

HAWAII CENTER FOR AIDS (HICFA)

CLINICAL STUDY PROTOCOL: **H046**

**IMPACT OF METFORMIN ON CD4 T CELL NEGATIVE
CHECKPOINT RECEPTORS IN INDIVIDUALS WITH
CHRONIC HIV**

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Funding: **HICFA Discretionary Funds**

Version Number: **H046 V 4.0 (July 26, 2021)**

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
ART	Antiretroviral Therapy
CAC	Coronary Artery Calcium
CBC	Complete Blood Count
CLIA	Clinical Laboratory Improvement Amendments
CRF	Case Report Form
CVD	Cardiovascular Disease
DXA	Dual Energy Absorptiometry
ER	Extended Release
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HAHC	Hawaii Aging with HIV Cohort
HICFA	Hawaii Center for AIDS
HIPAA	Health Insurance Portability and Accountability Act
IATA	International Air Transportation Association
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IRB	Institutional Review Board
NCR	Negative checkpoint receptor
NP	Neuropsychological
OHRP	Office for Human Research Protections
PBMC	Peripheral blood mononuclear cell(s)
PD-1	Programmed death receptor-1
PI	Principal Investigator
PID	Patient identifier number
QA	Quality Assurance
QC	Quality Control
qd	Daily
SAE	Serious Adverse Event
SID	Subject identifier number
SOP	Standard Operating Procedures
Sub-I	Sub-Investigator(s)
TIGIT	T cell immunoreceptor with Ig and ITIM domains
TIM-3	T-cell immunoglobulin and mucin-domain containing-3
UP	Unanticipated Problem
US	United States
WBC	White Blood Cell count

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

The principal investigator, co-investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of this clinical trial have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Principal Investigator: Cecilia M. Shikuma MD

Signed: _____ Date: _____

PROTOCOL SUMMARY

Title: **H046: IMPACT OF METFORMIN ON CD4 T CELL NEGATIVE CHECKPOINT RECEPTORS IN INDIVIDUALS WITH CHRONIC HIV**

Study Description: Non-diabetic, aviremic HIV-infected individuals on antiretroviral therapy (ART) randomized to metformin therapy or to observation for 72 weeks. Assessment of change over 72 weeks in T cell negative checkpoint receptors (PD-1, TIGIT and TIM-3), body composition (fat and lean tissue), body strength, neuropsychological performance and subclinical atherosclerosis as measured by carotid intima-media thickness (cIMT) . Blood to be banked for future analyses. The overall goal of this study is to evaluate metformin as adjunctive therapy to minimize the risk of chronic age-related complications in HIV.

Sub-studies: As optional procedures, this study will ask participants to undergo inguinal lymph node biopsies and/or rectal biopsies at entry and at week 72 (final) study visit

Objectives: Primary Objective: To determine the impact of metformin on CD4 T cell negative checkpoint receptor PD1+TIGIT+ expression over 72weeks

Secondary Objectives:

(1) To assess the safety and tolerability of metformin when given to non-diabetic HIV-infected individuals on potent antiretroviral therapy (ART)

(2) To assess the impact of metformin over 72 weeks on

- Body composition (total, peripheral and truncal fat, and lean tissue) by dual energy absorptiometry (DXA)
- Physical strength (Hand Grip)
- Global and domain-specific neuropsychological (NP) test performance
- Depression questionnaire
- Subclinical atherosclerosis as assessed by cIMT

(3) To bank blood (PBMC and plasma) to be utilized for future research to understand the mechanistic action of metformin

Substudy Objectives: To assess changes in levels of CD4+ T cell intracellular HIV DNA and RNA within lymph nodes and rectal tissue.

Endpoints: Primary Endpoint: Change in CD4 T cell PD-1+TIGIT+ receptor expression over 72 weeks.

Secondary Endpoints:

Number of grade II or higher adverse events (AE)

Change over 72 weeks in

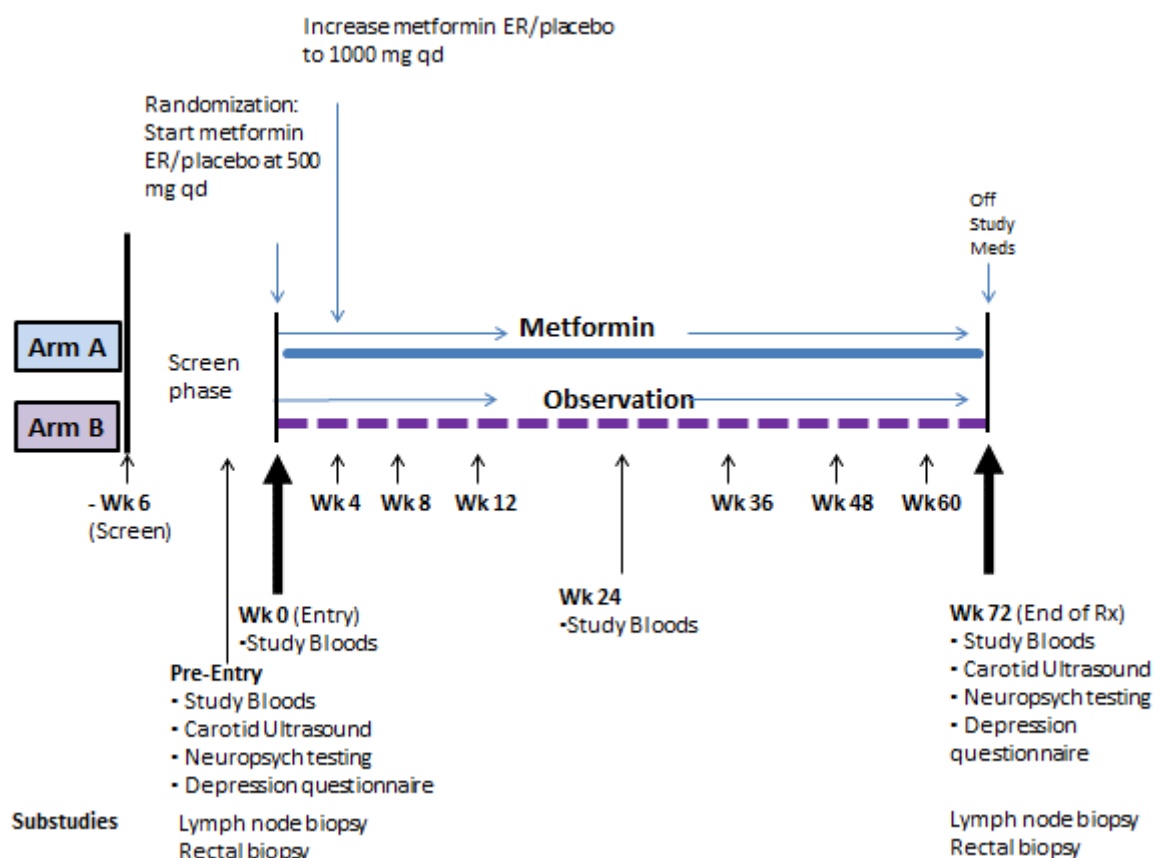
- DXA assessments: total, truncal and peripheral fat and total lean tissue
- Physical strength measurement: Hand Grip
- Global and Domain-specific neuropsychological test performance
- Depression questionnaire scale

- Carotid intima-media thickness

Substudy Endpoints: Change over 72 weeks in rectal and lymph node CD4 T cell intracellular HIV DNA and RNA

Study Population:	60 non-diabetic subjects (30 subjects/arm) with chronic HIV infection on stable suppressive ART
Phase:	Phase IV trial
Description of Sites/FacilitiesEnrolling	Single site study at the Hawaii Center for AIDS (HICFA), University of Hawaii
Description of StudyIntervention:	Metformin 500 mg Extended Release (ER) qd increasing to 1000 mg ER qd at week 4 and continued to week 72.
Study Duration:	4 year estimated time from opening of study to completion of clinical aspects of the study
Participant Duration:	Up to 6 weeks for screening followed by 72 weeks on study

SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Metformin is a drug used as first line therapy for type 2 diabetes. The drug was approved by the FDA for use in individuals with diabetes in 1995, and has **short term¹ as well as long term (>10 years)² documented record of safety**. Diarrhea leads to discontinuation of study medication in 0.6% of patients treated with extended release metformin³. Additionally, the following adverse reactions have been reported in $\geq 1.0\%$ to $\leq 5.0\%$ of individuals given metformin: abdominal pain, constipation, abdominal distention, dyspepsia/heartburn, flatulence, dizziness, headache, upper respiratory infection, and taste disturbance³. Metformin may decrease vitamin B12 levels⁴. Serious adverse events are infrequent and generally limited to lactic acidosis, reported in approximately 0.03 cases/1000 patient-years³ and typically only in persons with renal or hepatic insufficiency or other contraindications².

Metformin is also now known to have substantial pleiotropic cellular effects resulting in anti-aging, cardio- and neuro-protective, and anti-neoplastic benefits which appear to be independent of its ability to lower glucose⁵⁻⁷. Systemic review of animal models of myocardial infarctions found that metformin limits infarct-size and improves cardiac function⁸. While the overall evidence is still unclear in human studies, one review found that data emerging from both clinical trials and real-world registry is in favor of a protective effect of metformin with respect to both coronary events and progression to heart failure⁹. In Alzheimer's Disease, metformin was associated with improved executive functioning in a randomized placebo-controlled crossover study¹⁰. Perhaps most impressive is its potential efficacy against cancer observed in diabetic patients. Despite significant heterogeneity in studies, a recent meta-analysis of 47 independent studies involving 65,540 cancer cases in diabetic patients found that metformin reduced cancer incidence by 31% and cancer mortality by 34%¹¹. Another meta-analysis found diabetics taking metformin compared to non-metformin therapies to have lower cardiovascular (CVD) and lower all-cause mortality⁷.

