PROTOCOL

MET-IH

Assessment of metformin for restoration of immune homeostasis in HIV+ and HIV- individuals with a history of injection drug use

CLINICAL TRIAL SPONSORED BY

National Institute on Drug Abuse (NIDA)
Department of Health and Human Services (DHHS)
Bethesda, Maryland, USA

University of Alabama at Birmingham
Birmingham, Alabama, USA

March 25, 2026
MET-IH
Version 7.0

Protocol Signature Page

Assessment of metformin for restoration of immune homeostasis in HIV+ and HIV- individuals with a history of injection drug use

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with US Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice E6(R2); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (eg, US National Institutes of Health, NIDA) and institutional policies.

Principal Investigator: _____Ellen F. Eaton, MD_____

Signed: _____ Date: _____

Protocol Number: NIDA-UAB-01

Protocol Version: Version 7.0

Protocol Date: March 25, 2026

Acronyms and abbreviations

Ab	antibody
AE	adverse event
Ag	antigen
ALT	alanine aminotransferase
AFAB	assigned female sex at birth
AMAB	assigned male sex at birth
ANOVA	analysis of variance
AoU	Assessment of Understanding
BMI	body mass index
BP	blood pressure
CBC	complete blood count
CDM	Clinical Data Manager
CE	European Conformity (Conformité Européenne)
CI	confidence interval
CRF	case report form
DM	diabetes mellitus
EAE	expedited adverse event
eGFR	estimated glomerular filtration rate
EIA	enzyme immunoassay
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GINA	Genetic Information Nondiscrimination Act
GPP	Good Participatory Practices
HCV	hepatitis C virus
Hgb	hemoglobin
HHS	United States Department of Health and Human Services
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IM	intramuscular
IND	Investigational New Drug (application)
INR	international normalized ratio
IRB	Institutional Review Board
MAAE	medically attended adverse event
MedDRA	Medical Dictionary for Regulatory Activities
MET	Metformin
MO	Medical Officer

NIDA	National Institute on Drug Abuse
NIH	National Institutes of Health
NSAID	nonsteroidal anti-inflammatory drug
OUD	opioid use disorder
PBMC	peripheral blood mononuclear cell
PI	Principal Investigator
POPIA	Protection of Personal Information Act
PWID	People who inject drugs
PWH	Person with HIV-1
RA	Regulatory Authority
RCT	Randomized Control Trial
SAE	serious adverse event
SD	standard deviation
SICF	sample informed consent form
DSMB	data safety monitoring board
DSMP	data safety monitoring plan
SOP	standard operating procedures
SSP	study specific procedures
STI	sexually transmitted infection
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	upper limit of normal
USP	US Pharmacopeia
WBC	white blood cell

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Executive Summary

Title

Assessment of metformin for restoration of immune homeostasis in HIV+ and HIV- individuals with a history of injection drug use (MET-IH)

Design

This is a single site randomized controlled interventional trial. The purpose of this randomized clinical trial (RCT) is to determine the potential of metformin to reduce systemic inflammation and mitigate immune dysregulation among people who inject drugs (PWID). In the trial, adults with a history of recent injection drug including opioid and amphetamine use with (PWH) or without HIV and elevated systemic inflammation as determined by CRP ≥ 1 mg/L, will be recruited, and randomized 1:1 to 16 weeks of metformin or placebo treatment, during which time they will receive Jynneos (MPOX) and Capvaxvie (PCV21, S. pneumoniae) vaccinations. All participants will be followed to 6 months post-enrollment.

Study products and administration

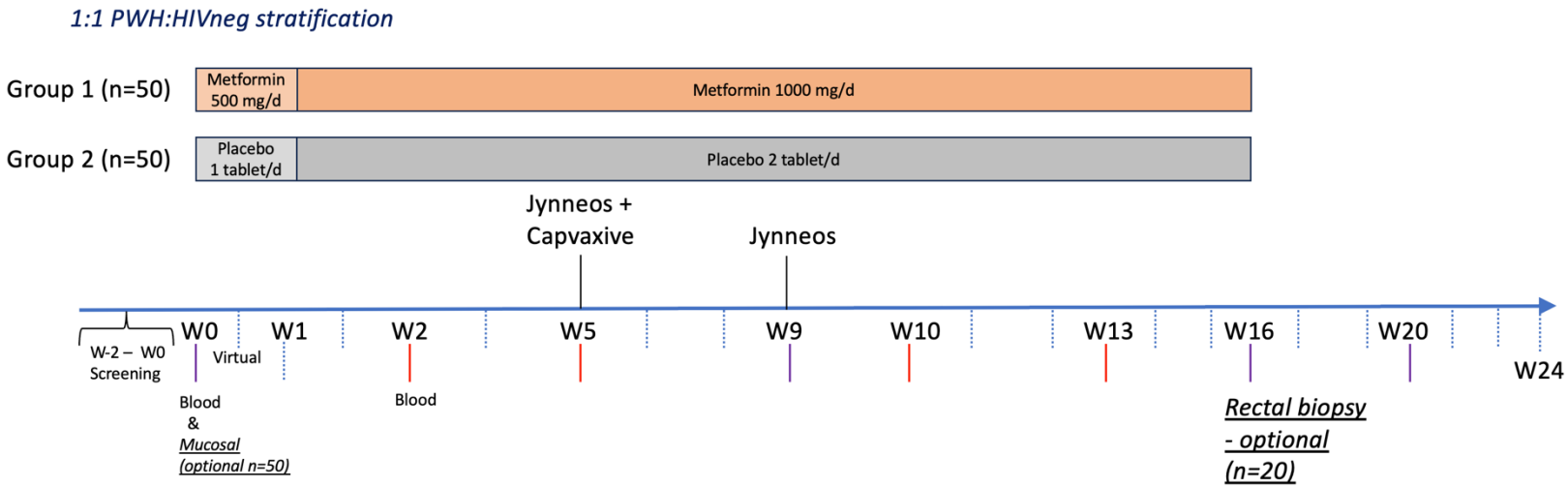
- **Metformin**: 500 mg metformin extended-release (ER) once a day for 1 week, followed by 1000 mg once a day for 15 weeks.

Study participants

100 volunteers with or without HIV, and a history of injection drug use in the Birmingham, AL metro area, aged 19 to 64 years

Study plan

Figure 0-1 Study plan



Eligible participants, ~1:1 stratified by HIV status, will be randomized 1:1 to receive either metformin (Group 1) or placebo (Group 2) starting at week (W) 0. At W1, participants will increase dosage and continue taking placebo or metformin for 112 days. At W5 participants will be immunized consistent with MPOX (Jynneos) and pneumococcal (Capvaxive, PCV21) vaccines. At W 0 (enrollment), 9, 16, and 20 visits participants will have the option of providing rectal secretion samples. Rectal biopsy samples will be collected at W16 from HIVneg participants that opt-in to this sampling.

Table 0-1 Treatment Schema

Group	N	Week 0 - 1 (Day 0-7)	Week 1 - 16 (Day 8-112)
1 (Metformin)	50	Metformin ER, 500 mg OD, orally	Metformin ER, 1000 mg OD, orally
2 (Placebo)	50	Placebo, one tablet OD, orally	Placebo, two tablets OD, orally
Total	100		

Both groups will open to enrollment concurrently.

Duration per participant

6 months of scheduled in-person clinic and virtual visits

Estimated total study duration

48 months

Study site

The study will be conducted at the University of Alabama at Birmingham.

1 Hypothesis and Study Objectives

1.1 Hypothesis

Compared to placebo (Group 2), metformin (MET) treatment (Group 1) will result in a decrease in systemic inflammation and improvement in antibody responses to vaccination among people who inject drugs (PWID).

1.2 Primary Objective and Endpoint

Objective: To assess if metformin treatment results in reduction in serum C-reactive protein (CRP) compared to placebo treatment.

Endpoint: Change in serum C-reactive protein from baseline to week 16.

Justification: CRP is a biomarker for systemic inflammation and is elevated in many PWID and PWH. Previous studies have demonstrated reduction of CRP with MET treatment.

1.1 Secondary Objectives and Endpoints

a. Secondary Objective: To assess if metformin treatment results in a decrease in B cell exhaustion compared to placebo treatment.

   Secondary Endpoint: Change in peripheral blood exhausted B cells from baseline to week 16.

b. Secondary Objective: To assess if metformin treatment results in a decrease in T cell exhaustion compared to placebo treatment.

   Secondary Endpoint: Change in peripheral blood exhausted T cells from baseline to week 16.

c. Secondary Objective: To assess if metformin treatment improves antibody response to vaccination.

   Secondary Endpoint: Change in vaccine-specific IgG antibody titer from baseline to week 9 and baseline to week 13.

Justification: Alterations in B cells, T cells, and antibody responses to vaccines are evident in many PWID and PWH. Previous studies have demonstrated improvement in B cell, T cell, and Ab responses with MET treatment

1.2 Exploratory Objective and Endpoints

Exploratory Objective: To assess if metformin treatment results in a decrease in mucosal inflammation compared to placebo treatment.

Exploratory Endpoint: Change in oral and/or genital mucosal inflammatory cytokines from baseline to week 16. Differences in rectal lymphocyte characteristics at week 16.

Justification: Substance use, including amphetamine use is associated with increased mucosal inflammation and related negative health consequences. As MET has been shown to decrease systemic inflammation, we seek to assess its effect on the mucosa.

2 Introduction

The aim of this study is to determine the potential of metformin (MET) to reduce systemic inflammation, mitigate immune dysregulation, and improve vaccine responses among people who inject drugs (PWID).

To achieve these goals, this study will:

1) Enroll adults with a history of injection drug including opioid and/or amphetamine use with or without HIV (PWH) and elevated systemic inflammation as determined by CRP ≥ 1 mg/L, who will then be treated with MET or placebo, and immunized with MPOX and Pneumococcal vaccines.

2) Assess the ability of MET treatment to 1) reduce systemic inflammation, as measured by CRP, 2) reduce exhausted B cell and exhausted T cells, 3) improve antibody responses to vaccines, and 4) reduce mucosal inflammation.

This study will determine the clinical potential of MET to mitigate immunopathology and improve immune responses to pathogens in PWID.

2.1 Study Rationale

We and others have described significant immune alterations in people with HIV (PWH) and individuals that inject opioids and/or stimulants including increased systemic inflammation and lymphocyte dysregulation, including among B cells. This heighted inflammatory state and distorted B cell function likely contribute to increased risk and severity of infections and inflammatory comorbidities including cardiovascular, kidney, liver, and autoimmune diseases. Although anti-retroviral therapy (ART) suppresses HIV, its ability to restore immune homeostasis is incomplete, resulting in accelerated immunological aging and associated co-morbidities; subsequently development of adjunctive interventions to mitigate these complications is ongoing. Among PWH, those who inject drugs are particularly vulnerable to inflammatory and infectious sequalae, driven by related factors including challenges in ART adherence, maintaining viral suppression, and engagement in care. Active substance use, intoxication, and related behaviors associated with injection drug use can impact HIV viral dynamics and immune competency. Among people who inject drugs (PWID) that do not have HIV, there is a high risk for acquiring HIV, clear indications of reduced effectiveness of HIV vaccines, and altered viral dynamics in the acute stage of HIV infection; these unique factors highlight the challenges of HIV vaccine development for PWID. Thus, there is an unmet need for interventions that can restore immune homeostasis in PWID to improve responses to pathogens and minimize inflammatory-related co-morbidities.

Metformin (MET), the most widely prescribed drug for type 2 diabetes (T2D), may be such an intervention. FDA approved in 1994, MET is a first-line treatment

for T2D and is used off-label for a variety of metabolic-related conditions and used by >20 million people in the US annually. Animal and human studies have demonstrated the ability of MET to reduce inflammation and enhance vaccine responses, likely mediated in part through direct and indirect lymphocyte regulation through mTOR inhibition. Our central hypothesis is that MET can mitigate the inflammation and immune dysregulation that is prevalent in PWID including among PWH, restoring immune homeostasis and responsiveness. This will be addressed through a randomized, two-group, placebo controlled, clinical trial of MET, stratified by HIV status, in individuals with a history of injection drug use including opioids and stimulants and with elevated systemic inflammation. Assessment of MPOX and pneumococcal vaccine responses during this trial will allow examination of adaptive responses to pathogens of concern within these populations and serve as a surrogate for assessing their potential response to a future HIV vaccine. Vaccine outcomes will provide a metric to complement the integrated behavioral and high-resolution functional analysis of systemic and mucosal inflammation and immunity, providing mechanistic insights relevant to HIV prevention and pathogenesis.

