Application for Review of Human Research: IRB Protocol Summary
Biomedical Research
Section II

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PROTOCOL TITLE

1. Full Title

Targeting the Cholinergic Pathway in HIV-associated Inflammation and Cognitive Dysfunction

2. Brief Title

Effect of galantamine on inflammation and cognition

BRIEF DESCRIPTION

This study tests whether galantamine (GAL) reduces HIV-related inflammation and cognitive deficits. In this double-blind placebo-controlled crossover study, HIV-infected individuals (N=120; 60 smokers and 60 non-smokers) will be randomized to 12 weeks of GAL or placebo, followed by a 4-week washout, then 12 weeks of GAL or placebo (arms switched). Outcomes are monocyte/macrophage and T cell activation and neurocognitive performance.

STUDY SPONSORSHIP

1. Funding Sponsor

National Institutes of Health

2. Primary Sponsor

Rebecca Ashare, Ph.D.

ClinicalTrials.gov IDENTIFIER

NCT03384784

PROTOCOL ABSTRACT

Although anti-retroviral therapy (ART) enhances life expectancy and overall quality of life (QoL), HIV-infected individuals are increasingly vulnerable to non-AIDS-related diseases including HIV-associated neurocognitive disorders (HAND). Inflammation is a primary mechanism in the pathogenesis of HAND and tobacco use may further exacerbate inflammation. Conversely, nicotine alone has anti-inflammatory effects suggesting that stimulating the cholinergic pathway via pharmacological treatment [e.g., galantamine (GAL)] may suppress inflammation and reverse or prevent neurocognitive deficits in HIV-1 infection. In this double-blind, placebo-controlled crossover study, HIV-infected individuals (N=120; 60

smokers, 60 nonsmokers) will be randomized to 12 weeks of GAL or placebo, followed by a 4-week washout, then 12 weeks of GAL or placebo (arms switched). All subjects will be stable on ART and the GAL dose will follow FDA guidelines. At the beginning and end of each treatment phase, inflammatory biomarkers and viral load will be assessed. Monocyte transcriptomics will also be assessed on a subset of the sample (n=60; 30/group). Neurocognition and clinical outcomes (e.g., QoL) will be measured at baseline and at 4-week intervals during each treatment phase. The primary outcomes are monocyte/macrophage and T-cell activation (CD16, CD163, and CCR2 expression; plasma CCL2 [MCP-1], sCD14; CD38/HLA-DR on CD8 cells) and neurocognitive performance (processing speed, verbal learning/memory, executive function). Exploratory outcomes include monocyte gene expression patterns and broad plasma cytokine analysis. This study will provide insight into the interactions among nAChR activation, HIV immune activation and pathogenesis, and tobacco use and has translational and therapeutic implications that could improve health outcomes among HIV-infected individuals.

OBJECTIVES – Main Study

1. Overall Objectives

Aim 1: To test whether a pharmacological approach to target cholinergic function reduces neurocognitive consequences of HIV-infection. We predict that galantamine (vs. placebo) will improve neurocognition, including working memory, executive function, verbal learning/memory, and processing speed.

Aim 2: To examine whether a pharmacological approach to target cholinergic function mitigates HIV-related inflammation. We predict that galantamine (vs. placebo) will reduce markers of residual inflammation (monocyte CD16, CD163, and CCR2 expression; plasma CCL2 [MCP-1] and sCD14; CD38/HLA-DR on CD8 cells).

Aim 3: To evaluate the interaction between cholinergic modulation via GAL and chronic tobacco use. We predict that the effects of galantamine on residual inflammation and neurocognitive performance will be significantly greater among HIV+ smokers, compared to HIV+ non-smokers.

2. *Primary Outcome Variables*

The primary outcomes are neurocognitive function and inflammatory markers following 4 weeks of treatment with GAL vs. PLA (within-subjects; order counterbalanced).

3. *Secondary Outcome Variables*

Aim 4: To define monocyte gene expression patterns in HIV+S (vs. HIV+NS) and identify pathways altered by GAL. Given the central role of M/M in HIV residual inflammation and HAND, we will identify global patterns of monocyte activation in HIV/ART subjects that are impacted by chronic tobacco use and the effect of immunomodulation by galantamine, by analyzing monocyte transcriptomic patterns in HIV+ smokers and HIV+ non-smokers, and changes with galantamine treatment, in a subset of the population (n=60; 30 per group).

