Contemporary Clinical Trials Communications 21 (2021) 100704

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: http://www.elsevier.com/locate/conctc

Design and implementation of a prospective cohort study of persons living with and without HIV infection who are initiating medication treatment for opioid use disorder

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ARTICLE INFO

Keywords:
Opioid use disorder
HIV
Medication treatment for opioid use disorder
Buprenorphine
Methadone

ABSTRACT

Background: Opioid use disorder (OUD) negatively impacts the HIV continuum of care for persons living with HIV. Medication treatment for OUD (MOUD) may have differential biological effects in individuals with HIV and OUD. To address the question of modulation of immune responses by MOUDs, we describe state of the art systems biology approaches to carry out the first prospective, longitudinal study of persons with and without HIV infection with OUD initiating MOUD.

Methods: A prospective cohort study of persons with DSM-5 diagnosed OUD who are living with and without HIV infection and initiating treatment with methadone or buprenorphine is underway to assess biological effects of these medications on immunobiological outcomes.

Results: We describe the recruitment, laboratory, and statistical methods of this study as well as the protocol details. Of those screened for enrollment into the study, 468 (36%) were eligible and 135 were enrolled thus far. Retention through month 6 has been high at 80%.

Conclusions: This study will use state of the art systems biology approaches to carry out the first prospective, longitudinal studies of persons living with and without HIV with DSM-5 OUD initiating treatment with MOUD.

1. Introduction

In 2017, over 2 million Americans reported having an opioid use disorder (OUD) involving heroin or prescription pain medications. From October 2018–2019, over 47,000 individuals died of opioid overdoses in the United States [1]. Although there have been decreases in overall opioid deaths, there were increases in overdose deaths due to synthetic opioids, showing the need for a multifaceted approach to combat this epidemic [2]. The most effective treatment for OUD is one of three FDA-approved medications for opioid use disorder (MOUD): methadone, buprenorphine or extended-release naltrexone (XR-NTX) [3–6]. These medications have been shown to reduce relapse to opioid use, reduce HIV transmission, and improve other health outcomes [7,8]. However, the availability and type of MOUD is often limited [9], and there are disparities with regards to receipt of available MOUD based on location (less in rural areas of the United States), income level (less in those below poverty level), and by race and ethnicity (less receipt in racial and ethnic minorities) [10].

There are currently no guidelines for the specific patient-preference or matching of MOUD type, and none related to HIV status. The possibility that MOUDs may have differential biological effects in individuals with OUD remains unresolved. This may be particularly relevant in the immune system, which is critical for defense from infections and whose cells types (B and T cells, Natural Killer (NK) cells and macrophages) express the μ, δ and κ opioid receptors at the gene expression level in mice and humans [11]. Studies have shown opioid receptor agonists

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https://doi.org/10.1016/j.conctc.2021.100704
Received 14 July 2020; Received in revised form 15 October 2020; Accepted 1 January 2021
Available online 6 January 2021

such as morphine or methadone inhibit the respiratory burst in human monocytes [12], phagocytosis in murine macrophages [13–15], NK cell function, and potentiate HIV replication in monocytes/macrophages [16–18]. In lymphocytes, morphine has been reported to inhibit IFN-γ and IL-2 production [19], and is associated with impaired B cell antibody production [20,21] and depletion of murine B and T cell subsets [22].

Two of the most commonly used forms of MOUD, methadone, a full opioid agonist, and buprenorphine, a partial μ opioid receptor agonist and κ opioid receptor antagonist, differ in their recognition by opioid receptors, but the potential for differential immunologic effects, particularly in the context of HIV infection—remains unclear. For example, methadone treatment of human T cells strongly induced IL-4 gene expression, while buprenorphine induced substantially lower levels of IL-4 mRNA and protein [23]. Methadone, but not buprenorphine treatment of a human lung epithelial cell line enhanced influenza virus replication [24]. Recent studies indicate that buprenorphine has an anti-inflammatory effect in inhibiting CCL2-dependent human monocyte chemotaxis [25], and studies in animal models indicate that buprenorphine may be associated with decreased levels of immunosuppression [26].

To address the question of whether modulation of immune responses by MOUDs differ depending on choice of agent, we will use state of the art systems biology approaches to carry out the first prospective, longitudinal studies of HIV-positive and negative adults with DSM-5 OUD initiating treatment with methadone or buprenorphine. In addition to addressing these biological questions, this cohort will also provide insights into the subset of persons with OUD who are actively seeking and starting treatment.

2. Methods

2.1. Study design

Project MAT BIO (The Impact of HIV Infection on Immunologic, Transcriptomic, and Metabolomic Signatures of Medication-Assisted Therapy for Opioid Addiction) is an NIH-sponsored prospective cohort study of persons with DSM-5 diagnosed OUD with and without HIV initiating treatment with methadone or buprenorphine.

