DETAILED PROTOCOL

EFFECTS OF BICTEGRAVIR-EMTRICITABINE-TENOFOVIR ALAFENAMIDE ON CORONARY FLOW RESERVE IN STABLE HIV PATIENTS (B/F/TAF-CFR) – PILOT STUDY

Open-label, multicenter, single-arm study to Evaluate the Safety and Efficacy of Switching from Regimens Consisting of Abacavir/Lamivudine/Dolutegravir (ABC/3TC/DTG) to the Bictegravir/ Emtricitabine/Tenofovir Alafenamide (B/F/TAF) Fixed-Dose Combination (FDC) in Virologically-Suppressed HIV-Infected Adult Subjects

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1. BACKGROUND AND SIGNIFICANCE

The endothelium provides a permeability barrier for the vasculature, maintains a nonthrombogenic surface, regulates vascular tone and tissue flow, and inhibits vascular smooth muscle cell growth. In the presence of inflammatory cytokines, endothelial cells are activated which facilitates leukocyte adhesion and migration into the vessel wall, production of prothrombotic substances, and vasoconstriction, thereby creating a proatherogenic and procoagulant environment. Consequently, inflammation is a key mediator of a constellation of abnormalities that initiate and accelerate the progression of atherosclerosis.

HIV-specific immune dysregulation promotes endothelial and immune cell activation. Indeed, antiretroviral (ART)-naive HIV positive patients have higher levels of endothelial immune activation markers (e.g., IL-6, VICAM-1 and others) than non-HIV controls. ART-naïve HIV positive patients also show evidence of endothelial-cell dysfunction, as assessed by brachial artery reactivity. Treatment with ART has been shown to reduce biomarker evidence of endothelial cell activation in observational and randomized trials, and some of these drugs also appear to improve endothelial function. However, most prior evidence of the direct biologic effect of HIV viral infection and its changes with therapy on coronary vascular function has been indirect through the measurement of circulating biomarkers or brachial artery reactivity.

Positron emission tomography (PET) imaging allows precise and reproducible quantification of myocardial blood flow, thereby providing a direct assessment of coronary vascular health. Coronary flow reserve (CFR, calculated as the ratio of peak hyperemic myocardial blood flow over that at rest) is emerging as a powerful quantitative prognostic imaging marker of clinical cardiovascular risk. CFR provides a robust and reproducible clinical measure of the *integrated* hemodynamic effects of epicardial coronary artery disease (CAD), diffuse atherosclerosis, vessel remodeling, and microvascular dysfunction resulting from endothelial cell dysfunction on myocardial tissue perfusion across the entire coronary circulation. These processes have direct relevance to the underlying vascular pathobiology in patients with HIV infection. Consequently, quantitative CFR provides a unique opportunity to examine the potential impact of novel therapies on the biology of the disease and its association with cardiovascular outcomes. By testing the fundamental concept of whether novel ART therapies in HIV can lead to improved coronary blood flow and myocardial tissue perfusion, TAF-CFR would provide important mechanistic insights of the capabilities of TAF therapy to improve key determinants of clinical risk.

JUSTIFICATION FOR THE USE OF CFR AS A SURROGATE MARKER OF CLINICAL RISK

Noninvasive quantification of coronary flow reserve improves diagnosis and risk assessment in non-HIV patients with CAD. Noninvasive quantification of coronary blood flow and flow reserve has been the research focus of our group for over 20 years. Our group has developed and validated methodology for flow quantification and disseminated this technology for broad access and use. We and others have demonstrated that CFR is a quantitative unique phenotyping tool to assess vascular health and pre-clinical atherosclerosis which, in higher risk patients, can reveal flow-limiting coronary artery stenosis, thereby improving the accuracy of myocardial perfusion imaging in the diagnostic evaluation of known or suspected CAD [1-4]. More recent data support the notion that coronary vascular dysfunction, as quantified by reduced CFR, is highly prevalent among patients with known or suspected CAD[2, 5], increases the severity of inducible myocardial ischemia (beyond the effects of upstream coronary obstruction)[6] and sub-clinical myocardial injury[7], and identifies patients at high risk for serious cardiac adverse events, including cardiac death[8-11]. Thus, CFR is a robust measure of the *integrated* hemodynamic effects of epicardial CAD, diffuse atherosclerosis, vessel remodeling, and microvascular dysfunction on myocardial perfusion. In the presence of increased oxygen demand, a decreased CFR

reflects an imbalanced supply-demand relationship that may lead to myocardial ischemia, subclinical LV systolic and diastolic dysfunction, clinical symptoms and, ultimately, death. There is also emerging evidence that a reduced global CFR may help identify patients who benefit most from revascularization[12].

Systemic inflammation is associated with coronary vascular dysfunction. There is increasing evidence that coronary vascular dysfunction is associated with systemic inflammation and may precede or coexist with high-risk coronary atherosclerosis. Compared to healthy subjects, patients with rheumatoid arthritis or systemic lupus erythematosus, without significant CAD on invasive angiography, have impaired CFR to a degree directly related to disease duration[13]. In patients with cardiac syndrome X (CSX) with exertional angina and ST-segment depression on exercise stress testing but normal luminal coronary angiography, only those with elevated high sensitivity C-reactive protein (hsCRP, >3 mg/L) have reduced CFR[14]. Among patients presenting with acute coronary syndrome and nonobstructive CAD by angiography, reduced CFR (assessed by invasive Doppler flow velocity monitoring) is associated with higher frequency of thin-cap fibroatheroma, greater plaque burden, and significantly higher levels of hsCRP, despite similar amounts of epicardial disease by luminal area and fractional flow reserve measurements[15]. These results reinforce previous observations[16-18] that inflammation is associated with impaired coronary vasoreactivity and, in the appropriate patient population, may be a better marker for poor outcomes related to diffuse and/or microvascular CAD than are conventional ischemic assessments.