2.2 Background

Immune dysregulation amplifies the HIV and opioid syndemic. Among PWH, HIV-associated co-morbidities driven in-part by accelerated inflammaging and not fully mitigated by anti-retroviral therapy (ART), continue to be a major health care burden(1, 2). These outcomes are further exacerbated in PWID(3, 4); with PWH that were presumed infected through injection drug use having the highest rates of all death among PWH even after adjustment for ART, including AIDS, non-AIDS infection, non-AIDS malignancy and heart/vascular deaths(5-7). Despite the increasing availability of ART for treatment and prevention, and encouraging declines in the incidence of new HIV infections overall, new HIV infections among PWID remain stable in the US(8). Particularly concerning is the current wave of the opioid epidemic is characterized by dominant synthetic opioid use (e.g. fentanyl) and its frequent co-use with illicit stimulants(9), primarily methamphetamine, and our understanding of immune dysregulation among PWID may now be underestimated. The differing pharmacology of fentanyl compared to heroin may further alter its impact on immunity, as it is more lipophilic(10) and is associated with a shorter intoxication compared to heroin(11), thus contributing to a higher frequency of injections per individual than was typically observed with heroin(11), and with the increased co-stimulant use(12, 13) may further drive inflammatory and infectious co-morbidities. In assessing the immunological and infectious complications associated with injection drug use, it has been challenging to disentangle the impact of the action itself and associated behaviors, from the substances themselves; however, it is clear that many of the co-morbidities associated with PWID are driven in part by increased inflammation and immune dysregulation, suggesting that interventions to restore immune homeostasis may have the potential to improve outcomes for these individuals.

B cells: Critical mediators of pathogen-specific immunity compromised by HIV and injection drug use. B cells, defined in humans by the expression of CD19, are essential members of the adaptive immune response most notably for their production of antibody (Ab); responsible for protection from infectious pathogens, mediating autoimmune diseases, and driving inflammatory processes. Naive B cells develop from the bone marrow, and in response to antigen encounter (e.g., pathogen), undergo a differentiation process into various effector and memory subsets that have specialized localization and functions. This includes B cells that can produce inflammatory (e.g., TNF-α) or regulatory cytokines (e.g., IL-10, TGF-β), and subsets that are specialized in production of secreted Ab, including short-lived plasmablasts and long-lived plasma cells. The B cell deficits among PWH is well described by us and others, and although some peripheral B cells subsets return to near normal frequencies with ART, damage to the IgM memory/marginal zone-like B cell compartment, vital for responses to S. pneumoniae, persists(14-21). We have previously found that people who inject heroin have a remarkable 2-fold increase in total B cells (**Fig 2-1A**), including expansion of several activated B cell phenotypes such as cells expressing CD95

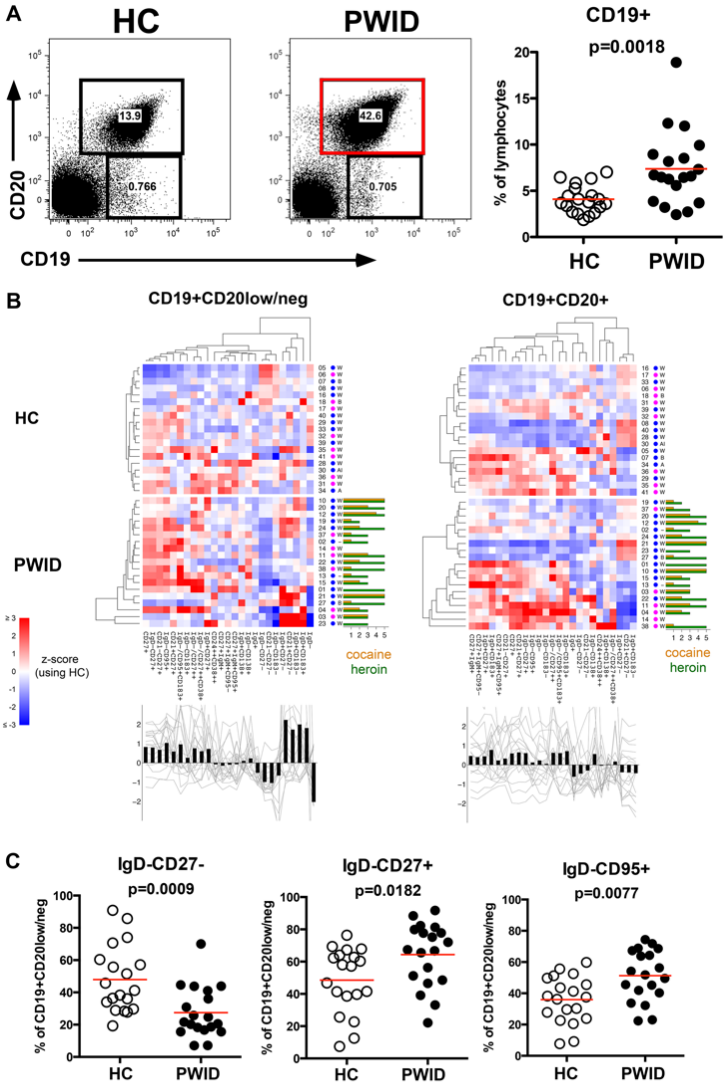


Figure 2-1. B cell dysregulation among PWID. Peripheral blood B cells from HIV- HCV- PWID (n=19) and healthy controls (HC) (n=19) were analyzed by flow cytometry. (**A**) Representative samples gated on live, CD14-CD3-CD4- lymphocytes and frequency of total B cells. (**B**) B cell phenotypic profiles of individuals with heroin and cocaine use metric indicated. With severity of cocaine and heroin use indicated. (**C**) Activated B cell subsets among CD19+CD20low/neg B cells.

and CD183 (CXCR3) (**Fig 2-1B**)(22). The CD19+CD20low/neg population which is typically enriched for plasmablasts and pre-plasmablasts was phenotypically distinct, with increased CD27 and CD95 expression consistent with an activated B cell phenotype (**Fig 2-1C**). Although PWID are likely to have more frequent infectious insults and subsequent antigen-specific B cell expansion, the extent of B cell activation and expansion observed suggests that bystander activation, induced by inflammatory mediators (e.g., cytokines and TLR stimulation) is occurring. Such chronic bystander activation may deplete the naïve B cell pool and impact the ability of the B cells to respond appropriately upon encountering a pathogen. This B cell dysregulation likely underlies the numerous reports of decreased Ab response to vaccination, including Hepatitis A/B and S. pneumoniae among PWID and PWH(23-28).

Systemic inflammation: a potential driver of B cell dysregulation and inflammatory diseases. Injection drug use has been associated with various inflammatory conditions including hypertension, cardiovascular disease, and kidney disease(29-32) as well as increased auto-reactive antibodies(33), suggestive of systemic inflammation and possible alteration in B cell tolerance. Our analysis of the plasma of individuals that inject heroin found significantly increased plasma TGF-α (~10-fold; an EGFR ligand), TNF-α (~5-fold, a potent inflammatory cytokine); IL-8 (~10-fold, a chemokine important for neutrophil migration, induction of phagocytosis and angiogenesis), and sCD40L (~5-fold; a potent inflammatory mediator produced primarily by platelets, which induces proliferation and class-switching of B cells) (**Fig 2-2**)(22). We also found plasma LPS (bacterial endotoxin) in several PWID, suggestive of bacterial infection or increased gut permeability. CRP was significantly increased in PWID, with 78% having CRP >3 mg/L (**Fig 2-3**). We performed global plasma proteomics analysis

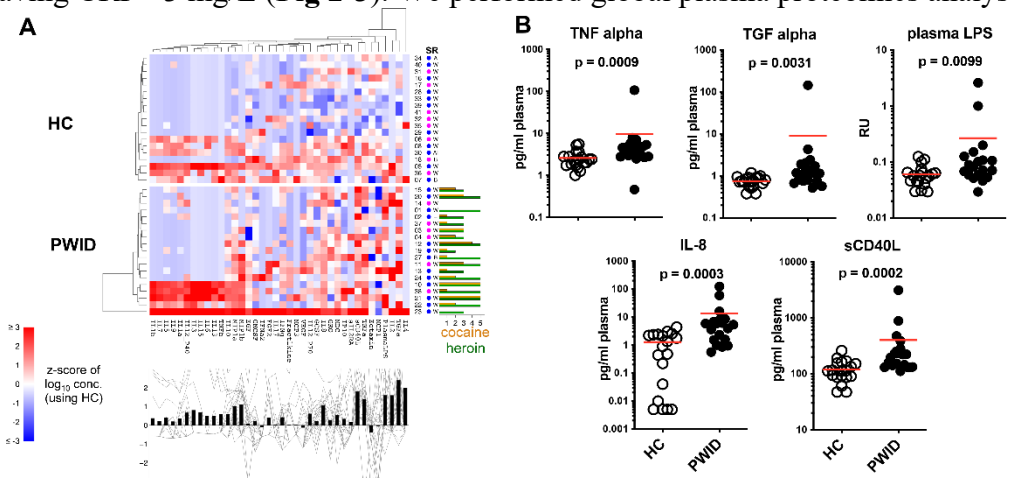


Figure 2-2. Plasma profile of PWID. Plasma cytokines, chemokines and growth factors were measured (**A**). (**B**) Select plots of individual analytes, each symbol represents an individual participant, the red lines indicate mean.

on a subset of PWID, those with the most distinct profiles from healthy controls (HC) to identify other factors and processes that may be altered and impact the B cell compartment. Our mass-spectrometry based data-independent acquisition (DIA-MS) approach, which is a next generation quantitative proteomics technique that is ideally suited to analyze sample cohorts with minimal bias(34), determined the abundance of 721 proteins, providing exceptional resolution of the plasma proteome. The plasma

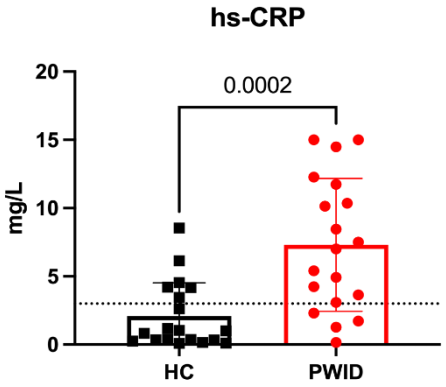


Figure 2-3. Plasma CRP in PWID. Plasma high sensitivity (hs) CRP was determined by ELISA. Each symbol represents an individual participant. Dashed line = 3 mg/L.

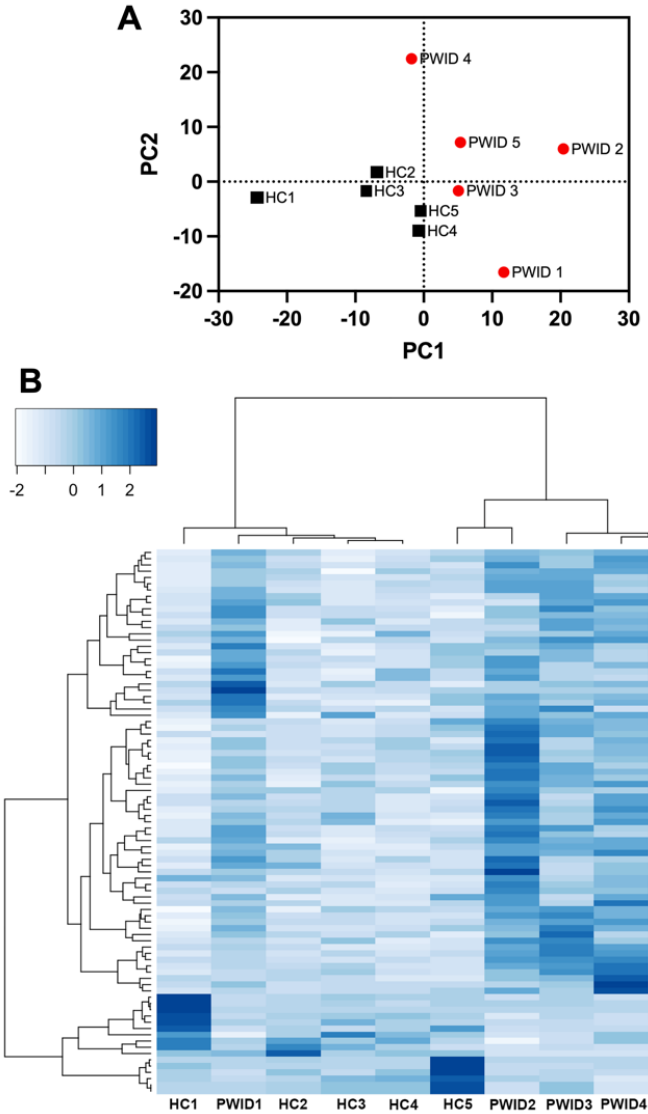
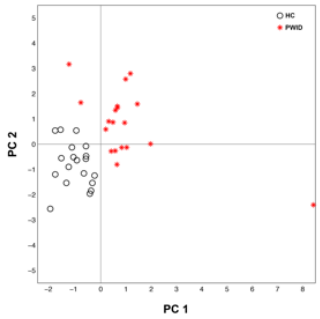


Figure 2-4. Plasma proteomics profile of PWID. Plasma from healthy controls (HC n=5) and individuals who inject heroin (PWID n=5) was analyzed by DIA-MS. (**A**) PCA of samples based on expression of all proteins. (**B**) Heatmap of significantly differentially expressed proteins. ·protein of interest

proteomes of PWID were highly distinct from HC, and 86 proteins were significantly differently expressed (**Fig 2-4**).