2.2. Ethical oversight

Institutional Review Boards (IRB) at Yale University and the Connecticut Department of Correction (CTDOC) reviewed and approved all study procedures. Additional protections were provided by the Office of Human Research Protections (OHRP) at the Department of Health and Human Services, and a Certificate of Confidentiality (CoC) was obtained.

2.3. Research goals

Since there are no clinical guidelines for the selection of MOUD, the main goal of this research study is to assess the biologic effects on immune responses and chronic inflammation of methadone vs. buprenorphine in persons with and without HIV. Our focus is to elucidate differences in immunologic, transcriptomic and metabolomic signatures that differentiate HIV-positive vs. HIV-negative persons with OUD initiated on methadone vs. buprenorphine treatment, since these are the most commonly used forms of MOUD available.

2.4. Sample size and power calculations

These analyses are intended be exploratory and hypothesis-generating, therefore, sample size and power were not calculated. We aim to enroll 200 persons with OUD starting MOUD (50 HIV negative starting methadone, 50 HIV positive starting methadone, 50 HIV negative starting buprenorphine, and 50 HIV positive starting buprenorphine).

3. Study procedures

3.1. Recruitment and screening

Recruitment began in March 2017 and was temporarily paused from March 13, 2020 through June 22, 2020 due to the COVID-19 pandemic and resulting restrictions set by Yale University regarding ongoing research. During this time, follow-up interviews were completed with currently enrolled participants, but blood draws were unable to be done. Recruitment will continue through December 2020 to allow for at least 3 months of follow-up for all participants. Participants starting MOUD at an approved study site are screened on-site and enrolled the day they are to begin MOUD. Approved study sites include: the APT Foundation, Yale Community Health Care Van, Nathan Smith Clinic, and the Substance Abuse Treatment Unit (SATU), a part of Addiction Services of the Connecticut Mental Health Center (CMHC), all in New Haven, CT, Connecticut Addiction Medicine in Harford CT, and facilities of the Connecticut Department of Corrections.

Screening questions to determine eligibility are incorporated into REDCap (Research Electronic Data Capture) [27]. Those who meet study inclusion criteria are invited to participate with study staff when they are enrolled at their treatment site and are asked to sign a release of information (ROI) form to allow study staff to speak with them confidentially.

3.2. Eligibility

Inclusion criteria include: 1) Age >18 years; 2) Able to speak English or Spanish; 3) Meets DSM-5 criteria for an opioid use disorder; 4) Able to give verbal and written informed consent; 5) Presenting at approved study site for treatment of opioid use disorder; 6) Initiating methadone or buprenorphine for OUD; 7) Consent to HIV and HCV rapid onsite testing; 8) If HIV+, prescribed antiretroviral therapy (a proxy for HIV viral suppression <200 copies/mL, until we can obtain HIV viral load).

Exclusion criteria include: 1) suicidal ideation or plans for self-harm; 2) displays threatening behavior towards staff (clinic or research staff); 3) requires a narcotic pain medicine prescription (through 04/18/2017, then removed) 4) pregnant or breastfeeding; 5) not willing to use contraceptives; 6) already maintained on MOUD; 7) self-report of fever in the past 2 weeks; 8) has an immunosuppressive condition other than HIV; and 16) newly diagnosed with HIV infection.

3.3. Informed consent and enrollment

Participants undergo a written informed consent process. Research staff assess willingness to participate in the study, including study procedures such as rapid HIV tests, and study blood draws for each visit. Research staff make sure that all participants have any questions related to the study answered and understand the study prior to signing the consent form. After receiving informed consent from the research participant, a study staff member collects location information from the research participant. This includes their full name, aliases (if any), address, phone number, an alternative contact and their contact information. Participants then undergo the baseline interview assessment (in REDCap) and blood draw. Participants consent to having 70–80 cc of blood collected for study analyses.

3.4. Baseline and follow-up visit procedures

All enrolled participants undergo baseline assessments and blood draws. Follow-up interviews and blood draws are done for each study visit at day 7, day 14, and at month 1, 3, and 6 after baseline. Please refer to Table 1 for the study activities, measure, and the study timeline.

Table 1
Study activities and measures.