These effects against age-related complications are potentially of particular interest to individuals with chronic HIV. In the U.S., 45% of individuals infected with HIV are over 50 years of age, and it is estimated that 70% will be over the age of 50 years by 2030¹². CVD, diabetes, non-AIDS-defining malignancies, dementia, osteoporosis and other chronic age-related conditions are more prevalent at all ages and occur approximately 10 years earlier than in their uninfected counterparts¹³ suggesting that there is an extra 'hit' in these individuals that accentuate aging^{12,14-16}. Associations between HIV immunologic factors such as nadir CD4 count and CD4/CD8 ratio and non-AIDS complications support the hypothesis that downstream effects of HIV may be playing a role in the higher risk of these age-related non-AIDS complications^{12,15,17,18}. Whether the adjunctive use of metformin may mitigate this high burden of age-related complications have not been studied.

There have been at least 8 clinical trials of metformin in HIV-infected individuals designed to study its impact on lipodystrophy, diabetes and metabolic syndrome in this population¹⁹⁻²⁶. These studies have demonstrated that metformin has similar safety and efficacy as a diabetic drug in this population; however, no study has investigated the impact of metformin on HIV age related complications.

Negative Checkpoint Receptors (NCR) and Chronic HIV Immune negative checkpoint receptors (NCR) are co-inhibitory receptors which down-modulate immune responses to prevent hyper-immune activation, minimize collateral damage and maintain peripheral self-tolerance. Overexpression of these molecules is associated with T-cell exhaustion and dysfunctional T cell effector responses and the inability to contain and control cancers and various chronic infections including HIV.

NCR increases during chronic HIV in both CD4 and CD8 T cells and include: PD-1 (programmed death receptor-1)^{27,28}, CTLA-4 (cytotoxic T-lymphocyte-associated protein 4)²⁸, TIGIT (T cell immunoreceptor with Ig and ITIM domains)²⁹, and LAG-3 (lymphocyte activation gene 3)³⁰. A recent evaluation of 7 different immune checkpoint molecules (PD-1, CTLA-4, LAG-3, TIGIT, TIM-3, CD160 and 2B4) identified PD-1, LAG-3 and TIGIT co-expressing CD4 T cells as highly enriched for integrated HIV DNA and strongly associated with the size of the viral reservoir even after adjusting for nadir and current CD4 T cells³⁰. Such data provides substantial rationale that a decrease in the population of cells harboring high levels of NCR may reduce the level of HIV infected cells during ART. It is possible that reduction on the level of HIV infected cells during ART may also translate to a decrease in HIV-associated immune activation that is believed to drive the age related complications of HIV.

Very little data exists about the role of NCR in age or age-related complications. However, there is growing interest in the role of NCR as therapeutic targets in chronic complications such as in atherosclerosis³¹. In mice, age associated T cell exhaustion was distinguishable by Tim-3 and/or PD1 expression, suggesting that prolonged expression of NCR may result in aging associated immune dysregulation³²⁻³⁵.

Age-related Complications of HIV The Hawaii Aging with HIV Cohort – Cardiovascular Disease (HAHC-CVD) Cohort Study (R01HL095135 Shikuma C, PI) recruited and longitudinally followed approximately 160 HIV+ participants age ≥ 40 years on ART over 4 years. The median age of this cohort on entry was 52yrs, 88% were males and 63% were Caucasian. The median CD4 count was 507 cells/mm³ and plasma HIV RNA was <50 copies/ml in 84.4% of the participants on entry. Various age-related disease

assessments were performed, including fasting bloods for lipid and glucose parameters, coronary artery calcium (CAC) as a marker of subclinical atherosclerosis, neuropsychological (NP) testing to assess neurocognitive performance and dual energy absorptiometry (DXA) to assess body composition. We utilized banked blood specimens from this study to examine the relationships between CD4 T cell NCR and various age-related co-morbidities.

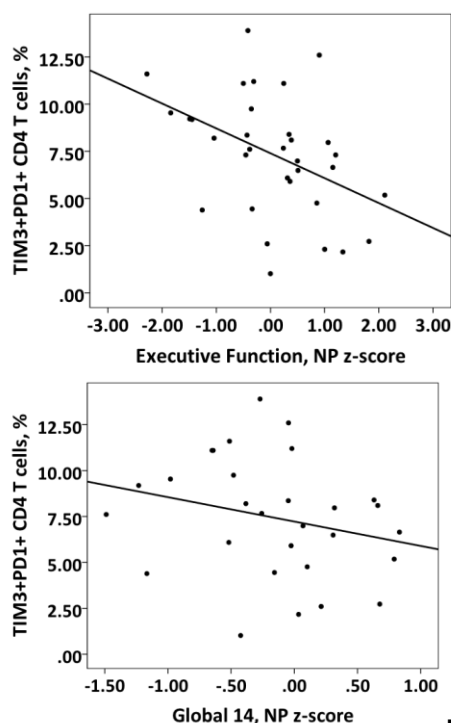


Figure 2. Higher frequencies of TIM3+PD1+ CD4 T cells correlate with lower cognitive performance. Executive function (top) and global function (bottom) are shown.

Relationship of NCR expressing CD4 T cell with neurocognitive impairment. Age- and education-adjusted composite z-scores of global and domain-specific neuropsychological (NP) function were available within this cohort and cross-indexed with the frequency of NCR expressing CD4 T cells. Higher frequencies of NCR positive CD4 T cells correlated with age- and gender-adjusted z-scores for two NP subdomains (executive function and working memory) known to be impaired in HIV-associated cognitive disease. A strong relationship was found between higher frequencies of PD1+TIM3+ CD4 T cells and worsening executive function ($\rho = -0.476$, $p = 0.005$) and poorer working memory performance ($\rho = -0.407$, $p = 0.019$). A trend towards significance was seen for global NP (NPZglobal14) performance z-scores ($\rho = -0.318$, $p = 0.093$) (Figure 2). Analyses excluding individuals with

detectable viral loads gave similar results.

Relationship of NCR expressing CD4 T cells to CVD: Data on coronary artery calcium (CAC) assessments were available in our cohort at entry and 2 years later. CAC was present in 51% of the population at entry and cross-sectionally no association was seen between NCR expressing CD4 T cells and CAC at entry. However, dichotomizing participants into those whose CAC levels remained stable (no change over 2 years) or for those whose CAC levels increased, higher CD4 T cell NCR predicted progression and continued increase of calcium deposits in the coronary arteries by logistic regression adjusting for Framingham class: PD1+ CD4 T cells (odds ratio 1.125, $p=0.04$), TIGIT+ CD4 T cells (odds ratio 1.26,

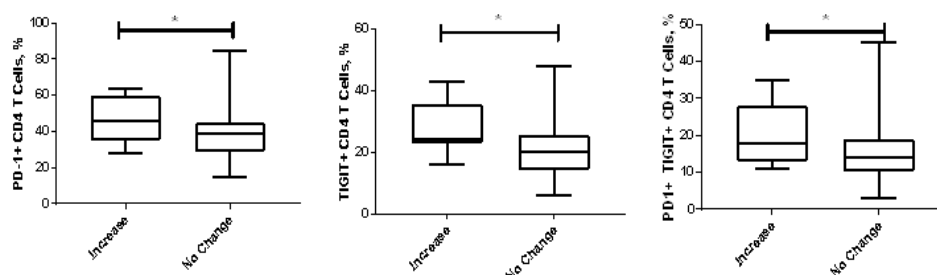


Figure 3: Percent negative checkpoint receptor expression associated with 2-year change in CAC dichotomized as no change in CAC over 2 years vs an increase in CAC.

$p=0.03$), and PD1+TIGIT+ CD4 T cells (odds ratio 1.27, $p=0.04$) (Figure 3).

NCR expressing CD4 T cells and Fat Tissue Loss Changes in body composition including changes in fat content occur with aging, are linked to frailty and may impact physical function and mortality. We assessed the relationship between entry T cell NCR and 2 year change in body composition by DXA. Multivariable linear regression adjusted for age, gender and undetectable plasma HIV RNA showed total fat loss over 2 years was associated with higher frequencies of TIGIT+ CD4+ T cells ($\beta = -0.58$, $p=0.01$), PD-1+ CD4+ T cells ($\beta = -0.46$, $p=0.04$), and TIGIT+PD-1+ CD4+ T cells ($\beta = -0.61$, $p=0.008$). More peripheral fat loss was associated with higher frequencies of TIGIT+ CD4+ T cells ($\beta = -0.61$, $p=0.007$), PD-1+ CD4+ T cells ($\beta = -0.54$, $p=0.01$), and TIGIT+PD-1+ CD4+ T cells ($\beta = -0.67$, $p=0.004$). More truncal fat loss was associated with higher frequencies of TIGIT+ CD4+ T cells ($\beta = -0.56$, $p=0.02$) and TIGIT+PD-1+ CD4+ T cells ($\beta = -0.58$, $p=0.01$). **Overall, these data suggest that frequencies of NCR expressing CD4 T cells were associated with multiple non-infectious complications of aging: cognitive dysfunction, increase in accumulation of coronary artery calcium and loss of fat content.**

Randomized Pilot Study of Metformin We conducted a small 24-week clinical trial of metformin in 12 HIV infected individuals stable > 6 months on ART with plasma HIV RNA < 20 copies/mL, randomizing participants 1:1 to either receive metformin 500 mg ER increasing to 1000 mg ER at week 4 or to an observation arm. The study excluded individuals with a known history of abnormal glucose metabolism.

Change in homeostatic model assessment - insulin resistance (HOMA-IR) over 24 weeks: As expected, even within this cohort of individuals without abnormal glucose metabolism, a decrease in HOMA-IR, albeit non-significant, was seen in subjects randomized to the metformin arm vs the observation arm [median (interquartile range) change in metformin arm over 24 weeks: -0.5 (-0.9, 0.1) vs change in observation arm: 0.5 (-0.1, 1.2); p value 0.13].