Many inflammatory factors were elevated including sCD14, sCD44, and LPS Binding Protein (LBP), consistent with previous reports(35, 36) (37). Additionally, many complement components and platelet/coagulation related factors including Pro-Platelet Basic Protein (PPBP) and Platelet Factor 4 (PF4), were elevated that have not been previously appreciated. These results are consistent with a chronic systemic inflammatory phenotype in PWID that may contribute to the observed B cell dysregulation. The elevated systemic inflammation in PWID that we observed is consistent with previous studies(15, 38-42), and may contribute to co-morbidities as chronic inflammation is associated with cardiovascular disease, kidney disease, accelerated aging, and neurodegenerative diseases, all of which are increased among PWID(43-48). Our recent systematic review of opioid use disorder (OUD) related biomarkers(49) highlights that systemic, peripheral, and chronic inflammation is generally evident with OUD.

<u>Need to understand and improve vaccine responses in PWID.</u> Previous studies have reported reduced vaccine immunogenicity among PWID, including for HCV, tetanus, influenza, and HIV (24, 50, 51). With the increased incidence of severe and chronic infections, inflammatory co-morbidities, and likelihood of diminished vaccine responses, a metric of B cell homeostasis and responsiveness would be advantageous in tailoring treatments to this population and monitoring clinical outcomes. In a small subset of PWID, using conventional univariate and



Ranked using SVM weights	Feature	HC (mean)	PWID (mean)	P (univariate)
1	sCD40L (pg/ml)	120.2	249.5	0.0004
2	IgG4 (RU)	192.7	308.6	0.0310
3	CD19+CD20+ (% of lymphocytes)	3.8	7.2	0.0036
4	TGF-α (pg/ml)	0.8	1.4	0.0056
5	TNF-α (pg/ml)	2.6	4.1	0.0016
6	MIP-1β (pg/ml)	4.4	13.2	0.0330
7	IgD-CD27- (% of 19+20low)	48.0	27.6	0.0015
8	MonoHex Ceramide d18.1/16:0 (ng/ml)	0.04	0.07	<0.0001
9	gp140_IgM (RU)	634.9	1055.0	0.0529
10	MonoHex Ceramide d18:1/22:0 (ng/ml)	0.01	0.012	0.0269

Figure 2-5. PWID B cell and inflammatory core metric. A supervised recursive feature elimination approach was utilized to identify the top ten features (of 216 total) that when combined, classified the samples as PWID or HC with 100% accuracy using 10-fold cross-validation. Left. PCA plot of individual participants based on the ten features. Right. Identified features.

multivariate machine learning analysis of 216 plasma and cellular features we identified 10 core systemic inflammatory and B cell parameters that distinguished PWID from healthy individuals (Fig 2-5). In a complementary meta-analysis of 9 different Phase 1-2 HIV vaccine trials, to identify host features at baseline that were predictive of their antibody response following vaccination(52), we utilized SuperLearner, which estimates the performance of multiple machine learning

models, creating an ensemble analysis(53). We identified host features at baseline that were predictive of their Ab response following HIV vaccination.(52) This revealed several features associated with immune activation including white blood cells (WBC), neutrophils, and platelets that provide value in predicting vaccine response in this generally healthy population (Fig 2-6). It is anticipated these features may be further informative in PWID given their elevated systemic inflammation. An anticipated outcome of this study is to build upon the emergent data of PWID and vaccine responsiveness to monitor B cell homeostasis with the long-term goal of establishing clinically informative metrics that can be validated in future studies.

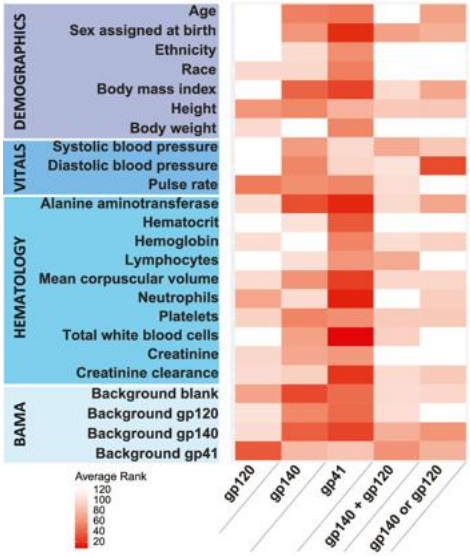


Figure 2-6. Baseline determinants of antibody response to HIV vaccines. SuperLearner based analysis of 25 baseline features for predictors of IgG response to HIV vaccine components (gp120, gp140, gp41) among 831 individuals was performed and average rank of feature across analysis approaches indicated.

This study will evaluate the impact of MET treatment on the immunogenicity of the FDA-approved MPOX (Jynneos) and Pneumococcal conjugate (PCV21, Capvaxive) vaccines. Specifically, IgG antibody responses will be evaluated as secondary outcomes. Evaluation of responses to these vaccines enable the assessment of both T-cell dependent (Jynneos) and T-cell independent (PCV21, Capvaxive) B cell responses in PWID, and contribute to understanding of immunity to pathogens relevant to this population.

2.3 Metformin: Clinical use and potential adjunctive therapy for PWID.

Numerous studies have cataloged the elevated inflammation and immune dysregulation in PWID and PWH, which was a critical first step in defining vulnerabilities in these populations; but now the field must advance to the development of interventions that can decrease inflammatory and infection-related co-morbidities in these individuals. Metformin (dimethylguanide), the third most prescribed drug in the US, prescribed to >20 million individuals in the US annually, with an average cost of $0.17/day(54, 55), represents a potential and practical intervention to mechanistically define and treat the immune deficits in PWID and PWH. Its mechanism of action is not fully elucidated but appears to involve adenosine monophosphate–activated protein kinase (AMPK) activation and increased cytosolic redox due to inhibition of glycerol-3-phosphate dehydrogenase (GPD2), processes that can result in direct and indirect effects on lymphocyte regulation including through mTOR inhibition(56-59). Indeed, mTOR activation is a major regulator of T cell and B cell exhaustion(60-63), such as observed in PWID and PWH. Numerous human studies in diverse populations have shown MET can reduce systemic inflammation including decreasing

CRP(64-68), and within 4 weeks of MET start(68). Several human studies, although with small sample sizes (n=7-15) have demonstrated the ability of MET to enhance Ab responses to influenza vaccine in T2D and non-diabetic older adults, likely related to its ability to mitigate B cell and T cell exhaustion(69-74). MET has been shown to improved COVID-19 outcomes(75-79). Several studies have evaluated MET in PWH, although not in the context of substance use. The LILAC trial, which evaluated MET in non-diabetic virologically suppressed and stable PWH, showed modest reduction in plasma analytes associated with systemic inflammation and peripheral blood activated T cells, and a more substantial MET effect in the gut, with reduction in activated mTOR and HIV-RNA/HIV-DNA ratios in colon-infiltrating CD4+ T cells, and increase of anti-inflammatory associated gut bacteria(56, 80). These were encouraging results, and it is likely that the beneficial effects of MET may have been more striking if the PWH cohort had been pre-selected for those with increased systemic inflammation. A reduction in exhausted peripheral blood CD4+ and CD8+ T cells occurred with MET treatment of non-diabetic PWH on ART(73), and perhaps is linked with the effects of MET on the increased gut inflammation and gut permeability that are thought to drive in-part the systemic inflammation observed in PWH and PWID(22, 81, 82). Our preliminary results indicate MET can improve B cell responsiveness to stimulation in vitro, including B cells from PWID, enhancing IgM and non-IgM (i.e. IgG/IgA) memory B cell development (**Fig 2-7**). Observing that metformin (MET) enhances memory B cell development *in vitro* (**Fig 2-7**), we evaluated the influence of MET on the transcriptome of B cells (**Fig 2-8**). MET treatment resulted in 44 differentially expressed genes, notably the upregulation of HIC1 which positively regulates plasma cell development. As expected MET resulted in significant inhibition of the mTOR pathway, but no significant effect on the AMPK pathway was

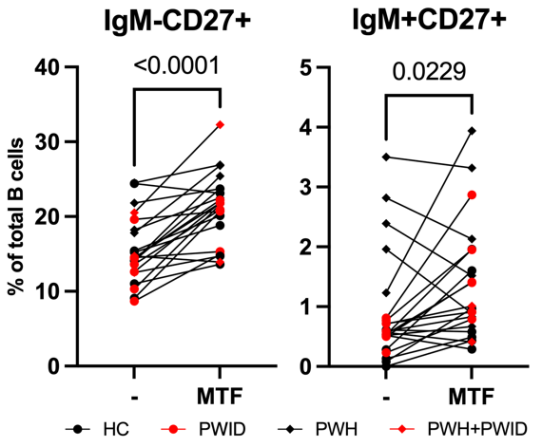


Figure 2-7. MET enhances memory B cell development. B cells from HC (n=6), PWID (n=6), PWH (n=7), and PWH+PWID (n=3) were stimulated with anti-IgM & CD40L *in vitro* for 4 days in the presence of 1 mM MET and analyzed by flow cytometry.

observed (not shown). Further assessment of MET on the regulation of the B cell response is ongoing.

Beyond the potential immune-related benefits of MET in PWID, emerging results from animal studies suggest MET may have beneficial effects in the setting of

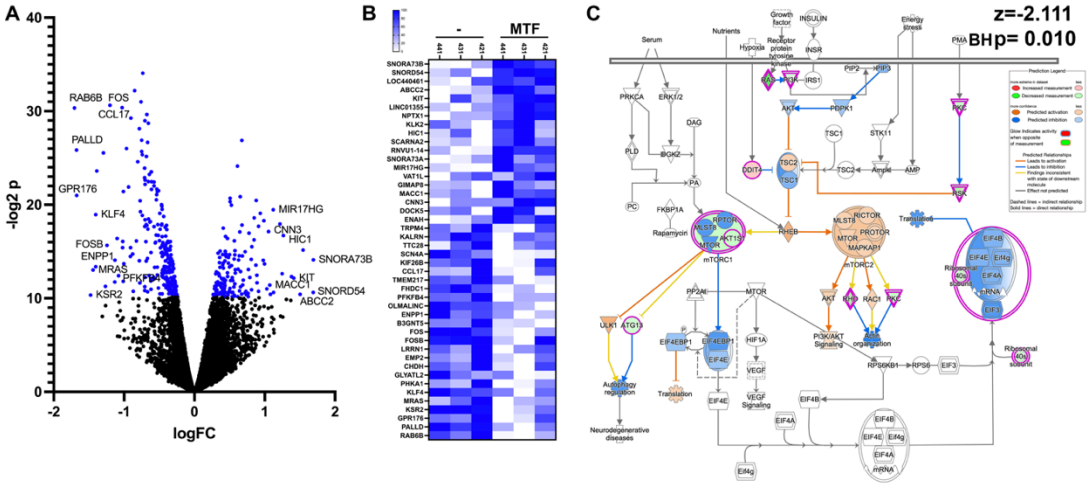


Figure 2-8. MET regulation of B cell responsiveness. Total PBMC from 3 PWID (w/o HIV or HepC) were stimulated with IL-2, CD40L, and α-IgM for 4 d in the presence of 1mM MET and total B cells isolated by flow sorting and gene expression determined by RNA-Seq. (**A**) Volcano plot of RNA-Seq data. (**B**) Heatmap of differentially expressed genes. (**C**) mTOR signaling pathway IPA analysis.

stimulant use, including decreasing cocaine seeking via an AMPK-mediated process in rats(83), neuroprotective and anti-inflammatory effects in methamphetamine treated rats(84), and reduction in methamphetamine-mediated gut microbiome dysbiosis, anxiety, and depression in mice(85). Subsequently, we will be testing MET in participants with a history of methamphetamine use. Overall, there is substantial evidence suggesting MET may have beneficial effects in PWID, justifying its evaluation in the proposed comprehensive bio-behavioral proof-of-concept mechanistic randomized clinical trial. Additionally, ongoing efforts to develop long-acting injectable MET(86, 87) could further elevate the long-term clinical potential impact of MET for at-risk populations.

Need to understand mucosal inflammation in PWID

Increased mucosal inflammation and altered mucosal lymphocyte function in PWID remains poorly defined and likely contributes to increased risk of HIV acquisition(88), and in PWH exacerbation of associated co-morbidities(89). This mucosal inflammation seems to be in-part driven by the impact of infection and drug use on gut permeability and microbiome dysregulation(81, 82, 90-94). In addition to the well-established associations of rectal/genital inflammation and

HIV risk; oral inflammation, which is known to be substantial in individuals with methamphetamine use(95), a growing segment of PWID, likely drives the numerous oral pathologies observed. Oral inflammation is associated with the greater incidence and severity of periodontal disease(96, 97) and oropharyngeal cancers(98) that is observed in PWH. The potential for MET to reduce mucosal inflammation in PWID is substantiated by previous studies demonstrating its ability to reduce mucosal inflammation including in the oral cavity(99-102), and in PWH that MET reduces the frequency of CD4+ T cells and their expression of phosphorylated mTOR in the colon(56), and that it is suggested to mitigate gut microbiome dysbiosis to reduce mucosal inflammation(80, 81). Therefore, we posit that MET reduces oral and genital mucosal inflammation in PWID, which could mitigate HIV infectivity and associated co-morbidities.