BACKGROUND – Main Study

HIV-Associated Neurocognitive Disorders (HAND) Persist Despite ART and Impair Quality of Life. The widespread use of antiretroviral therapy (ART) has greatly improved survival rates for those diagnosed with HIV/AIDS (Brugnaro *et al*, 2015; Deeken *et al*, 2012; Palella *et al*, 2006). Despite this achievement, rates of new HIV infections have remained constant, increasing the number of people living with HIV/AIDS (2011; Hall *et al*, 2008; Palella *et al*, 1998). Between 2001 and 2008, the percentage of adults living with HIV who are aged 50 or older has increased from 17% to 31% and the rates continue to rise (Mahy *et al*, 2014; Prevention., 2010). Although ART enhances life expectancy and quality of life, HIV-infected individuals are increasingly vulnerable to non-AIDS-related diseases including cardiovascular disease, bone disease, frailty, and HIV-associated neurocognitive disorder (HAND) (D'Abramo *et al*, 2016; Leng and Margolick, 2015; Nasi *et al*, 2016; Palella *et al*, 2006; Rubinstein *et al*, 2014; Vaccher *et al*, 2014). Identifying novel treatments to address HAND, in particular, has become a critical priority, given its prevalence and impact (Nahvi and Cooperman, 2009; Pacek and Cioe, 2015). HAND is classified into three categories: HIV-associated dementia (HAD), mild neurocognitive disorder (MND), and asymptomatic neurocognitive impairment (ANI) (Antinori *et al*, 2007; Robertson and Yosief, 2014). Since the advent of ART, the incidence of HAD, the most severe form of HAND, has substantially decreased (Heaton *et al*, 2010; Heaton *et al*, 2011; Sacktor *et al*, 2016). Yet, MND and ANI persist despite ART, and 25-47% of HIV-infected people exhibit deficits in multiple cognitive domains including memory, verbal fluency, processing speed, and executive function (Heaton *et al*, 2015; Sacktor *et al*, 2016). *These cognitive deficits are associated with functional*

disabilities including unemployment, difficulty driving, and poor ART adherence (Doyle *et al*, 2013; Schouten *et al*, 2011; Thames *et al*, 2013). Two common factors across these comorbidities are persistent chronic inflammation, that often incompletely reverses with ART, and tobacco use.

Inflammation is a Primary Mechanism Contributing to HAND.

HAND in the pre-ART era was driven by systemic immune activation, reflected in an expanded population of CD16+ monocytes believed to transmigrate into the brain, and neuroinflammation, reflected by the accumulation of activated and/or infected brain macrophages/microglia (M/M) and elevated inflammatory markers in the CNS (Gannon *et al*, 2011; Gonzalez-Scarano and Martin-Garcia, 2005; Yadav and Collman, 2009). In the post-ART era, a common feature underlying many comorbidities including HAND, is persistent inflammation that does not completely reverse with viral suppression (Butler *et al*, 2011; Hunt *et al*, 2016; Lederman *et al*, 2013). In blood, persistent inflammation is reflected by monocyte and T cell markers including sCD14, sCD163, MCP-1/CCL2, monocyte CD16; CD8 T cell CD38/HLA-DR, activated coagulation products, and other cellular and soluble markers (Kamat *et al*, 2012; Lyons *et al*, 2011). Importantly, these monocyte, T cell and other soluble markers are associated with cognitive deficits observed in HIV+/ART-treated individuals (Burdo *et al*, 2013; Lyons *et al*, 2011; Schrier *et al*, 2015; Tiraboschi *et al*, 2015). In the CNS, persistent neuroinflammation is reflected by CSF inflammatory markers (McGuire *et al*, 2015; Saylor *et al*, 2016). Moreover, expression levels of genes involved with immune regulation (e.g., TNF-α, IL-1β) are correlated with HAND severity (Venkatachari *et al*, 2016). Thus, HAND in the post-ART era may be driven by persistent systemic inflammation (particularly monocytes) and neuroinflammation, which may be targeted by nicotinic receptor (nAChR) modulation.

Tobacco Use Exacerbates Inflammation and Cognitive Dysfunction.