Anti-inflammatory therapies can modulate CFR. There is preliminary evidence that anti-inflammatory therapies lead to improved CFR. Indeed, treatment with statins in patients with dyslipidemia has been shown to improve CFR in a way that is modified among carriers of a specific polymorphism that reduces IL-1beta expression[19]. Short-term inhibition of IL-1 activity with the IL-1 receptor antagonist anakinra in rheumatoid arthritis patients without perfusion abnormalities has been shown to improve echocardiographic measures of LV myocardial deformation (by speckle tracking) and CFR (by Doppler flow velocity in the left anterior descending artery (LAD))[20]. Finally, treatment with methotrexate led to reduced clinical scores of disease severity and improved CFR (as measured by echo Doppler flow velocity in the LAD) in patients with early rheumatoid arthritis, with no effect on common carotid intima-medial thickness[21].

Coronary vascular dysfunction is a powerful predictor of clinical risk. Emerging data have consistently shown that CFR measurements by PET can distinguish patients at high risk for serious adverse events, including cardiac death[8-11]. We recently reported that reduced global CFR is independently associated with higher rates of cardiac and all-cause mortality in a large cohort of patients with and without DM[8, 22]. Relatively preserved CFR identifies patients with known or suspected CAD who have significantly lower risk of cardiac death, regardless of traditional semi-quantitative measures of stress-induced ischemia. Conversely, reduced CFR identifies patients at significantly higher risk of cardiac death, even among those without objective evidence of ischemia, probably due to diffuse atherosclerosis and/or microvascular dysfunction. PET measures of CFR improved risk stratification beyond comprehensive clinical assessment, LVEF and semi-quantitative measures of myocardial ischemia and scar, and led to clinically meaningful risk-reclassification of ~50% of intermediate risk patients[8]. Diabetics with impaired vs. preserved CFR experienced substantially higher cardiac mortality (7.6%/year vs. 1.3%/year, relative to 4.2%/year vs. 0.4%/year in nondiabetics, respectively, both $p<0.0001$)[22]. Importantly, diabetic patients without known CAD and with impaired CFR experienced a rate of cardiac death comparable to, and possibly higher than, that for nondiabetic patients with known CAD (Fig. 3). Measures of CFR integrate the hemodynamic effects of focal epicardial coronary stenosis, fluid dynamic effects of diffuse atherosclerosis, and the effects of coronary microvascular dysfunction, all of which are prevalent among diabetics[23-25], and affected by systemic inflammation. These observations have implications for the

classification of DM as a coronary disease risk equivalent[26]. *Specifically, only among diabetics with impaired vascular function is prognosis comparable to nondiabetic patients with known CAD.* Thus, differing levels of vascular health among previously studied cohorts may account for inconsistencies in relative mortality rates of diabetics without CAD and nondiabetics with CAD[27-29].

Quantitative CFR is a modifiable imaging biomarker of disease. There is consistent evidence supporting quantitative CFR as a powerful marker of vascular health and, as such, a useful marker to improve diagnosis and risk stratification and to monitor response to treatment, particularly in the context of clinical trials. The accuracy of quantitative noninvasive PET measures of myocardial blood flow and flow reserve by PET has been extensively validated in experimental animals[30] and humans[31-34]. The reproducibility of this technique is also well-established[33, 34]. PET is considered *the gold standard* for quantifying coronary blood flow and flow reserve. There is extensive data supporting its use in diagnosis of CAD[4, 35-37]. *The data discussed above demonstrate that the presence of abnormal CFR as measured by PET provides incremental risk stratification that leads to significant and meaningful risk-reclassification of intermediate risk patients[5, 9-11], especially those with DM[22]*. Furthermore, quantitative CFR as a measure of coronary vascular dysfunction is a *modifiable* imaging biomarker, which has been tested in the context of clinical trials [19, 38-46].

Abacavir is a nucleoside reverse transcriptase inhibitor used as part of treatment regimens for HIV. An association between abacavir and increased myocardial infarction was first reported in 2008[47]. Since then, most but not all studies have had similar findings[48]. As a result, HIV treatment guidelines state that ABC should be used with caution in patients with CV disease or at high CV risk. While the mechanism by which ABC increases CV risk is not definitively known, hypotheses include its effect on platelet aggregation, leukocyte adhesion, and systemic inflammation[48].

Bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) is a single pill regimen that was approved by the FDA in February 2018 for treatment of HIV. The marketed name of the drug is Biktarvy. In two phase 3 comparative clinical trials, including one with ABC/3TC/DTG, it was found to be non-inferior to dolutegravir-containing regimens in terms of virologic outcomes[49, 50]. B/F/TAF was also well tolerated, with few discontinuations for adverse events. As a result, B/F/TAF is an ideal non-abacavir containing regimen to assess the effect of removing ABC on coronary flow reserve.

2. SPECIFIC AIMS

2.1 Hypothesis and Objectives

The *primary hypothesis* of the B/F/TAF-CFR study is that reducing endothelial cell activation and dysfunction using *bictegravir/Emtricitabine/Tenofovir Alafenamide (B/F/TAF)* in place of abacavir/lamivudine/dolutegravir (ABC/3TC/DTG) will quantitatively improve coronary flow reserve as measured by PET over 24 weeks, in stable higher risk asymptomatic patients with HIV.