2.3.1 The safety of study product administration

Metformin was approved by the FDA in 1994 for the treatment of type 2 diabetes, and metformin ER (i.e. Glucophage XR) was approved by the FDA in 2000 for the treatment of type 2 diabetes. Additionally, it has been used off-label for treatment of various conditions (103) including polycystic ovarian syndrome, gestational diabetes, adjunct therapy in type 1 diabetes, and prevention of type 2 diabetes in prediabetes. It has been evaluated in numerous clinical trials in non-diabetic populations including for lymphoma(104), schizophrenia(105), Alzheimer's disease(106-108) (109-111), periodontal disease(112), and in people with HIV(73).

We do not anticipate there are any additional safety risks of metformin to PWID and PWH who meet the inclusion and exclusion criteria for this study.

The most common adverse reactions (>5%) associated with metformin ER are: diarrhea (9.6%) and nausea/vomiting (6.5%). And are more evident with doses of >1000 mg per day.

Rare risks associated with metformin ER include lactic acidosis. Postmarketing cases of metformin-associated lactic acidosis have resulted in death, hypothermia, hypotension, and resistant bradyarrhythmias. The onset of metformin associated lactic acidosis is often subtle, accompanied only by nonspecific symptoms such as malaise, myalgias, respiratory distress, somnolence, and abdominal pain. Metformin associated lactic acidosis was characterized by elevated blood lactate levels (>5 mmol/Liter), anion gap acidosis (without evidence of ketonuria or ketonemia), an increased lactate/pyruvate ratio; and metformin plasma levels generally >5 mcg/mL.

Risk factors for metformin-associated lactic acidosis include renal impairment, concomitant use of certain drugs (e.g. carbonic anhydrase inhibitors such as topiramate), age 65 years old or greater, having a radiological study with contrast, surgery and other procedures, hypoxic states (e.g., acute congestive heart failure),

excessive alcohol intake, and hepatic impairment. Subsequently, the inclusion/exclusion criteria, monitoring, and counseling of participants have been designed to exclude these risk factors and mitigate the potential risk of metformin-associated lactic acidosis within this trial.

Other risks associated with metformin ER include decrease in vitamin B12 to subnormal levels ~7% of patients that appeared to be rapidly reversable with discontinuation of metformin ER. Subsequently, normal vitamin B12 levels at study enrollment are an inclusion criterion, and vitamin B12 monitoring are included in this trial to mitigate this potential risk.

Hypoglycemia is a rare, but potential risk associated with metformin ER. Hypoglycemia does not occur in patients receiving metformin alone under usual circumstances of use, but could occur when caloric intake is deficient, when strenuous exercise is not compensated by caloric supplementation, or during concomitant use with other glucose-lowering agents (such as sulfonylureas and insulin) or ethanol. Elderly, debilitated, or malnourished patients, and those with adrenal or pituitary insufficiency or alcohol intoxication are particularly susceptible to hypoglycemic effects. Hypoglycemia may be difficult to recognize in the elderly, and in people who are taking beta-adrenergic blocking drugs. Subsequently, inclusion/exclusion criteria, monitoring, and counseling of participants have been designed to exclude these risk factors and mitigate the potential risk of metformin-associated hypoglycemia within this trial.

2.3.2 Other potential risks

Blood collection (phlebotomy) may cause pain, bruising, fainting, and, rarely, infection at the site where the blood is taken.

Optional rectal biopsy. Flexible sigmoidoscopy, the method used to take the rectal samples, may cause mild discomfort and pressure while the sigmoidoscope is moved along inside of the colon. There is a very slight risk of intestinal perforation, which carries the potential for peritonitis requiring additional medical intervention including surgery. These risks are associated with the sigmoidoscopy itself and are most commonly encountered when there is underlying colon abnormalities. The biopsies themselves pose minimal additional risk.

Other risks: The medical tests performed as part of this research protocol may result in new diagnoses or abnormal values without clinical significance (“false positives”). Depending on the medical findings and consequences of being provided with the results of these tests, the study participant may view this as either a risk or a benefit. Any such information will be shared and discussed with the participant and, if requested by the participant, may be forwarded to the primary health care provider for further workup and management.

Participants in this study risk experiencing discrimination or other personal problems that may result from study participation itself; these are known collectively as negative social impacts. The study team is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with trial.

3 Study Design

MET-IH is a 6 month, two-group, single blinded, placebo controlled, randomized study of MET treatment of people with a history of injection drug use, with or without HIV. Participants will be stratified ~1:1 with or without HIV, and randomized 1:1 to 16 weeks of treatment with MET (Group 1) or placebo (Group 2). Systemic and mucosal inflammation and antibody response to vaccines will be assessed.

Persons aged 19-64 years with a history of injection drug use will be randomized to receive MET (Group 1, n=50) or placebo (Group 2, n=50), for a total of 100 participants.

4 Selection and Enrollment of Participants

4.1 Inclusion Criteria

4.1.1 Provision of signed and dated informed consent form

4.1.2 Stated willingness to comply with all study procedures and availability for the duration of the study

4.1.3 Aged 19 to 64 years old

4.1.4 Weight of at least 110 lbs

4.1.5 Body Mass Index 18.5-40. Enrollment of individuals with BMI >40, whom the study team assesses are in good health, may be considered by PSRT approval.

4.1.6 Willing to receive Jynneos and Capvaxive vaccines

4.1.7 Ability to take oral medication and be willing to adhere to the metformin treatment regimen

4.1.8 Injection opioid, amphetamine, and/or cocaine use with past 10 years (self-report)

4.1.9 Use of non-prescription opioid, amphetamine, and/or cocaine within the past 30 days (self-report)

4.1.10 Clinically confirmed urine drug screen for opioid, amphetamine, and/or cocaine within past 30 days

4.1.11 CRP ≥ 1 mg/L

4.1.12 glucose 70-180 mg/dL, non-fasting

4.1.13 HbA1c <6.4%

4.1.14 CD4 > 200 cells/ml

4.1.15 If PWH, HIV viral load < 200 copies / ml

4.1.16 If PWH, on anti-retroviral therapy (ART) for > 12 months

4.2 Additional Inclusion Criteria for All Participants Providing Rectal Biopsy:

1. HIV negative, as determined by fourth generation HIV screening test.

2. Willing to refrain from occasional over-the-counter use of aspirin and NSAID use for 72 hours before and after each study biopsy visit. NSAIDS should also be held for 72 hours after biopsy.

3. Participants who were born female: negative serum or urine pregnancy test performed within 7 days prior to procedure

4. Willing to abstain from insertion of anything (e.g., drug/medication, penis, object, sex toy, or enema including take-home enema) into the anorectum for 72 hours before study drug dose and until 72 hours after each flexible sigmoidoscopy with biopsy collection, or one week after the study drug dose, whichever is later

5. PT, PTT, and CBC within normal range performed within 30 days prior to procedure.

6. Negative rectal swab for gonorrhea and chlamydia performed within 30 days prior to procedure

7. Clinical abdominal exam performed within 42 days prior to the procedure and without any clinical findings which in the opinion of the clinician represents a contraindication to the biopsy procedure.

4.3 Exclusion Criteria

4.3.1 Inability to give informed consent

4.3.2 Refusal or inability to have blood drawn

4.3.3 Bleeding disorder diagnosed by a doctor (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

4.3.4 pregnant or nursing

4.3.5 diabetes mellitus

4.3.6 history of severe renal impairment and/or eGFR <60 mL/min/1.73m2 > 10 days

4.3.7 creatine clearance <60 mL/min

4.3.8 history of liver disease

4.3.9 ALT/AST >3X upper limit of normal

4.3.10 Total Bilirubin >1.4 mg/dL

4.3.11 Albumin <3.5 g/dL

4.3.12 Prothrombin >1.5X upper limit of normal

4.3.13 AUDIT-C ≥8

4.3.14 hemoglobin <9.0 g/dL

4.3.15 absolute neutrophil <1,000/ml

4.3.16 platelet <100,000/ml

4.3.17 history of acute or chronic metabolic acidosis

4.3.18 anion gap >14 mEq/L OR

anion gap >12 mEq/L when serum bicarbonate < 22 mEq/L

4.3.19 serum lactate >2.2 mmol/L

4.3.20 serum vitamin B12 <250 pg/ml

4.3.21 history of chronic diarrhea

4.3.22 currently taking metformin or other diabetes medication

4.3.23 history of myocardial infarction, endocarditis, stroke, heart failure, chronic obstructive pulmonary disease, or sepsis

4.3.24 furosemide, nifedipine, ranolazine, vandetanib, or cimetidine use (current or within past 30 days)

4.3.25 active hepatitis B infection

4.3.26 Hepatitis C infection within 6 months of study entry. If previous history of hepatitis C, less than 6 months after completion of treatment with direct-acting antivirals

4.3.27 previous receipt of Jynneos or pneumococcal vaccine within past two years (self-report)

4.3.28 previous history of severe allergic reaction to metformin (self-report)

4.3.29 previous history of severe allergic reaction to previous dose of Jynneos or following exposure to any component of Jynneos (self-report)

4.3.30 previous history of severe allergic reaction to any component of Capvaxive or to diphtheria toxoid (self-report)

4.3.31 Donations of blood in the 8 weeks prior to enrollment which, combined with expected volumes to be drawn for this study, would exceed 450 mL in an 8 week period.

4.3.32 Participant has any medical, psychiatric, or social condition, or occupational or other responsibility that, in the judgment of the investigator, would interfere with, or serve as a contraindication to the planned procedure(s).

4.4 Additional Exclusion Criteria for All Participants Providing Rectal Biopsy:

1. Abnormality of the colorectal mucosa, or significant colorectal symptom(s), which in the opinion of the clinician represents a contraindication to biopsy

(including but not limited to history of C. difficile colitis or enterocolitis within 3 months prior to procedure, presence of unresolved injury, infectious or inflammatory condition of the local mucosa, and presence of hemorrhoids)

2. Thrombocytopenia, platelets < 125,000 / mm3

3. History of coronary artery disease, myocardial infarction, chronic obstructive pulmonary disease, chronic renal failure, decompensated cirrhosis, or any other condition that in the opinion of the investigator will compromise ability to participate in the study.

4. Currently on anti-coagulation therapy (e.g. Coumadin/Warfarin).

5. Receipt of any of the following medications within 30 days prior to procedure: systemic steroids (inhaled or nasal steroid therapy is permitted), interleukins, systemic interferons (e.g., local injection of interferon alpha for treatment of human papilloma virus is permitted) or systemic chemotherapy.

6. History of intolerance, sensitivity, allergy, or anaphylaxis to benzodiazepines or other narcotics that may be used during the procedure

7. Insertion of any foreign object into the anus within 3 days prior to procedure

8. Participant has any medical, psychiatric, or social condition, or occupational or other responsibility that, in the judgment of the investigator, would interfere with, or serve as a contraindication to the planned procedure(s).

5 Study Treatment

5.1 Regimens, Administration and Duration

5.1.1 Regimens/Administration

Participants will be randomized 1:1 to:

- Group 1: Metformin ER, 500 mg by mouth once per day with evening meal for Day 0 to Day 6, followed by Metformin ER, 1000 mg by mouth once per day with evening meal for Day 7 to Day 112

- Group 2: Placebo, one tablet by mouth once per day with evening meal for Day 0 to Day 6, followed by Placebo, two tablets by mouth once per day with evening meal for Day 7 to Day 112

5.1.2 Duration

Total study duration for all groups will be 6 months.

5.2 Study Product Description, Storage, and Preparation

Metformin ER (MET) is supplied as white to off-white tablets, each containing 500 mg of metformin hydrochloride as the active ingredient. For the purposes of this study, MET will be over-encapsulated to accommodate a matching placebo.

Store MET at 20°–25° C (68°–77° F); excursions permitted to 15°–30° C (59°–86° F). [See USP Controlled Room Temperature.]

The placebo will be cellulose in identical capsules used to over-encapsulate MET.

Store placebo at 20°–25° C (68°–77° F); excursions permitted to 15°–30° C (59°–86° F).

Both MET and placebo will be dispensed in light-resistant containers

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Acquisition/Distribution

Metformin ER tablets, cellulose powder, and gelatin capsules are commercially available and will be purchased in bulk by UAB Hospital Investigational Pharmacy Services (IDS). The metformin ER tablets and cellulose powder will be encapsulated using gelatin capsules by IDS and distributed to the site pharmacist.

5.3.2 Study Product Accountability

The site pharmacist is required to account for all study products. An accountability record must be used to document receipt, management, and final disposal of study product. The site pharmacist must also maintain a record of storage conditions for the study products.

5.4 Concomitant Medications

Whenever a concomitant medication or study agent is initiated or a dose changed, investigators must review the concomitant medication's and study agent's most recent package insert, to obtain the most current information on drug interactions, contraindications, and precautions.