CD4 Negative Check-Point	Change over 24 Weeks		P-Value
	Observation (N = 6)	Metformin (N = 6)	
Singles			
TIGIT+	2.7 (1.8, 3.4)	-0.5 (-2.9, 1.8)	0.1039
PD1+	2.0 (0.4, 3.7)	-1.6 (-4.7, 0.2)	0.0260
TIM3 +	0.5 (0.1, 0.6)	-0.1 (-1.7, 3.6)	1.0000
Doubles			
TIGIT+ PD1+	0.8 (0.2, 1.2)	-0.9 (-2.9, -0.1)	0.0022
TIGIT+ PD1-	1.5 (0.7, 3.1)	0.4 (0, 1.7)	0.4848
TIGIT- PD1+	1.3 (0, 2.6)	-0.7 (-1.8, 0.3)	0.0776
TIGIT- PD1-	-3.6 (-4.2, -3.4)	1.2 (-2.9, 4.7)	0.0931
Triples			
TIGIT+ PD1+ TIM3+	0.3 (0.07, 0.6)	-0.9 (-1.3, -0.1)	0.0411
TIGIT+ PD1+ TIM3-	0.7 (0.4, 1.5)	-0.2 (-2.0, 0.2)	0.0043
TIGIT- PD1+ TIM3+	0.4 (0.2, 1.4)	-0.2 (-0.7, 1.1)	0.2403
TIGIT- PD1+ TIM3-	1.1 (0.1, 2.2)	-0.4 (-1.0, 0.2)	0.0260
TIGIT+ PD1- TIM3+	0.3 (0.02, 0.6)	0.5 (-0.2, 1.3)	0.5887
TIGIT+ PD1- TIM3-	0.9 (0.3, 2.4)	0.5 (0.4, 0.9)	0.9372
TIGIT- PD1- TIM3+	-1.0 (-1.9, -0.2)	-0.05 (-0.4, 0.2)	0.0476
TIGIT- PD1- TIM3-	-3.4 (-3.7, -2.9)	0.6 (-4.6, 1.8)	0.3939

CD8 Negative Check-Point	Change over 24 Weeks		P-Value
	Observation (N = 6)	Metformin (N = 6)	
Singles			
TIGIT+	0.4 (0.3, 2.1)	1.0 (-1.8, 5.8)	0.7857
PD1+	0 (-0.9, 0.9)	-0.7 (-4.2, -0.1)	0.4848
TIM3+	1.0 (-2.9, 2.8)	1.8 (0.4, 2.3)	0.8182
Doubles			
TIGIT+ PD1+	0.2 (0.1, 0.9)	-0.8 (-2.6, 1.2)	0.3095
TIGIT+ PD1-	0.6 (-0.4, 2.0)	1.0 (-1.2, 2.7)	0.9372
TIGIT- PD1+	-0.2 (-1.0, 0.1)	0.6 (-1.8, 1.3)	0.3939
TIGIT- PD1-	-0.5 (-1.1, 0.1)	-1.5 (-4.0, 8.4)	0.6991
Triples			
TIGIT+ PD1+ TIM3+	-0.1 (-0.3, 1.0)	0.2 (-1.7, 2.2)	0.9372
TIGIT+ PD1+ TIM3-	0.4 (0.3, 0.8)	-0.7 (-1.2, 1.1)	0.3939
TIGIT- PD1+ TIM3+	0.1 (-0.04, 0.2)	1.0 (0.6, 1.5)	0.3095
TIGIT- PD1+ TIM3-	-0.3 (-1.0, 0.3)	0.05 (-1.7, 1.5)	0.8182
TIGIT+ PD1- TIM3+	0.3 (-1.5, 1.0)	-0.1 (-1.3, 0.6)	0.8182
TIGIT+ PD1- TIM3-	1.3 (-0.9, 1.7)	0.6 (-0.8, 2.7)	1.0000
TIGIT- PD1- TIM3+	-0.2 (-1.1, 0.4)	0.7 (-1.1, 4.7)	0.4848
TIGIT- PD1- TIM3-	-1.8 (-2.7, 0.5)	-3.2 (-4.4, 4.6)	0.6234

Table 1. 24-week Change in NCR Expression on CD4 (left) and CD8 (right) T cells in Observation vs Metformin Arms; Comparison of 24-week change in the observation arm vs change in metformin performed by Mann-Whitney U

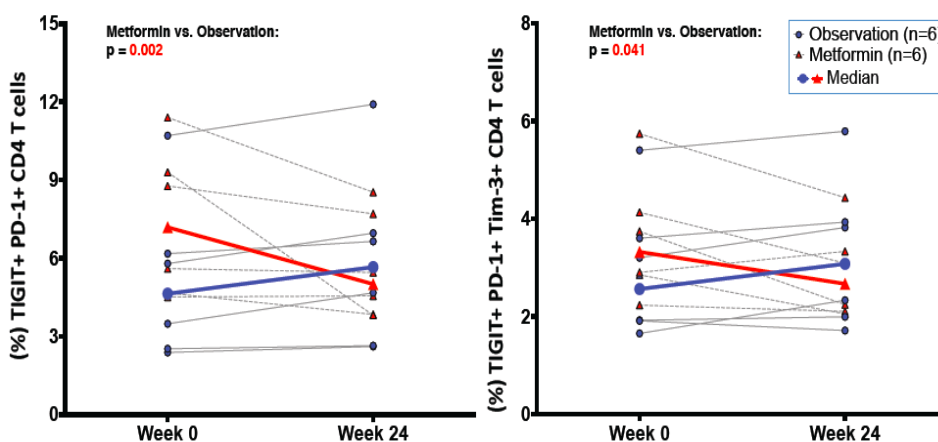


Figure 3. Change in the frequencies of dual TIGIT+ PD1+ (left) and Triple PD1+TIGIT+ Tim-3+ expressing CD4 T cells from entry to week 24 shown for each individual and as medians in the observation vs metformin arms.

single PD-1+, double TIGIT+PD-1+ and multiple triple combinations including the triple combination of TIGIT+PD-1+TIM-3+ cells. Among all the NCRs types that showed statistically significant differences, we observed the strongest standardized effect sizes by PD-1+TIGIT+ (1.87) and TIGIT+PD-1+TIM-3+ (1.94) for dual and triple categories, respectively. Changes in these NCR-expressing CD4 T cells are shown figuratively in Figure 3. In contrast there were **NO** significant differences in 24-week change in any

Change in Negative Checkpoint Receptor expressing CD4 T cells 24-week changes in NCR on CD4 and CD8 T cells in metformin vs observation arms are shown in Table 1. Compared to observation, metformin led to significant 24-week decreases in the frequencies of CD4 T cells expressing

single, double, or triple NCR expressing CD8 T cells population as shown in Table 1. **In summary, our preliminary data suggests that use of metformin results in decreases in NCR expressing CD4 T cells but not in CD8 T cells.**

2.2 RATIONALE

Metformin is a drug used as first line therapy for type 2 diabetes. Metformin is also now known to have substantial pleiotropic cellular effects resulting in anti-aging, cardio- and neuro-protective, and anti-neoplastic benefits which appear to be independent of its ability to lower glucose. In a previous small pilot study of 12 aviremic participants with chronic HIV on antiretroviral therapy (ART), we found that the addition of metformin over 24 weeks led to a significant decrease in the frequency of CD4 T cells expressing multiple negative checkpoint receptors, specifically PD-1 and two 2nd tier NCR, TIGIT and TIM-3, when compared to the observation group.

In a separate study, we have reported that cross-sectionally higher frequencies of PD-1+TIM-3+ CD4 T cell levels correlate with higher frequencies of activated and senescent T cells, and with worse neuropsychological impairment in executive function and working memory. In the same cohort study, higher frequency of CD4 T cells bearing NCR correlated with 2 year decrease in total and peripheral fat as measured by dual energy absorptiometry and with 2 year increase in coronary artery calcium.

The primary aim of this study is to first confirm our finding that metformin leads to a decrease in CD4 T cell NCR in a larger cohort over a longer extended period of time. As secondary aims, we will assess the potential benefits of decreasing CD4 T cell NCR, looking at clinical parameters that may change over 72 weeks of therapy: body composition by DXA; strength measurements; neuropsychological performance and depression, and cIMT. We will bank blood, and as funding allows also assess the mechanistic pathway mediating the effect of metformin on CD4 T cell NCR and the immunologic consequences of metformin on other immune cells including CD8 T cell and monocyte phenotype and function.

HICFA has previously conducted studies approved by the UH Committee on Human Subjects for both lymph node biopsies (CHS# 24020) and rectal biopsies (CHS# 22007). As higher levels of intracellular HIV DNA and RNA are found in association with exhausted CD4 T cells with higher levels of NCR, we hypothesize that improvement in CD4 T cell NCR will translate to lower levels of intracellular HIV DNA and RNA within this CD4 T cell viral reservoir.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

As previously mentioned, metformin has a long track record of both short term and long-term safety, with the most common side effects related to GI complaints including diarrhea. In extremely rare cases in the setting of renal or liver failure, metformin may lead to episodes of lactic acidosis syndrome. Individuals with renal or liver failure will not be enrolled into this study. There is potential for excessive bleeding and of infection with both lymph node biopsies and rectal biopsies. In lymph node biopsies, in addition to infection, there is a risk of development of keloids, seromas, and wound dehiscence. In rectal biopsies, bowel perforation may occur on rare occasions.

2.3.2 KNOWN POTENTIAL BENEFITS

Our data to date suggests that high levels of CD4 T cell NCR are related to several age-related complications in individuals with chronic HIV. This includes neurocognitive impairment, loss of fat, and possibly sub-clinical CVD. If metformin decreases CD4 T cell NCR levels, it is possible that use of metformin may also improve various age related chronic complications in individuals with chronic HIV.

Should examination of intracellular HIV DNA and RNA within CD4 T cells from lymph nodes and rectal biopsies show a decrease in HIV DNA/RNA following metformin, it is possible that metformin may have value as an adjunctive therapeutic modality for HIV cure.