Abundant evidence suggests that chronic smoking increases the risk of neurocognitive dysfunction and global cognitive impairment in the general population (Durazzo *et al*, 2012; Paul *et al*, 2006; Weiser *et al*, 2010) and among HIV-infected individuals (Bryant *et al*, 2013; Durazzo *et al*, 2007). Our preliminary data also show that HIV-infected smokers (HIV+S) perform worse on working memory and attention tasks compared to HIV-uninfected smokers (Harrison *et al*, under review). Tobacco smoking induces inflammatory markers including CRP, IL-6, MCP-1, and activated macrophages (D'Hulst A *et al*, 2005; Sopori, 2002; Spira *et al*, 2004; Stampfli and Anderson, 2009; Wannamethee *et al*, 2005) and may downregulate expression of genes involved with regulation of immune function (Spira *et al*, 2004), suggesting a mechanism by which smoking exacerbates HAND. Indeed, higher levels of CRP and sCD14 have been observed in both smokers with and without HIV, compared to nonsmokers (Kooij *et al*, 2016). Chronic tobacco exposure may also compromise the integrity of the blood-brain barrier and increase the likelihood of monocyte transmigration into the brain and/or direct CNS exposure to tobacco constituents, which, in turn, enhances the risk for neurodegeneration (Manda *et al*, 2010). *Taken together, evidence suggests that tobacco use may increase the risk of HAND through effects on inflammation.*

Modulation of Nicotinic Receptors and their Role in Inflammation and Neurocognition.

Despite the adverse effects of tobacco use on neurocognition and inflammation, nicotine alone, the main psychoactive ingredient in cigarettes, may have opposite effects. Nicotine attenuates pro-inflammatory cytokines IL-1β, IL-6, TNF-α (Wei *et al*, 2015) and inhibits NFkB-signaling (Sugano *et al*, 1998). Many of nicotine's anti-inflammatory properties are attributed to its activation of the α7 nAChRs (Baez-Pagan *et al*, 2015; Wei *et al*, 2015; Wittebole *et al*, 2007). For instance, activation of α7 nAChRs suppresses abnormal activation of microglia and expression of CD14, ICAM-1, CD40, and TNF-α (De Simone *et al*, 2005; Hamano *et al*, 2006; Suzuki *et al*, 2006), and the α7 nAChR antagonist mecamylamine reverses these effects (Hamano *et al*, 2006). Nicotine also has neuroprotective effects (Evans and Drobes, 2009; Heishman *et al*, 2010; Levin *et al*, 2006; Swan and Lessov-Schlaggar, 2007) and improves attention, learning, and memory in patients with schizophrenia (Jones *et al*, 2012; Lieberman *et al*, 2013; Winterer *et al*, 2013) and Alzheimer's disease (Chen *et al*, 2006; Leiser *et al*, 2009; Srivareerat *et al*, 2011). Nicotine attenuates inflammation-induced cognitive deficits (Wei *et al*, 2015), indicating that *enhancing cholinergic function may be an important therapeutic strategy to mitigate the effects of inflammation on HAND*.

Interactive Effects of HIV and Nicotine on Inflammation.

Mounting evidence indicates important interactions between the cholinergic system and HIV-1 pathogenesis on neurocognition and inflammation. *In vitro* evidence suggests nicotine and HIV work synergistically to alter synaptic plasticity and neuronal cells (Atluri *et al*, 2014). For instance, the HIV-1 envelope glycoprotein (gp120), which activates M/M (Gonzalez-Scarano *et al*, 2005; Hong and Banks, 2015; Yadav *et al*, 2009) and has neurotoxic effects (Haughey and Mattson, 2002), is also reported to bind to nAChRs (Bracci *et al*, 1992). Moreover, stimulation of the cholinergic pathway, particularly α7 nAChRs, suppresses gp120-induced inflammation (Loram *et al*, 2010) and reverses neurocognitive deficits produced by gp120 (Nesil *et al*, 2015) in rats. Nicotine alters gene expression profiles in HIV-1 infected microglia (Rock *et al*, 2008) and in rodent models of HIV (Yang *et al*, 2016). Although nicotine upregulates some genes (e.g., TGF-β1, IL-4,

CCR2, CXCR6, IL-1α) and downregulates others (e.g., TNF, CCL2, IL-8, IL-10, CXCR4, IRF4) (Rock *et al*, 2008; Yang *et al*, 2016), nAChR antagonists reverse these changes in gene expression (Rock *et al*, 2008). Thus, while tobacco cessation remains a critical priority to increase healthy outcomes among HIV+ individuals, evaluating the effects of cholinergic activation on inflammation is essential to identifying novel and effective therapeutic strategies to reduce HAND.

Galantamine's Effects on Inflammation, Cognition, and Cholinergic Function.