Study Activity	Study Time Point					
	Baseline	Day 7	Day 14	Month 1	Month 3	Month 6
Screening for eligibility	X					
Consent	X					
Obtain or update locating information	X	X	X	X	X	X
Study blood sample	X	X	X	X	X	X
Research Interview	X	X	X	X	X	X
Demographic questions	X					
Housing Questions	X			X	X	X
Current and past medical history	X				X	X
Current medications	X	X	X	X	X	X
HIV questions (medications, adherence, etc.)						
MOUD questions (type, dose, changes, etc.)	X	X	X	X	X	X
Mental health diagnosis and treatment questions	X				X	X
Alcohol Use Disorders Identification Test (AUDIT)	X					
Addiction Severity Index (ASI) legal questions	X					
Alcohol, Smoking and Substance Involvement Screening Test (ASSIST v3.0)	X				X	X
Opioid Craving Scale	X	X	X	X	X	X
Patient Health Questionnaire (PHQ-9)	X			X	X	X
WHO Quality of Life-BREF (WHOQOL-BREF)	X			X	X	X
HIV Risk Behaviors		X			X	X
Mini International Neuropsychiatric Interview (MINI) v7.0.2	X					
Time Line Followback (TLFB)	X	X	X	X	X	X
Clinical Tests - on site						
Rapid HIV test	X					X
Rapid HCV test	X					X
Urine toxicology screen	X	X	X	X	X	X
Pregnancy test	X	X	X	X	X	X
Breathalyzer	X	X	X	X	X	X
Clinical Lab Tests						
HIV-1 RNA level[a]	X				X	X
CD4 count[a]	X				X	X
HCV RNA level[b]	X					X
Compensation for participation						
Interview	X	X	X	X	X	X
Study blood sample	X	X	X	X	X	X

Abbreviations: MOUD = Medication for Opioid Use Disorder; HCV=Hepatitis C Virus.
[a] for those with HIV or positive rapid HIV test.
[b] for those with HCV or a positive rapid HCV test.

Rapid HIV and HCV tests are administered to participants at baseline. A rapid test for HIV (OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test) [28] and HCV test (OraQuick® HCV test) is performed on all consented participants. For these tests, participants receive information on the procedure, meaning of test results, and an explanation of the window period during which an HIV antibody test might be negative [29]. A reactive HIV or HCV test is followed by a confirmatory blood test conducted by Quest Diagnostics, using HIV and HCV viral load (VL) tests with reportable ranges of 20 copies/mL to 10,000,000 copies/mL and 15 IU/ml to 100,000,000 IU/ml, respectively. Repeat HIV and HCV rapid tests are done at month 6 for those who tested negative at baseline.

Participants meet with study staff at each scheduled visit to complete the interview, phlebotomy, urine drug screens (Abbot, formerly Redwood Toxicology), urine pregnancy tests for female participants, and alcohol breathalyzer assessment (Alco-Sensor III breathalyzer). Participants who stop MOUD or switch treatment are followed until the end of the study and receive the same assessments as other participants. Participants who return to drug use are referred to New Haven's syringe services program (or other local program), which provides safe injection equipment, naloxone, and has opioid as well as other substance use disorder treatment programs.

4. Covariate and outcome measures

4.1. Research interviews

Measures, outcomes and study timeline are depicted in Table 1. Measures from the research interviews include demographic information, housing status, current and past medical history, current medications, MOUD questions, mental health diagnoses, Alcohol Use Disorders Identification Test (AUDIT) [30], ASI (Addiction Severity Index) legal questions [31], Alcohol, Smoking and Substance Involvement Screening Test (ASSIST v3.0) [32], Opioid Craving Scale [33], Patient Health Questionnaire (PHQ-9) [34], WHO Quality of Life-BREF (WHOQOL--BREF) [35], HIV risk behaviors, and the Mini International Neuropsychiatric Interview (MINI) v7.0.2 [36]. For those with HIV, information on HIV medications and medication adherence questions is obtained. Table 1 summarizes questions for participants at each timepoint.

4.2. Substance use outcomes and MOUD retention

The timeline follow back (TLFB) asks about opioid use for every calendar day, and can be used to assess both prior and recent opioid use. Urine toxicology screens are conducted at every visit, and the ASSIST [32] asks about drug use and frequency of drug use. Variables that will be used to assess drug use include: 1) time to first opioid relapse, from the timeline follow back (TLFB, a self-report of drug use [37]), 2) urine toxicology screen results, and the ASSIST (drugs used in past 3 months). From the TLFB data, median time to relapse and Kaplan-Meier time--to-event analysis will be performed, and significance will be tested using log rank and Wilcoxon statistics. The number of days of opioid use per month will be calculated from baseline (30 days before enrollment) and for each time point. TLFB data will be compared to both drug urine screen results and answers to the ASSIST questionnaire. Retention on MOUD is assessed at every study visit. Participants are asked if they are still on the same form of MOUD as when they started the study. If they are not, they are asked if they stopped MOUD altogether or switched MOUD, and reasons why.

4.3. Whole blood immune profiling by mass cytometry

Profiling of immune cell frequency and functional status is determined by multiparameter immune profiling using cytometry by time-of-flight (CyTOF) according to our established methods [38,39]. Surface markers on freshly isolated heparinized blood are labeled with metal-conjugated antibodies (Fluidigm, South San Francisco, CA) prior to fixation for optimal labeling. To detect viability, cells were resuspended in 5 μM cisplatin (Enzo Life Sciences) for 5 min and quenched with 100% FBS. Samples are labeled with the antibody cocktail for 30 min on ice, fixed (BD FACS Lyse), and frozen at −80 °C. Intracellular

labeling is conducted on permeabilized cells (BD FACS Perm II) before overnight in iridium interchelator (125 nM; Fluidigm) in 2% paraformaldehyde. Samples are run on a Helios 2 CyTOF instrument at a flow rate of 30 μL/min and a minimum of 100,000 events is collected. Raw FCS files are bead normalized and live cells are gated using the Cytobank platform following exclusion of debris (DNA^{lo}), dead cells ($cisplatin^{hi}$), and doublets.