5.4.1 Prohibited Medications

1. Non-study product diabetic medication
2. Glyburide
3. Furosemide
4. Nifedipine
5. Ranolazine
6. Vandetanib
7. Cimetidine

5.4.2 Precautionary Medications

Interactions between the following medications and metformin have been previously noted, typically at metformin dose >1,000 mg/day, however commonly used together. Initiation and continuation of metformin for individuals taking these medications is at the discretion of study physicians.

1. Dolutegravir
2. Lamivudine

6. Clinical and Laboratory Evaluations

6.1 Schedule of Activities

Table 6.1-1 Schedule of Activities

Visit number	V0	V1	V2	V3	V4	V5	V6
Study week		0	0.5	1	2	3	5
Study Day (Extended Window Days)	-14 to -3	0	2-4	7-10	12-16	19-23	30-40 (+7)
Procedure	Screen	Enrollment /MET start		MET increase			Vac 1
In person (IP) / Virtual (V)	IP	IP	V	V	IP	V	IP
Study Procedures							
Assessment of Understanding		x					
Informed Consent	X						
Medical History	X						
Physical Exam	X	X					X
Concomitant medications	X	X			X		X
ASSIST		X					X
AUDIT-C	X	X			X		X
BRAID		X					
Health Survey Questionnaire	X	X	X	X	X	X	X
Adverse Events		X			X		X
MAAEs/SAEs		X			X		X
Adherence assessment/ metformin/placebo dispense		X			X		X
Acidosis / Hypoglycemia awareness counseling	X	X	X	X	X	X	X
Naloxone / overdose counseling		X					X
Vaccination							X
Clinical Labs							
Pregnancy test (urine or serum)	X	X			X		X
HIV-1/2 antibody[&]	X						
HBsAg/anti-HCV	X						
CRP	X	X			X		
CD4 T cell count	X				X		
HIV PCR viral load*	X	X			X		X
Complete blood count (CBC)/Differential	X	X			X		X
Comprehensive metabolic panel	X	X			X		X
HbA1c	X				X		
Serum vitamin B12	X				X		
Lactate	X	X			X		X
Prothrombin	X				X		
Urine drug screen and Urine EtG	X	X			X		X
Research Samples							
Blood (volume)		X (51 ml)			X (34 ml)		X (34 ml)
Saliva		X					
Rectal secretion^		X					
Rectal biopsy^[&]							

**PWH only*
[&]PWH excluded
^optional

Table 6.1-1 Schedule of Activities- continued

Visit number	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15**	**V16**
Study week	6	8	9	10	11	12	13	14	15	16
Study Day (Extended Window Days)	38-40	54-58	61-65 (+ 7)	68-72 (+ 7)	75-79	82-86	89-93 (+ 7)	96-100	103-107	110-114 (+ 7)
Procedure			Vac 2							MET stop
In person (IP) / Virtual (V)	V	V	IP	IP	V	V	IP	V	V	IP
Study Procedures										
Assessment of Understanding										
Informed Consent										
Medical History										
Physical Exam			X	X			X			X
Concomitant medications			X	X			X			X
ASSIST				X			X			
AUDIT-C			X	X			X			X
BRAID										
Health Survey Questionnaire	X	X	X	X	X	X	X	X	X	X
Adverse Events			X	X			X			X
MAAEs/SAEs			X	X			X			X
Adherence assessment/ metformin/placebo dispense			X	X			X			X
Acidosis / Hypoglycemia awareness counseling	X	X	X	X	X	X	X	X	X	X
Naloxone / overdose counseling				X						X
Vaccination			X							
Clinical Labs										
Pregnancy test (urine or serum)			X				X			X
HIV-1/2 antibody[&]							X			
HBsAg/anti-HCV										
CRP			X				X			X
CD4 T cell count			X							X
HIV PCR viral load*			X							X
Complete blood count (CBC)/Differential			X				X			X
Comprehensive metabolic panel			X				X			X
HbA1c			X				X			
Serum vitamin B12			X							X
Lactate			X				X			X
Prothrombin			X							X
Urine drug screen and Urine EtG			X	X			X			X
Research Samples										
Blood (volume)			X (34 ml)	X (51 ml)			X (34 ml)			X (51 ml)
Saliva			X							X
Rectal secretion^										X
Rectal biopsy^[&]										X

**PWH only*
[&]PWH excluded
^optional

Table 6.1-1 Schedule of Activities- continued

Visit number	**V17**	**V18**	**V19**	**V20**	**V21**	**V22**	**VPD**
Study week	17	19	20	21	22	24	
Study Day (Extended Window Days)	117-121	131-135	138-142 (+ 7)	145-149	152-156	166-170	
Procedure							
In person (IP) / Virtual (V)	V	V	IP	V	V	V	IP
Study Procedures							
Assessment of Understanding							
Informed Consent							
Medical History							
Physical Exam			X				X
Concomitant medications			X				X
ASSIST			X				X
AUDIT-C			X				X
BRAID			X				X
Health Survey Questionnaire	X	X	X	X	X	X	X
Adverse Events			X				X
MAAEs/SAEs			X				X
Adherence assessment/ metformin/placebo dispense							X
Acidosis / Hypoglycemia awareness counseling	X	X	X	X	X	X	X
Naloxone / overdose counseling			X				X
Vaccination							
Clinical Labs							
Pregnancy test (urine or serum)			X				X
HIV-1/2 antibody[&]			X				X
HBsAg/anti-HCV			X				X
CRP			X				X
CD4 T cell count			X				X
HIV PCR viral load*			X				X
Complete blood count (CBC)/Differential			X				X
Comprehensive metabolic panel			X				X
HbA1c			X				X
Serum vitamin B12			X				X
Lactate			X				X
Prothrombin			X				X
Urine drug screen and Urine EtG			X				X
Research Samples							
Blood (volume)			X (34 ml)				X (34 ml)
Saliva			X				X
Rectal secretion^							
Rectal biopsy^[&]							

**PWH only*
[&]*PWH excluded*
^*optional*

6.2 Timing of Study Activities

6.2.1 Screening Activities

Screening activities to determine eligibility must be completed within 45 days, and prior to study enrollment unless otherwise specified. In addition to data being collected on participants who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured in a Screening Failure Results form.

6.2.2 Enrollment Activities

Enrollment study activities must occur after screening activities unless otherwise specified. Study treatment should be initiated as soon as possible after enrollment activities have been completed and within 3 days of enrollment.

6.2.3 Post-Enrollment Activities

For all post-enrollment study activities, if a participant is unable to attend or complete a visit within the specified window, the visit can be completed during the extended window of an additional 7 days as indicated in SOA. If a visit cannot be completed within the specified window or extended window, every attempt should be made to bring or enable the participant in for an out-of-window visit rather than skip the visit, unless the next visit window has opened.

If a participant experiences an acute inflammatory condition within 14 days prior to a scheduled vaccination visit (V6 and V9), the vaccination should be postponed for an additional 7 days from the initial scheduled visit. Examples of inflammatory conditions that would justify delaying vaccination visit include an infection requiring hospitalization, a systemic viral illness such as an influenza-like illness, a severe drug hypersensitivity reaction, myocardial infarction, fever on the day of visit (defined as T° >38.5°C), and major trauma.

6.2.4 Event-Driven Activities

<u>Activities for Randomized Participants Who Do Not Start Study Treatment</u>

All eCRFs must be keyed for the period up to and including the enrollment visit.

<u>Premature Treatment Discontinuation Activities</u>

Participants who prematurely discontinue/modify any component of their study regimen should ideally complete the premature discontinuation study activities (listed in the (SOA)) prior to treatment discontinuation or within 7 days of discontinuation. The premature discontinuation study activities are listed in the SOA. Participants who discontinue the study

treatment will still be encouraged to remain in study follow-up through 24 weeks. Every reasonable effort should be made for the premature treatment discontinuation to include an in-person visit, however if not possible due to participant availability, completion of virtual visit elements should be attempted.

Premature Study Discontinuation

Participants who prematurely discontinue from the study will have the study discontinuation activities performed prior to being taken off the study (listed in the SOA).

6.3 Instructions for Study Activities

All clinical and laboratory information required by this protocol is to be present in the source documents.

All stated evaluations are to be recorded on the eCRF unless otherwise specified.

The protocol team and/or study monitoring entity may determine that additional source data associated with procedures or evaluations performed per protocol should be entered into eCRFs so that the data can be used for analysis or to otherwise assist with interpretation of study findings. In such cases, sites will be officially instructed to enter the additional data into eCRFs from available source documentation.

6.3.1 Documentation of HIV-1 status

For participants with previous diagnosis of HIV-1, HIV-1 status should be confirmed from EHR, provider documentation, or confirmatory licensed rapid HIV or HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit at any time prior to study enrollment.

For participants without previous diagnosis of HIV-1, HIV-1 status should be determined by confirmatory licensed rapid HIV or HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit, and as appropriate confirmed by a licensed Western blot or a second antibody test by a method other than the initial rapid HIV and/or E/CIA, or by HIV-1 antigen, plasma HIV-1 RNA viral load.

6.3.2 Medical History

The medical history must include all signs and symptoms regardless of grade for all clinical events and other diagnoses regardless of grade within the past 30 days. In

addition, the following diagnoses should be recorded regardless of when the diagnosis was made:

- HIV status
- Diabetes mellitus (exclusionary)
- Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) (exclusionary)
- Pregnancy or nursing (exclusionary)
- Severe renal impairment (exclusionary)
- Liver disease (exclusionary)
- Acute or chronic metabolic acidosis (exclusionary)
- Chronic diarrhea (exclusionary)
- Coronary heart disease, cardiovascular disease, myocardial infarction, stroke, heart failure (exclusionary)
- Chronic obstructive pulmonary disease (exclusionary)
- Sepsis (exclusionary)
- Acute or chronic Hepatitis B virus (exclusionary)
- HCV status, including history of spontaneous clearance or cure (exclusionary, if active)
- Blood clots or clotting disorders (exclusionary)
- Cerebrovascular accident
- Orchiectomy or chemical castration
- MPOX and Pneumococcal vaccination history (verbal history accepted)

Any allergies to any medications and their formulations must also be documented.

6.3.3 Medication History

A medication history must be present, including start and stop dates. The table below lists the medications that must be included in the history.

Medication Category	Complete History or Timeframe
Diabetes-related medications	Complete history, as available
Antiretroviral therapy	Complete ART history, as available
Glyburide, Furosemide, nifedipine, ranolazine, vandetanib, ocimetidine	Within 4 weeks prior to study enrollment
Immune-based therapy	Complete history, as available
Study treatment	Complete history
Prescription drugs for treatment of opportunistic infections	Within 4 weeks prior to study enrollment
Prescription drugs for prophylaxis of opportunistic infections	Within 4 weeks prior to study enrollment
Anti-coagulation therapy	Within 4 weeks prior to study enrollment
Medications for opioid use disorder	Complete history, as available
Prescription drugs (other)	Within 4 weeks prior to study enrollment
Diet or weight loss therapy, either prescribed or over the counter	Within 4 weeks prior to study enrollment
Alternative therapies	Within 4 weeks prior to study enrollment
Dietary supplements	Within 4 weeks prior to study enrollment
Other category	Within 4 weeks prior to study enrollment

6.3.4 Clinical Assessments

Complete Physical Examination

A complete physical examination at enrollment to include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac examination; abdominal examination; and examination of the lower extremities for edema. The complete physical examination will also include signs and symptoms, diagnoses, and vital signs (temperature, pulse, respiration rate, and blood pressure).

Targeted Physical Examination

A targeted physical examination at study visits after enrollment is to include vital signs (temperature, pulse, respiration rate, and blood pressure), and is to be driven by any previously identified or new adverse event, that the participant has experienced since the last visit or at this visit.

Concomitant Medications

Post-enrollment, all new or discontinued concomitant medications must be recorded, including but not limited to:

- Glyburide, furosemide, nifedipine, ranolazine, vandetanib, and cimetidine
- Anti-diabetic medications, antipsychotics, antidepressants, anticonvulsants/mood stabilizers, medications for opioid use disorder, dietary supplements, and weight loss agents (including over the counter)
- Any drugs with anti-inflammatory properties
- Any weight loss drugs (including non-prescription)
- Antiviral therapy

Study Treatment Modifications

Record all study drug modifications including initial doses, participant-initiated, investigator-initiated, and/or protocol-mandated modifications, inadvertent and deliberate interruptions of more than 2 consecutive days since the last visit, and overdose. Study drug (metformin) dose may be decreased by study doctor due to tolerability. Record any permanent discontinuation of treatment.

6.3.5 Alcohol Use Disorders Identification Test - Consumption (AUDIT-C)

The AUDIT-C will be administered using Qualtrics at screening per the SOA.
AUDIT-C ≥ 8 is exclusionary.

6.3.6 Alcohol, Smoking and Substance Involvement Screening Test (ASSIST)

The ASSIST will be administered using Qualtrics at visits per the SOA.

6.3.7 Behavioral Risk Assessment for Infectious Diseases (BRAID)

The BRAID will be administered using Qualtrics at visits per the SOA.

6.3.8 Health Survey Questionnaire (HSQ)

The HSQ will be administered using Qualtrics at visits per the SOA.