Consistent with this RFA (#11 under Research Objectives), we will evaluate a pharmacological therapeutic that targets cholinergic function and attenuates inflammation, in order to mitigate HIV-related adverse health consequences, including neurocognition. Although α7 nAChRs are important for neurocognition and inflammation, the rapid desensitization of these receptors (Briggs and McKenna, 1998; Quick and Lester, 2002) limits the therapeutic potential of α7 agonists, including nicotine (Echeverria *et al*, 2016; Kalkman and Feuerbach, 2016). Indeed, chronic tobacco use leads to upregulation of nAChRs (Benwell *et al*, 1988; Breese *et al*, 1997) which may be due to increased trafficking of nAChRs to the cell surface and increased receptor assembly and/or maturation (Govind *et al*, 2009). Thus, alternative methods of modulating cholinergic function must be explored. Galantamine (GAL), which is an FDA-approved medication for treating the cognitive symptoms of Alzheimer's disease (Birks, 2006; Brodaty *et al*, 2005; Lockhart *et al*, 2009), modulates cholinergic function in two ways. First, as a cholinesterase inhibitor (AChEI), it increases acetylcholine (ACh) by inhibiting the enzyme that degrades ACh, acetylcholinesterase (Ashare and Schmidt, 2014; Sofuoglu *et al*, 2013). Second, as a positive allosteric modulator (PAM) of the α7 nAChRs (Maelicke and Albuquerque, 2000; Samochocki *et al*, 2003), GAL modifies the conformation of the receptor to enhance the likelihood that it will open and increases the efficacy of an agonist by decreasing the desensitized state of the receptor (Uteshev, 2014). Thus, GAL enhances cholinergic function without resulting in receptor desensitization caused by typical nAChR agonists (Freitas *et al*, 2013). In addition to its pro-cognitive effects, GAL has anti-inflammatory properties in rodents (Gowayed *et al*, 2015; Pavlov *et al*, 2009; Satapathy *et al*, 2011) and humans (Reale *et al*, 2005). Indeed, GAL suppresses markers of inflammation important in the neuropathogenesis of HAND, including TNF-α, IL-6, and CCL2 (MCP-1) (Gowayed *et al*, 2015; Pavlov *et al*, 2009; Satapathy *et al*, 2011) and GAL's pro-cognitive effects may be mediated, in part, by its modulation of microglial nAChRs (Takata *et al*, 2010). In a rodent model of HIV, acute treatment with GAL and nicotine synergistically attenuated gp120-induced inflammation (Giunta *et al*, 2004). As shown in **Figure 1**, we propose that among all ART-treated individuals, GAL will enhance neurocognition (**Aim 1**) and reduce inflammation implicated in the pathogenesis of HAND (**Aim 2**). *Given the opposing effects of nicotine versus tobacco smoking, we will evaluate whether chronic tobacco use (i.e., exposure) modulates GAL's effects on inflammation and neurocognition* (**Aim 3**). Lastly, we will analyze transcriptomic patterns of monocytes and changes with GAL treatment in a subset of the sample (**Aim 4**). This innovative approach has the potential to clarify the mechanisms that underlie persistent inflammation despite ART, evaluate a potential therapeutic that could improve health outcomes among HIV-infected individuals, and identify those most likely to benefit.

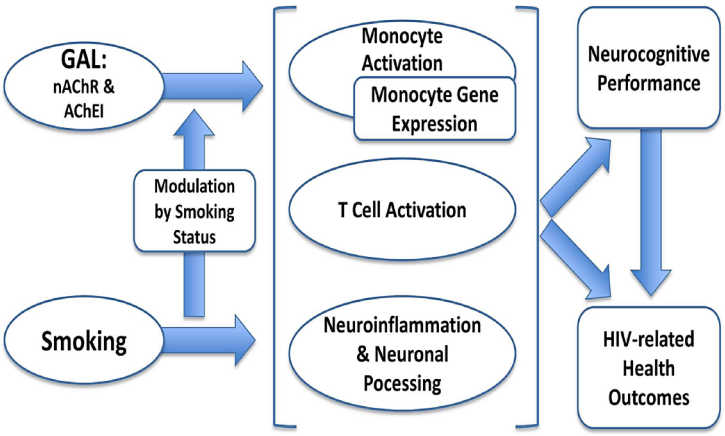


Figure 1. Hypothesized model of cholinergic modulation of HIV-related inflammation and neurocognitive performance.