3 OBJECTIVES AND PURPOSE

Primary Objective:

To determine the impact of metformin on CD4 T cell PD1+ TIGIT+ negative checkpoint receptor expression over 72 weeks:

Secondary Objectives

1. To assess the safety and tolerability of metformin when given to non-diabetic HIV-infected individuals on potent ART
2. To assess the impact of metformin over 72 weeks on
 - Body composition by dual energy absorptiometry (DXA)
 - Total, peripheral and truncal fat
 - Lean tissue
 - Physical functional performance (strength, balance and gait)
 - Hand grip
 - Neuropsychological performance
 - Global NP performance
 - Domain-specific (Learning and Memory, Executive Function, Working Memory and Psychomotor Speed) NP performance
 - Depression (BDI and CES)
 - Carotid Intima-media thickness
3. To bank blood (PBMC and plasma) to be utilized for future research to understand the mechanistic action of metformin

Objectives: Tissue
Substudies

To determine the impact of metformin on lymph node and rectal tissue HIV viral reservoir (HIV DNA and HIV RNA)

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

A 72 week, single site, open label, randomized, Phase IV trial of metformin [extended release 500 mg daily advancing to 1,000 mg daily at week 4] vs observation in non-diabetic, aviremic participants with chronic HIV on potent antiretroviral medications. Changes in T cell NCR and clinical changes in body composition; strength, and neuropsychological performance will be assessed over 72 weeks. Research bloods will be drawn and banked for future studies.

4.1.1 PRIMARY ENDPOINT

Change in CD4 T cell PD-1+TIGIT+ NCR expression over 72 weeks

4.1.2 SECONDARY ENDPOINT

Changes over 72 weeks in:

Body composition parameters -

- **Total fat**
- **Peripheral fat**
- **Truncal fat**
- **Lean Tissue**

Physical performance parameters -

- **Hand grip**

Neuropsychological performance

- **Global NP performance**
(Domain-specific NP performances):
- **Learning and Memory**
- **Executive Function**
- **Working Memory**
- **Psychomotor Speed**

Depression

- **BDI**
- **CES**

Subclinical Atherosclerosis

- **Carotid intima-media thickness**

4.1.3 SUB-STUDY ENDPOINTS

Changes over 72 weeks in tissue (lymph node and rectal tissue)

- **intracellular HIV DNA**
- **intracellular HIV RNA**

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

- HIV+
- On suppressive ART stable for > 1 year
- Plasma HIV RNA <50 copies/mL within 6 months of entry, with no HIV RNA > 200 copies/mL within the past 6 months prior to entry
- Age >40 years
- Ability and willingness to provide written informed consent

5.2 PARTICIPANT EXCLUSION CRITERIA

- Uncontrolled chronic medical condition or cancer
- Acute illness within 2 weeks of entry
- Diagnosis of diabetes by history, fasting blood glucose >126, or by HgbA1c > 6.5
- Chronic, uncontrolled diarrhea
- Known hypersensitivity or contraindication to metformin use
- Current presence of hepatitis C including currently on or intent to start therapy for hepatitis C within the 72 week duration of study.
- Serum B12 level below the reference normal range as listed by the commercial laboratory (Diagnostic Laboratory Services)
- Pregnancy, or intent to become pregnant or nursing an infant
- Any immunomodulator, HIV vaccine, any other vaccine, or investigational therapy within 30 days of study entry.
- Current uncontrolled coronary artery disease or NYHA Class 3 or 4 congestive heart failure
- History of liver cirrhosis
- Current use of zidovudine, stavudine or didanosine
- The following lab values
 - Hemoglobin < 9.0 g/dL
 - Absolute neutrophil count < 1000/ μ L
 - Platelet count < 50,000/ μ L
 - AST (SGOT) and ALT (SGPT) > 5x upper limit of normal (ULN)
 - Calculated creatinine clearance (Cockcroft and Gault) < 50 ml/min
- Active or recent past history (within past 2 years) of illicit substance or alcohol use or abuse which, in the judgment of the Investigator, will interfere with the patient's ability to comply with the protocol requirements
- Patients, who, in the opinion of the Investigator, are unable to comply with the dosing schedule and protocol evaluation or for whom the study may not be advisable

Additional Exclusions for Lymph Node Sub-study:

- History of recurrent staph infections
- History of active hepatitis B or C
- More stringent laboratory criteria as follows:
 - Hemoglobin < 12 g/dL
 - Platelet Count < 100,000/ μ L
- PT/PTT > upper limit of normal
- Contra-indication to use of local lidocaine

- History of bleeding disorder or medical condition/requirement for meds that increase bleeding tendencies
- Tendency to form keloids
- Requirement for continued use of glucocorticoids or other drugs with immunomodulator effects
- Current active illicit substance or alcohol use or abuse which, in the judgment of the Investigator, will interfere with the patient's ability to comply with the protocol requirements or to properly care for inguinal wound.
- Patients who, in the opinion of the investigator, are not willing or are unable to comply with wound evaluation and care.

Additional Requirements for Rectal Biopsy Sub-study

- History or current presence of anal fistula
- History of gastrointestinal (GI) cancer or chronic GI pathology such as ulcerative colitis
- More stringent laboratory criteria as follows:
 - Hemoglobin < 12 g/dL
 - Platelet Count < 100,000/ μ L
- PT/PTT > upper limit of normal
- Inability or unwillingness to comply with bowel prep requirements or follow-up

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Participants will be recruited using the standard recruitment methods utilized by HICFA. It is anticipated that many of the participants will come from HICFA's HIV Clinic (Clint Spencer Clinic). Information about the study will be posted in the Clinic's waiting area, and the study may be discussed with clinic patients or research patients who have agreed to be contacted for future research opportunities. Information about the study will also be circulated to various other clinics caring for HIV infected individuals on Oahu and to AIDS Service Organizations. All advertisement material will be approved by the UH Human Subjects Committee prior to its use.

Participants will be given \$40 at each mandated study visit, \$40 for neuropsychological testing, \$50 for cIMT testing, \$220 for each lymph node biopsy procedure, and \$120 for each rectal biopsy procedure.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Each participant may discontinue study drug and/or withdraw completely from the study for any reason. The date and reason for termination or withdrawal will be documented.

Premature Termination of Study Drug for a Study Participant may occur for the following reasons:

- Possible drug-related AE or toxicity which, in the judgment of the PI or Sub-I, is considered to not be in the subject's best interest
- Request by the subject to terminate study drugs
- Subject cannot make scheduled visit(s)
- Subject does not provide blood sample(s)

Withdrawal completely from the study may occur for the following reasons:

- Failure to comply with the provisions of the protocol
- Request by the subject to withdraw.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Participants may withdraw or terminate involvement at any time for any reason, and that request will be honored.

No discontinuation visit is necessary. However, participants who terminate or withdraw for toxicity reasons will be evaluated as per the protocol for that toxicity until the toxicity is resolved.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated at the discretion of the study team. Study participants will be informed of study suspension or termination and whether the circumstances that led to this decision confers possible added risk to study participants.

Regulatory authorities including the IRB, the Food and Drug Administration (FDA), and ClinicalTrials.gov will be notified as warranted.

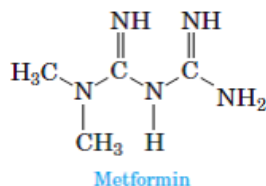
6 STUDY AGENT

6.1 STUDY AGENT AND CONTROL DESCRIPTION

6.1.1 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Metformin Extended-Release Tablets are oral antihyperglycemic drugs used in the management of type 2 diabetes. Metformin hydrochloride (N,N-dimethylimidodicarbonimidic diamide hydrochloride) is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents.

The structural formula is as shown:



Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5 \cdot HCl$ and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pKa of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68. The extended-release tablets to be used in this study contain 500 mg of metformin hydrochloride as the active ingredient.

All drug packaging, labels, storage, and handling will meet applicable state and federal regulations and ICH guidelines.

6.1.2 PRODUCT STORAGE AND STABILITY

Metformin should be stored at 20°–25° C (68°–77° F); excursions are permitted to 15°–30° C (59°–86° F).

6.1.3 PREPARATION

Study medication to be dispensed to participants will be prepared by the research pharmacist.

6.1.4 DOSING AND ADMINISTRATION

Metformin ER in this study will be dispensed to the study participant by the research pharmacist or research nurse.

6.1.5 ROUTE OF ADMINISTRATION

Study drug will be taken orally.

6.1.6 STARTING DOSE AND DOSE ESCALATION SCHEDULE

Participants randomized to the metformin arm will initiate the drug at 500 mg ER daily with dinner and if tolerated, will then increase dosage to 1000 mg ER daily.

6.1.7 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

The toxicity grading system that will be used is the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014, located at:

https://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf

Dose adjustments are allowed for toxicity management purposes at the discretion of study team such as for GI intolerance.

6.1.8 DURATION OF THERAPY

The study medication will be given for a duration of 72 weeks (+/- 4 weeks).

6.1.9 TRACKING OF DOSE

Subjects will be asked to bring back medication bottles to each visit and pill counts will be performed to assess compliance.

6.1.10 DEVICE SPECIFIC CONSIDERATIONS

Not applicable.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

Drug Accountability

The pharmacist will be responsible for ensuring adequate accountability of all used and unused study drug. This includes ordering and acknowledgment of receipt of study product (quantity and condition), subject dispensing records and returned or destroyed study product. Accountability records will document quantities including lot number, date dispensed, subject identifier number, subject initials and the initials of the person dispensing the medication. Study drug will not be supplied to any person other than the PI or Co-I listed on the FDA 1572 and designated staff.

Unused Study Drug

Upon study completion, unused vials of study drug will be destroyed at the research unit (verified by witness). Original drug accountability records must be maintained in the site's records.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

7.1.1.1 Medical History

Medical history will be obtained by interview from subject and will include past medical, psychiatric, and surgical histories; past alcohol, tobacco, or illicit substance abuse histories; current prescription and over-the-counter medications; medication allergies; and current or past participation in a research study within 12 weeks prior to the Screening Visit. HIV specific history will also be obtained to include date of HIV diagnosis, nadir CD4 count, date of initiation of ART, and, as possible, timing of previous ART medications taken.