6.3.9 Adherence Assessment

The adherence questionnaire will be administered per the SOA and the pill count eCRF will be completed per the SOA.

NOTE: Site staff or investigators should review the results of the adherence assessments during the visit, and counsel participants on adherence if any concerns become apparent.

6.3.10 Acidosis and Hypoglycemia Awareness Counseling

Participants will be counseled on signs and symptoms of acidosis as indicated in SSP including confusion, difficulty talking, difficulty walking, memory loss, feeling intoxicated in the absence of drinking alcohol.

Participants will be educated on signs and symptoms of hypoglycemia as indicated in SSP including tremor, palpitations, anxiety, sweating, hunger, and dizziness, weakness, drowsiness, confusion or altered mental status.

6.3.11 Naloxone and Overdose Counseling

Participants will be provided naloxone and educated on overdose awareness as indicated in SSP including information on the Never Use Alone hotline and indications for naloxone for overdose reversal. Participants will be counseled on how to assess for signs of overdose, notify emergency medical services (911), and how to administer intranasal naloxone. Naloxone, and naloxone and overdose counseling can be provided outside of SOA at the discretion of study team.

6.3.12 Vaccination

At week 5 post enrollment visit (V6), participant will receive MPOX (Jynneos) vaccine and pneumococcal (Capvaxvie, PCV21) vaccine.

Jynneos is administered **subcutaneously**.

Capvaxvie is administered intramuscularly.

At week 9 post enrollment visit (V9), participant will receive second MPOX (Jynneos) vaccine.

6.3.13 Clinical Laboratory Evaluations

At screening, enrollment, and post-enrollment all laboratory values must be recorded on the eCRF per the SOA.

Hematology
Complete blood count (CBC) with differential to include: hemoglobin, hematocrit, white blood cell count [WBC], differential WBC, absolute neutrophil count (ANC), platelets. Prothrombin, absolute CD4+ T cell count.

Liver Function Tests
Total and direct bilirubin, AST [SGOT], ALT [SGPT], alkaline phosphatase, total protein, albumin

Chemistry
Glucose, Sodium, potassium, chloride, phosphate, bicarbonate, serum creatinine, and blood urea nitrogen, anion gap, serum lactate, serum vitamin B12, HbA1c, C-reactive protein

Calculated Creatinine Clearance
Calculated creatinine clearance will be estimated by the CKD-EPI equation (a calculator is available at: https://qxmd.com/calculate/calculator_251/egfr-using-ckd-epi).

Pregnancy Test
For participants capable of becoming pregnant: serum or urine β-HCG (urine test must have a sensitivity of <25 mIU/mL) performed per SOA.

Virological
HBV surface antigen. HCV antibody followed by HCV RNA testing when HCV antibody testing is positive.

For individuals without documented HIV infection, HIV enzyme immunoassay (EIA) or chemiluminescent microparticle immunoassay (CMIA).

For individuals with documented HIV infect, plasma HIV-1 RNA.

Urine Drug and Ethylglucuronide (EtG) Test
The presence of the following substances: amphetamines, benzodiazepines, buprenorphine, cocaine, ecstasy (MDMA), marijuana (THC), methadone, methamphetamines, opiates/morphine, oxycodone, ethyl glucuronide (EtG), fentanyl, synthetic marijuana (K2), and tramadol will be recorded on eCRF.

6.3.14 Stored Plasma/PBMC/Serum

Per the SOA, plasma, viable PBMCs, and serum samples will be collected for clinical laboratory evaluations and research assays. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal. The total blood volume drawn for each participant will not exceed 100 mL per visit, nor 500 mL in any 56-day (8-week) period, per American Red Cross guidelines for blood donation <https://www.redcrossblood.org/donate-blood/how-to-donate/eligibility-requirements.html>.

6.3.15 Stored Mucosal Secretion Samples

Saliva
Per the SOA, all participants will provide unstimulated saliva ~1 ml. Participants will be instructed to rinse their mouth using distilled water and after 2 minutes instructed to refrain from talking, sit comfortably, and accumulate saliva in their mouth before drooling into the collection tube until ~ 1 ml is collected.

Optional Rectal secretions
Per SOA, all participants will be offered the option of providing rectal secretion samples. For those participants Rectal fluid sampling (both sexes): For participants born female, a pregnancy test must be performed and be negative prior to any rectal

mucosal sampling. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Rectal secretion sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, if still within the visit window. In addition, rectal sampling will not be performed (or may be deferred to a later date if still within the visit window) if there is a contraindication to rectal secretion sampling, such as an active infection or inflammation of the colorectal area [such as a herpes simplex virus (HSV)-2 outbreak or inflamed hemorrhoids or colitis/diarrhea] or if the participant has any active genital tract infection (GTI).

For 72 hours prior to sample collection, participants should abstain from:

 Receptive anal sex,

 Insertion of any foreign object or substance into the anus (including but not limited to cleaning products [creams, gels, lotions, pads, etc.], lubricant, enemas, and douching even with water), and

 Using perianal or intra-anal steroid or other anti-inflammatory cream in or around the anus.

Rectal fluid sampling will be performed by licensed and trained clinician using an anoscope and two pre-moistened sponges (e.g. Weck-Cel). The sponges will be inserted ~2-3 cm into the rectum and left in place for 5 minutes and then removed.

Optional Rectal Biopsy

Per SOA, participants who have not been diagnosed with HIV will be offered the option to provide a rectal biopsy sample. A pre-procedural cleansing douche will be performed prior to baseline flexible sigmoidoscopies. The cleansing douche must be only tap water because it is demonstrated to be non-toxic to colorectal mucosa and doesn't have any impact on the ex-vivo explant HIV challenge assay. Colorectal tissue biopsy collection will be performed via flexible sigmoidoscopy by an experienced surgeon at UAB; up to 15 biopsies will be collected via a flexible sigmoidoscope. Collected biopsies will be used for the following: tissue dissociation and isolation of mucosal mononuclear cells (MMC) for phenotypical analysis; ex vivo biopsy challenges with HIV-1 and subsequent p24 viral replication analysis and spatial transcriptomics.

6.4 Drug Use Measures

6.4.1 Urine Drug Screen

Urine Drug Screens (UDS) are collected at each in-person study visit. However, to minimize participant and staff burden we will accept non-study drug screen result from a test that was performed as part of standard of care or otherwise present in the participant's medical record. Such extracted urine drug screen results may not include all substances tested using the study provide urine drug test cup. The study provide UDS will test for the presence of the following substances: amphetamines, benzodiazepines, buprenorphine, cocaine, ecstasy (MDMA), marijuana (THC), methadone, methamphetamines, opiates/morphine, oxycodone, ethyl glucuronide (EtG), fentanyl, synthetic marijuana (K2), and tramadol.

6.4.2 Self-report and EHR Extracted Substances Use Measures

Screening measures
Eligibility is determined by either study-conducted UDS or EHR extracted UDS results. Additional substance use measures for screening are collected by self-report.

Enrollment and Post-Enrollment Substance Use Measures
Alcohol and Drug use severity will be assessed with The Alcohol Use Disorders Identification Test-C (AUDIT-C) and the ASSIST. Results of AUDIT-C will be used to exclude those with unhealthy alcohol use as defined by an AUDIT C Score of 8 or greater. The ASSIST Survey will be used to identify substance use including opioids, stimulants, and other substances.

7 Study Procedures

All required clinic and laboratory procedures for each study visit are summarized in SOA.

Optional rectal secretion sampling will be offered to all eligible participants.

Optional rectal biopsy sampling will be offered to only eligible participants without HIV-1 infection.

7.1 Definition of "study day" and "study visit"

Enrollment equals "study day 0" and is defined as the day of the first study-product administration. A study visit may be conducted remotely, such as via phone, text message, email, or other electronic means, in lieu of, or in combination with, in-person visits. Procedures for a study visit can be completed over multiple days and should be completed within the visit window.

7.2 Screening (V0)

Screening for eligibility will be performed after informed consent has been obtained and properly documented before enrollment. Screening evaluations and sample collection include medical history review, physical exam, review of concomitant medications, AUDIT-C, health survey, and any clinical laboratory tests that are needed to confirm eligibility, as detailed in the Schedule of Activities. Counseling on lactic acidosis and hypoglycemia awareness will be done. Urine drug screen and urine EtG will be performed. Persons of pregnancy potential will be given a pregnancy test. Screening procedures may occur over one or more visits. Additional assessments of health may be conducted at screening based on clinical judgment.

Screening may be split into a two-step process over multiple visits. If a participant is deemed not eligible prior to completing all screening elements, remaining screening elements need not be completed.

Individuals deemed not eligible will be informed that they do not meet the eligibility criteria for the study and will be referred for appropriate medical care, if necessary.

A single out of range laboratory test may be repeated once 3-10 days following initial test at the discretion of the PI to determine eligibility.

Potential participants may be rescreened once at the discretion of the PI.

7.3 Enrollment visit (V1)

An AoU will be completed and documented prior to enrollment. Records that document the reason screened participants did not enroll will be kept.

If a participant has been screened and been found eligible, they will progress to the enrollment visit, to occur 3 to 28 days following the screening visit.

The definition of enrollment in this study is the point of randomization. That is, if a site successfully randomizes a participant in the randomization system, that participant is considered enrolled.

All visit-specific administrative and regulatory, clinical, behavioral, and laboratory procedures outlined in the Schedule of Events (Table 6.1-1) should be conducted at this visit.

Metformin (25 x 500 mg) or placebo (25 tablets) sufficient for 16 days will be dispensed by study pharmacist. Participants will be provided nutrition bars and reminded to take study drug with food at evening meal.

Optional rectal secretion.

7.4 Post enrollment follow-up virtual visit (V2)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 2-3 days after enrollment visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 2 days, study coordinator will call and text study participant.

7.5 Post enrollment follow-up virtual visit (V3)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 7 days after enrollment visit that includes, reminder to increase metformin (or placebo) dose, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 2 days, study coordinator will call and text study participant.

7.6 Post enrollment visit (V4)

Two weeks following initiation of metformin (or placebo), participant will have an in-person visit.

All visit-specific administrative and regulatory, clinical, behavioral, and laboratory procedures outlined in the Schedule of Activities should be conducted at this visit.

Metformin (70 x 500 mg) or placebo (70 tablets) sufficient for 35 days will be dispensed by study pharmacist. Participants will be provided nutrition bars and reminded to take study drug with food at evening meal.

7.7 Post enrollment follow-up virtual visit (V5)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 19-23 days after enrollment visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.8 Vaccination visit (V6)

Five weeks following enrollment, participant will have an in-person visit.

All visit-specific administrative and regulatory, clinical, behavioral, and laboratory procedures outlined in the Schedule of Activities should be conducted at this visit.

Participant will receive Jynneos and Capvaxvie immunizations.

Metformin (84 x 500 mg) or placebo (84 tablets) sufficient for 42 days will be dispensed by study pharmacist. Participants will be provided nutrition bars and reminded to take study drug with food at evening meal.

7.9 Post vaccination virtual visit (V7)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 7 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product and vaccination, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If

questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.10 Post vaccination virtual visit (V8)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 21 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product and vaccination, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.11 Vaccination visit (V9)

Four weeks following first vaccination visit, participant will have an in-person visit.

All visit-specific administrative and regulatory, clinical, behavioral, and laboratory procedures outlined in the Schedule of Activities should be conducted at this visit.

Participant will receive Jynneos immunization.

Metformin (34 x 500 mg) or placebo (34 tablets) sufficient for 17 days will be dispensed by study pharmacist. Participants will be provided nutrition bars and reminded to take study drug with food at evening meal.

7.12 Post vaccination visit (V10)

On week following second vaccination visit, participant will have an in-person visit.

All visit-specific administrative and regulatory, clinical, behavioral, and laboratory procedures outlined in the Schedule of Activities should be conducted at this visit.

Metformin (70 x 500 mg) or placebo (70 tablets) sufficient for 35 days will be dispensed by study pharmacist. Participants will be provided nutrition bars and reminded to take study drug with food at evening meal.

7.13 Virtual visit (V11)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 7 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product and vaccination, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.14 Virtual visit (V12)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 14 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product and vaccination, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.15 In person visit (V13)

Four weeks following second vaccination visit, participant will have an in-person visit.

All visit-specific administrative and regulatory, clinical, behavioral, and laboratory procedures outlined in the Schedule of Activities should be conducted at this visit.

Metformin (70 x 500 mg) or placebo (70 tablets) sufficient for 35 days will be dispensed by study pharmacist. Participants will be provided nutrition bars and reminded to take study drug with food at evening meal.

7.16 Virtual visit (V14)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 7 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.17 Virtual visit (V15)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 14 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.18 Metformin cessation visit (V16)

Sixteen weeks following study enrollment, participant will have an in-person visit.

All visit-specific administrative and regulatory, clinical, behavioral, and laboratory procedures outlined in the Schedule of Activities should be conducted at this visit.

Any remaining study product will be collected.

Optional rectal secretion.

Optional rectal biopsy. Rectal biopsy can occur week 13 – week 16, prior to cessation of study product (metformin or placebo).