BACKGROUND – Recruitment & Retention Pilot Study

Nearly one in three clinical trials closes prematurely due to under-enrollment. Reports of clinical trials consistently state that initial approaches to recruitment are rarely successful, take longer and are more costly than planned, and the pool of participants is overestimated. Unfortunately, many studies implement recruitment strategies without taking a systematic approach to identifying the most efficient and cost-effective approaches to enrolling subjects. With the increasing ubiquity of cell phones, text messaging (SMS) interventions have the potential to increase reach and reduce costs. In the United States, it is estimated that 85 to 91% of adults (18 and over) own a mobile phone, and these rates are observed across low- and high-income individuals. SMS interventions for improving adherence to antiretroviral treatment in people living with HIV as well as smoking cessation have demonstrated efficacy. However, few studies have explicitly examined strategies to optimize the use of SMS to enhance clinical trial enrollment. Behavioral economic strategies, including information provision and incentives, may represent useful approaches to overcoming barriers to clinical research and

ultimately advancing science. Information provision includes utilizing descriptive and injunctive norms, personalization, and reciprocity. Framing messages to shape social norms regarding research participation may increase engagement. For example, in addition to "personal medical benefit", patients cite "contributing to research that could help other people" and "giving something back in return" as the most important reasons to participate in clinical research. Another common strategy for improving recruitment and retention is to offer incentives, including monetary payments or other rewards that target motivation. For instance, contingency management (CM), where tangible reinforcement is provided in close temporal proximity to a participant performing a target behavior (e.g., on-time attendance) is highly efficacious in engendering target behaviors. Although information provision and incentives are effective strategies for behavior change, they may target different aspects of motivation: intrinsic (i.e., the behavior itself is purposive) vs. extrinsic motivation (i.e., the prospect of gaining the incentive motivates the behavior), respectively. Although numerous studies comparing intrinsic vs extrinsic strategies to enhance motivation have yielded inconsistent results, a recent meta-analysis suggested intrinsic and extrinsic factors may act synergistically. Thus, we propose to employ information provision and incentive strategies independently and in combination to evaluate the optimal approach for recruiting and retaining subjects in clinical research studies.

OBJECTIVES - Recruitment & Retention Pilot Study

Aim 1: To evaluate the effects of information provision and incentives, alone and in combination, on study enrollment rates.

Hypothesis: Behavioral economic interventions will (Information provision & contingency management) will produce higher rates of enrollment compared to standard recruitment.

CHARACTERISTICS OF THE STUDY POPULATION

1. Target Population

One hundred and twenty (60 smokers and 60 nonsmokers) adults living with HIV 30 years of age or older who are stable on ART regimens will complete the study. Smokers will report smoking at least 5 cigarettes per day and provide a breath CO sample greater than 5 ppm at Intake and at the beginning of each treatment period. Non-smokers will report smoking fewer than 100 cigarettes in their lifetime, or, less than 5 pack years of smoking and no cigarettes smoked in the last year. They will self-report no current use of any tobacco or nicotine product and will provide a CO sample of less than 3 ppm at Intake and at the beginning of each treatment period. If CO sample does not reflect self-report, the PI will be consulted to determine eligibility. Participants in the smoking and non-smoking groups will be matched according to key demographics such as age, race, education and history of substance use.

2. Accrual

For this trial, we expect to screen ~2000 patients, 77% of whom will be current smokers (HIV+S n=1540 and HIV+NS n=460). Based on our recent GAL trial (Ashare *et al*, 2016) and the present inclusion/exclusion criteria, we expect ~30% of these patients to be eligible via phone screen (n = ~462 and 138 for HIV+S and HIV+NS, respectively). Of these, we expect 60% to attend the Intake visit and enroll in the study (n=~277 and 83 for HIV+S and HIV+NS, respectively). Because we anticipate differences in rates of smokers and nonsmokers, efforts will be made to oversample nonsmokers. To be conservative, given the possible health problems evident in this population of smokers, we will project less than 20% of participants will withdraw from the trial and more than 80% of subjects will complete. We will enroll 150 subjects (75/group) to account for attrition. This will require enrolling 4 subjects per month over the course of a 38-month recruitment period.