7.1.1.2 Physical Examination

The subject's weight (kg) and height (cm) will be recorded in the CRF.

Vitals signs will include blood pressure, heart rate, respiratory rate, and temperature. Vitals signs will be measured in the sitting position after 5 minutes of rest. If possible, the same automatic sphygmomanometer or method to measure blood pressure will be used on each subject for the duration of the study.

Targeted physical examination may include examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system as dictated by medical history and patient complaints.

7.1.2 STANDARD OF CARE STUDY PROCEDURES

Clinical lab results (e.g., hematology, chemistry) may be given to participants or their physicians for use in their routine clinical care.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Hematology: Complete blood count (CBC)

Chemistry: Chemistry to include sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), creatinine, blood urea nitrogen (BUN), aspartate aminotransferase (AST), aminotransferase (AST), alkaline phosphatase (ALK-P), total bilirubin, and glucose.

Vitamin B12: Vitamin B12 levels will be drawn on screening and for those on the metformin arm again at week 24.

T Cell Subset: T Cell Subset analyses to include CD4 and CD8 counts and CD4/CD8 ratio

Plasma HIV RNA

7.2.2 OTHER ASSAYS OR PROCEDURES

7.2.2.1 Blood T cell Negative Checkpoint Receptor Assay

Cryopreserved PBMC will be rapidly thawed and stained for viability with an aqua amine reactive dye, then incubated with a panels of conjugated anti- human monoclonal antibodies (mAbs) The following directly conjugated mAbs used in this study will be obtained from BD biosciences: anti-TIGIT (MBSA43), anti-PD-1 (Invitrogen), anti-TIm-3 (R&D Systems) and anti-LAG-3 (Biolegend). Data will be analyzed using Flowjo Software version 9.5 (Treestar, Ashland, Oregon).

7.2.2.2 Dual Energy Absorptiometry (DXA)

Body composition by DXA to include assessment of total fat, peripheral fat and truncal fat as well as lean tissue. DXA will be done using GE Lunar Whole body DXA available at the UH Clinics at Kaka’ako

7.2.2.3 Physical performance parameter

Evaluation of physical strength will be performed:

Hand grip

Hand grip will be assessed for both hands of subjects via a hand-held dynamometer. Grip strength will be performed 3 times and the highest value used as the criterion value.

7.2.2.4 Neuropsychological Test

The NP testing to be conducted will be the standard protocol utilized by HICFA

Domain Name	Components
Learning and Memory	CVLT Total
	CVLT Long-Delay Free Recall
	BVMT Total
	BVMT Delayed Recall
Executive Function	FAS
	Stroop
	Trail B
	Action
Working Memory	WAIS IV Digit Span Backward
	CVLT B
	WAIS IV Letter Number
Psychomotor Speed	WAIS IV Digit Symbol
	Trail A
	Grooved Pegboard Dominant
	Grooved Pegboard Non-Dominant
Global	ALL components used in Domains above, PLUS
	CalCap Choice
	CalCap Sequential
	Timed Gait

7.2.2.5 Depression

We will administer 2 validated and relatively simple depression questionnaires – the BDI-II (Beck’s Depression Index II) which is currently administered at our site to eliminate individuals with major depression prior to neuropsychological testing; and the CES-D (Center for Epidemiologic Studies Depression Scale), a questionnaire utilized by a major HIV cohort study (Women’s InterAgency HIV Study) to assess depression.

7.2.2.6 Carotid Intima Media Thickness

The carotid intima media thickness will be performed at entry and at the 72 week study visit. This ultrasound evaluation is a measure used to diagnose the extent of carotid atherosclerotic vascular disease. The test non-invasive, ultrasound test will measure the thickness of the inner two layers of the R carotid artery in the neck —the intima and media. The ultrasound will be conducted at the Cardiology Non-Invasive Laboratory at Queen’s Medical Center as a fee for service procedure.

7.2.2.7 Lymph Node Biopsy

The lymph node biopsy will be performed as an optional elective procedure at the UH Clinics at Kaka'ako by a certified general surgeon Dr. Fred Yost to be conducted at entry and again as part of the final week 72 study visit procedures. The procedure will be performed as was performed in our previous lymph node study ('H034 Lymph Node Fibrosis in HIV Disease' CHS #24020) utilizing sterile techniques with the patient in the supine position. No antibiotics will be given preoperatively. The skin directly anterior to the lymph node bed will be decontaminated with an iodine-based solution and draped. A 2:1 mixture of 1% lidocaine and 0.25% bupivacaine with epinephrine (1:100,000) will be injected into the skin and subcutaneous tissue for pain control. The lymph node will be excised intact without fragmentation. After the biopsy, hemostasis will be ensured and the incision closed primarily with 4-0 subcutaneous and 5-0 subcuticular Vicryl or Monocryl sutures (Ethicon, Somerville, NJ). The surgical site will be covered with a non-occlusive sterile dressing. Operative times, from skin sterilization to dressing placement, is anticipated to be between 30 to 60 min. All patients will be seen on day 7 and day 30 post-biopsy to ensure there are no complications.

7.2.2.8 Rectal Biopsy

The rectal biopsy will be performed at the UH Clinics at Kaka'ako also as an optional elective procedure at entry and again as part of the final week 72 study visit procedures. Participants may elect to participate in this sub-study separately or in addition to the lymph node sub-study. We have previously conducted rectal biopsies as part of a routine colonoscopy for HIV+ individuals over the age of 50 (H023 Effects of HIV infection on Gastrointestinal Mucosal Immunity – a Pilot Study' (CHS# 22007). In this study, approximately 20-25 rectal biopsies were obtained per procedure. For this metformin study, rather than conducting biopsies via a colonoscopy which visualizes the entire length of the colon, requires a dedicated endoscopy suite and an extensive bowel prep prior to the procedure, we propose to access the rectum immediately above the anus in a procedure similar to the anoscopy procedure routinely conducted at the UH Clinics in Kaka'ako by our anal dysplasia clinic. Specifically we propose to utilize a 13 cm disposable proctoscope complemented by a fiber optic illumination system to visualize the rectum and then obtain biopsies collecting a much smaller number of biopsies of not more than 12 biopsies.

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

7.2.3.1 Blood Draw

Collection of blood with the intent to bank specimens is an important component of the study. The quantity of blood to be drawn at each setting is defined in the Schedule of Events.

Whole blood will be obtained by venous puncture and processed by the Processing Laboratory ideally within 1 hour of collection. If processing will be delayed (2-3 hours), the collection tube should be placed on a slow rocking table until processed.

7.2.3.2 Plasma (EDTA tube)

Plasma is separated from whole blood collected in EDTA vacutainers.

1. Spin vacutainer at 900xg for 10 minutes at room temperature (RT) to separate plasma from blood cells.
2. Remove plasma and save tube to process white blood cell separation.
3. Transfer plasma to a labelled 15mL conical Falcon tube and centrifuge at 820xg for 10 minutes at 4°C.
4. Aliquot plasma into 1mL labelled cryovials (3 to 4 aliquots).

5. Cryovials are stored at -20°C for 24 hours before being transferred to long-term cryobox storage at -80°C or placed in liquid nitrogen Dewar to snap freeze.

7.2.3.3 Peripheral Blood Mononuclear Cells (PBMC)(EDTA tube)

1. Resuspend EDTA blood pellet after plasma separation with 2x DMSO freezing medium in 1:1 ratio and freeze in 1 mL aliquots at -80°C after counting total WBCs for use in chloramine analysis assay.
2. Follow standard Ficoll Hypaque procedure to separate PBMC from 10 mL of heparinized blood.
3. Perform a cell count of the isolated PBMC.
4. Add appropriate DMSO/FBS solution for a final concentration of 5×10^6 cells/mL.
5. Transfer 1mL aliquots (5×10^6 cells) to 1.25 mL cryovials. Store in Mr. Frosty at -80°C for at least 24 hours before transferring samples to long-term -140°C storage.

7.2.4 SPECIMEN SHIPMENT

There is no intent to ship blood at this time.

7.2.5 SCREENING

Screening Visit includes the following:

- Obtain informed consent of potential participant verified by signature of written consent form. If the participant's medical chart will be reviewed to determine eligibility or results of laboratory tests used for screening, informed consent will be obtained prior to record review in accordance with the Health Insurance Portability and Accountability Act (HIPPA)
- Record medical history including verification of plasma HIV to determine eligibility based on inclusion/exclusion criteria
- Record medication history to determine eligibility based on inclusion/exclusion criteria.
- Record focused general examination and vital signs to determine eligibility based on inclusion/exclusion criteria
- Record weight and height
- Collect blood for CBC, chemistry and B12 level to determine eligibility based on inclusion/exclusion criteria
- Schedule study visits for subjects who are eligible and available for the duration of the study

7.2.6 ENROLLMENT/BASELINE

After the Screening Visit and prior to the Entry Visit, the following will be performed to confirm participant's study eligibility:

- Review and verify all inclusion/exclusion criteria have been met
- Confirm all Screening Visit Procedures are complete

Entry Visit (Visit 1, Day 0) into the study will occur within 6 weeks of screening. **Participants to come in fasting state.** The visit will include the following which may be performed in multiple visits:

- Ask participant if any other questions arose between Screening and Entry visits.
- The pharmacist to randomize study participant to metformin arm or to observation only arm.
- Record changes to medical and medication history which occurred between Screening and Entry visits.

- Record focused general examination and vital signs to determine if any changes arose between Screening and Entry visits.
- Obtain fasting blood for clinical labs (CBC, chemistries) and for research in quantity as specified in the Schedule of Events.
- Whole body DXA for measurement of body composition
- Hand grip
- NP testing
- Depression assessment
- cIMT testing
- If participating in the lymph node and rectal biopsies, biopsies to be done PRIOR TO dispensing of study medications
- If study participant has been randomized to the metformin arm, study medication (metformin ER 500 mg) to be dispensed after completion of all entry procedures. The nurse will review with the participant the potential side effects of metformin. The participant will be asked to call if troubling symptoms arise.