The primary objective of the B/F/TAF-CFR study is to test the hypothesis that in stable HIV patients treated with abacavir/lamivudine/dolutegravir STR regimens, a switch to a single tablet regimen (B/F/TAF) will improve coronary flow reserve and myocardial tissue perfusion, as assessed by quantitative PET imaging. This is a prospective, single arm study in which we will test that treatment B/F/TAF orally daily for 24 weeks will result in improved global coronary flow reserve in response to adenosine, reflecting coronary vasodilator function, as compared to baseline.

2.2 Primary Endpoint:

Change (from baseline) in global coronary flow reserve, as measured by PET imaging at 24 weeks after initiation of B/F/TAF therapy.

2.3 Secondary Endpoints:

2.3.1. Change (from baseline) in peak-stress global myocardial blood flow (in mL/min/g) at 24 weeks after initiation of B/F/TAF;

2.3.2. Change (from baseline) in peak-stress global coronary vascular resistance (in mm Hg/mL/min/g) at 24 weeks after initiation of B/F/TAF;

2.3.3. Change in serum biomarkers of inflammation (hs-CRP, IL-6, sCD163 and sCD14), myocyte injury and strain (hs Troponin, NT-proBNP) at 24 weeks after initiation of B/F/TAF.

3. SUBJECT SELECTION

3.1. Study Design

Open label, multicenter, uncontrolled, single arm pilot study. Patients with stable HIV currently treated with abacavir/lamivudine/dolutegravir STR regimens will be eligible for the B/F/TAF-CFR study. PET scans will be performed after enrollment while on the abacavir/lamivudine/dolutegravir STR regimen and at 24 weeks after the switch to B/F/TAF regimen. Patients will be encouraged to remain on stable medical therapy throughout the enrollment period.

3.2. Study group: HIV patients on stable therapy with abacavir/lamivudine/dolutegravir STR regimens.

3.2.1. Inclusion criteria:

We will recruit patients with HIV on abacavir/lamivudine/dolutegravir STR regimens for at least 1 year fulfilling the following inclusion criteria:

(1) age ≥ 45 years for men and ≥ 55 years for women;

(2) at least one coronary risk factor including smoking, dyslipidemia, hypertension, obesity (BMI >30) or diabetes, or a calculated 10-year risk of heart attack of 7.5% or higher;

(3) HIV RNA < 200 copies/mL at last clinical measurement, done within the past 12 months prior to screening, with no intervening HIV RNA > 200;

(4) Screening HIV RNA < 50 copies/mL, HGB >10 g/dl, or Glomerular Filtration Rate (GFR) >30 that do not preclude the use of Biktarvy.

3.2.2. Exclusion criteria:

(1) patients not fulfilling inclusion criteria;

(2) unstable HIV disease or other medical condition that, in the opinion of the investigator, would interfere with the conduct of the study;

(3) history of cardiomyopathy (LVEF <40%) or significant valvular heart disease;

(4) cirrhosis;

(5) end stage renal disease on dialysis;

(6) uncontrolled hypertension (defined as SBP >200 or DBP >110);

(7) pregnancy and breast-feeding;

(8) Patients requiring medications contraindicated with the components of B/F/TAF;

(9) Patients on active treatment for severe asthma or severe COPD;

(10) Hepatitis B;

(11) Any reported use of methamphetamine, cocaine and heroin within the past 6 months; if such use has been reported, a negative toxicology screen will be required before entry.

3.2. Source of subjects and recruitment methods

A total of 30 evaluable patients will be required. This will be a collaborative effort between 4 sites in the Boston area: (1) *Brigham and Women's Hospital, BWH* (PI: Paul Sax, MD), (2) *Boston Medical Center* (PI: Nina Lin, MD), (3) *Tufts Medical Center* (PI: Laura Kogelman, MD)~~,~~ and (4) *Massachusetts General Hospital* (PI: Virginia Triant, MD). All patients will be referred for PET imaging to BWH, with BWH serving as the Core Imaging Lab and Data Coordinating Center (PI: Marcelo Di Carli, MD).

4. SUBJECT ENROLLMENT

4.1. Procedures for obtaining informed consent

The Principal Investigator, co-investigator, or qualified PI designee at each site will obtain consent during the study-related hospital visit prior to any study-related procedures. Consent of subjects who do not speak English will be obtained and documented following the procedures outlined by IRB policy at each recruiting site. The informed consent process will also be documented and added to the research files for this study.

4.2. Methods of Enrollment

Potential study subjects will be recruited from medical clinics at BWH, MGH, BMC, and Tufts Medical Center. Screening procedures will include queries for potentially eligible subjects from lists of patients who are followed at each site. Investigators at each site will evaluate whether a patient might be a candidate for the study and follow site-specific protocols for approaching patients and invite them into the study. We will make every effort to mitigate any undue influence on patients to participate in this study, including providing the informed consent document to the patient to take home. The patient will then be instructed to reach out to the study team or other healthcare providers with questions at any time. The PI or PI designee at each site will review the informed consent form with the patient and answer any additional question the patient may have. All PET/CTs will occur at BWH. Treatment assignment and monitoring will be performed by each site. Additional procedures, particularly at the Baseline and End of Treatment visits may be performed at the local site or at BWH, for the convenience of the subjects.

4.3. Treatment assignment

Study subjects will all receive one tablet daily of bictegravir 50 mg/emtricitabine 200 mg/tenofovir alafenamide 25 mg.

5. STUDY PROCEDURES

5.1. Study visits and parameters to be measured

This is a collaborative, multi-center, single arm trial. After giving written informed consent to participate in the study and fulfilling criteria for inclusion, patients will be switched to B/F/TAF and followed up for 24 weeks. Patients will undergo rest and vasodilator-stress PET/CT scan for measurement of coronary flow reserve at baseline (before the switch to B/F/TAF), and again after completion of 24 weeks of B/F/TAF. Safety labs will be assessed at baseline at the 45-day study visit and at the end of the study at the Day 168 visit. There will be follow up phone calls at day 14, day 126, and one week after the end of study at day 174. Follow up calls will be used to assess changes in medical history and for any adverse event.