7.19 Post cessation virtual visit (V17)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 7 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.20 Post cessation virtual visit (V18)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 21 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.21 Final in person visit (V19)

Four weeks following metformin (or placebo) cessation, participant will have an in-person visit.

All visit-specific administrative and regulatory, clinical, behavioral, and laboratory procedures outlined in the Schedule of Activities should be conducted at this visit.

Optional rectal secretion.

7.22 Virtual visit (V20)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 7 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.23 Virtual visit (V21)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 14 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.24 Final virtual visit (V22)

Participant will receive a link to Qualtrics-based questionnaire via text message and email 28 days after previous clinic visit that includes, abbreviated assessment of tolerance of study product, review of lactic acidosis and hypoglycemia awareness counselling, general health questions, and phone number of study coordinator and option to request call from study coordinator. If questionnaire is not completed within 7 days, study coordinator will call and text study participant.

7.25 Other visits

Interim/unscheduled visits may be conducted to allow for repeat testing of laboratory results, conduct missed procedures/assessments, conduct safety assessments and safety labs.

7.26 Total blood volume

Required blood volumes per visit are shown in SOA. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal. The total blood volume drawn for each participant will not exceed 500 mL in any 56-day (8-week) period, per American Red Cross guidelines for blood donation <https://www.redcrossblood.org/donate-blood/how-to-donate/eligibility-requirements.html>.

7.27 Visit windows and missed visits

The schedule of visits and evaluations performed at each visit and visit windows are shown in the Schedule of Activities. The procedures for documenting missed visits and out-of-window visits are described in the SSP. If the missed visit is one that requires safety assessments or safety labs, study staff should attempt to bring the participant in for an interim visit as soon as possible. If the participant does not respond to call or text, the study team will attempt to reach them via their emergency contact.

7.28 Early termination visit

If a participant terminates participation in the study early for any reason, the PI should consider if the following assessments are appropriate: CBC with differential, serum chemistry, physical examination, and, if indicated, pregnancy test and assessment for new or unresolved AEs/intercurrent illnesses. If the PI has questions regarding a termination visit, they should consult with the PSRT.

7.29 AE contact

If indicated, the participant may be asked to come in for a clinical assessment, which may also include referrals for an AE assessment.

Record the participant's responses to questions regarding any occurrence of the following events since the last visit/contact:

- SAEs
- AEs
- Medically attended AEs (MAAEs), defined as any AEs leading to an unscheduled visit to a healthcare professional, which are reported regardless of their relationship to the study product(s)

- Pregnancies and outcomes, including congenital anomalies/birth defects.

All such events will be recorded and AEs will be assessed for relationship to study products.

If the site learns the participant is deceased, the site must attempt to learn the cause and date of death.

8 Adverse Events and Study Monitoring

8.1 Definition of Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or diagnosis that occurs in a study participant during the conduct of the study REGARDLESS of the attribution (i.e., relationship of event to medical treatment/study product/device or procedure/intervention). This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition.

8.2 Adverse Event Collection Requirements for This Protocol

All AEs must be recorded on the eCRFs if any of the following criteria have been met.

- All Grade ≥3 AEs
- All AEs that led to a change in study treatment/intervention regardless of grade
- All MAAEs (defined as any AEs leading to an unscheduled visit with a healthcare professional in person or virtually/remotely)
- All AEs meeting serious adverse event (SAE) definition or expedited adverse event (EAE) reporting requirement
- The following AEs will be recorded at Grade ≥1:
    - Diarrhea
    - Nausea/vomiting
    - Change in laboratory values
- The following AEs will be recorded at Grade ≥2:
    - Fatigue
    - Headache
    - Unintentional Weight Loss
    - Dyspnea
- The following will be recorded regardless of grade:

- Coronary heart disease or other cardiovascular disease
- Cancer (exclusive of basal/squamous cell skin cancer)
- Diabetes mellitus or pre-diabetes
- Any vascular events, including arterial events (strokes and myocardial infarctions) or deep venous thrombotic events (venous thromboembolism, deep venous thrombosis, retinal vein thrombosis, and pulmonary embolism)
- Lactic acidosis
- Hypoglycemia
- New HIV diagnosis

All AEs that are reported must have their severity graded. To grade AEs, refer to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, which can be found on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

Serious Adverse Events (SAEs)

An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above).

8.3 Expedited Adverse Event Reporting

All serious adverse events must be reported to the IRB according to the regulatory requirements. All serious adverse events must be followed to satisfactory resolution or until the investigator deems the event to be chronic or not clinically significant, the event is considered to be stable, or the participant is lost to follow-up.

8.4 Study Monitoring

Members of the protocol team will monitor the conduct and safety of the study via regular pooled summaries of accrual, baseline characteristics, treatment and study discontinuation, and data completeness.

During the study, the safety and tolerability of the study medication will be monitored by toxicity reports presenting laboratory and clinical data. The subset of the team receiving these reports (as per the specifications in the DSMP) will discuss these reports on regularly scheduled conference calls or by e-mail.

8.5 Serious adverse events (SAEs)

The term "serious adverse event" (SAE) is defined in 21 CFR 312.32 as follows:

"An adverse event or suspected adverse reaction is considered 'serious' if, in the view of either the investigator or the sponsor, 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
- 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."

"Life-threatening" refers to an AE that, at occurrence, represents an immediate risk of death to the participant. A hospital admission for an elective procedure is not considered an SAE.

8.6 Safety monitoring

8.6.1 Protocol Safety Review Team (PSRT)

The PSRT comprises:

- Medical Officer (MO), Sonya Heath, MD
- Study PI: Ellen Eaton, MD, MSPH
- Study Co-PI: James Kobie, PhD
- Clinic Director: Paul Goepfert, MD
- Lead Study Nurse: Heather Logan, DNP, CRNP

The clinician members of the PSRT are responsible for decisions related to participant safety. The PSRT will review study safety information on a weekly basis through 6 weeks after the last participant completes study product. After this timepoint, less frequent safety reviews may be conducted at the discretion of the PSRT.

8.6.2 Data Safety Monitoring Board (DSMB)

The DSMB is a multidisciplinary group consisting of biostatisticians, clinicians, and experts in HIV and substance use research that, collectively, has experience in the conduct and monitoring of trials. Members of the DSMB are not directly affiliated with the protocol under review.

The DSMB reviews safety data (including cumulative AEs, laboratory safety data, and individual SAE reports) approximately every 4 months. The DSMB conducts additional special reviews at the request of the PSRT.

Study investigators will receive DSMB summary minutes. DSMB summary minutes will be provided to Institutional Review Board (IRB)/Ethics Committee (EC) and any applicable Regulatory Authority (RA) as appropriate.

8.7 Safety reviews

8.7.1 Safety evaluations

Safety pause and prompt PSRT AE review

The PSRT (see Section 8.6.1) will closely monitor participant safety. The trial can be paused at any time for any reason by the PSRT. When a trial is placed on safety pause, all enrollment will be held until further notice. The AEs that will lead to a safety pause or prompt the PSRT AE review are summarized in Table 8-7. Enrollment and/or study product administration may be suspended for safety concerns other than those described in the table or before pause rules are met, if, in the judgment of the PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 10.1.

Table 8-7 Pause Rules

Event / relationship to study product	Severity grade	Study Team Actions	PRST Actions
SAE Related	4	**Phone**: 205-934-3411 and page Dr. Eaton and Dr. Heath immediately **Email:** metihpsrt@uabmc.edu **Submit** CRFs immediately	Immediate pause for PSRT review
SAE Related	3, 2, or 1	**Email:** metihpsrt@uabmc.edu **Submit** CRFs immediately	Prompt PSRT AE review and consideration of pause
AE Related	4 or 3	**Email**: metihpsrt@uabmc.edu **Submit** CRFs immediately	Prompt PSRT AE review and consideration of pause

Unrelated Participant Death: During working hours (local time) study team will email the PSRT upon learning of any unrelated participant deaths. The study team will also email the PSRT and immediately submit CRFs.

8.7.2 Plan for review of safety pause rules

For all safety pauses, the PI will notify the PSRT, NIDA, DSMB, and IRB.

Once a trial is paused, the PSRT will review safety data and decide whether the pause can be lifted or whether permanent discontinuation of study product is appropriate, consulting the DSMB if necessary.

If an immediate PSRT notification or prompt PSRT AE review is triggered, the study team will notify the PSRT as soon as possible during working hours (local time) or, if the information was received during off hours, by the morning of the next workday. If a prompt PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause will occur.

The study team will submit to the IRB/EC and any applicable RA protocol-related safety information. The study team must also follow all applicable RA reporting requirements.

8.8 Study termination

This study may be terminated early by the determination of the PSRT, the National Institutes of Health (NIH), the United States Department of Health and Human Services (HHS) Office for Human Research Protections (OHRP), or the DSMB. In addition, the conduct of this study may be terminated by the determination of the IRB/EC and any applicable RA.

9 Clinical Management Issues

9.1 Toxicity

9.1.1 General instructions for management of Grades 1-4 AEs are provided in this section. Refer to section 9.2 and further for information on specific toxicities.

9.1.2 Grade 1 or 2

Participants who develop a Grade 1 or 2 AE or toxicity thought to be related to study-supplied metformin may continue metformin at the discretion of the principal investigator and will be followed carefully until resolution or stabilization.

If the principal investigator or participant chooses to discontinue any study-supplied metformin, the site should notify the medical monitor within 72 hours. The premature treatment/study discontinuation evaluations must be completed, and then the participant may remain in follow-up off study treatment.

9.1.3 Grade 3 or 4

For Grade 3 or 4 AE or toxicity that is related to study-supplied metformin, metformin must be discontinued, participants must be followed carefully, and the medical monitor should be notified within 72 hours.

The participant should be followed carefully until resolution or stabilization.

The premature study discontinuation evaluations must be completed, and then the participant may remain in follow-up off study treatment

9.1.4 Serious Adverse Event

For Grade 3 or 4 SAE (as defined in section 8.2) that is related to study-supplied metformin, metformin must be permanently discontinued and the medical monitor should be notified within 72 hours. The participant should be followed until the AE has decreased to Grade ≤2. The premature study discontinuation evaluations must be completed, and then the participant may remain in follow-up off study treatment

9.2 Hyperlactatemia /Lactic Acidosis

The following case definition of symptomatic hyperlactatemia/lactic acidosis will be used in this protocol:

New, otherwise unexplained and persistent (≥2 weeks) occurrence of one or more of the following symptoms:

- Nausea and vomiting
- Abdominal pain or gastric discomfort
- Abdominal distention
- Elevated liver function tests
- Unexplained fatigue
- Dyspnea
- Concern for hyperlactatemia/lactic acidosis should be confirmed by measurement of serum lactate level at the discretion of the principal investigator. If a lactate level >2.2 mmol/L is confirmed, participants should immediately discontinue their study medications and the medical monitor should be contacted to plan further treatment.

10 Criteria for Discontinuation

10.1 Permanent and Premature Study Product Discontinuation

Under certain circumstances, study product will be permanently discontinued for an individual participant. Specific events that will result in stopping product for an individual participant include:

- Drug-related toxicity (see section 9.1)
- Request by participant to terminate study product
- Clinical reasons believed life-threatening by the physician, even if not addressed in section 9.0 of the protocol.
- Requirement for prohibited concomitant medications.
- At the discretion of the IRB, NIDA, Office for Human Research Protections (OHRP), other government agencies as part of their duties, medical monitor, or principal investigator.
- SAE that is subsequently considered to be related to metformin
- Grade 3 or Grade 4 AE assessed as related to metformin
- glucose outside of range (70-180 mg/dL, non-fasting), that is not resolved within 1 week
- HbA1c >6.4%
- eGFR <60 mL/min/1.73m2
- creatine clearance <60 mL/min
- ALT/AST >3X upper limit of normal, or >2X increase of ALT/AST from baseline
- Total Bilirubin >1.4 mg/dL
- Albumin <3.5 g/dL
- Prothrombin >1.5X upper limit of normal
- serum bicarbonate <22mEq/L
- anion gap >14 mEq/L OR

anion gap >12 mEq/L when serum bicarbonate <22 mEq/L

- serum lactate >2.2 mmol/L
- Pregnancy (regardless of outcome).
- PI or medical monitor assessment that it is not in the best interest of the participant to continue receiving study product.
- A participant that was previously subjected to study product discontinuation due to previous (not current) criteria for discontinuation may re-enroll in the study at the discretion of the PI.

10.2 Premature Study Discontinuation

- Failure by the participant to attend 3 consecutive in-person clinic visits.
- Participant repeatedly does not adhere to study products as prescribed, per the principal investigator's discretion.
- Request by the participant to withdraw.
- Failure/inability of participant to attend study visits.
- Request of the primary care provider if she or he thinks the study is no longer in the best interest of the participant.
- At the discretion of the IRB, NIDA, Office for Human Research Protections (OHRP), other government agencies as part of their duties, medical monitor, or principal investigator.

