}$ for rivaroxaban when administered alone is 5.7 to 16.3 hours when administered in combination with strong CYP3A4/P-gp inhibitors. As such, 5 half-lives (4 days) would be adequate for >97% drug elimination. DRV has a half-life of 7 hours when co-administered with COBI. It takes ~5 half-lives for a drug to achieve steady state. Thus, administering DRV/COBI for 6 days is sufficient to achieve steady state. Participants will undergo periodic serial PK blood draws on days 1, 7, and 13. Matched PD parameters will also be collected on the same days.

Rivaroxaban, when dosed orally once daily as per the FDA label, reaches t_{max} at 2 to 4 hours postdose. As such, frequent sampling is necessary to closely capture t_{max} value.

Figure 1: Study Schematic



Study Timeline

Screening: Days -89 to 0

Baseline visit: Days -6 to 0

PK visits: Days 1, 7, 13

- Blood samples for plasma rivaroxaban PK: at times 0 (predose), 1, 2, 3, 4, 6, 8, 10, and 24 hours postdose. Rivaroxaban is dosed once a day in subjects. It has linear PK properties, which can also predict PK parameters in multiple dosing.
- Safety labs drawn with predose sample on days 1, 7, and 13

End-of-study-visit: 7±3 days after administration of final study dose

Total study duration (excluding screening): 17 to 23 days

3.2 Study Endpoints

3.2.1 Primary Endpoints

The following are the primary endpoints for rivaroxaban:

- $AUC_{0-\infty}$,
- AUC_{0-24},
- C_{max},
- t_{max},
- $t_{½}$,
- CL/F,
- V/F,
- C_{min}.

3.2.2 Secondary Endpoints

- The PD parameters of interest are:
  - $AUEC_{0-24}$ for FXa, aPTT, and PT/INR
  - ER_{max} for FXa, aPTT, and PT/INR
- AEs and abnormal laboratory values, as graded according to the Division of AIDS (DAIDS) AE and the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials AE table (total bilirubin only).

4 STUDY POPULATION

4.1 Rationale for Subject Selection

Healthy volunteers will be studied, as opposed to patients with HIV infection, in order to eliminate the influence of confounding variables (medications, HIV infection, etc.) on our study results. In addition, HIV patients are often on concomitant medications, which may interact with the study drugs being examined, and thus the final interpretation of study results could be affected.

4.2 Recruitment Plan

This study is being conducted at a single site, the National Institutes of Health (NIH) Clinical Center (CC). Up to 40 participants will be screened for a total of up to 12 healthy, HIV-negative volunteers to enroll and complete this open-label study. Participation of all ethnic groups and genders will be actively encouraged. This will be done through the Office of Patient Recruitment, which recruits participants for volunteer studies conducted at the NIH CC. There is an active effort to recruit minorities and women through outreach programs in the Washington, DC metropolitan area. This study is not designed to assess the influence of gender, age, and/or ethnicity on the drug-drug interaction (if observed) between oral anticoagulants (rivaroxaban) and COBI or DRV/COBI.

4.2.1 Recruitment of NIH Staff or Family Members of Study Team Members

NIH staff and family members of the study team may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to participate in the research will have an effect, either beneficial or adverse, on the participant's employment or position at the NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable and unidentifiable manner.

The NIH investigator will provide and request that the NIH staff member review the *Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* and the *Leave Policy for NIH Employees Participating in NIH Medical Research Studies (NIH Policy Manual 2300-630-3)*. Considerations for consent of staff is described in section 16.1.1.

4.3 Subject Inclusion Criteria

A subject will be considered eligible for this study only if all of the following criteria are met:

1. Adults between the ages of 18 to 65 years.
2. Body mass index between 18 to 30 kg/m^2.
3. Judged to be healthy based on medical history, physical examination, vital signs and clinical laboratory tests (liver function tests (LFTs: alanine transaminase [ALT], aspartate transaminase (AST), total bilirubin ≤ upper limit of normal [ULN] (with the exception of participants with Gilbert's syndrome); albumin within normal limits (WNL)], eGFR>90 mL/min/1.73 m^2, PLT>150,000/µL, hemoglobin (Hgb)≥12 g/dL, aPTT≤ULN, PT≤ULN, INR≤ULN.
4. Subject agrees to storage of specimens for future research.
5. Negative serum or urine pregnancy test for females of child-bearing potential.
6. For female subjects, willing to avoid pregnancy by (a) practicing abstinence or (b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant during the study period. (baseline visit up to end of study day 20±3)
7. Willing to avoid engaging in activities such as contact sports, including extreme sports, that may increase the risk of bleeding through body injury or bruising, during the study period (baseline visit up to end of study day 20±3)
8. Willingness to forgo drinking alcohol during the study period (baseline visit up to end of study day 20±3)
9. Able to provide consent.

4.4 Subject Exclusion Criteria

A subject will be ineligible for this study if one, or more, of the following criteria are met:

1. HIV infection, as determined by standard serologic or virologic assays for HIV infection.
2. Laboratory evidence of active or chronic hepatitis A, B or C infection.
3. History or presence of any of the following:
   a. any major medical conditions that requires daily frequent medication or potentially impairs medication absorption, metabolism and elimination
   b. any other condition that may interfere with the interpretation of the study results, or not be in the best interest of the subject in the opinion of the Investigator.
4. Current participation in an ongoing investigational drug protocol or use of any investigational drug within 30 days (based on last dose received) prior to receipt of any study drugs/medications.
5. History or presence of the following:
   a. bleeding/hematologic disorders (e.g., anemia, hemophilia, etc.),

b. serious/major bleeding event (intracranial, gastrointestinal (GI), as assessed by subject interview), or
6. current increased risk of bleeding
7. for female subjects, menorrhagia
8. Planned invasive or surgical procedure within (prior to or following) 28 days of study participation.
9. Therapy with any prescription, over-the-counter, herbal, or holistic medications, including hormonal contraceptives by any route, within 5 half-lives of the agent prior to receipt of any study medications will not be permitted with the following exception: Intermittent or short-course therapy (<14 days) with prescription, vaccines or over-the-counter medications will be reviewed by investigators on a case-by-case basis for potential drug interactions.
10. Inability to obtain venous access for sample collection.
11. Inability to swallow whole capsules and/or tablets.
12. Pregnant female.
13. Breastfeeding female.
14. The presence of persistent diarrhea or malabsorption that could interfere with the subject's ability to absorb drugs.
15. Illicit drug or alcohol use
16. Use of nicotine-containing tobacco products, including cigarettes, vaping and chewing tobacco.
17. Known hypersensitivity to rivaroxaban, COBI, or DRV.
18. History of documented hypersensitivity or allergy to sulfa.
19. Organ transplant recipient.

Co-enrollment guidelines: Co-enrollment in other trials is restricted, other than enrollment on observational studies. Study staff should be notified of co-enrollment as it may require the approval of the investigator.