Figure 1. Outline of Procedures

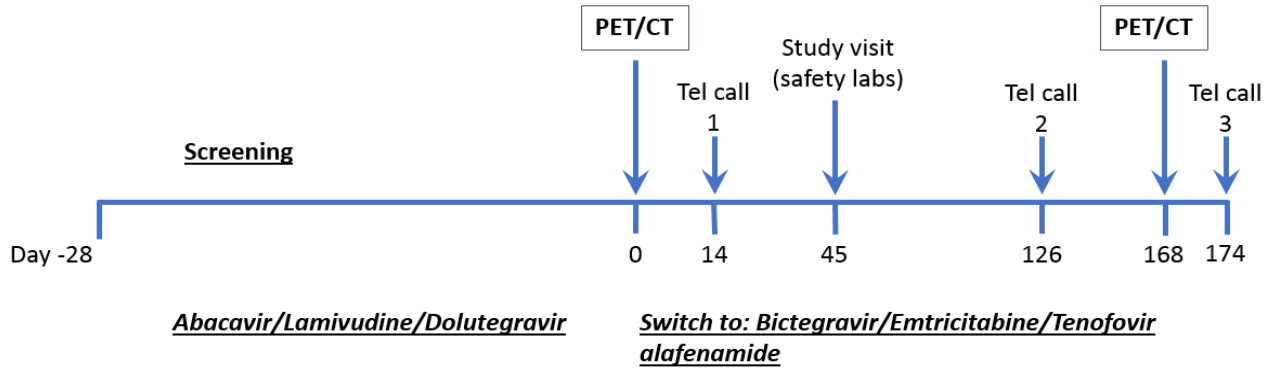

Table 1. Schedule of Assessments

Procedure	Screening (-28 to -1 days)	Baseline (day 0)	Tel F/U 1 (day 14)	Study Visit (day 45)	Tel F/U 2 (day 126)	End of Treatment (day 168)	Tel F/U 3 (day 174)
Informed Consent	X						
Inclusion/Exclusion Criteria	X	X					
Demographics		X					
Medical History		X	X	X	X	X	X
Medication History		X	X	X	X	X	X
Vital Signs	X	X		X		X	
Physical Exam		X		X		X	
Pregnancy Test [+]	X	X				X	
Lab Tests (Clinical)	X	X		X		X	
Lab Tests – Research Only (to store)		X				X	
ECG		X				X	
PET/CT		X				X	

Study Drug Dispensed		X		X			
Study Drug Pill Count / Diary Collection				X		X	
AE Monitoring		X	X	X	X	X	X

***Telephone follow-up calls and study visits will be +/- 7 days to allow for scheduling flexibility.**

****Day 45 study visit will be +/- 14 days to allow for scheduling flexibility.**

*****Day 168 study visit will be +/- 7 days to allow for scheduling flexibility and to ensure adequate supply of study drug.**

[+]Screening Pregnancy test may be urine or serum

Screening (-28 to -1 days)

- All screening assessments will occur within 28 days prior to start of medication switch
- Obtain informed consent
- Note whether subjects have fasted for 4 hours prior to the visit
- Review inclusion and exclusion criteria to ensure the patient is eligible
- Obtain vital signs, including weight and height, systolic and diastolic blood pressure (BP), heart rate (HR)
- Perform laboratory tests, including pregnancy test (if WOCBP) and hepatitis B. Laboratory tests (excluding pregnancy is WOCBP) are valid within one year of the screening visit).

Baseline (Day 0, just prior to study medication initiation)

- Verify clinical stability since screening evaluation
- Confirm that subjects have withheld caffeine for 12 hours and fasted for 4 hours prior to imaging tests
- Confirm that subjects have withheld beta blockers, calcium channel blockers and arterial vasodilators for 24 hours prior to the PET scan
- Obtain interim medical history and concomitant medications review
- Obtain vital signs, including weight and height, systolic and diastolic blood pressure (BP), heart rate (HR), and oxygen saturation (O_2 sat)
- Obtain 12-lead ECG
- Complete physical exam
- Perform laboratory tests, including serum pregnancy test prior to any imaging
- Perform PET/CT scan
- Study medication initiation must occur *within 10 days* of the baseline evaluation
- Provide subjects with study drug and diary to take until next study visit (Day 45)
- Monitor for AEs. Educate patients on most common side effects of the new drug (Table 3). They are encouraged to immediately contact study investigators/staff by email and/or phone for any new or concerning symptoms.

Day 14 (Telephone follow-up call 1)

- Contact all subjects by telephone approximately 2 weeks after medication switch
- Assess changes in medical history or medication changes
- Monitor for AEs

Day 45 (study visit)

- Assess drug compliance via pill count
- Verify clinical stability since screening evaluation
- Confirm that subjects have fasted for 4 hours prior to imaging tests
- Obtain interim medical history and concomitant medications review
- Obtain vital signs, including weight and height, systolic and diastolic blood pressure (BP), heart rate (HR), respiratory rate (RR), and oxygen saturation (O_2 sat)
- Complete physical exam
- Perform laboratory tests
- Collect drug remaining study drug and first diary to assess compliance
- Prior to leaving, patients will be supplied with study drug and a new drug diary
- Monitor for AE; subjects are again educated about the most common side effects. They are encouraged to immediately contact study investigators/staff by email and/or phone for any new or concerning symptoms.