11 Statistical Considerations

11.1 General Design Issues

The purpose of this randomized clinical trial (RCT) is to determine the potential of metformin to reduce systemic inflammation and mitigate immune dysregulation among people who inject drugs (PWID). The study's primary objective is to evaluate if in people with a history of injection drug use does MET result in a change (decrease in CRP) in systemic inflammation as measured by CRP compared to placebo over 20 weeks. It is of secondary interest to compare MET versus placebo for change in B cell exhaustion, T cell exhaustion and improvement in antibody responses to vaccination over 20 weeks.

11.2 Outcome Measures

11.2.1 Primary Outcome Measures

Change (absolute) in CRP (mg/L) from enrollment to week 16.

11.2.2 Secondary Outcome Measures

11.2.2.1 Change (percentage) in peripheral blood exhausted B cells (% of total B cells) from enrollment to week 16.

11.2.2.2 Change (percentage) in peripheral blood exhausted T cells (% of total T cells) from enrollment to week 16.

11.2.2.3 Change (absolute) in vaccine-specific IgG antibody titer from enrollment to week 9.

11.2.2.4 Change (absolute) in vaccine-specific IgG antibody titer from enrollment to week 13.

11.2.3 Exploratory Outcome Measures

11.2.3.1 Change in oral and/or genital mucosal inflammatory cytokines from enrollment to week 16.

11.2.3.2 Differences in rectal lymphocyte characteristics at week 16.

11.3 Randomization and Stratification

All eligible participants will be randomized using a 1:1 ratio to one of two study groups: MET or placebo, using permutated blocks. Participants will be stratified at randomization by sex assigned at birth and by HIV infection status. Participant enrollment targets are 50% participants assigned female sex at birth and 50% persons with HIV.

11.4 Sample Size and Accrual

The proposed sample size is 100 participants (50 per arm). It is anticipated that the initial 10 participants will be enrolled within 4 months, followed by a 2-month planned safety pause in new enrollment, and then commencing with enrollment of ~3-4 participants per month, with final enrollment expected to be completed by M36, and final study visit completed by M42.

Based on previous studies that evaluated our planned endpoints in response to MET, a sample size was determined For the Primary Objective using a 2-way ANOVA with an interaction between treatment (MET/Placebo) and HIV status (+/-) with change in CRP of 1.56 from week 0 to 16(64, 65). Allowing for a significant interaction at the a=0.10 level; an adjusted a=0.0167, for the 3 direct comparisons of interest: HIV+/MET vs HIV+/Placebo, HIV-/MET vs HIV-/Placebo, and HIV+/MET vs HIV-/MET; has 80% power to detect a change in CRP of 1.56 (SD 2.12) with 80.1% power with an n=80 (n=100, with 20% attrition). Without the interaction, comparing only MET vs Placebo and HIV+ vs HIV-, an a=0.025, has power of 84.0%, or 80.3% power for a D=1.49, or D=1.56 with an increased SD=1.56 (JMP v17). Use of repeated measures mixed model will allow for use of results collected for all participants that terminate prior to week 16, the mixed model approach should also provide additional protection by leveraging within participant correlation. In addition, adjustment for *a priori* covariates known to be associated with CRP will also serve to increase power. **Table 11-4-1** shows detectable differences for Secondary Outcomes: exhausted B cell(69) and T cell(73) subsets, from baseline to WK16, as well as differences in IgG plasma antibody response to vaccination(69, 70) from WK5 to WK9 (Capvaxvie, PCV21) or WK13 (Jynneos). Exploratory Objectives are not powered for confirmatory statistical comparisons, however, the goal with this research is to estimate variables associated with mucosal inflammation and immunity. The sample size of 20-50 for these analyses was selected based upon feasibility but aligns well with statistical recommendations for pilot trials to determine precise estimates(113).

Secondary Endpoints	Δ	SD	Power
B cell exhaustion	4.04 %	6.3	80.9
T cell exhaustion	1.67 %	2.6	81.0
Ab IgG titer	119	187	80.3

Table 11-4-1. Power considerations for secondary endpoints. α=0.05

11.5 Data and Safety Monitoring

The Data Safety Monitoring Board (DSMB) will undertake reviews of interim data to ensure safety of study participants and to possibly recommend termination or modification of the study. At DSMB reviews, data will be considered as detailed in section 8.6.

At each interim review, the DSMB will review unblinded summaries, by randomized arm, of study status and, including loss to follow-up (LTFU), treatment discontinuations, and safety, including adverse events. Interim efficacy analyses are not planned.

There are no pre-specified stopping guidelines for this study, but the DSMB should consider possible modifications or termination on bases of safety concerns or operational futility. With regards to safety, if 20% or more of participants in Group 1 (metformin treatment) have treatment-related ≥Grade 2 AEs, this would be concerning to the study team. With respect to operational futility the DSMB should consider the overall and within arm LTFU rates. As a benchmark, an overall LTFU rate of 40% or higher would be cause for concern, particularly if a higher rate is observed in Group 1 (metformin treatment).

11.6 Analyses

They study statistician, Dr. Hartzes will analyze the data using SAS or SPSS for quantitative analytical software.

Participant characteristics will be presented as mean±SD, mean (median) and n (%), as applicable. Baseline comparability and early terminations will be assessed by parametric and nonparametric single-factor (treatment group) ANOVA or chi-square analyses, as applicable. Any baseline differences will be assessed for degree of imbalance and included as model covariates, as appropriate. The primary endpoints will be analyzed with linear mixed model regression, adjusting for CRP at baseline, HIV status (the stratification variable), with participant as a repeated effect. The effects considered will be treatment (MET/Placebo), HIV status, and the interaction between treatment and HIV status, creating 4 groups. If the significance level for the interaction does not correspond to an alpha value of 0.10 or less for Type I error, it will be removed to assess the differences between MET and placebo and HIV status as main effects (at an adjusted a=0.025). The study is not powered for the interaction but rather for the 2 main-effect comparisons. If the interaction is significant, the 3 direct comparisons of interest: HIV+/MET vs HIV+/Placebo, HIV-/MET vs HIV-/Placebo, and HIV+/MET vs HIV-/MET, will be compared at an adjusted a=0.0167. Fixed effects for primary endpoints will also include an interaction between treatment arm and time. This will allow estimation of the mean difference in change between arms and 95% confidence intervals. Using a unified analytic approach allows us to make sure that the structure of the different analyses is parallel, by including covariates in the same way, and reduces the complexity of comparing the results across different variables. If there are a large number of potential confounders at baseline, we will use the propensity score approach to ensure that

the estimates are unbiased while minimizing the degrees of freedom required. Sex will be considered as a biological variable, through efforts to equally recruit study participants and post-hoc analysis of the association of sex and immunological variables. While not powered for confirmatory subgroup analyses, the sample size of 100 should allow for investigation of binary covariates such as polysubstance use features, and exploratory endpoints such as PROs, and association with primary and secondary endpoints. The mixed model will also allow for estimation and assessment of time-vary covariates(113). Due to the exploratory nature of select advanced ‘omics analyses, a less conservative FDR of 0.01 will be used to generate hypotheses and identify areas for further investigation. Analyses will be carried out using SAS and R, p-values<0.05 will be considered meaningful, unless otherwise noted.

12 Pharmacology Plan

Blood will be stored to measure concentrations of MET at enrollment, week 5, week 16, and week 20 (or premature discontinuation) from Group 1 to inform study adherence interpretation.

13 Data Collection and Monitoring

13.1 Records to Be Kept

eCRF screens will be used for data entry. Participants must not be identified by name on any data submitted to the DSMB. Participants will be identified by the patient identification number (PID).

13.2 Role of Data Management

The Principal Investigator is responsible for overseeing the data collection process, ensuring that all required information is captured accurately, efficiently, and timely throughout the trial. All source documents should be completed in a manner to ensure the Principles for Data Integrity are upheld.

13.3 Clinical Site Monitoring and Record Availability

Clinical site monitoring is conducted to ensure that the rights and well-being of participants are protected, that the reported trial data are accurate, complete and verifiable, and the conduct of the trial is in compliance with the currently approved protocol/amendments and applicable regulatory requirements.

Monitoring for this study will be performed by the UAB Center for Clinical and Translational Science (CCTS) Clinical Research Support Program and may be conducted onsite or remotely. Monitoring visits will occur after the first 5 enrollments and quarterly thereafter, and may include but are not limited to, review of regulatory files, accountability records, case report forms, consent documents, medical and laboratory reports, study product storage records, training records, and protocol and GCP compliance. Upon conclusion of the monitoring visit, monitoring reports will be distributed to the Principal Investigator and relevant site staff.

13.4 Reporting Protocol Deviations

The principal investigator and staff are responsible for identifying and reporting deviations. If protocol deviations are identified, corrective actions are to be developed by the site and implemented promptly. Protocol deviations must be reported to the IRB/EC per their guidelines.

Refer to the SSP for the definition of protocol deviation and instructions for completing the study protocol deviation eCRF.

14 Participants

14.1 IRB Review and Informed Consent

This protocol and the informed consent document (Appendix A) and any subsequent modifications will be reviewed and approved by the IRB or PI for oversight of the study. A signed consent form will be obtained from the participant (or parent, legal guardian, or person with power of attorney for participants who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant and this fact will be documented in the participant's record.

14.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB/EC, NIDA, OHRP, or other local, US, and non-US regulatory entities as part of their duties.

14.3 Study Discontinuation

The study may be discontinued at any time by the PI, IRB/EC, NIDA, OHRP, or other country-specific government agencies as part of their duties to ensure that research participants are protected.

15 Data Sharing, Public Access, and Publications

This study will comply with the NIH Data Sharing Policy and Implementation Guidance (https://sharing.nih.gov/data-management-and-sharing-policy). Investigators will register and report results of the trial in ClinicalTrials.gov, consistent with the requirements of the Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration (https://grants.nih.gov/policy-and-compliance/policy-topics/clinical-trials/reporting/nih-policy).

Primary data for this study will be available to the public in the NIDA data repository, per NIDA policy (https://datashare.nida.nih.gov/).

The primary outcome(s) publication will be included along with study underlying primary data in the data share repository, and it will also be deposited in PubMed Central (https://pmc.ncbi.nlm.nih.gov/) per NIH Policy (https://sharing.nih.gov/public-access-policy).

We will disseminate findings through abstracts for presentation at academic conferences, manuscripts for publication in interdisciplinary high-impact peer-reviewed journals, and grant proposals for subsequent studies. We anticipate this project will result in the production of at least three manuscripts.

We will also disseminate findings from the study to members of the community so that trial results can inform policies and procedures. We will seek guidance from community stakeholders on the most effective ways to share findings with members of their organizations and the community at-large.

16 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the US CDC and US National Institutes of Health.

All dangerous goods and materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CRF 42 Part 72. Please refer to instructions detailed in International Air Transport Association (IATA) Dangerous Goods Regulations.

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18 Protocol Amendment Summary of Changes Table

A summary of amendment history is provided below.

Protocol Amendment 6 (Protocol Version 7.0/25MAR2026)

Key Changes Included in Amendment 6
Section 4.1 Adjusted CRP inclusion criteria
Clarifications throughout to reconcile referenced CRP inclusion criteria

Protocol Amendment 5 (Protocol Version 6.0/03NOV2025)

Key Changes Included in Amendment 5
Section 18 Added section: Amendment Summary of Changes Table
Section 6.1 SOA: Added extended window to V6, V9, V10, V13, V16, V19
Section 6.2.3 added text to address extended window visits.
Section 7.2 Screening (V0) added allowances and instructions for conducting a split screening visit
Sections 7.6, 7.8, 7.11, 7.12, 7.15 Increased study drug product dispensed at V4, V6, V9, V10 to account for extended visit windows
Minor clerical corrections and edits throughout

Protocol Amendment 4 (Protocol Version 5.0/24JUL2025)

Key Changes Included in Amendment 4
Section 5.1 Corrected inconsistency in study drug product (metformin or placebo) treatment duration (120 days>112 days (16 weeks)).
Section 7.2 Added the ability to repeat a single out of range laboratory test once 3-10 days following initial test at the discretion of PI to determine eligibility.
Section 7.25 Added section for clarification of interim/unscheduled visits.

Protocol Amendment 3 (Protocol Version 4.0/23MAY2025)

Key Changes Included in Amendment 3
Executive Summary & Section 3 Corrected age range to 19-64
Sections 4.1 & 4.3 Adjusted HbA1c and anion gap inclusion and exclusion criteria.
Section 10.1 Adjusted permanent and premature study product discontinuation due to adjustment of inclusion/exclusion criteria.
Section 10.1 Added criteria for re-enrollment of participants that met discontinuation criteria under prior protocol versions.

Protocol Amendment 2 (Protocol Version 3.0/01APR2025)

Key Changes Included in Amendment 2
Section 6.3.10 Added instruction regarding naloxone counseling and provision
Minor clerical corrections and edits throughout

Protocol Amendment 1 (Protocol Version 2.0/24FEB2025)

Key Changes Included in Amendment 1
Section 4.1.3 Inclusion Citeria: Age changed from 18-64 years old to 19-64 years old.
Section 6.1 SOA: Updated blood volumes to – slightly reduced to match anticipated tubes to be used.
Minor corrections and edits throughout