4.5 Justification for Exclusion of Special Populations

4.5.1 Exclusion of Pregnant Women

Pregnant people are excluded from this study to reduce the confounding effects of physiological changes during pregnancy on PK parameters of interest. Pregnant women may also develop anemia as a result of frequent PK blood draw. Furthermore, the effects of rivaroxaban on the developing human fetus have not been well established. There are no adequate and well-controlled studies of rivaroxaban in pregnant women. Animal studies show that rivaroxaban crosses the placenta.[22]

Prezcobix is not recommended during pregnancy due to substantially lower exposures of DRV and COBI during pregnancy. In a clinical trial of 7 pregnant women receiving Prezcobix in combination with a background regimen, there were no new clinically relevant safety findings compared with known safety profile of Prezcobix in HIV-1 infected adults. There is insufficient data with Prezcobix in pregnant individuals from the Antiretroviral Pregnancy Registry (APR).

DRV/RTV in combination with a background regimen is one of the recommended HIV regimens for pregnant women at a dose of DRV/RTV 600/100 mg BID or 800/100 mg once daily (QD), depending on certain clinical scenarios. Following exposure of darunavir-containing regimens during pregnancy, there was no difference in rate of overall birth defects for darunavir (2.7%)

compared with the background rate for major defects of 2.7% in a U.S reference population of the Metropolitan Atlanta Congenital Defects Program. Studies in animals did not show evidence of embryotoxicity or teratogenicity.

There are insufficient numbers of pregnancies with exposure to cobicistat that have been reported to the APR to estimate the rate of birth defects. In pregnant rats, increases in post-implantation loss and decreased fetal weights were observed at a maternal toxic dose of 125 mg/kg/day of COBI, while no malformations were noted at doses up to 125 mg/kg/day of COBI. When COBI was administered to pregnant rabbits, no maternal or embryo/fetal effects were noted at 100 mg/kg/day, which was a 3.8-times higher systemic exposure than human exposures at the recommended daily dose of COBI.

4.5.2 Exclusion of Breastfeeding Women

Because there is an unknown but potential risk for AEs in nursing infants secondary to exposure of the mother with rivaroxaban, breastfeeding participants are excluded from study participation.

4.5.3 Exclusion of Children and Persons >65 Years-old

This study intends to investigate the PK/PD properties and safety profiles of agents in an adult population, as opposed to children, as age-related metabolic and transport processes in children differ from those of adults. The safety and effectiveness of rivaroxaban has not been established in the pediatric population.[22] Additionally, there is greater than minimal risk, with no benefit in the pediatric population.

As in children, changes in metabolic processes as a result of aging may confound findings from participants older than 65 years. As this is a preliminary study, there is no potential benefit as a population to justify their participation. However, future investigators may include this population as well.

4.5.4 Exclusion of Adults Who Cannot Consent

Adults who are unable to provide informed consent are excluded from enrolling in this study. If a participant permanently loses the capacity to provide ongoing consent, then they will be withdrawn from the study (section 12.9).

5 STUDY AGENT/INTERVENTIONS

5.1 Disposition, Dispensation and Accountability

Study agents will be distributed and accounted for by the NIH pharmacy according to standard pharmacy procedures.

5.2 Formulation, Packaging and Labeling

Each bottle will be individually labeled with the patient identification number, dosing instructions, recommended storage conditions, the name and address of the manufacturer, and that the agent should be kept out of reach of children.

5.3 Assessment of Participant Compliance with Study Agents

Participants will receive a memory aid card to log their doses of COBI and DRV/COBI, as well as to record AEs between study visits. This memory aid card will not be used as a source document. Each participant will receive telephone contact to remind them of appropriate

adherence and/or of upcoming appointments. Additionally, participants will be instructed to bring all study agent bottles to each study visit for pill count.

5.4 Study Agent Storage and Stability

All study agents should be stored and dispensed from the NIH CC Pharmacy according to standard pharmacy procedures.

5.5 Preparation, Administration and Dosage of Study Agents

Participants will receive once-daily dose of COBI (Tybost) 150 mg for 6 days (days 2 to 7) and once-daily dose of DRV/COBI (Prezcobix) 800/150 mg for 6 days (days 8 to 13). Participants will also receive single doses of rivaroxaban (Xarelto) 10 mg on days 1, 7, and 13 at the day hospital.

Each tablet of Xarelto (Janssen Pharmaceuticals, Inc [Titusville, New Jersey]) contains 10 mg of rivaroxaban. Participants will be given rivaroxaban with food at 0800±2 hours on days 1, 7, and 13 at the day hospital.

Each tablet of Tybost (Gilead Sciences, Inc [Foster City, CA]) contains 150 mg of COBI. Participants will be instructed to take one tablet daily at the same time (0800±2 hours) every day with food at home on non-PK/PD days. At the 24-hour PK visit day, participants will be instructed to bring their study agent supply to the NIH Day Hospital or OP-8 Infectious Diseases Clinic.

Each tablet of Prezcobix (Janssen Pharmaceuticals, Inc [Titusville, New Jersey]) contains 800 mg of DRV and 150 mg of COBI. Participants will be instructed to take one tablet daily at the same time (0800±2 hours) every day with food at home on non-PK/PD days. At the 24-hour PK visit day, participants will be instructed to bring their study agent supply to the NIH Day Hospital or OP-8 Infectious Diseases Clinic.

5.6 Concomitant Medications and Procedures

All concomitant prescription and nonprescription (including over-the-counter [OTC]) medications taken during study participation will be recorded in the Clinical Research Information Management System of the NIAID (CRIMSON). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

5.7 Prohibited Medications and Procedures

To minimize potential drug interactions or AEs in healthy volunteers, therapy with any prescription, OTC, herbal, or holistic medications, except for occasional use of acetaminophen (no more than 2 g/24 hours), loperamide, or antihistamines (on non-PK/PD days), will not be permitted throughout the study period (baseline visit up to end of study visit Day 20±3) unless discussed with and approved by the principal investigator (PI). In addition, subjects are not allowed to drink alcohol during the study period (baseline visit up to end of study visit Day 20±3).

6 STUDY SCHEDULE

A flowchart of the study schedule is provided in Table 2. A table of the study schedule is provided in Appendix B, blood volumes are listed in Appendix C, and study initiation calendars in Appendix D.