Day 126 (Telephone follow-up call 2)

- Contact all subjects by telephone approximately 18 weeks after medication switch
- Assess changes in medical history or medication changes
- Monitor for AEs

Day 168 (End of Treatment)

- Assess drug compliance via pill count
- Verify clinical stability since screening evaluation; including specific inquiry regarding common dose-related side effects associated with B/F/TAF
- Confirm that subjects have withheld caffeine for 12 hours and fasted for 4 hours prior to imaging tests
- Confirm that subjects have withheld beta blockers, calcium channel blockers and arterial vasodilators for 24 hours prior to the PET scan
- Obtain interim medical history and concomitant medications review
- Obtain vital signs, including weight and height, systolic and diastolic blood pressure (BP), heart rate (HR), and oxygen saturation (O_2 sat)
- Obtain 12-lead ECG
- Complete physical exam
- Perform laboratory tests, including serum pregnancy test prior to any imaging
- Collect remaining study drug and second drug diary to assess compliance
- Perform PET/CT scan

- Monitor for AEs

Day 174 (Telephone follow-up call 3)

- All patients will be contacted by telephone at approximately 7 days following administration of the last dose of study drug for AE monitoring.

5.2. Drug to be used

B/F/TAF is indicated for treatment of people with HIV-AIDS. Patients enrolled in the study will be switched from a HIV regimen containing abacavir/lamivudine/dolutegravir to B/F/TAF for 24 weeks. In a comparative clinical trial, B/F/TAF was non-inferior to ABC/3TC/DTG in virologic outcomes and showed a significantly lower rate of patient-reported nausea. In Study GS-US-380-1489, a double-blind, multicenter, active-controlled, randomized controlled non-inferiority trial of ABC/3TC/DTG vs B/F/TAF in treatment naïve adults, both regimens were well tolerated. Adverse events leading to study discontinuation were noted in no participants in the bictegravir, emtricitabine, and tenofovir alafenamide group and 1% of participants in the dolutegravir, abacavir, and lamivudine group. Adverse events related to study drug were less common with bictegravir, emtricitabine, and tenofovir alafenamide than with dolutegravir, abacavir, and lamivudine (26% [n=82] vs 40% [n=127]), the difference being driven by a higher incidence of drug related nausea in the dolutegravir, abacavir, and lamivudine group (5% [n=17] vs 17% [n=55]; $p<0.0001$).

5.3. Concomitant medications

Only patients on stable HIV therapies will be included in the study. Because concurrent medications can potentially affect coronary blood flow and flow reserve (e.g., antihypertensive and oral hypoglycemic agents, statins), only patients on stable treatment for hypertension, diabetes and/or dyslipidemia will be included in the study. The following medications will be allowed provided they are neither initiated nor discontinued during the study period:

- Aspirin;
- Angiotensin-converting enzyme inhibitors and/or Angiotensin Receptor Blockers;
- β-blockers;
- HMG –CoA Reductase Inhibitors (statins);
- Nitroglycerin and long-acting nitrates;
- Oral anti-diabetic medications and Insulin;
- Stable hormone replacement therapy

5.4. Procedures

Positron Emission Tomography (PET): Regional and global myocardial blood flow will be assessed using PET imaging. Clinical and research cardiac PET studies are routinely performed at BWH. The PET imaging team at BWH under Dr. Di Carli's leadership consist of experienced imaging specialists and have a track record in research studies using PET. Di Carli has extensive experience in performing these procedures, quantifying myocardial blood flow and flow reserve, has published extensively on this topic, and runs an active core laboratory with experience in multi-center trials using imaging endpoints. PET scans will be performed using a whole body PET/CT scanner. Study participants will be asked to refrain from drinking caffeine-containing beverages and taking theophylline-containing medications for 12 hours before the PET study. Calcium channel blockers, beta blockers, and arterial vasodilators will be withheld for 24 hours prior to the PET scan. Studies will be performed after 4 hours of fasting.

Assessment of myocardial blood flow and coronary flow reserve: Regional myocardial blood flow (MBF) will be measured at rest and during vasodilator-stress with adenosine using ^{13}N-ammonia as the flow tracer. 13N-ammonia is used in clinical and research studies and has been validated for the quantification of myocardial blood flow and coronary flow reserve. After transmission imaging and beginning with the intravenous bolus administration of ^{13}N-ammonia (~20 mCi), list mode images will be acquired for 19 minutes. The patient will then undergo a standard infusion of adenosine (140 mcg/kg/min) for 4 mins or regadenoson bolus injection (0.4 mg). Two mins into the adenosine infusion or one minute after the regadenoson bolus, a second dose of ^{13}N-ammonia will be administered, and PET imaging collected in the same manner. Heart rate, blood pressure, and 12-lead electrocardiogram will be recorded at baseline, every minute during the infusion, and during 5 minutes after completion of the adenosine infusion or regadenoson injection.

Analysis of PET data: Absolute MBF (in ml/g/min) will be computed from the dynamic rest and stress images using commercially available software (Corridor4DM; Ann Arbor, Michigan) and previously validated methods [33, 34]. Automated factor analysis will be used to generate blood pool (arterial input function) and tissue time-activity curves. Regional and global rest and vasodilator-stress MBF will be calculated by fitting the ^{13}N-ammonia time-activity curves to a two-compartment tracer kinetic model as described previously [33, 34]. Per-patient global coronary flow reserve (CFR) will be calculated as the ratio of MBF at peak vasodilator-stress over that at rest for the entire left ventricle. This method for quantitation of MBF is highly reproducible. In the PET core laboratory at BWH, the intra-class correlation coefficient for CFR among four readers is 0.94 (95%CI 0.88-0.98), indicating excellent reproducibility[5].