3. Key Inclusion Criteria

Eligible subjects will be males and females:

1. At least 30 years old
2. Diagnosed with HIV-1 infection
3. On stable ART regimens (no changes to treatment within 4 weeks of Intake visit)
4. Viral load of less than or equal to 200 copies/mL completed 12 months prior to enrollment
5. CD4 counts greater than 200 completed 12 months prior to enrollment (if most recent)

6. If current or past diagnosis of bipolar disorder, eligible if:
    a) No psychotic features
    b) MADRS: total score less than 8 (past 4 weeks), suicidal item score less than 1 (past 4 weeks)
    c) Y-MRS: total score less than 8 (past 4 weeks), irritability, speech content, disruptive or aggressive behavior items score less than 3 (past 4 weeks)
    d) No psychiatric hospitalization or Emergency Room visits for psychiatric issues in the past 6 months
    e) No aggressive or violent acts or behavior in the past 6 months
7. Able to communicate in English and provide written informed consent
8. Will be residing in the geographic area for at least 7 months
9. Not currently trying to quit smoking
10. **Smoking Status**
    a) **Smokers** (HIV+S) will report at least 5 instances of smoking per day, on average for the past year and provide a breath CO sample greater than 5 ppm at Intake and at the beginning of each treatment period.
    b) **Non-smokers** (HIV+NS) will report smoking fewer than 100 cigarettes in their lifetime, or, less than 5 pack years of smoking and no cigarettes smoked in the last year. They will self-report no current use of any tobacco or nicotine product and will provide a CO sample of less than 3 ppm at Intake and at the beginning of each treatment period. If CO sample does not reflect self-report, the PI will be consulted to determine eligibility.

4. Key Exclusion Criteria

Subjects who present with and/or self-report the following criteria will not be eligible to participate in the study.

Smoking Behavior

1. Current enrollment or plans to enroll in another smoking cessation program in the next 7 months.
2. Regular (daily) use of electronic cigarettes, chewing tobacco, snuff, snus, cigars, cigarillos, or pipes.
3. Current use or plans to use nicotine substitutes (gum, patch, lozenge, e-cigarette) or smoking cessation treatments in the next 7 months.

Alcohol/Drug Use

1. Current untreated and unstable diagnosis of substance abuse or dependence (if past use and if receiving treatment and stable for at least 30 days, eligible)
2. Positive urine drug screen for cocaine, methamphetamines, PCP, barbiturates, ecstasy (MDMA), at Intake or Lab visits. Those who screen positive for amphetamines, benzodiazepines, methadone, oxycodone, and/or opiates (low level cut-off 300 ng/mL) and who are prescribed these medications will be reviewed on a case-by-case basis by the study physician and PI (see Measures and Table 1 for details). Participants believed to have a false-positive result on the drug screen may continue in the study, with investigator approval.

Medical/Psychiatric Conditions

1. Women who are pregnant, planning a pregnancy or lactating
2. Current diagnosis of unstable and untreated major depression (if stable for at least 30 days, eligible)
3. Current or past diagnosis of psychotic disorder
4. Cancer diagnosis within the past 6 months (except basal cell carcinoma)
5. Major heart disease or stroke within the past 6 months
6. Uncontrolled hypertension (SBP greater than 160 or DBP greater than 100).
7. Medical conditions contraindicated for use with galantamine:
    a. Diagnosis of Alzheimer's disease or dementia
    b. Epilepsy or other seizure disorder
8. Bladder outflow obstruction
9. Active HCV co-infection (if cured, requires study physician approval)

10. Liver function tests more than 20% outside of the normal range; Gamma-glutamyl Transpepsidase (GGT) values more than 20% outside of the normal range. If Albumin/Globulin ratios are 20% outside of normal range the abnormal value will be evaluated for clinical significance by the Study Physician and eligibility will determined on a case-by-case basis.
11. Renal disease or renal dysfunction (e.g., serum creatinine levels greater than 1.5 X upper limit of normal). Those with moderate hepatic impairment or creatinine clearance 9 to 59 mL/min shall not exceed the 16mg/day dose.
12. Peptic ulcer disease (requires study physician approval)
13. Suicide risk as indicated by at least one of the following on the Columbia Suicide Severity Rating Scale (the PI &/or PM (LCSW) will be consulted to assess safety and determine eligibility in cases close to the eligibility cutoffs):
    a. Current suicidal ideation (within 30 days of enrollment)
    b. Two or more lifetime suicide attempts or episodes of suicidal behavior
    c. Any suicide attempt or suicidal behavior within 2 years of enrollment