Table 2: Study Schedule and Procedures

	Screening (Days -89 to 0) • Signing of informed consent • Vital signs • Medical/medication history • Physical examination • Laboratory tests (including HIV testing, viral markers for hepatitis screen [hepatitis B surface antigen, anti-hepatitis C antibody, anti-hepatitis A IgM], serum or urine pregnancy test for females of child-bearing potential, a basic chemistry [acute care panel], hepatic panel, mineral panel, complete blood count with differential [CBC/diff], aPTT, PT/INR) • Blood draws and serum or urine pregnancy test may be repeated as clinically indicated at the discretion of the medically responsible investigator within 14 days of initial screening • Counsel female subjects on ways to avoid pregnancy by a) practicing abstinence or b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant
	Baseline (Days -6 to 0) • Medical/medication history • Vital signs • Physical examination • Safety labs[a] (if not within 6 days of screening) • Serum or urine pregnancy test (if of child-bearing potential, if not within 6 days of screening) • Counsel female subjects on ways to avoid pregnancy by a) practicing abstinence or b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant
Phase 1	**Day 1: Serial 24-hr PK/PD sampling**[b,c] • Vital signs • Serum or urine pregnancy test (if of child-bearing potential, which must be negative to proceed with the study) • Counsel female subjects on ways to avoid pregnancy by a) practicing abstinence or b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant • Medical/medication history • Eat a standardized breakfast • Insertion of intravenous (IV) catheter • Administer rivaroxaban 10 mg with 240 mL of water at 0800 (±2 hrs) • Serial 24-hr blood PK/PD samples • Safety labs[a] • Assessment of AEs

	• Dismiss from NIH CC following 10-hr blood draw and removal of IV catheter • Counsel subjects not to engage in activities that would increase the risk of bleeding, such as contact sports • Participant will be provided the option to stay overnight or to return the next day for the 24-hr blood draw.
Phase 2	**Day 2: 24-hr trough and COBI dosing** • Return to NIH CC for 24-hr postdose PK sample • Medical/medication history • Vital signs • Blood sample for pharmacogenomic testing • Administer COBI 150 mg at 0800 (±2 hrs) with a well-balanced meal after collection of 24-hr PK/PD sample • Assessment of AEs • COBI 150 mg, 6-day supply (9 tablets) dispensed from NIH CC Pharmacy (+3 tablets to allow for minor scheduling variances) • Receive memory aid log (to serve as reminder of dosing schedule, and document of self-reported AEs) • Counsel female subjects on ways to avoid pregnancy by a) practicing abstinence or b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant
	Day 2 – 6: COBI dosing days • COBI 150 mg once daily at 0800 (±2 hrs) with food • Administration recorded on the provided dosing log
	Day 7: Serial 24-hr PK sampling[b] • Vital signs • Serum or urine pregnancy test (if of child-bearing potential, which must be negative to proceed with the study) • Counsel female subjects on ways to avoid pregnancy by a) practicing abstinence or b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant • Medical/medication history • Review memory aid card and conduct COBI pill count • Eat a standardized breakfast • Insertion of IV catheter • Administer rivaroxaban 10 mg COBI 150 mg with 240 mL of water at 0800 (±2 hrs) • Serial 24-hr blood PK/PD samples • Safety labs[a] • Assessment of AEs • Collect any remaining COBI and return to NIH CC Pharmacy for discard • Dismiss from NIH CC following 10-hr blood draw and removal of IV catheter

	• Counsel subjects not to engage in activities that would increase the risk of bleeding, such as contact sports • Participant will be provided the option of staying overnight or to return the next day for the 24-hr blood draw
Phase 3	**Day 8: 24-hr trough and DRV/COBI dosing** • Return to NIH CC for 24-hr postdose PK/PD sample • Medical/medication history • Vital signs • Administer DRV/COBI 800/150 mg at 0800 (±2 hrs) with a well-balanced meal after collection of 24-hr PK/PD sample • Assessment of AEs • DRV/COBI 800/150 mg, 6-day supply (9 tablets) dispensed from NIH CC Pharmacy (+3 tablets to allow for minor scheduling variances) • Counseling female subjects on ways to avoid pregnancy by a) practicing abstinence or b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant
	Day 8 – 12: DRV/COBI dosing days • DRV/COBI 800/150 mg once daily at 0800 (±2 hrs) with food • Administration recorded on the provided dosing log
	Day 13: Serial 24-hr PK sampling[b] • Vital signs • Serum or urine pregnancy test (if of child-bearing potential, which must be negative to proceed with the study) • Counsel female subjects on ways to avoid pregnancy by a) practicing abstinence or b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant • Medical/medication history • Review memory aid card and conduct DRV/COBI pill count • Eat a standardized breakfast • Insertion of IV catheter • Administer rivaroxaban 10 mg and DRV/COBI 800/150 mg with 240 mL of water at 0800 (±2 hrs) • Serial 24-hr blood PK/PD samples • Safety labs[a] • Assessment of AEs • Collect any remaining DRV/COBI and return to NIH CC Pharmacy for discard • Dismiss from NIH CC following 10-hr blood draw and removal of IV catheter • Counsel subjects not to engage in activities that would increase the risk of bleeding, such as contact sports • Participant will be provided the option to stay overnight or to return the next day for the 24-hr blood draw

	Day 14: 24-hr trough • Collection of 24-hr postdose PK/PD sample • Medical/medication history • Vital signs • Assessment of AEs • Counsel female subjects on ways to avoid pregnancy by a) practicing abstinence or b) using effective non-hormonal and/or barrier methods of birth control, as well as avoid breast feeding or providing breast milk to infant
	End of Study Visit (Day 20 ±3 days) • Medical/medication history • Vital signs • Physical examination • Safety labs • Assessment of AEs

[a] Safety labs will be collected at baseline, with the time 0 (predose) PK/PD sample (days 1, 7, and 13) and at the end of study visit. Assessments will include serum or urine pregnancy test for females of child-bearing potential; a basic chemistry (acute care panel including Na, K, CO_2, anion gap, BUN, SCr, eGFR, glucose, albumin), hepatic (including AST, ALT, alkaline phosphatase, total bilirubin, direct bilirubin), and mineral panel (Ca, Mg, Phos) and complete blood count with differential [WBC, RBC, Hgb, HCT, PLTs, differential]. PD measurements will be time matched with PK collection for both the purpose of PK correlation as well as safety assessment on days 1, 2, 7, 8, 13, and 14. PD measures include anti-factor Xa, aPTT, and PT/INR. Additional safety labs will be assessed at the discretion of the medically responsible investigator, without additional blood draw.

[b] 24-hr plasma PK/PD sampling times will occur at the following times: 0 (predose), 1, 2, 3, 4, 5, 6, 8, 10, and 24 (±2) hours postdose. The participant will be discharged after collection of the 10-hour postdose sample, to then return for the 24-hour postdose sample collection the following day. PD blood samples will be collected at the same times as PK samples, but in a separate tube.

[c] Day 1 will always be scheduled for a Wednesday or Thursday to ensure that other study visits also occur on weekdays

6.1 End-of-Study Visit (7±3 days after administration of final study dose)

Participants will return to the NIH CC for a final follow-up visit 7 (±3) days after the end of final study drug. AEs will be assessed, vital signs measured, and blood for safety labs. Participants who can become pregnant will have a serum or urine pregnancy test. Participation ends after this visit.

6.2 Early Termination Visit

If participants terminate the study early, they will be asked to return for drawing end-of-study labs (7±3 days after the final dose of any study drug[s]) and will receive a physical exam and AE assessment.

6.3 Pregnancy and Follow-up Visit

Participants who become pregnant while on study will discontinue study agents and procedures and will be instructed to follow-up with their physician for prenatal care; they will also be followed to delivery for purposes of safety monitoring for this study.