4.4. Peripheral blood mononuclear cell (PBMC) isolation

Blood samples are collected in sodium heparin CPT tubes (BD Biosciences) from participants according to our standard procedures [40,41]. CPT tubes are mixed 5 times by inverting, carefully transferred to a centrifuge (Legend XT, Thermo Fisher Scientific), and centrifuged for 20 min at room temperature without brake at 1500 RCF. Buffy coats containing peripheral blood mononuclear cells (PBMC) at the interface are carefully collected to 50 ml RPMI medium with 10% Fetal Calf Serum (FCS). Samples are centrifuged to pellet cells and the supernatant are discarded. Lymphocyte pellets are re-suspended in 50 ml RPMI containing 10% FBS. A 20-μL volume of suspended PMBC is mixed with equal volume Trypan Blue solution (Thermo Fisher Scientific) and incubated at room temperature for about 2 min. A 20-μL cell suspension is immediately counted using a hemocytometer under light microscopy. Percent viability is determined by the formula (Number of total cells counted – Number of Blue cells counted) x 100. After initial experiments using freshly isolated PBMCs, remaining PBMCs are cryopreserved in fetal bovine serum containing 10% DMSO.

4.5. In-vitro stimulation of monocytes in human PBMC using toll like receptor (TLR) ligands

About 0.5×10^6 of total PBMC in 200 μL per well of 96-well plate are incubated in the presence of TLR ligands. Of the 18 h of total incubation time, Brefeldin A is added to each well for last 6 h. Various concentration of TLR ligands (Invivogen, CA) were as follows: TLR1/2 ligand Pam3Csk4 (10 μg/ml), TLR2/6 ligand LTA (2 μg/ml), TLR3 ligand PolyI:C (10 μg/ml), TLR4 ligand LPS (1 μg/ml), TLR5 ligand Flagellin (5 μg/ml) and TLR7/8 ligand R848 (0.5 μg/ml).

4.6. Platelet activation using TLR ligands

About 8 ml of blood is collected in acid citrate dextrose (ACD) tubes (Cat. Number 364606, BD Biosciences) for platelet isolation, taking care that the ACD tube is not drawn first during phlebotomy (shear forces for the first tube drawn into the Vacutainer tube could result in platelet activation). Samples are kept at room temperature and centrifuged at 240 g using a bench top centrifuge (Thermo Fisher Scientific) for 20 min without brake. The straw-colored platelet rich plasma (PRP) at the top of the red pellet is carefully transferred to a 15 ml conical tube for RNA extraction and in vitro stimulation assays.

PRP (100 μL) is dispensed per well of a 96 well plate containing TLR 2/6 ligand LTA (2 μg/ml), Invivogen, CA), TLR4 ligand LPS (1 μg/ml, Invivogen, CA), TLR7/8 ligand R848 (0.5 μg/ml, Invivogen, CA), TLR9 ligand CPG ODN (1 μM final concentration, Invivogen, CA) and PMA (40.5 μM final concentration, (Thermo Fisher Scientific, CA) in 100 μL of M199 media. After 20 min of incubation, samples are centrifuged at 800 g on a bench top centrifuge (Legend XT, Thermo Fisher Scientific) fitted with plate adapters. The supernatant is discarded and platelet pellet is washed with 1x FACS buffer (1x PBS containing 2% FCS). Washed pellets are stained using a cocktail of antibodies against cell surface markers as listed below.

4.7. Flow cytometry

On the day of recruitment, freshly isolated PBMCs are surface stained with anti–CD14-phycoerythrin Texas Red (clone TüK4; Life Technologies), anti–CD16-phycoerythrin–cyanine 7 (clone 3G8; BioLegend), anti–CD11c-allophycocyanin (clone B-ly6; BD Biosciences), and anti–CD11b-allophycocyanin-cyanine 7 (clone ICRF44; Affymetrix eBioscience). Cells are fixed in Cytofix buffer (BD Biosciences) and stored at −80 °C in freezing medium until analysis. On the day of flow cytometry analysis, samples are briefly thawed at 37 °C, washed, and permeabilized with BD Perm/Wash buffer. A cocktail of anti–interleukin 6 (IL-6) fluorescein isothiocyanate (clone MQ2–1385; Affimetrix eBioscience), anti–tumor necrosis factor (TNF) α Alexafluor 700 (MAB11; BD Biosciences), anti-interleukin 12 (IL-12) PE (C11.5, BD Bioscience) and anti–interleukin 10 (IL-10) Pacific Blue (JES3–9D7; Affymetrix eBioscience) in 1X Perm/Wash buffer is used to stain for intracellular cytokines. Samples are washed and analyzed together using either a Fortessa instrument (Becton Dickinson) or CytoFlex LX instrument (Beckman Coulter) fitted with an automated sampler in 96-well plates.