7.2.7 FOLLOW-UP STUDY VISITS

Week 4 Study Visit (Metformin arm only) At this visit:

- Update medical history and medication list
- Record AEs as reported by participant or observed by investigator
- Record vital signs and weight and conduct targeted PE as warranted
- Collect blood for CBC and chemistries
- Increase metformin dosage to 2 tablets (total of 1000 mg metformin ER) daily with dinner

Week 12 Study Visit (Metformin arm only) At this visit,

- Update medical history and medication list
- Record vital signs and weight
- Record AEs as reported by participant or observed by investigator
- Collect blood for CBC and chemistries
- Collect blood for research
- (For Observation arm participants: update medical information by phone)

Week 24 Study Visit (ALL patients; participants to come in fasting state) At this visit

- Update medical history and medication list
- Record vital signs and weight
- Record AEs as reported by participant or observed by investigator.
- Collect blood for CBC and chemistries including fasting glucose and insulin; draw B12 if participant on metformin.
- Collect blood for research.

Week 36 and 60 Study Visit (Metformin arm only) At this visit,

- Update medical history and medication list
- Record vital signs and weight
- Record AEs as reported by participant or observed by investigator
- Collect blood for CBC and chemistries

(For Observation arm participants: update medical information by phone)

Week 48 (ALL participants; participants to come in fasting state) At this visit:

- Update medical history and medication list
- Record vital signs and weight
- Record AEs as reported by participant or observed by investigator.
- Collect blood for CBC and chemistries
- Collect blood for research

7.2.8 FINAL STUDY VISIT

Week 72 (Final Visit) (ALL participants; participants to come in fasting state) At this visit:

- Update medical history and medication list
- Record vital signs and weight
- Record AEs as reported by participant or observed by investigator.
- Collect blood for CBC and chemistries
- Collect blood for research
- Whole body DXA for measurement of body composition
- Strength assessment
- NP testing
- Depression assessment
- cIMT testing
- Lymph node biopsy and/or rectal biopsies if participating in sub-studies. Biopsies to be done PRIOR TO discontinuation of metformin if on the metformin arm.

7.2.9 LYMPH NODE SUB-STUDY VISITS

LYMPH NODE SUB-STUDY Additional Study Visits are required specifically for the lymph node sub-study. These are meant to be safety visits and will occur after the lymph node biopsy at entry and again after the lymph node biopsy at week 72 of study.

Day 7 (1 week) post lymph node biopsy. Lymph node biopsies are scheduled at entry and again at week 72 of study

- Record any adverse signs or symptoms
- Record vital signs and weight
- Evaluation of lymph node biopsy wound

4 Weeks post lymph node biopsy

- Record any adverse signs or symptoms
- Record vital signs and weight
- Evaluation of lymph node biopsy wound

7.2.10 SCHEDULE OF EVENTS TABLE

For Participants on the Metformin Arm:

SCHEDULE OF EVENTS: METFORMIN ARM									
	Screen	Week 0 (Entry)	Week 4	Week 12	Week 24	Week 36	Week 48	Week 60	Week 72
Clinical Signs and Symptoms	x	x	x	x	x	x	x	x	x
Height	x								
Weight	x	x	x	x	x	x	x	x	x
Vital Signs	x	x	x	x	x	x	x	x	x
Targeted Physical Exam	x	x	x	x	x	x	x	x	x
Safety Labs (CBC with diff, chem) [8-13 ml]	x	x	x	x	x	x	x	x	x
T Cell Subset [6 ml]		x					x ²		x
HIV RNA [6 ml]	x ¹	x			x ²		x ²		x
Fasting Glucose/Insulin ³ [6 ml]		x			x		x		x
Vitamin B12 [blood volume included with chem]	x				x		x		
NP Testing		x							x
Depression Assessment		x							x
Hand grip		x							x
DXA		x							x
cIMT		x							x
Pregnancy Test ⁴	x								
PT/PTT for LN and Rectal bx within 4 wks of bx [3 ml]	x								x
LN Biopsy ⁵ (optional)		x							x
Rectal Biopsy ⁶ (optional)		x							x
RESEARCH Bloods (volume to be drawn)		100 cc		40 cc	100 cc		40 cc		100 cc

¹Lab assay to be done within 6 months prior to entry visit

²May use labs done 6 months before or after visit

³Patient will need to be fasting for these lab tests

⁴If female of child-bearing age; pregnancy tests should also be done prn whenever pregnancy is suspected.

⁵There are extra study visits at day 7 and week 4 post procedure for participants participating in the lymph node sub-study

⁶Call patient the day following rectal biopsy to assess for any adverse signs or symptoms

For Participants on the Observation Arm:

SCHEDULE OF EVENTS: OBSERVATION ARM								
	Screen	Week 0 (Entry)	Week 12	Week 24	Week 36	Week 48	Week 60	Week 72
Clinical Signs and Symptoms	x	x		x		x	x	x
Height	x							
Weight	x	x		x		x		x
Vital Signs	x	x		x		x		x
Targeted Physical Exam	x	x		x		x		x
Safety Labs (CBC, chem) [8-13 ml]	x	x		x		x		x
T Cell Subsets [6 ml]		x				x ²		x
HIV RNA [6 ml]	x ¹	x		x ²		x ²		x
Fasting Glucose/Insulin ³ [6 ml]		x		x		x		x
Vitamin B12 [blood vol included with chem]	x							
NP Testing		x						x
Depression Assessment		x						x
Hand grip		x						x
DXA		x						x
Pregnancy Test ⁴	x							
PT/PTT for LN bx within 4 wks of bx [3 ml]	x							x
LN Biopsy (Optional) ⁵		x						x
Rectal Biopsy(Optional) ⁶		x						x
Phone Interview			x		x		x	
RESEARCH Bloods (volume to be drawn)		100 cc		100 cc		40 cc		100 cc

¹Lab assay to be done within 6 months prior to entry visit

²May use labs done 6 months before or after visit.

³Patient will need to be fasting for these lab tests

⁴If female of child-bearing age; pregnancy tests should also be done prn whenever pregnancy is suspected.

⁵There are extra study visits at day 7 and week 4 post procedure for participants participating in the lymph node sub-study

⁶Call patient the day following rectal biopsy to assess for any adverse signs or symptoms

7.3 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

There are no prohibited medications for entry into this study. However there are medications known to cause drug-drug interactions. Therefore, the participant's current medications will be carefully reviewed from the standpoint of safety both prior to enrollment and in study follow-up.

During the study, participants will be requested to bring new medications to the attention of the study team before taking the new medication to assess its potential impact on safety. Whether discontinuation or alteration of dosage of study medication is warranted will be decided on a case to case basis by the study team.

All concomitant prescription medications (i.e., medication that can only be prescribed by a licensed physician) taken during study participation will be recorded on the CRF at Screening and subsequent visits. Medications reported in the CRF may be concomitant prescription medications, over-the-counter medications, and non-prescription medications.

7.4 PROHIBITED OR PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

Known drug interactions none which rises to the level of prohibited medications include the following per the FDA package insert³⁶: Furosemide, Nifedipine, Ranolazine, Vandetanib, Cimetidine and Dolutegravir.

Dolutegravir is noteworthy as it is an antiretroviral medication used as part of HIV antiretroviral regimens. Dolutegravir increases metformin levels; however it was been reported that the combination of metformin and dolutegravir are well tolerated³⁷. While interaction exists, the impact has been reported to be minor and dose adjustment not likely to be necessary^{37,38}.

7.5 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable.

7.6 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable.

7.7 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Metformin is a drug readily available by prescription. Metformin will not be provided to any participant outside the context of the study or following the closure of the study.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study drug. Collection of AEs will begin at the start of the study drug infusion and continue until the Final Visit.

AEs may be volunteered spontaneously by the subject or discovered as a result of general questioning by the study staff, by physical examination, or through procedures or laboratory testing. At each evaluation, the subject will be asked, "Have you experienced any problems or changes in your health since the last evaluation?" In addition, open-ended queries will help elicit information about possible AEs. All AEs will be recorded on the source documents and subsequently the CRF. For all AEs, the PI or Sub-I must pursue and obtain information adequate to determine the outcome of the AE and to assess whether it meets the criteria for classification as a serious AE requiring immediate notification. Follow-up of the AE is required until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

To avoid vague, ambiguous, or colloquial expressions, all AEs should be recorded in standard medical terminology on the CRF and on the medical record rather than in the subject's own words. Each AE will also be described in terms of duration, frequency, severity, association with the study drug, assessment of possible causes, actions taken, and outcome, using choices given on the CRF. Specific guidelines for PI or Sub-I classification of AEs by severity and relationship to study drug are given below.

The toxicity grading system that will be used to grade the AE is the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014, located at: https://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

Serious adverse event or serious suspected adverse reaction (SAE) is an AE or suspected adverse reaction that is considered "serious" if, in the view of either the PI or Sub-I, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

OHRP considers unanticipated problems (UP) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being

studied;

- Related or possibly related to participation in the research (“possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This study will use the OHRP definition of UP.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

The following guidelines will be used to describe AE severity.

- **Mild (Grade 1)** – An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- **Moderate (Grade 2)** – An event that is sufficiently discomforting to interfere with normal everyday activities.
- **Severe (Grade 3)** – An event that prevents normal everyday activities.
- **Life-Threatening (Grade 4)** - An event that causes extreme limitation in activity or an event where significant medical intervention/therapy is required.
- **Death Related to AE (Grade 5)**- An event that results in a fatal outcome.