7 STUDY PROCEDURES/EVALUATIONS

Blood draw: The amount of blood drawn for research purposes will be within the limits allowed for adult research participants by the NIH CC (Medical Administrative Policy 95-9, Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf). Blood samples will be used for the following:

- Screening labs (see section 6).
- 24-hour plasma PK/PD of rivaroxaban
- Pregnancy testing
- Safety labs: safety panel (acute care, hepatic and mineral), CBC/diff. These safety labs are the same as the safety labs listed in section 6, Study schedule (Table 2)
    - Results of safety lab assessments will be reviewed as they become available, with appropriate clinical follow-up through resolution as necessary for any abnormal results.

Pharmacogenomics: A single blood sample (10 mL) on day 2 will be collected for pharmacogenomic testing (targeted sequencing).

Urine: Urine samples will be used for pregnancy testing.

8 POTENTIAL RISKS AND BENEFITS

8.1 Potential Risks

Potential research-related risks to study participants include adverse effects of study medications, alone or in combination (rivaroxaban, COBI and/or DRV/COBI) and AEs associated with blood collection by venous catheter insertion or venipuncture in the face of anticoagulation.

8.1.1 Rivaroxaban

In clinical trials and post-marketing surveillance, the most frequent AEs with rivaroxaban were bleeding complications.[22] In the ROCKET-AF trial, whereby 7111 subjects with nonvalvular AF received rivaroxaban (15 mg or 20 mg orally [PO] QD) to reduce the risk of stroke and systemic embolism, the bleeding rate (percent per year) was 3.6% for major bleeding (defined as overt bleeding associated with a decrease in Hgb≥2g/dL, a transfusion ≥2 units of packed red blood cells or whole blood, bleeding at a critical site, or with a fatal outcome) compared to 3.5% with warfarin. Among the major bleeding categories, GI bleeding rate (upper GI, lower GI, and rectal bleeding) was the highest in rivaroxaban group (2%) compared to warfarin group (1.2%). The risk of bleeding was increased in subjects who were ≥75 years and those with reduced CrCl (<50 mL/min). Drug discontinuation due to bleeding events was higher in the rivaroxaban group than in warfarin group (4.3% vs. 3.1%). The incidence of discontinuation for non-bleeding adverse events was similar in both treatment groups.

In EINSTEIN trial, whereby 6962 subjects received rivaroxaban (15 mg PO BID×21 days, then 20 mg PO QD) to reduce the risk of recurrence of DVT and/or PE, major bleeding events occurred in 1% of subjects in the rivaroxaban group compared to 1.7% in the enoxaparin/VKA group. Other bleeding categories include clinically relevant non-major bleeding of 8.6% (compared to 8.7% with enoxaparin/VKA). For any bleeding event, 28.3% was reported in rivaroxaban group versus 28% in enoxaparin/VKA group.

Drug discontinuation due to bleeding events was higher in rivaroxaban group than in enoxaparin/VKA group (1.7% vs. 1.5%).

In the RECORD trial (Prevention of Venous Thromboembolic Events in Patients Undergoing Major Orthopedic Surgery of the lower Limbs), whereby 4487 subjects received rivaroxaban (10 mg PO QD) for prophylaxis of DVT following hip or knee replacement surgery, any bleeding event was reported in 5.8% of subjects (compared to 5.6% in enoxaparin group), with major bleeding events reported at 0.3%.

Rivaroxaban should be discontinued at least 24 hours before invasive/surgical procedures. In case of overdose, there is the potential of hemorrhagic complications. Andexanet alfa, a specific anti–factor Xa agent for the reversal of rivaroxaban activity is available.[21] In PK studies (doses ranging from 2.5 to 80 mg), rivaroxaban was safe and tolerable.[26,27] In a food effect PK study, of the 24 healthy subjects who received rivaroxaban 10 mg PO×1, 10 subjects experienced treatment-related AEs. All drug related AEs were of mild intensity (subcutaneous bleeding, headaches, pain in both knees, neck pain, epistaxis), with exception of two moderate AEs (drug-related headache and non–drug-related lipase elevation). In the same study, in subjects who received rivaroxaban 20 mg PO×1, 9/23 subjects experienced treatment-related AEs of mild intensity. All treatment-related AEs were resolved by the end of the study.

The risk of bleeding will be minimized to subjects in the current investigation, as subjects will receive rivaroxaban 10 mg as 3 separated single doses.

(Refer to Appendix E for Xarelto® prescribing information.)

8.1.2 Cobicistat

COBI is generally well tolerated. In clinical trials, the most frequent adverse reaction after prolonged administration of COBI, albeit in conjunction with other ARV medications, were GI events (nausea, diarrhea).[44(p2),45,46] COBI has been show to increase SCr (mean change 0.14±0.13 mg/dL), usually within the first 2 weeks of therapy, due to the inhibition of tubular secretion of creatinine; glomerular function is not believed to be affected.[45–47] Patients who experience a confirmed increase in SCr>0.4 mg/dL from baseline should be closely monitored.[30] In PK studies, COBI was tolerable.[41,47,48] Most AEs judged to be related to study medications were mild to moderate. Three exceptions include: a single subject who reported left upper extremity discoordination (grade 3) during daily doses of COBI, a single subject who reported rash (grade 3) during daily doses of COBI with DRV, and a single subject who reported a severe rash during daily doses of COBI with atazanavir.[41,44,47] All AEs resolved upon discontinuation of study drug. Commonly reported AEs included nervous (headache, somnolence, abnormal dreams) and integumentary (rash) system AEs, which resolved by the completion of the study. Because subjects in the current investigation will receive COBI limited to 6 days, the risk to subjects is expected to be slightly above minimal.

(Refer to Appendix F for Tybost® prescribing information.)

8.1.3 Darunavir/Cobicistat

The fixed-dose tablet combination of DRV and COBI was FDA-approved in January 2015.[35] Prior to approval, DRV coadministered with RTV (DRV/RTV) and COBI had been used separately in the clinical setting. In clinical trials and post-marketing surveillance of DRV coadministered with RTV, the most frequent AEs occurring in ≥5% of individuals were diarrhea (9%), headache (7%), rash (6%), and abdominal pain (6%).[35] Long-term studies with the coformulation of DRV/COBI have shown similar AEs to those observed with DRV/RTV and COBI alone.[35,40] In short-term PK studies, DRV/COBI has been well tolerated, with similar AEs to those reported in long-term clinical studies.[41,42] Mild-to-moderate rash usually occurs within the first four weeks of starting therapy, but typically resolves with continued administration.[35] Warnings with the use of DRV/COBI include drug-induced hepatitis (0.5%), liver failure (notably in patients with pre-existing liver dysfunction), severe skin reactions accompanied by fever and/or transaminase elevation (0.4%), Stevens-Johnson Syndrome (<0.1%), and elevation of SCr due to inhibition of tubular secretion by COBI.[35] This increase in SCr is not associated with an actual decrease in glomerular renal function. However, patients who experience an increase over 0.4 mg/dL from baseline should be monitored. Additionally, patients with a known sulfa allergy should be monitored after initiating DRV/COBI as DRV contains a sulfonamide moiety. Incidence of rash between subjects with and without a history of sulfonamide allergy was similar in clinical trials. Other serious AEs associated with long-term use of protease inhibitors include new onset of diabetes mellitus, exacerbation of pre-existing diabetes mellitus, hyperglycemia, fat redistribution, and increased bleeding in patients with hemophilia.