Coordination of ancillary study and central imaging analyses. BWH will serve as the coordinating center and central imaging core laboratory for the proposed PET scans in the pilot study. Dr. Di Carli runs a very active core laboratory and, as mentioned above, has extensive experience in managing multi-site imaging trials. In this investigator-initiated study, BWH will sub-contract all clinical centers in the ancillary study.

For the central analysis of the PET images, all imaging data will be stripped of patient identifiers and assigned a trial-specific identifier to maintain compliance with US regulatory requirements regarding protection of patient privacy. Drs. Di Carli and Sax will organize a bi-monthly teleconference including investigators and research coordinators from each clinical site to monitor, discuss, and troubleshoot recruitment and other issues that may arise during the conduct of the clinical trial.

5.5. Data to be collected and timing of data collection

At each of the study visits, a medical history with symptom assessment, medication review, vital signs, physical exam, and blood samples will be obtained. As described above, additional data from the PET/CT scan as well as EKG assessment will be obtained at baseline and at the end of treatment period. One week after completion of the trial, telephone follow-up will be conducted to assess for any adverse events.

Laboratory analysis: Clinical labs will be collected at the Screening, Baseline, Day 45, and End of Treatment visits as follows:

- Screening: Comprehensive metabolic panel, lipid panel, hs-CRP, HbA1c, CBC, HIV RNA, D-Dimer, Hepatitis B Surface Antigen
- Baseline: hs-troponin and NT-proBNP
- Day 45: Comprehensive metabolic panel, lipid panel, hs-CRP, HbA1c, CBC, HIV RNA, D-Dimer

- End of Treatment: Comprehensive metabolic panel, lipid panel, hs-CRP, HbA1c, CBC, HIV RNA, D-Dimer, hs-troponin, NT-proBNP
- In addition, pregnancy tests will be performed (for women of child-bearing potential) at Screening and before each PET scan.

6. BIOSTATISTICAL ANALYSIS

6.1. Identification of clinical sites participating in the proposed ancillary study. BWH, MGH, Boston Medical Center and Tufts Medical Center will participate in this pilot study.

Based on the power calculations above and assuming a 20% effect size, the study will need to recruit approximately 30 patients, approximately 10 patients per site. BWH may recruit up to 20 patients. We believe that the proposed study is reasonable and attainable. Dr. Sax is an experienced clinical investigator with a successful track record in HIV clinical trials. Di Carli is a recognized expert in quantitative PET and nuclear cardiology imaging, has published widely on these topics, and has extensive experience in conducting clinical trials using imaging endpoints[51-54].

6.2. Power Analysis:

The table below shows the sample size needed to reach 85% power for an effect size of 20-30% relative improvement in coronary flow reserve from baseline using a standard deviation of 0.7 at baseline and follow-up and a correlation of 0.7 between baseline and follow-up measurements. Effect sizes were calculated from a baseline mean CFR of 1.74, which is a conservative value derived from our previous measurement of CFR in patients with HIV referred for myocardial perfusion imaging with PET:

Table 2. Power calculation

Effect Size	Mean CFR difference (TAF – Baseline)	SD	Total Sample Size (80% power)	Total Sample Size (85% power)	Total Sample Size (90% power)
20%	0.35	0.7	23	26	30
25%	0.43	0.7	16	18	21
30%	0.51	0.7	12	14	15

Using a total sample size of 30 evaluable patients and a two-tailed alpha of 0.05, there will be 85% power to detect an effect size of 20% relative improvement in coronary flow reserve from baseline, estimating a ~15% attrition rate between time of baseline and final PET. The 20% relative improvement in CFR is the effect size previously demonstrated in statin trials.

6.3. Study endpoints

Primary Endpoint: Change (from baseline) in coronary flow reserve, as measured by PET imaging at 24 weeks post medication switch.

Secondary Endpoints:

(1) Change (from baseline) in peak-stress global myocardial blood flow (in mL/min/g) at 24 weeks after initiation of B/FTAF;

(2) Change (from baseline) in peak-stress global coronary vascular resistance (in mm Hg/mL/min/g) at 24 weeks after initiation of B/FTAF;
(3) Change in serum biomarkers of inflammation (hs-CRP, IL-6, sCD163 and sCD14), myocyte injury and strain (hs Troponin, NT-proBNP) at 24 weeks after initiation of B/F/TAF.

6.4. Statistical methods

To test *the central hypothesis*, that reducing endothelial cell activation and dysfunction by substituting B/F/TAF for ABC/3TC/DTG will quantitatively improve coronary flow reserve as measured by PET over 24 weeks, we will perform a paired t-test to assess the ΔCFR between baseline and follow-up for each subject.

If a significant change in CFR after the switch is observed, we will perform secondary analyses of association between other markers of inflammation also implicated in atherosclerosis (hs-CRP, IL-6, sCD163 and sCD14) with ΔCFR. Significance of associations with other inflammatory markers and ΔCFR will be adjusted using the Bonferroni correction.

7. RISKS AND DISCOMFORTS

7.1. Blood sampling and intravenous catheter

The risks and discomforts of drawing blood from a vein include the possibility of discomfort, pain, redness, bruising, or bleeding at the site of the blood draw, occasional feeling of lightheadedness, and rarely, infection or fainting.

There are minor risks and discomforts associated with placement of an intravenous line for the PET scan, including the possibility of bleeding, pain, inflammation (redness and swelling), and leaking of contrast agent outside of the vein at the site of the IV. These problems are usually self-limited or require only minor treatments, such as application of an ice-pack or slight pressure for a few minutes.