8.2.2 RELATIONSHIP TO STUDY AGENT

For all collected AEs, the PI or Sub-I who examines and evaluates the participant will determine the AE’s causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related**– There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant’s clinical condition, other concomitant events). Although an AE may rate only as “possibly related” soon after discovery, it can be flagged as requiring more information and later be upgraded to “probably related” or “definitely related,” as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication)

and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.2.3 EXPECTEDNESS

Dr. Shikuma and Co-Investigators will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care. During each study visit or phone call, research nurse coordinator, PI, or Sub-I will query the participant for unsolicited and solicited AEs. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the CRF throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI or Sub-I will record all reportable events with start dates occurring any time after informed consent is obtained until 7 days for non-serious AEs and 30 days for SAEs after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

All AEs will be recorded on the source documents and subsequently the CRF. For all AEs, the PI or Sub-I must pursue and obtain information adequate to determine the outcome of the AE and to assess whether it meets the criteria for classification as a serious AE requiring immediate notification. Follow-up of the AE is required until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

To avoid vague, ambiguous, or colloquial expressions, all AEs should be recorded in standard medical terminology on the CRF and on the medical record rather than in the subject's own words. Each AE will also be described in terms of duration, frequency, severity, association with the study drug, assessment of possible causes, actions taken, and outcome, using choices given on the CRF. Specific guidelines for Investigator classification of AEs by severity and relationship to study drug outlined in [Sections 8.2.1, 8.2.2, 8.2.3](#) and [8.3](#).

When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes will be recorded separately (with distinct onset dates).

AE will be reported to the IRB. The PI or designee is responsible for assessing each event and signing off on the AE report.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

The PI, Sub-I, or research nurse coordinator is responsible for recording and reporting SAEs. The following procedure will be followed for reporting and handling SAEs:

The research nurse coordinator will notify the PI or Sub-I immediately on becoming aware of a potential SAE. After business hours, PI or Sub-I can be reached via pager system at 808-522-4000 or 808-566-5036. PI or Sub-I is responsible for assessing each event and will determine if the event meets the criteria for an SAE, the SAE severity, its relationship to the study intervention, and if the event was expected. PI or Sub-I assessment will be communicated to the research nurse coordinator who will record the event on the SAE form. Because each federal and local have their own reporting requirements, SAE reports will be filed in accordance with their respective time requirements. The SAE will be followed until satisfactory resolution or the PI or Sub-I believes the situation is stable or chronic. Any other supporting documents or follow-up reports will be completed and submitted if warranted. The IRB will be notified of any unexpected fatal or life-threatening SAE within 7 calendar days of receipt notification.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. PI or designee will be responsible for reporting UPs to the IRB. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- All UPs, whether they are SAEs or not, will be reported to the IRB within the institution's specific time frame of the PI or Sub-I becoming aware of the event.

8.4.4 EVENTS OF SPECIAL INTEREST

Not applicable.

8.4.5 REPORTING OF PREGNANCY

Not applicable.

8.5 STUDY HALTING RULES

As metformin has a long history of both short term and long term safety, it is not anticipated that there will be any circumstances that would necessitate a 'halt' to the study. A halt to the study will be considered by the study team if new information becomes available or adverse events connected with

the study suggest that continuation of the study or its sub-study (ies) is not in the best interest of the participants. Should this happen, the PI or Sub-I will inform the IRB within 24 hours of the decision and will provide the IRB with written documentation detailing the circumstances leading to this decision. The PI or Sub-I will notify all regulatory bodies and ClinicalTrials.gov the FDA of the disposition of the study.

8.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of the PI from first patient screening through study completion.

9 CLINICAL MONITORING

Clinical site monitoring will be conducted to ensure the safety of the participants, to ensure that the rights and well-being of human subjects are being protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

- A HICFA research physician or HICFA nurse practitioner is available for each patient visit. Every participant seen during the week is presented at Friday staff meetings with all HICFA research physicians, research nurses and the research pharmacist in attendance. At this meeting, any concerning adverse events are discussed and all labs are reviewed.
- This is a single-site study and independent audits will not be conducted.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

<u>Primary hypotheses</u>	Compared to controls, oral metformin given for 72 weeks will result in a reduction in frequency of dual PD-1 ⁺ TIGIT ⁺ CD4 T cells
<u>Secondary hypotheses</u>	Metformin given over 72 weeks is safe and well tolerated in aviremic HIV-infected individual patients on potent ART Compared to controls, oral metformin given for 72 weeks will lead to ○ Preservation of total and peripheral fat ○ Less increase in truncal fat. ○ Preservation of total lean tissue. ○ Increased preservation of hand grip strength ○ Lower increase in carotid intima-media thickness ○ Improvement in global NP performance ○ Improvement in domain-specific NP performance (Learning and Memory, Executive Function, Working Memory and Psychomotor Speed) ○ Improvement in depression (BDI-II and CES) scores
<u>Sub-Study hypotheses:</u>	Compared to controls, oral metformin given for 72 weeks will result in ○ Decrease in intracellular HIV DNA in CD4 T cells within rectal lymphoid tissue ○ Decrease in intracellular HIV DNA in CD4 T cells within lymph node tissue

10.2 ANALYSIS DATASETS

The analysis dataset will include all subjects enrolled into the study with data from entry to the final week 72 study visit.

10.3 DESCRIPTION OF STATISTICAL METHODS

10.3.1 GENERAL APPROACH

This is a prospective, randomized, 72 week study of metformin vs observation in aviremic individuals with chronic HIV on potent antiretroviral therapy. Subjects will be randomized 1:1 to receive metformin vs observation.

For descriptive statistics, continuous data will be presented as a mean with standard deviation and range. Categorical data will be presented as a percentage. Descriptive statistics will include all participants and sorted by study group (i.e., subjects on metformin versus subjects on observation).

For inferential tests, p-value < 0.05 will be used for statistical significance. Sample size will be limited to the use of co-variables in the statistical model. Tests of normality will be performed on continuous data and transformation applied where applicable.

10.3.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

As the primary analysis for Aim-1, we will perform Mann–Whitney U tests to compare changes of the frequencies in PD1+ TIGIT+ expression on CD4 T cells between metformin and observation arms, from baseline to 72 weeks. Since we expect to see a greater reduction in the metformin arm, one-sided tests will be used to evaluate the hypotheses at 5% type 1 error rate. As secondary analyses for Aim-1, we will also test changes from baseline to 24 and 72 weeks using Mann–Whitney U tests in all single, double, and triple CD4 cell NCR combinations in comparing the metformin and observation arms. Furthermore, generalized linear models (GLM) will be used to identify the significance of metformin effect while adjusting for the effects of multiple potential risk factors that may affect the outcome variable. Possible risk factors such as age, gender, duration of HIV infection, and nadir CD4 count, will be included in the analysis.

10.3.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

AEs are categorical data. AEs will be summarized as frequencies and percentages within metformin-treated subjects versus observation subjects and presented in a table. Chi-squared test or Fisher's exact test will be used to test for differences in frequencies between metformin treated subjects versus observation subjects. P-value < 0.05 will be used for statistical significance.

For hypotheses involving body composition, strength, and NP test performance, we will perform Mann–Whitney U tests to compare changes in various parameters between metformin and observation arms. P-value < 0.05 will be used for statistical significance.

10.3.4 SAFETY ANALYSES

The number of adverse events will be tabulated by grade and symptom/lab parameter.

10.3.5 ADHERENCE AND RETENTION ANALYSES

The number of subjects who discontinue study drug, withdraw completely from the study, or are lost to follow-up will be summarized in a table. The specific reasons will also be presented.

10.3.6 BASELINE DESCRIPTIVE STATISTICS

Baseline characteristics, including demographics and HIV associated immunologic parameters will be presented in a table with standard descriptive summary statistics collectively and by treatment group.

10.3.7 PLANNED INTERIM ANALYSES

Considering the abundance of data on the short term and long term safety of metformin, interim analyses for the purpose of safety is believed to not be necessary. Because the intent of this study is to assess the degree of improvement in CD4 T cell NCR over 72 weeks and whether there is concomitant improvement that might be demonstrated in some clinical parameters, the study team believed that the study should be carried to completion. As there is no intent to terminate the study early, no interim efficacy analysis is believed to be necessary.

10.3.8 ADDITIONAL SUB-GROUP ANALYSES

Sub-group analysis by age, sex, race/ethnicity will be performed as possible within the relatively small sample size of this study.

10.3.9 MULTIPLE COMPARISON/MULTIPLICITY

Not applicable.

10.3.10 TABULATION OF INDIVIDUAL RESPONSE DATA

Individual participant data will be listed by measure and time point.

10.4 SAMPLE SIZE

The sample size estimation for the proposed study is based on the effect of metformin on the frequency of NCR expression on CD4 T cells. We will evaluate changes of PD1+ TIGIT+ expression on CD4 T cells from entry to 24 and 72 weeks, by comparing metformin and the observation arms. Based on the 24 weeks long pilot study conducted using 12 subjects (6-metformin and 6-observation arms) very high standardized effect sizes $d=1.87$ and $d=1.94$ were observed for change in PD1+ TIGIT+ expression. However, we calculated the sample size for a conservative effect size of 0.8, which require a total of 44 patients to achieve 80% power to reject the null hypotheses under 5% type 1 error rate. To allow 20% dropout rate, a total 56 subjects will be required. However, we will recruit a total of 60 subjects with equal allocation, 30 per each group.

10.5 MEASURES TO MINIMIZE BIAS

10.5.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

After signing the Informed Consent Form (ICF), the subject is assigned a unique patient identifier number (PID). At the time of enrollment (randomization), the subject is assigned a unique subject identifier number (SID). PIDs and SIDs are not re-assigned. Screening Numbers and Subject Numbers are not re-assigned. Subjects are randomly allocated (1:1) to active medication (metformin) or observation.

Randomization will occur only after written informed consent has been obtained from the subject or representative, and subject eligibility has been confirmed. A study enrollment form will be sent to the research pharmacist to initiate the randomization process and assign the participant to study medication or to observation. The research pharmacist will obtain the randomized treatment allocation for the subject and the pharmacist or designated staff will prepare the study medication if indicated.

10.5.2 EVALUATION OF SUCCESS OF BLINDING

Not applicable.