The above-mentioned serious reactions are reported in patients on chronic therapy after 24 to 96 weeks on daily dosing of DRV/COBI. These patients are infected with HIV-1, are on other drugs that may contribute to the serious reactions, and often have other co-morbidities. As subjects in this study will be healthy volunteers and exposure to DRV/COBI will be limited to 6 days, the risk to subjects is expected to be slightly above minimal. The first dose of DRV/COBI will be administered by a nurse under direct observation, and subjects will be observed for 0.5 to 1 hour, should any immediate reactions occur. In addition to assessing AEs during PK/PD days, close monitoring will be provided through phone calls in between PK/PD study visits, as well as safety follow-up (7±3 days after last study drug).

(Refer to Appendix G for Prezcobix® prescribing information.)

8.1.4 Blood draw and IV catheter

The risks of drawing blood include pain, bruising, bleeding, and, rarely, fainting or infection. Insertion of an IV catheter for collection of blood can also result in inflammation of the skin and vein.

8.1.5 Pharmacogenomic testing

There are no foreseeable risks with pharmacogenomic testing. Results of pharmacogenomic testing will not be clinically validated or become a part of the participant's medical record at the NIH, and will not be returned to participants. Research records containing this information are maintained in a secure manner as described in section 16.2.

8.2 Potential Benefits

This is not a therapeutic trial; therefore, study participants will not experience direct benefits from their participation. However, the results of this study may help in the care of patients receiving these or similar medications in the future.

9 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS, AND DATA

9.1 Intended Use

Samples, specimens, and data collected under this protocol will be used to assess plasma concentrations of rivaroxaban. PD markers in plasma of these drugs will also be assessed. Genetic testing will be performed. Method development for drug assays (rivaroxaban) and sample processing will be performed by the Clinical Pharmacokinetics Research Unit (CPRU) lab. Assay for PD markers will be performed by the Department of Laboratory Medicine (DLM) here at NIH.

9.2 Storage

Samples collected for PK assessments will be stored at −80°C in a locked freezer in the CPRU lab. Other samples collected under this study may be stored at the National Cancer Institute at Frederick Central Repository and at DLM. Samples and data will be stored using codes assigned by the investigators or their designees. All stored computer data will be password protected. Only investigators will have access to the samples and data.

9.3 Tracking

Samples acquired under this protocol will be tracked using a database located on a password-protected computer. Data will be stored and maintained in CRIMSON database.

9.4 Disposition at the Completion of the Protocol

In the future, other investigators (both at NIH and outside) may wish to use these samples and/or data for research purposes. If the planned research falls within the category of "human subjects research" on the part of the NIH researchers, IRB review and approval will be obtained. This includes the NIH researchers sending out coded and linked samples or data and getting results that they can link back to their subjects.

9.5 Loss or Destruction

Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of a reportable event will be reported to the NIH IRB according to HRPP Policy 801.

Additionally, participants may decide at any point not to have their samples stored. In this case, the PI will destroy all known remaining samples and report what was done to both the participant and to the IRB. This decision will not affect the individual's participation in this protocol or any other protocols at NIH.

10 DATA SHARING PLAN

Human data generated in this study will be shared for future research as follows:

- De-identified data in an NIH-funded or approved public repository.

- Identified data in the Biomedical Translational Research Information System (BTRIS, automatic for activities in the CC).
- De-identified or identified data with approved outside collaborators under appropriate agreements.
- Through publications and/or public presentations.

Data will be shared before, at the time of or shortly after publication.

This study is not expected to generate the amount of genetic data that triggers the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research.

11 REMUNERATION PLAN FOR PARTICIPANTS

Participants will be compensated for their time and inconvenience per study visit as described in the following table:

Table 3: Compensation Schedule

Compensation schedule	
Screening	$50
Baseline	$50
Days 1-2	$400
Days 7-8	$400
Days 13-14	$400
Day 20	$50
Total	**$1350**

If the participant requires additional clinical follow-up outside of scheduled study visits based on medical advisory investigator discretion, then the participant may be compensated an amount consistent with NIH CC policies and guidelines for additional follow-up visits/procedures. Subjects who do not complete all study procedures will receive compensation that has been prorated based on the extent of their participation. Volunteers who complete all study procedures will be compensated a maximum total of $1350. Payment will be issued after the final study visit in each arm. Travel and/or lodging expenses may be provided as per PI discretion.

12 ASSESSMENT OF SAFETY

Information regarding expected side effects from the study drugs is taken from the manufacturers' package inserts and published studies. The risk to volunteers is considered to be slightly above minimal since exposure to rivaroxaban will be limited to 3 single doses; exposure to COBI and DRV/COBI will be limited to 6 days each respectively. The protocol does involve blood drawing.

12.1 Toxicity Scale

The Investigator will grade the severity of each AE according to the "DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" most recent Version 2.1, July 2017, which can be found at: https://rsc.tech-res.com/docs/default-source/safety/division-of-aids-(daids)-table-for-grading-the-severity-of-adult-and-pediatric-adverse-events-corrected-v-2-1.pdf?sfvrsn=2.

Some Grade 1 lab parameters on the DAIDS Toxicity Table fall within the NIH lab reference range for normal values. These normal values will not be reported as Grade 1 AEs.

Total bilirubin will be graded according to the "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials", as indicated in the table below. The full table can be accessed through the following link: http://www.fda.gov/downloads/BiologicsBloodVaccines/ucm091977. Only the total bilirubin measure (not direct or indirect bilirubin individually) will be used to determine whether pausing rules have been met. Direct and indirect bilirubin measures will still be collected as AEs.

Table 4: Toxicity Grading Scale for Total Bilirubin, According to the "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Trials"

Serum Test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Total bilirubin – when accompanied by any increase in LFTs (eg, AST, ALT) increase by factor.	1.1-1.25 x ULN	1.26-1.5 x ULN	1.51-1.75 x ULN	> 1.75 x ULN
Total bilirubin – when LFTs (eg, AST, ALT) are all normal; increase by factor.	1.1-1.5 x ULN	1.6-2.0 x ULN	2.0-3.0 x ULN	> 3.0 x ULN
ALT = alanine transaminase; AST = aspartate transaminase; LFT = liver function test; ULN = upper limit of normal.				

All other laboratory and clinical AEs that occur in a subject will be assessed for severity and classified into one of the categories below:

- Grade 1 (Mild): Event requires minimal or no treatment and does not interfere with the subject's daily activities.
- Grade 2 (Moderate): Event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Grade 3 (Severe): Event interrupts a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Grade 4 (Life threatening): Any adverse drug experience that places the subject or participant, in the view of the Investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.
- Grade 5 (Death)

12.2 Recording/Documentation and Reporting Adverse Events

At each contact with the participant, starting from the time the informed consent form is signed through the end of the final study visit, information regarding AEs will be elicited by appropriate questioning and examinations. All events, both expected/unexpected and related/unrelated, will be immediately documented in the subject's medical record/source document, recorded in CRIMSON, and reported as outlined below. Source documents will include progress notes, laboratory reports, consult notes, phone call summaries, survey tools, and data collection tools.