7.2. Vasodilator stress

Vasodilator-stress with regadenoson and adenosine, as will be performed in this study, has been used routinely for many years for evaluation of known or suspected coronary artery disease in conjunction with myocardial perfusion imaging. During the infusion of the vasodilator stress agent, the patient may experience flushing, chest pain/pressure, nausea, or lightheadedness. If significant symptoms are present, patients will be given aminophylline (reversal agent) IV to relieve these symptoms. There will be continuous monitoring of heart rate, blood pressure, and 12-lead ECG throughout the infusion and recovery. These procedures are routinely performed in patients with CAD, as those in the CIRT trial, and are considered safe.

Regadenoson stress as performed in this study has been used routinely for many years for evaluating patients with known or suspected CAD. The most common side effects associated with the regadenoson bolus include: flushing, chest pain/pressure, shortness of breath, palpitations, headache, mild hypotension and heart block. These side effects are usually mild and self-limiting. If they are severe in intensity, aminophylline IV (1 mg/kg) will be given as per standard protocol.

Subjects with a history of seizures may receive adenosine. The side effects associated with this product include: facial flushing, headache, sweating, palpitations, chest pain, hypotension, shortness of breath/dyspnea, chest pressure, hyperventilation, head pressure, lightheadedness, dizziness, tingling in arms, numbness, apprehension, blurred vision, burning sensation, heaviness in arms, neck and back pain, nausea, metallic taste, tightness in throat, and pressure in groin. Adenosine is administered as a continuous infusion over 4 minutes and administered in a dose of 0.14mg/kg/min. The half-life of adenosine is less than 10 seconds. If the patient is having symptoms the infusion can be stopped, no further medication is administered, and the symptoms would stop within seconds because the medication would already be cleared from the heart. Therefore, no reversal medication is needed.

Rarely, an aminophylline IV injection may be used to reverse side effects of regadenoson infusion. Aminophylline is generally well tolerated; possible side effects of aminophylline may include nausea, headache, restlessness, convulsions, rapid breathing, a rapid heart rate, and allergic reactions such as rash.

7.3. Radiation risk

The estimated whole body effective radiation dose from each of the ^{13}N-ammonia PET scan is ~3-4 mSv[55], comparable to the annual radiation dose from background radiation sources in North America. Each subject will undergo 2 PET studies during the trial for a total dose of ~6-7 mSv, significantly lower than the average radiation exposure associated with a single conventional myocardial perfusion imaging study using single-photon emission computed tomography (SPECT)[55].

7.4. Drug side effects and toxicities

The most common side effects with the use of B/F/TAF are nausea, diarrhea, and headache, as described in Table 3 which is based on the results of the phase III trials[49, 50].

Table 3. Drug side effects

	B/F/TAF group (n=314)	**DTF/ABC/3TC group (n=315)**
Any adverse event (AE)	265 (84%)	283 (90%)
Grade 3 or 4 AE	19 (7%)	25 (8%)
Drug-related AE	82 (26%)	127 (40%)
Drug-related serious AE	0	4 (<1%)
Any AE leading to study drug discontinuation	0	4 (1%)*
AE occurring with ≥5% incidence in either group		
Nausea	32 (10%)	72 (23%)
Diarrhea	40 (13%)	41 (13%)
Headache	36 (11%)	43 (14%)
Upper respiratory tract infection	20 (6%)	34 (11%)
Nasopharyngitis	23 (7%)	29 (9%)
Fatigue	19 (6%)	27 (9%)
Syphilis	12 (4%)	25 (8%)

Insomnia	14 (4%)	20 (6%)
Arthralgia	11 (4%)	19 (6%)
Vomiting	12 (4%)	17 (5%)
Cough	20 (^%)	8 (3%)
Bronchitis	10 (3%)	16 (5%)
Abdominal pain	9 (3%)	16 (5%)

Data are in n (%). B/F/TAF=bictegravir, emtricitabine, and tenofovir alafenamide. DTG/ABC/3TC= dolutegravir, abacavir, and lamiduvine. *Chronic pancreatitis and steatorrhea (n=1), nausea and generalized rash (n=1), depression (n=1), and thrombocytopenia (n=1).[49, 50]

7.5. Reproductive risks

Pregnant or breastfeeding patients will be excluded from this study.

A female is considered of child-bearing potential unless:

- Permanently sterile (includes hysterectomy, bilateral oophorectomy, or bilateral salpingectomy)
- Medically documented ovarian failure
- Post-menopausal, defined as ≥ 60 years of age with cessation of menses for ≥ 12 months.

7.6. Unknown Risks:

There may be other risks and side effects that are not known at this time.

8. POTENTIAL BENEFITS

8.1. Potential benefits to participating individuals

There are no direct benefits for the patient from taking part in this study. The proposed study will test the important question of whether reducing endothelial cell activation and dysfunction using B/F/TAF will quantitatively improve coronary flow reserve, a sensitive measure of myocardial ischemia. Thus, this pilot study will provide important and unique mechanistic insights of the capabilities of novel HIV therapies to improve key determinants of clinical risk (improve coronary blood flow and reduce ischemia) and, in so doing, improve patient symptoms and quality of life, as well as outcomes.

8.2. Potential benefits to society

Data collected in this pilot trial may provide important mechanistic insights into presumed cardiovascular benefits from novel HIV therapies, as well as into the pathophysiology of microvascular dysfunction in HIV-AIDS.

9. MONITORING AND QUALITY ASSURANCE

9.1. Safety monitoring

The PI of the study will monitor the overall safety of the study and will report any adverse events to the IRB, per local institutional guidelines. The PI will also monitor this study to ensure that subjects' human rights, safety, and well-being are protected, that the study is properly conducted in adherence to the current protocol, and study data reported by the investigator/subinvestigator are accurate, complete and verifiable with study-related records such as source documents.