10.5.3 BREAKING THE STUDY BLIND/PARTICIPANT CODE

Not applicable.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Source data is defined as information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the study. CRF will be designed by the research nurse coordinator and will serve as source documents for some of the required data except for the source data from participant's medical records, hematology, chemistry, and EKG results.

The research nurse coordinator and research pharmacist will maintain the source data, CRF, and all regulatory documents in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants. The participant's research records will be stored in a locked file cabinet in the research unit's main office. PI, Co-I, research nurse coordinator, research pharmacist, and research staff working on the protocol or data analyses will have access to all documents and will adhere to all requirements for subject confidentiality as outlined in the ICF. QUALITY ASSURANCE AND QUALITY CONTROL

This section indicates the plans for quality management, the system for assessing the quality of processes within the study. Quality management will encompass quality assurance (QA) and quality control (QC). QA is defined as all planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirement(s) (ICH E6 1.46). QC is defined as the operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial-related activities have been fulfilled (ICH E6 1.47).

The research unit has a written QA and QC management plan that will be used for this protocol.

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be clarified/resolved by the PI in collaboration with the research nurse coordinator and research pharmacist.

The PI, research nurse coordinator, and research pharmacist will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices, GLP).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by local and regulatory authorities.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 ETHICAL STANDARD

The PI will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and the ICH E6.

12.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form by the IRB must be obtained before any participant is enrolled.

Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

12.3 INFORMED CONSENT PROCESS

12.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

IRB-approved informed consent document describing in detail the study intervention, study procedures, and risks will be given to the participant and written documentation of informed consent will be required prior to starting intervention/administering study intervention.

The informed consent document for this study is submitted with this protocol.

12.3.2 CONSENT PROCEDURES AND DOCUMENTATION

For each study subject, a signed written informed consent will be obtained prior to any protocol-related activities. The process will be fully documented in the source documents.

The research nurse coordinator, PI or Co-I will explain orally the nature, duration, and purpose of the study, and the action of the drug (metformin) in such a manner that the subject is aware of the benefits, potential and other risks, inconveniences, or adverse effects that may occur because of their participation. They will be informed that the subject may withdraw from the study at any time. They will receive all information that is required by federal, state and local laws and regulations and ICH guidelines.

The participant will also be asked to read and review the IRB-approved informed consent document and to ask questions prior to signing. An opportunity will be given for the participant to discuss the study with their significant other and/or primary care physician or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures specifically for the study. The participant 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 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.

12.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators and research staff at HICFA. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without the permission of the Principal Investigator.

All subjects will be assigned a unique patient identifier number (PID) and at the time of enrollment (randomization), each subject will be assigned a unique subject identifier number (SID). PID and SIDs are not re-assigned. The log that links the subject's name to the PID is stored in a locked file cabinet. This information is also stored on a separate flash drive and not on a computer hard drive or server. The locked file cabinets housing these confidential documents will be stored in research unit's main office, which is in a 24-hour secured building. The only individuals who have access to these locked files are authorized personnel of the research staff directly involved in this study.

Representatives of the IRB or FDA may inspect all documents and records required to be maintained by the PI, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study.

If the study participant elects to have their clinical laboratory data sent to their primary care providers, they will be asked to sign a separate consent covering release of his/her data to their physician.

The study participant's contact information will be securely stored in a locked file cabinet for internal use during the study. At the end of the study, all records will continue to be kept in a locked file cabinet in the main office of the research unit for as long a period as dictated by local IRB and Institutional regulations.

A study database containing de-identified study participant research data only will be utilized for the purposes of statistical analysis and scientific reporting. The database will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by research staff will be secured and password protected.

12.4.1 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

Intended Use of Stored Human Samples, Specimens, or Data: The ability to conduct studies on the mechanisms underlying metformin's pleomorphic effects by accessing the banked specimens together with de-identified data is an integral part of this clinical study. Therefore, the willingness to have specimens stored for future use is part of the permission we are seeking from participants prior to entry into this study. Participants who are not willing to have their specimens stored and utilized for future studies related to HIV or metformin will not be enrolled into the study.

Storage of Stored Human Samples, Specimens, or Data: Blood samples will be stored in the research unit's processing laboratory. Access to stored samples will be limited to laboratory staff of the research unit. No genetic testing will be performed.

Tracking of Stored Human Samples, Specimens, or Data: De-identified data will be tracked using subject's unique identifier code. Data will be kept in password-protected computers.

12.5 FUTURE USE OF STORED SPECIMENS

Stored specimens may be used for research into the causes, diagnosis and treatment of HIV and its complications or the impact of metformin in the context of HIV.

13 DATA HANDLING AND RECORD KEEPING

13.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the PI, Co-I, research nurse coordinator, and research pharmacist at the site under the supervision of the PI. The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. ERASURE, OVERWRITE, OR USE OF CORRECTION FLUID OR TAPE ON THE ORIGINAL WILL NOT BE ALLOWED.

Copies of the CRF will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the CRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap and/or Microsoft Excel, a 21 CFR Part 11-compliant data capture system provided by the University of Hawaii John A. Burns School of Medicine. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

13.2 STUDY RECORDS RETENTION

Study documents will be maintained and retained in accordance with applicable federal, state and local laws, rules, regulations, guidelines and regulatory requirements.

13.3 PROTOCOL DEVIATIONS

The following describes the managing of Non-Compliance that may affect the rights or welfare of human subject participants.

13.3.1 DEFINITIONS

Non-compliance is defined as conducting research involving human participants in a manner that disregards or violates federal regulations and/or the institutional human research protection program policies governing such research.

Continuing Non-Compliance is defined as a pattern of non-compliance that suggests the likelihood that, without intervention, situations of non-compliance will recur, a repeated unwillingness to comply, or a persistent lack of understanding of how to comply. **Serious Non-Compliance** is defined as non-compliance that adversely affects the rights or welfare of human research participants.

Protocol Violation is defined as a type of non-compliance, any deviation or departure from the IRB-approved protocol or proposal that does not have prior approval by the IRB unless the change is necessary to remove an apparent immediate hazard to one or more study participants.

- **Minor Protocol Violation** (also known as "Protocol Deviation") is defined as a protocol violation that does not impact the safety or welfare of study participants, compromise the integrity of study data, or affect participants' willingness to participate in the study. Examples of protocol deviations include the following (not an all-inclusive list):
 - Use of an outdated version of the consent form if risks to participants described in the

- form do not differ from those described in the current form;
 - A study procedure conducted out of sequence;
 - A study visit conducted outside the required time period;
 - Failure to perform a required procedure, assessment, or lab test that, in the opinion of the PI, is unlikely to have an adverse impact on participant safety or welfare or the integrity of the data collected; and
 - Enrollment of more than the IRB-approved number of participants.
- **Major Protocol Violation** is defined as a protocol violation that may impact the safety or welfare of study participants, compromises the integrity of study data, or affects participants' willingness to participate in the study. Examples of major protocol violations include the following:
 - Failure to obtain informed consent, or obtaining informed consent from a participant after initiation of study procedures;
 - Using an outdated version of the consent form when risks to participants described differ from those described in the current consent form;
 - Performing a study procedure not approved by the IRB;
 - Modifying a study without prior IRB approval unless to remove an apparent immediate hazard to one or more study participants;
 - Enrollment of a subject who did not meet any inclusion/exclusion criteria;
 - Failure to perform a required procedure, assessment, or lab test that, in the opinion of the PI, may affect the safety or welfare of one or more participants or the integrity of the data collected;
 - A drug dispensing or dosing error;
 - A study visit conducted outside of the required time period with a potentially adverse effect on the safety or welfare of one or more study participants;
 - Failure to follow applicable federal regulations or IRB policies and procedures, including those for reporting Unanticipated Problems and Adverse Events;
 - Failure to follow an IRB-approved safety monitoring plan.

Corrective Action Plan (CAP) is defined as a plan developed in response to a protocol violation that outlines the steps to be taken to: (1) reduce the risk to participants affected by the violation, and (2) prevent a recurrence of the violation.

13.3.2 PROCEDURES FOR REPORTING PROTOCOL VIOLATIONS

Minor Protocol Violations will not require reporting to the IRB at the time of each occurrence per institution Standard Operating Procedures (SOP).

The PI will provide to the IRB a summary of minor or major protocol violations that occurred during the prior IRB approval period at the time of *continuing review*.

Timeframe of Reporting Major Violations. The PI will notify the IRB of major protocol violations no later than 24 hours after the PI becomes aware of the event by phone or email, and report the event to the IRB no later than 10 business days after discovery of the violation using the protocol violation report form by a member of the study team.

Changes to Remove a Hazard. If a protocol change has been initiated to remove an apparent immediate hazard to one or more study participants, the PI will report this change to the IRB no later than 5 business days after initiation of the change.

13.4 PUBLICATION AND DATA SHARING POLICY

13.4.1 USE OF STUDY FINDINGS

This study will be registered with www.clinicaltrials.gov and updated as study findings become available.

It is also anticipated that if beneficial impact of metformin is seen in this study, grant applications proposing further research into the potential use of metformin in HIV would be submitted.

13.4.2 PUBLICATION

It is anticipated that multiple publications will result from this study. The Investigative Team research-physicians (Drs. Shikuma, Chow and Shiramizu) will be responsible for assigning the primary author responsible for leading the writing of the manuscript, for developing publication procedures and resolving authorship issues.

14 STUDY ADMINISTRATION

14.1 STUDY LEADERSHIP

The clinical protocol will be implemented with Dr. Cecilia Shikuma as Principal Investigator and Drs. Dominic Chow and Bruce Shiramizu as Co-Investigators.

15 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial.

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VERSION HISTORY

Version	Date	Significant Revisions
1.0	Approval: 7/26/18	Original
2.0	Approval: 6/6/19	Several balance & mobility measurements deleted; compensation revised; inclusion HIV RNA criteria changed from <50 to <100 copies/mL; admin. revisions
3.0	Approved 9/18/19	Addition of cIMT and lengthening the study from 48 to 72 wks
4.0		HIV RNA inclusions criteria revision and increasing remuneration by \$20