<u>Medication</u>

1. Current use or discontinuation within the last 14 days of:
    a. Quit smoking medications including varenicline (Chantix), bupropion (Wellbutrin)
    b. Anti-psychotic medications (e.g., Zyprexa, Clozaril, Seroquel, Risperdal). If used to treat psychotic symptoms. Other uses may be eligible pending physician approval.
    c. Systemic Steroids (e.g., Prednisone).
    d. Alzheimer's disease medications (e.g., Acetylcholinesterase inhibitors (ACIs), Aricept/donepezil, Exelon/rivastigmine, Tacrine, or memantine)
    e. Irritable bowel syndrome medication (e.g., Dicylomine/Bentyl)
    f. Heart medications (e.g., quinidine).
    g. Muscle relaxants (e.g., Anectine/succinylcholine)
    h. Anti-seizure medications (e.g. Ativan, Banzel, Carbatrol, Dilantin, Lamictal, Gabitril, Lyrica, Neurontin, Tegretol, Topomax). If used to treat a seizure disorder or epilepsy. Other uses may be eligible.
    i. Urinary retention medications (e.g., Duvoid/bethanechol, Proscar/finasteride, Avodart/dutasteride, Dibenzyline/ phenoxybenzamine, Regitine/phentolamine)

2. Daily use of:
    a. Opiate-containing medications for chronic pain (Duragesic/fentanyl patches, Percocet, Oxycontin). Smokers who report taking opiate-containing medications on an “as-needed” basis will be instructed to refrain from use until their study participation is over and that they will be tested to ensure they have complied with this requirement.
    b. COPD medication (e.g., Atrovent/Ipratropium Bromide)
3. Known allergy to study medication.

Subjects will be instructed to refrain from using any study prohibited drugs/medications (both recreational and prescription) throughout their participation in the study.

5. Vulnerable Populations

Children, pregnant women, fetuses, neonates, or prisoners are not included in this research study.

6. Populations vulnerable to undue influence or coercion

Educationally or economically disadvantaged persons are included but not solely targeted for recruitment. Cognitively impaired persons are not included in the current study. Because of our recruitment efforts for this study, it is possible that University of Pennsylvania employees and students may be invited to participate. Status of participation in the study will be independent of the subject’s work or school activities.

7. Subject Recruitment

Participants will be recruited from the Infectious Disease practices at the Hospital of the UPENN, Presbyterian Hospital, and Pennsylvania Hospital. Dr. Frank will oversee the integration of this study into the clinics, ensuring access to participants, collection of medical data (through UPHS PennChart medical record reviews and laboratory result requests), and access to private consulting rooms for screening at the CFAR clinics. These practices see over 500 patients monthly and more than 200 new patients each year.

After obtaining the necessary training and clinic clearances to access PennChart for participating UPHS clinics, Research Assistants (RAs) will review the electronic medical records to identify potential subjects on a weekly basis (each site has patient smoking status indicated on the record). Individual medical records will be evaluated for eligibility based on the inclusion and exclusion criteria for this study. Daily clinic schedules will be ascertained and RAs will approach patients prior to or after consultation or treatment at the clinic. In addition to in-clinic recruitment, RAs will contact potentially eligible patients (after EMR review) by telephone based on their clinic provider's specified research contact preference. Providers may choose one of the following contact options: 1) all patients identified as initially eligible may be contacted 2) all patient records identified as initially eligible will be sent to the provider via PennChart for review and approval prior to contact 3) all patient records identified as initially eligible may be sent to the provider, who assumes full responsibility and discretion regarding the research contact (no contact may be made by the research staff). Those patients deemed eligible for contact will be contacted by telephone. RAs will introduce the research study and the collaboration between the researchers, infectious disease clinic, and the patient's provider. After assessing the patient's interest, HIV status, and smoking status, the patient will then be provided with additional study information and an opportunity to assess his/her intake eligibility based on a screening questionnaire. Research Recruitment Best Practice Advisories (BPAs) will also be integrated into electronic medical record recruitment. BPAs are designed to fire passive alerts within PennChart at the point of care, notifying providers that a patient may be eligible for a specific study. This specific BPA will evaluate if a patient meets specific criteria associated with the trial and will present the provider with the option to indicate if a patient is interested in participating in the study or not. This BPA will only present if the patient meets the initial screening criteria based on smoking status and problem list diagnoses and will only present in specified departments. This indication of interest only serves to trigger a notification to study staff that a patient has met initial screen criteria and further follow-up is requested to determine eligibility of the patient. This alert is a passive alert and will not interrupt the provider's workflow, but will be listed in the same section as other clinically warranted BPAs.