Safety data will be obtained at each study visit from the spontaneous reports by patients of adverse events, vital sign monitoring, physical examinations, 12-lead ECGs and clinical laboratory tests. In addition, telephone follow-up will be conducted to assess for any adverse events. All safety data will be reviewed by an HIV physician investigator (Dr. Paul Sax).

Patients with unexpected abnormalities on the baseline PET scan will be discussed with the patient's PCP/ID physician to decide whether the patient should participate in this pilot trial. The findings will be discussed with the patient and the patient's primary physician in order to facilitate an appropriate management plan. If a patient is excluded on safety grounds, we do not think that this will affect our ability to determine the effect of B/F/TAF on myocardial blood flow.

The safety of B/F/TAF will be assessed with respect to the following end points: (1) death from any cause, (2) the composite of death from any cause or any cardiovascular hospitalization, (3) frequency of symptomatic documented arrhythmia, (4) serious adverse events related to the study drug and clinically significant laboratory abnormalities.

9.2. Treatment Compliance

Subject compliance to treatment regimen will be assessed at each study visit by a count of remaining medication in each bottle. Subjects will be asked to complete a medication diary for additional compliance assessment. The diary will be compared to the drug count. A threshold of ≥80% will be used to define compliance with treatment assignment. Subjects determined to be noncompliant (<80% compliance) will be counseled regarding the importance of following dosing instructions but not withdrawn from the study.

9.3. Discontinuation criteria for individual subjects

Discontinued subjects are those who are enrolled in the study and for whom study treatment is terminated prematurely for any reason. The subject is free to withdraw from the study treatment and/or study for any reason and at any time without giving reason for doing so and without penalty or prejudice. The investigator is also free to terminate a subject's involvement in the study at any time if the subject's clinical condition warrants it.

Subjects will be discontinued if:

- Subject experiences a serious or intolerable adverse event
- Subject has severe disease and has to be intervened upon based on the imaging tests
- In the Investigator's opinion, the subject is non-compliant with the protocol requirements
- Subject's health would be jeopardized by continued participation
- Subject wishes to withdraw consent
- Investigator elects to end the study

9.4. Adverse event reporting guidelines

9.4.1 Definition of Adverse Events (AEs)

An adverse event (AE) is defined as any untoward medical occurrence in a subject administered a study drug, and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding),

symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug.

An abnormality identified during a medical test (e.g., laboratory parameter, vital sign, ECG data, physical exam) should be defined as an AE only if the abnormality meets one or more of the following criteria:

- Induces clinical signs or symptoms
- Requires active intervention
- Requires interruption or discontinuation of study medication
- The abnormality or investigational value is clinically significant in the opinion of the investigator

9.4.2 Definition of Serious Adverse Events (SAEs)

A serious AE is any untoward medical occurrence that, at any dose:

- Results in death,
- Is life threatening (an event in which the subject is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe),
- Results in persistent or significant disability/incapacity,
- Results in congenital anomaly, or birth defect,
- Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious)
- Other medically important events

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These events, including those that may result in disability, should also usually be considered serious.

9.4.3 Criteria for Causal Relationship to the Study Drug

Adverse events that fall under either "Possible" or "Probable" should be defined as "adverse events whose relationship to the study drugs could not be ruled out."

Causal relationship to the study drug	**Criteria for causal relationship**
Not Related	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and/or in which other drugs, chemicals or underlying disease provide plausible explanations.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which

	could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Probable	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on re-administration (re-challenge) or withdrawal (de-challenge).

9.4.4 Criteria for Defining the Severity of an Adverse Event

The following standard with 3 grades is to be used to measure the severity of adverse events, including abnormal clinical laboratory values.

- Mild: No disruption of normal daily activities
- Moderate: Affect normal daily activities
- Severe: Inability to perform daily activities

9.4.5 Reporting of Serious Adverse Events (SAEs)

In the case of a serious adverse event (SAE), the investigator should submit a report to the IRB-per local institutional guidelines of the date the investigator first becomes aware of the problem as per Partners policy.

9.4.6 Follow-up to Adverse Events

All adverse events occurring during the study are to be followed up until resolved or judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized.

If during adverse event follow-up, the adverse event progresses to an "SAE," or if a subject experience a new SAE, the investigator must immediately report the information to the sponsor.

9.4.7 Procedure in Case of Pregnancy

If a woman becomes pregnant during the study dosing period or within 30 days from the discontinuation of dosing, the investigator will report the information to the IRB as if it is an SAE. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated fertility date, pregnancy result and neonatal data etc., should be included in this information.

The investigator will follow the medical status of the mother, as well as the fetus, as if the pregnancy is an SAE.

When the outcome of the pregnancy falls under the criteria for SAEs [spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly (including anomaly in a miscarried fetus)], the investigator should respond in accordance with the report procedure for SAEs. Additional information regarding the outcome of a pregnancy (which is categorized as an SAE) is mentioned below:

- "Spontaneous abortion" includes abortion and missed abortion.
- Death of an infant within 1 month after birth should be reported as an SAE regardless of its relationship with the study drug.

- If an infant dies more than 1 month after the birth, it should be reported if a relationship between the death and intrauterine exposure to the study drug is judged as ″possible″ by the investigator.
- In the case of a delivery of a living newborn, the ″normality″ of the infant is evaluated at the birth. ″Normality″ of the miscarried fetus is evaluated by visual examination unless test results which indicate a congenital anomaly are obtained prior to miscarriage.

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