An antibody cocktail for platelet activation assays includes CD61 FITC (Cat. Number 336404), CD40L PE (Cat. Number 310806, Biolegend), CD14 PE-CF594 (Cat. Number 562335, BD Biosciences), CD63 PercpCy5.5 (Cat. Number 353022, Biolegend), CD41Alexafluor 700 (Cat. Number 303728, Biolegend), CD62p PECy7 (Cat. Number 304922, Biolegend), CD45 APCCy7 (Cat. Number 3368516, Biolegend) and CD66b Pacific Blue (Cat. Number 561649, BD Biosciences). After incubation for 20 min, samples are washed with 1x FACS buffer followed by a paraformaldehyde (PFA) fixation step involving BD Cytofix buffer for 10 min at room temperature. Samples are washed with 1X FACS buffer again to remove the PFA and finally re-suspended in 1 x FACS buffer for flow cytometry analysis using either a Fortessa (Becton Dickinson) or CytoFlex LX instrument (Beckman Coulter) fitted with an automated sampler in 96-well plates. FCS files generated by the BD FACSDiva software or CytExpert software analyzed using FlowJo software (FlowJo, LLC).

4.8. Whole blood RNA extraction

ACD tubes are centrifuged at 800 g on bench top centrifuge (Thermo Fisher Scientific) for 20 min without brake. The top clear plasma is collected to a separate 15 ml conical tube and aliquoted as 2 ml vials for further analysis. The red pellet containing leukocytes are incubated for 20 min in 1x RBC lysis buffer (Cat. Number 00-4333-57, Thermo Fisher Scientific) at room temperature. Following first step lysis, pellets are incubated again in 1x RBC lysis buffer (Cat. Number 00-4333-57, Thermo Fisher Scientific) at room temperature for an additional 20 min. Following the second RBC lysis samples are washed with complete RPMI media. To the pellet containing leukocytes and neutrophils, 700 μL of QIAsol lysis reagent ((cat. 217004, Qiagen) is added and mixed by pipetting at least 10 times to ensure proper cell lysis. Lysed cells are immediately frozen at −80 °C until further extraction using QIAcube instrument (Qiagen).

4.9. QIAcube RNA extraction protocol

All RNA samples are extracted using the miRNeasy kit (cat. 217004, Qiagen) following the instructions provided using a QIAcube (Qiagen). Briefly, samples lysed in QIAzol reagent are incubated for 5 min at room temperature. To each sample about 140 μL of chloroform is added and shaken vigorously and left at room temperature for about 5 min. Subsequently, samples were centrifuged at 4 °C at 12,000×*g* for 15 min. The upper aqueous phase containing the RNA species is carefully transferred to a 2 ml collection tube (cat. 990381) Qiagen without touching the interphase and placed in the QIAcube for extraction. For every sample, a rotor adapter is prepared with a RNeasy mini spin column at position L1 and a 1.5 ml collection tube at position L3 and placed in the QIAcube rotor. All reagents are prepared by adding 100% ethanol (44 ml to buffer RPE and 30 ml to buffer RWT) prior to extraction and placed in respective positions in the reagent rack in the QIAcube (100% ethanol in position 3, Buffer RWT in position 4, buffer RPE in position 5 and RNase-

free water in position 6). RNA extraction is carried out by the recommended protocol (FIW-50-001-J_FW_MB and PLC program version FIW-50-002-G_PLC_MB) available from the QIAcube web portal. RNA samples with RIN values above 7.0 are used for gene expression analysis.

5. Compensation for research participation

Participants were paid for contributing their time to research activities. Participants were compensated with gift cards for $25 for each study interview and $25 for agreeing to give a blood sample.

6. Analytic plan

6.1. Immunologic outcomes

The primary study outcome is whether type of MOUD (buprenorphine vs. methadone) is associated with immunologic markers of interest in HIV-positive and negative persons. These studies are intended to be exploratory and hypothesis-generating, and stipulate validation of a hypothesis arising from this work. We will create development and validation cohorts by each timepoint randomly dividing new participants by HIV and MOUD status. In this way, the impact of changes in clinical care or other factors over time will be balanced. Next, within each HIV and MOUD stratum, we will use spline models [42] to describe the immunologic marker of interest. Spline models have been shown to have less bias and are an alternative to standard linear, curvilinear, or categorical analyses of exposures and confounders [43]. This will allow us to determine if the immunologic markers have visually different progressions.