Source documents will be reviewed in a timely manner by the research team. All reportable AEs that are identified will be recorded in CRIMSON. The start date, the stop date, the severity of each reportable event, and the PI's judgment of the AE's relationship and expectedness to the study agent/intervention will also be recorded in CRIMSON. If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

12.3 Reporting Procedures

12.3.1 Investigator Assessment of Adverse Events

The medical advisory investigator (MAI) and the PI will assess all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade*)* [using the specified toxicity tables] and **Causality** (relationship to study agent and relationship to research) according to the following guidelines.

12.3.1.1 Causality

Causality: likelihood that the event is caused by the study agent(s) will be assessed considering the factors listed under the following categories:

- Definitely related
    - reasonable temporal relationship
    - follows a known response pattern
    - clear evidence to suggest a causal relationship
    - there is no alternative etiology
- Probably Related
    - reasonable temporal relationship
    - follows a suspected response pattern (based on similar agents)
    - no evidence of a more likely alternative etiology
- Possibly Related
    - reasonable temporal relationship
    - little evidence for a more likely alternative etiology
- Unlikely Related
    - does not have a reasonable temporal relationship
      OR
    - good evidence for a more likely alternative etiology
- Not Related
    - does not have a temporal relationship

      OR

    - definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

12.3.2 Reporting to the Medical Monitor

A medical monitor (MM) has been appointed for oversight of safety in this clinical study (see Safety Oversight, section 13.)

12.3.3 Reporting Procedures to the NIH IRB

- **Assessment of Safety**

AEs and other reportable events are defined in Policy 801: Reporting Research Events.

- **Reporting Procedures**

Unanticipated problems, non-compliance, and other reportable events will be reported to the NIH IRB according to Policy 801.

- **Reporting to the NIAID Clinical Director**

The PI will report UPs, major protocol deviations, and deaths to the NIAID Clinical Director according to institutional timelines.

12.4 Pregnancy

Although pregnancy itself is not an AE, events occurring during pregnancy, delivery, or in the neonate (e.g., congenital anomaly/birth defect) may be AEs or SAEs.

In the event a participant or female partner of a participant becomes pregnant, prenatal care will not be provided by the study and the following steps will be taken:

- Discontinue the study agents and procedures but continue to follow-up until delivery for safety.
- Enroll in ARV pregnancy registry (APR).
- Report to the MM and the IRB as required.
- Advise research participant to notify the obstetrician of study participation and study agent exposure.

12.5 Type and Duration of the Follow-up of Participants after Serious Adverse Events

SAEs that have not resolved by the end of the follow-up period will be followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the participant is lost to follow-up), then the reason a final outcome could not be obtained will be recorded by the investigator in CRIMSON.

12.6 Pausing Rules for An Individual Participant

Pausing is the suspension of administration of study agent to a single participant. The pausing criteria for a single participant in this study include any of the following:

- A participant experiences a ≥Grade 3 AE that is possibly, probably, or definitely related to a study agent
- A participant experiences a Grade 2 AE of transaminase elevations that is possibly, probably, or definitely related to the study agents.
- Any safety issue that the site investigator determines should pause administration of a study agent to a single participant.

12.6.1 Reporting a Pause

If a pausing criterion is met, then a description of the AE(s) or safety issue must be reported by the PI within one business day to the IRB and the MM by fax or email.

12.6.2 Resumption of a Paused Participant

A participant who experiences any AE that meets the pausing criteria, and is possibly related to study drugs will not be permitted to resume the study agents but will continue to be followed for safety. The PI, MAI, and MM will determine whether or not it is safe to resume administration of the study agent to the subject.

12.6.3 Discontinuation of Study Agent

A participant who does not resume study agent will continue to be followed for safety until AE resolution.

12.7 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agents administered for all participants and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration. The PI, MAI, and MM will determine if the study should be halted. The halting rules are:

- **One** (or more) subject(s) experiences the same or similar **Grade 4 or Grade 5** AE that is assessed as possibly, probably, or definitely related to rivaroxaban, COBI, or DRV/COBI
- **Two** (or more) subjects experience the same or similar **Grade 3** AE assessed as possibly, probably, or definitely related to rivaroxaban, COBI, or DRV/COBI, or
- **Three** (or more) subjects experience the same or similar **Grade 2** events possibly, probably or definitely related to rivaroxaban, COBI, or DRV/COBI (except: headache that requires administration of acetaminophen, diarrhea that requires the administration of loperamide, nausea that requires the administration of an anti-emetic, or rash).
- Any safety issue that the PI, MAI, and/or the MM determines should halt the study.

12.7.1 Reporting a Study Halt

If a halting rule is met, then a description of the AE(s) or safety issue must be reported by the PI within one business day to the IRB and the MM by fax or email.

12.7.2 Resumption of a Halted Study

The PI, MAI, and MM will convene to determine an appropriate course of action (which will include IRB notification and consultation) before the study is allowed to resume. The PI will notify the IRB of the decision on resumption of the study.

12.7.3 Discontinuation of Study Agent

Subjects who do not resume study agent will continue to be followed for safety, until AE resolution.

12.8 Study Discontinuation

The IRB, the NIAID, and the MM, as part of their duties to ensure that research participants are protected, may discontinue the study at any time. Subsequent review of serious, unexpected, and

related AEs by the IRB may also result in suspension of enrollment and further trial interventions/administration of study agent.

12.9 Premature Withdrawal of a Participant

An individual participant will be withdrawn for any of the following:

- An individual participant's decision. (The investigator should attempt to determine the reason for the participant's decision.)
- Participant loses ability to provide ongoing informed consent.
- Non-compliance with study procedures to the extent that it is potentially harmful to the participant or to the integrity of the study data.
- The investigator determines that continued participation in the study would not be in the best interest of the participant.

12.10 Replacement of Withdrawn Participants or Participants Who Discontinue Study Treatment

Participants who withdraw or are withdrawn from the study prior to day 13 will be replaced. If a participant is replaced, all the data collected from that participant will still be included for the safety assessment.

13 SAFETY OVERSIGHT

13.1 Safety Review and Communications Plan (SRCP)

A safety review and communication plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the MM, which delineates the safety oversight responsibilities of the PI and MM. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

13.2 Medical Monitor

A MM has been appointed for oversight of safety in this clinical study. The MM will be responsible for performing safety assessments as outlined in the SRCP.

The MM does not have direct involvement in the conduct of the study and has no significant conflicts of interests as defined by NIAID policy.

The MM will conduct independent safety monitoring and recommend appropriate action regarding AEs and other safety issues. The PI will send to the MM the safety reports specified in the SRCP.

After each MM review, a recommendation as to whether the study is to continue as is, be modified, or be terminated will be discussed with the PI.

In addition, deaths and immediately life-threatening SAEs must be reported to the MM within 1 business day after the PI becomes aware of the event. All other SAEs must be reported to the MM within 3 business days of PI awareness.