Because this is an observational study in which the providers will determine choice of MOUD, we will confirm that characteristics of participants stratified by HIV status and MOUD agent are comparable using parametric and non-parametric tests as appropriate. If it appears as if there are no widespread systematic differences, we will calculate propensity scores (PS) [44] for MOUD treatment for all participants and use a matching algorithm [45], in concert with appropriate calipers, to match one reference participant to each patient receiving a specific treatment for buprenorphine relative to methadone. The use of PS matching combined with longitudinal immunologic markers will reduce the sources of confounding in inferring the relationship with MOUD. PS allow for the assessment of whether the characteristics of those receiving a specific MOUD agent overlap enough with those not being treated with that agent, thereby yielding an unbiased estimate of the treatment effect from the data. Given a collection of covariates that are thought to reasonably capture the significant predictors of treatment use, the treatment effect estimated from the difference of pairs of experimental units matched by PS [46] is more likely to be approximately unbiased. We will employ the method based on nearest available Mahalanobis metric matching within calipers defined by the PS. The Mahalanobis distance is used to identify the specific unit from the treatment arm whose covariate information is most similar within the framework of a range of PS values. An important consideration in the usage of a PS-based model is the choice of specific variables from which to calculate the PS. The choice of variables included can affect the bias, variance, and mean squared error of an estimated treatment effect derived from comparison groups constructed via PS methods. Using these PS-matched subjects, we will use multivariable non-linear mixed effects models [47] and test for the optimal covariance structure to capture within person correlation over time. Analyses will adjust for variables contributing to improved model fit, potentially including gender, race, retention on MOUD and MOUD dose, SUDs in addition to OUD (and SUD severity), and comorbid medical conditions (including HCV). These models will test whether type of MOUD (buprenorphine vs. methadone) is associated with the immunologic marker(s) of interest. Regression analyses [48] will include checks of model assumptions and goodness of fit using residual analyses, influence diagnostics, and goodness-of-fit statistics.

Methods of handling missing data [49] will test missing completely at random (MCAR), at random (MAR), or missing not at random (MNAR). The nonlinear longitudinal regression models are unbiased when data are MCAR or MAR. The SASv9.4 multiple imputation procedure now has a MNAR statement that imputes missing values by using the pattern-mixture model approach. To assess the MAR assumption, sensitivity analyses will compare models by varying the level of informative missingness. We will fit the validation cohort to these regression models and will use both internal and external validation techniques to test reproducibility. Using jackknife methods [50], we will test whether $\geq$ 90% of observations fall within the confidence bands during internal validation. We will also perform external validation using a cohort of half of the participants and anticipate achieving 85% of observations falling within the original confidence bands for the validation cohort. Analyses will employ SAS® v9.4, and a type I error of 5% (two-sided) will test for statistical significance. For exploratory hypothesis generation there will be no adjustment for multiple comparisons, but once the set of hypotheses are determined, the primary will be tested at type I error of 5% and the secondary hypotheses will maintain a family-wise type 1 error of 5% using the Hochberg multiple comparison correction [51].

7. Recruitment, retention and baseline characteristics

Of those screened for eligibility thus far, 36% were eligible for enrollment. Reasons for ineligibility and refusal of study participation are detailed in Fig. 1. Given the low prevalence of HIV in Connecticut, we have had to adjust recruitment to balance groups by HIV status and MOUD type, and thus at times persons who were HIV-negative but otherwise eligible were not enrolled.

Of those eligible, 135 began enrollment from April 2017 (started the baseline interview and/or completed blood draw) through March 2020; 120 completed enrollment. Fifteen participants were dis-enrolled: 6 had no starting MOUD dose (and it was unclear if they started MOUD), 3 had incomplete baseline interviews, 2 did not start MOUD, 2 did not get a baseline blood draw, and 1 did not meet the eligibility criterion of having a HIV viral load <200 copies/mL.

Study retention has been 88% for day 7, 88% for day 14, 89% for month 1, 86% for month 3, and 80% for month 6. Retention on MOUD treatment for those who had an interview at each time point was 98% at Day 7, 94% at Day 14, 91% at Month 1, 80% at Month 3, and 78% at Month 6.

Thus far, we have enrolled 43 persons with HIV infection. Of the remaining 77 participants, all received a rapid HIV test, and all tested negative. Rapid HCV tests were completed for 63/120 (53%) participants; the remaining participants reported a prior HCV diagnosis. Of those tested, 14 had a preliminary positive HCV test and were referred for lab work and/or follow-up care. Of those who have had a month 6 interview, all consented to HIV and HCV rapid tests, and none had a new HIV or HCV diagnosis. Of those who have had follow-up interviews, we have obtained study samples from 95% of those who had a Day 7 interview, 91% at Day 14, 91% at Month 1, 93% at Month 3, and 90% at Month 6.

8. Discussion

This is the only study that we are aware of to date that is prospectively evaluating immunologic, transcriptomic and metabolomic signatures among HIV-positive and negative persons with OUD initiating MOUD. At present, there are no validated guidelines for deciding on the appropriate selection of one of the forms of MOUD based on patient matching, and this choice is largely dependent on clinical experience and practitioner preference. The possibility for differential biologic effects of MOUD agents on immune responses and chronic inflammation, particularly relevant in those with HIV infection, remain incompletely studied despite a substantial body of evidence for opioid-induced

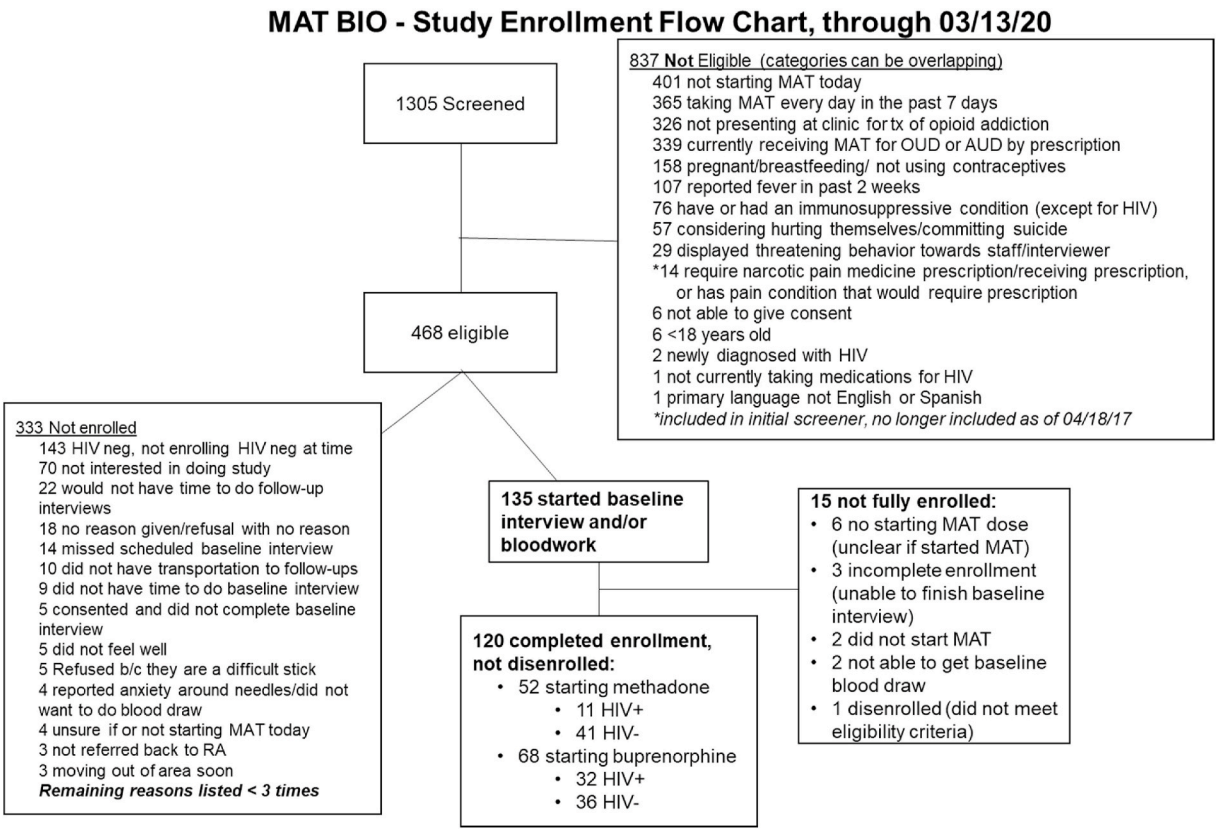

Fig. 1. MAT BIO - Study enrollment flow chart, through 03/13/20.

immunosuppression [21]. This study is hypothesis generating, but will test the hypothesis that methadone and buprenorphine will differentially affect the inflammatory dysfunction associated with opioid use that may be influenced by HIV serostatus. The findings from this study may show whether methadone or buprenorphine may be more suitable for persons with OUD based on HIV status and other factors.

Thus far, our recruitment of study participants has been consistent with our recruitment goals. However, the difficulty of finding persons with HIV and OUD starting MOUD has been a challenge. The CTDOC has assisted with our recruitment of PLWH, since persons with HIV and OUD are more concentrated in the criminal justice system than in the community [52,53].

Interview retention for this study has been excellent, with 80% retained at month 6. Studies of persons with SUDs and SUD treatment studies typically have higher attrition, as this population faces challenges that make it difficult to stay in both clinical care and research [54–58]. Retention on MOUD treatment has varied thus far, with only 78% retaining on a form of MOUD by the end of the 6-month follow-up period; however, this is greater than MOUD retention in other published MOUD studies [55,59,60]. Results from this study may not be generalizable to groups of persons starting MOUD outside of study settings, but instead to groups that remain maintained on MOUD. Return to drug use is common among persons with OUD [61], and accounts for some instances of stopping MOUD treatment. All participants who stopped MOUD were offered referrals to return to their original treatment program or another, based on their preference.