All non-SAE UPs and pregnancies will be reported by the PI to the MM at the same time they are submitted to the IRB.

The MM will be notified immediately if any pausing or halting rule is met, and the MM will provide a recommendation for continuation, modification, or termination of the study.

14 STUDY MONITORING

Accrual and safety data will be monitored by the PI and the medical advisory investigator, who will provide oversight to the conduct of this study. The PI will continuously evaluate implementation of the protocol for any unusual or unpredicted complications that occur and will review the data for accuracy and completeness.

The NIH CC's Quality Assurance Program will conduct study monitoring at least annually or more frequently as required. Participant consent documents, primary outcome and safety laboratory results, and diagnostic test results will be monitored for accuracy, correct dating, and agreement between case report forms and source documents. All regulatory reports, reviews and amendments, AEs and problem reports related to study, along with investigator credentials, training records, and the delegation of responsibility log will also be reviewed during monitoring visits. The PI will be responsible for reporting any problems to the IRB as defined in Section 12.4, Reporting Procedures.

15 STATISTICAL CONSIDERATIONS

15.1 Primary Study Hypothesis

In healthy volunteers receiving rivaroxaban concomitantly with COBI or DRV/COBI, significant COBI or DRV/COBI inhibition of efflux transporters (P-gp and BCRP) and CYP450 enzymes will be observed. Plasma levels of rivaroxaban will be significantly increased, requiring a dose adjustment. A significant increase in rivaroxaban levels that necessitates a dose adjustment is defined as a 50% or greater increase in the AUC geometric mean ratio (GMR) in combination with COBI or DRV/COBI compared to rivaroxaban alone (phase 2 to phase 1 and phase 3 to phase 1).

15.2 Secondary Study Hypothesis

In healthy volunteers receiving rivaroxaban concomitantly with COBI or DRV/COBI, we predict increase in FXa, PT, and aPTT, corresponding with increased systemic concentrations of rivaroxaban.

15.3 Sample Size Justification

Because AUC is likely to have a skewed distribution, we will transform the data using the natural logarithm Y=ln(AUC). Let Y_1 be ln(AUC) when rivaroxaban is given alone, and Y_2 be ln(AUC) when in combination with COBI or DRV/COBI. We will first compute a 90% confidence interval for the mean difference of Y_2-Y_1. That interval will be transformed back to the original (unlogged) scale to produce an estimate and confidence interval for the GMR. The GMR estimates the ratio of the median AUC when in combination with COBI or DRV/COBI to the median AUC of rivaroxaban alone.

To compute sample size/power, we must estimate the variance, V, of Y_2-Y_1. It can be shown using the mean and variance of the lognormal distribution that V can be estimated as $2\ln(1+CV^2)$, where CV is the intra-subject coefficient of variation. The CV of rivaroxaban is estimated to be 27%=0.27 based on available PK data.[20] This CV corresponds to a V of 0.1407 ($=2\ln(1+.27^2)$ for the log-transformed GMR of AUCs for rivaroxaban alone and in combination. A 50% relative increase on the original scale translates to mean difference of ln(1.50)=0.4055 for

log-transformed AUC. Therefore, power for this procedure is equivalent to that of a one-tailed paired t-test of the null hypothesis that the mean difference of Y_2-Y_1 is 0.4055, versus the alternative hypothesis that it exceeds 0.4055, when the variance is 0.1407.

If θ denotes GMR for combination vs. rivaroxaban alone, the procedure we use may be viewed in either of the following equivalent ways.

1. Test the null hypothesis that $\theta \leq 1.5$ versus the alternative hypothesis that $\theta > 1.5$ using a one-tailed test at $\alpha=0.05$. If the test rejects the null hypothesis, declare the interaction effect to be greater than 50%.
2. Compute the lower confidence limit of a one-sided, 90% confidence interval for θ (again by log transforming and then transforming back at the end). If the lower limit exceeds 1.5, declare the interaction effect to be greater than 50%.

Power for demonstrating a greater than 50% increase is shown in Table 5 for different true GMRs and sample sizes. A sample size of 12 participants provides 80% power to demonstrate at least a 50% increase in AUC if the true increase is 100% (true GMR=2). Power is very low if the true increase is 75%, but very high if the true increase is 125%.

Table 5: Power and Sample Size (n)

True GMR	n=10	n=11	**n=12**	n=13	n=14	n=15
1.75	.329	.354	.379	.402	.426	.448
2	.723	.765	**.800**	.831	.858	.880
2.25	.933	.954	.968	.978	.985	.990

A total of 12 subjects will be enrolled for this study.

15.4 Description of the Analysis

Rivaroxaban PK parameters will be determined using noncompartmental methods with Phoenix WinNonlin® (version 7.0; Pharsight Corporation, Mountain View, CA). C_{max}, t_{max}, and C_{min} for rivaroxaban will be obtained directly by visual inspection of the plasma concentration vs. time profiles. The apparent elimination rate constant (λ_Z) will be determined by calculating the absolute value of the slope of the log-linear regression of at least 3 points of the plasma concentration-time plot. The $t_{½}$ will be calculated as $0.693/\lambda_Z$. The area under the concentration vs. time curve from time zero to tau hours postdose ($AUC_{0–24}$, for rivaroxaban) will be calculated using the linear trapezoidal rule. CL/F will be calculated as 10 mg ÷ $AUC_{0\text{-}tau}$.

PK parameters ($AUC_{0–tau}$, C_{max}, t_{max}, $t_{½}$, CL/F, and C_{min}) for the single dose of anticoagulant (rivaroxaban 10 mg) will be compared alone and in combination with COBI or DRV/COBI. Paired t-tests will be used for comparison of the log-transformed AUC values between the combination and rivaroxaban alone. A one-sided p-value less than 0.05 will be claimed as statistically significant. GMRs (in combination vs. alone) with 90% confidence intervals will be calculated by exponentiating the limits of the log-transformed interval.

Primarily, the analysis will be performed in subjects who report no missing doses. Sensitivity analysis will be conducted in all participants using the linear mixed effects models for the repeated measures data. Sensitivity analysis will also be performed in subjects that are adherent to the planned course of dosing.

16 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

16.1 Informed Consent

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research participant. It is an ongoing conversation between the human research participant and the researchers which begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks, and benefits. Participants will be given the opportunity to ask questions and have them answered.

Informed consent will be obtained in person by a study team member authorized to obtain consent. The privacy of the subject will be maintained. Consenting investigators and the participant will be located in a private area (e.g., clinic consult room). The participants will sign the informed consent document prior to undergoing any research procedures. Consent will be documented with required signatures on the hard copy of the consent form. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The researcher will document the signing of the consent form in the participant's medical record. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

16.1.1 Considerations for Consent of NIH Staff or Family of Study Team Members

Informed consent will be obtained as detailed above and will comply with the requirements of NIH HRPP Policy 404 Research Involving NIH Staff as Subjects.

Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. When consent of that staff member is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on the staff member. Similarly, for family of the study team, a study team member unrelated to the participant will obtain their informed consent. When consent of that staff member's relative is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on them.

16.2 Participant Confidentiality

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized individuals may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB, NIAID, and Office for Human Subjects Research Protections (OHSRP).

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify

research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

17 DATA HANDLING AND RECORD KEEPING

17.1 Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from participants during study visits and telephone calls or will be abstracted from participants' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

17.2 Record Retention

The investigator is responsible for retaining all essential documents in accordance with regulatory and institutional requirements, International Council for Harmonisation/Good Clinical Practice guidelines, and the NIH Intramural Records Retention Schedule. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide notification of such intent to NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location.

Appendix A: Scientific References

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Appendix B: Schedule of Procedures/Evaluations

Evaluation	Study Day											Early Termination Visit
	Screening (Day -89 to 0)	Baseline (Day -6 – 0)	1	2	5±1	7	8	9±1	13	14	20±3	
Informed Consent	X											
Medical/Medication History	X	X	X	X		X	X		X	X	X	X
Vital Signs	X	X	X	X		X	X		X	X	X	X
Clinical Assessment[a]	X	X	X	X		X	X		X	X	X	X
Physical Examination	X	X									X	X
Telephone Reminder/Assessment[b]		X			X			X				
Acute Care Panel	X	X[c]	X			X			X		X	X
Hepatic Panel	X	X[c]	X			X			X		X	X
Mineral Panel	X	X[c]	X			X			X		X	X
CBC/diff	X	X[c]	X			X			X		X	X
aPTT, PT/INR	X	X[c]									X	X
Pregnancy Testing[d]	X	X[c]	X			X			X		X	X
Anti-HIV-1/2	X											
Viral Markers Hepatitis Screen	X											
Study Labs (Rivaroxaban PK/PD)			X[e]	X[f]		X[e]	X[f]		X[e]	X[f]		
Study Labs (Pharmacogenetic testing)				X[g]								

Study Drug (rivaroxaban) administered			X[h]			X[h]			X[h]			
Study Drug (COBI) Dispensed and Administered				X[i]								
Study Drug (DRV/COBI) Dispensed and Administered							X[j]					

CBC/diff = complete blood count with differential; aPTT = activated partial thromboplastin time; PT = Prothrombin time; INR = international normalized ratio; PK = pharmacokinetics; PD = pharmacodynamics; X = to be performed.

a Clinical assessment will involve AE assessments, medication reconciliations, and adherence assessments.
b Reminder phone calls for long study visit days 1, 7 and 13. Assessment of study medication tolerability will also be performed.
c Blood will only be collected at baseline if the baseline visit is more than 6 days after screening.

d Serum or urine pregnancy test must be negative to proceed in the study. Pregnancy test will be performed at every PK/PD visit prior to administration of rivaroxaban.

e Blood for plasma serial PK/PD time points. Rivaroxaban time points are 0 (predose), 1, 2, 3, 4, 5, 6, 8, 10 and 24 hours postdose. At each PK/PD time point, two samples will be drawn, one for PK and one for PD assessment (Anti factor Xa, aPTT, PT/INR). PK blood samples will be processed and stored by CPRU lab. PD blood samples will be processed and stored by DLM.

f 24-hour postdose plasma trough collection. PK blood samples will be processed and stored by CPRU lab. PD blood samples will be processed and stored by DLM.

g Sample collection for pharmacogenomics will be drawn during PK time point 24 (day 2). Send to Frederick as whole blood to be frozen and stored. NIH Contact: Cathy Rehm.

h On study day visits 1, 7 and 13, rivaroxaban will be dispensed from the pharmacy and administered to the participant at the OP8 clinic or Day Hospital. Study drug will be administered at 0800 ± 2 hours

i A 6-day supply (+ 3 additional tablets) of COBI 150 mg will be dispensed to the participant at Day 2 visit. Participant will be reminded to bring the COBI study medication supply to their next visit (Day 7). Participants will also be administered COBI 150 mg from the dispensed bottle under directly observed therapy (DOT).

j A 6-day supply (+ 3 additional tablets) of DRV/COBI 800/150 mg will be dispensed to the participant at Day 8 visit. Participant will be reminded to bring the DRV/COBI study medication supply to their next visit (Day 13). Participants will also be administered DRV/COBI 800/150 mg from the dispensed bottle under DOT.

Appendix C: Blood Volumes (mL) for Specimen Collection

Evaluation	Screening (-89 to 0 days)	Baseline[a] (-6 to 0)	1	2	7	8	13	14	20 ±3	Early Termination Visit
HIV-1/2 antigen/antibody testing	8									
Viral Markers Hepatitis screening	8									
Acute Care Panel[b]	4	4	4		4		4		4	4
Hepatic Panel	(4)	(4)	(4)		(4)		(4)		(4)	(4)
Mineral Panel	(4)	(4)	(4)		(4)		(4)		(4)	(4)
Pregnancy Testing	(4)	(4)	(4)		(4)		(4)		(4)	(4)
CBC/diff	3	3	3		3		3		3	3
aPTT, PT/INR	3	3							3	3
Study Labs (Rivaroxaban PK/PD)[c]			81	9	81	9	81	9		
Pharmacogenomics[d]				10						
Daily Volume (mL)	26	10	88	19	88	9	88	9	10	10
Cumulative Volume (mL)	26	36	124	143	231	240	328	337	347	

CBC/diff = complete blood count with differential; aPTT = activated partial thromboplastin time; PT = prothrombin time; INR = international normalized ratio; PK = pharmacokinetics; PD = pharmacodynamics.

[a]Blood will only be collected at baseline if this visit is more than 6 days after screening.

[b]A single volume of blood will be collected for acute care panel, hepatic panel, mineral panel and serum pregnancy testing.

[c] Rivaroxaban serial PK/PD time points are 0 (predose), 1, 2, 3, 4, 5, 6, 8, 10, and 24 hours postdose.

[d]Sample collection for pharmacogenomics. Send to Frederick as whole blood to be frozen and stored. NIH Contact: Cathy Rehm.

Appendix D: Study Initiation Calendars

I) **Study Start Day: Wednesday**

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
			Day 1	**Day 2**	Day 3	Day 4
Day 5	Day 6	**Day 7**	**Day 8**	Day 9	Day 10	Day 11
Day 12	**Day 13**	**Day 14**	Day 15	Day 16	Day 17	Day 18
Day 19	**Day 20**	Day 21	Day 22	Day 23	Day 24	Day 25
Days with study visits to the NIH are bolded.						

II) **Study start Day: Thursday**

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
				Day 1	**Day 2**	Day 3
Day 4	Day 5	Day 6	**Day 7**	**Day 8**	Day 9	Day 10
Day 11	Day 12	**Day 13**	**Day 14**	Day 15	Day 16	Day 17
Day 18	**Day 19**	Day 20	Day 21	Day 22	Day 23	Day 24
Days with study visits to the NIH are bolded.						

Appendix E: Xarelto® Prescribing Information

Please refer to attachment.

Appendix F: Tybost® Prescribing Information

Please refer to attachment.

Appendix G: Prezcobix® Prescribing Information

Please refer to attachment.

Appendix H: Prezista® Prescribing Information

Please refer to attachment.