All participants consented and agreed to have rapid HIV and HCV tests at baseline, showing acceptability to rapid tests and receiving results. Among participants, there were no new diagnoses of HIV, but several diagnoses of past or current HCV that warranted further testing. Frequent testing and treatment for both HIV and HCV are critical among persons with SUDs, and thus engaging this population in frequent testing outside of research settings is important.

For whole blood profiling by CyTOF, we have optimized the panel of antibodies to identify cell subsets. We define subsets by labeling with cell lineage markers for specific immune cell types including CD11b, CD3, CD4, CD8, CD19, CD14, CD56 and HLA-DR. Using this panel, we identify 23 populations including granulocytes, T cells, B cells, monocytes, NK cells, dendritic cells, platelets, and several functional subsets. The antibody panel for phenotyping has been validated for lineage markers to define immune cell subsets as well as shared markers of cellular activation. We have processed whole blood samples from study subjects for immune profiling [39].

Immunophenotypic characterization of monocytes and blood derived platelets has been carried out in flow cytometry-based assays. Specifically, monocyte subsets such as classical monocytes ($CD14^{+}$ $CD16^{-}$), inflammatory monocytes (CD14 + $CD16^{+}$), CD11c + monocytes, and CD11b + monocytes are evaluated for expression of cytokines (IL-6, TNFα, IL-12 and IL-10) and co-stimulatory molecules such as CD80, CD86, CD150 and CD62L upon TLR ligand challenge. Monocyte immunophenotypes established by flow cytometry-based assays will be integrated with network-based gene clusters being evaluated by whole blood gene transcriptomics platform.

The impact of MOUD on thromboinflammation is being assessed by immunophenotypic characterization of blood derived platelets at baseline and in response to stimulation with TLR ligands and PMA. Platelets in peripheral blood are immunophenotyped by expression of CD41 and CD61 and TLR induced surface expression of p-selection molecule CD62p, multivesicular associated protein CD63 and TNF superfamily member protein CD154 (CD40L).

CyTOF sample acquisition is conducted for in-depth immune profiling by intracellular labeling with antibodies in batches of samples with all timepoints from a subject. We quantify levels of 13 functional markers of cell status including cytokines and chemokines such as MIP-1β, TNF-α, IFN-γ and CXCR4 which are detected within each of the 23 cell subsets. CyTOF immune profile analysis employs an unsupervised

deep learning model that performs several tasks for data analysis including clustering, batch correction, visualization, denoising, and imputation. Multivariable linear regressions will be fit to the outcomes of cell proportions and cytokine expression using predictors of group, stimulation type, and their interaction, with age and gender included as covariates. We conduct this detailed analysis using both manual gating and an unsupervised deep learning model following our recent methods [39]. Data from immune profiles will be integrated with other immune elements in the study for a full understanding of factors associated with MOUD and HIV status.

Data generated by flow cytometry based immuno-characterization, plasma metabolomics, whole blood transcriptomics and CyTOF will be integrated to create a subdomain of laboratory generated data structure. Laboratory data will be merged with the clinical data subdomain including demographic information to ultimately create a unique data structure that will be utilized in big-data-analysis pipelines. We anticipate that this data structure will be extremely valuable to predict MOUD outcomes in future studies.

Ending the opioid and HIV epidemics will require actions taken by physicians and other healthcare professionals, researchers, public health experts, policymakers, and funders. The research conducted for this project may help determine whether methadone or buprenorphine is a more suitable treatment for OUD based on biologic effects, HIV status, or other factors, and can aid future research and treatment protocols to better treat OUD. In the United States, approximately 1 in 15 new HIV diagnoses are among persons who inject drugs, with numbers of new infections increasing alongside the opioid epidemic [62]. MOUD has been shown to improve HIV viral suppression, and in combination with needle and syringe exchange programs, reduce HIV transmission among IDU [5,63]. Strategies that combine MOUD and HIV treatment, with special attention to the most suitable forms of MOUD, can help to end both the OUD and HIV epidemics.

Declaration of competing interest

The authors have no conflicts of interest to report related to this research.

Acknowledgments

We thank the APT Foundation, Yale Community Health Care Van, Nathan Smith Clinic, and the Substance Abuse Treatment Unit (SATU), a part of Addiction Services of the Connecticut Mental Health Center (CMHC), all in New Haven, CT, Connecticut Addiction Medicine in Harford CT, and facilities of the Connecticut Department of Corrections for their help with referrals, recruitment, and MOUD and other clinical services. This research was funded by the National Institute on Drug Abuse (R01 DA043337, Springer and Shaw) and for career development by the National Institute on Drug Abuse (K02 DA032322 for Springer) and National Institute on Aging (K24 AG042489 for Shaw). The funders were not involved in the research design, analysis or interpretation of the data or the decision to publish this manuscript.

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