If our accrual rate is lower than anticipated based on our feasibility data, Dr. Metzger and the CFAR CAB will use their connections with community-based HIV/AIDS organizations in the Delaware Valley region to promote the trial and enhance accrual rates. The CFAR CAB, comprised of people with HIV/AIDS or professionals in the treatment of substance use among those with HIV, has numerous linkages to community-based organizations that can be used to enhance participant recruitment should that be necessary. We will also advertise the study using poster, newspaper, and internet based advertisements (this includes in-app advertising, Craigslist, and Twitter). Information about the study will be available on the CIRNA and iConnect websites. In addition, research staff will attend HIV/AIDS community events and community clinic intake hours to provide information about the study, distribute recruitment materials, and collect participant contact information via a secure, web-based data collection service. Additionally, we will place recruitment data requests with electronic research data warehouses such as Penn Data Store, PennOmics, and PennSeek to obtain lists of pre-consented potential participants. ResearchMatch, a national health volunteer registry that was created by several academic institutions and supported by the U.S. National Institutes of Health as part of the Clinical Translational Science Award (CTSA) program, will also be utilized. ResearchMatch has a large population of volunteers who have consented to be contacted by researchers about health studies for which they may be eligible.

RAs and UPHS medical personnel will screen patients from UPENN and community-based HIV/AIDS clinics to identify potentially eligible and interested participants by phone or in person. Participants recruited through other methods will also be screened by phone or in person. Participants who are eligible and interested in the study will complete the Intake session (Week -2) with research staff. The participant will review and sign an informed consent and HIPAA form, complete

eligibility and baseline assessments, and be scheduled for a Week 0 visit. The participant's eligibility will be confirmed by Drs. Frank and Ashare.

To ensure a high level of retention and adherence in this trial we will: 1) educate subjects about the benefits of protocol compliance; 2) schedule in-person sessions at convenient times (e.g., evenings); 3) provide modest financial compensation for session completion and transportation costs.

Referral Bonus Program
Participants who achieve their final study visit (Week 28) will be given the opportunity to receive a small bonus for referring others to the program. If the person who is referred completes the initial eligibility phone screen, regardless of outcome, the study participant will be awarded $20 per referral, for a maximum of 3 referrals ($60).

Social Media
Methods of social media: Twitter & Social Networking Applications (Grindr & Growlr). Content: 140 Characters or less advertisement - "HIV+? You may be eligible for a research study at UPenn. Time & travel compensated. Call 215-746-7155." Method for posting: Twitter (Center for AIDS Research Twitter accounts); Social Networking Applications (direct message through application sent directly by vendor).

STUDY DESIGN

1. Phase
n/a.

2. Design
Main Study. This is a randomized placebo-controlled cross-over study with one within-subjects factor of medication (GAL vs. PLA; order counterbalanced) and one between-subject factor of smoking status (HIV+ smoker vs. HIV+ non-smoker). Smokers (n=60) will be defined as those who smoke at least 5 cigarettes per day for the past year; non-smokers (n=60) will be those who report smoking fewer than 100 cigarettes lifetime and who self-report no current use of any tobacco or nicotine product, confirmed with carbon monoxide (CO) and saliva cotinine (self-reported smokers only). Subjects will complete this 28-week study, which consists of two 12-week treatment phases separated by a 4-week washout period. The dosing regimen follows FDA-approved guidelines for GAL extended release (ER), which recommends 8 mg per day for the first 4 weeks and increasing to 16 mg per day for the next 4 weeks before increasing to 24 mg (Aronson *et al*, 2009; Brodaty *et al*, 2005). During each phase, M/M activation markers (and T cell activation markers), soluble inflammatory biomarkers, neurocognition, viral load, clinical outcomes (e.g., medication adherence, health-related quality of life), and monocyte transcriptomics will be assessed at baseline (week 0 and 16) and at the end of each phase (weeks 12 and 28). Monocyte transcriptomics will be assessed on a subset of the sample (n=60; 30/group). In addition, neurocognition, and clinical outcomes will be assessed after 4 weeks at each intermediate dose (weeks 4, 8, 20, and 24). Subjects will also receive a phone call after 2 weeks at each dose to assess for potential side effects or contraindications. The primary outcome for Aim 1 is neurocognitive performance (a composite score comprised of processing speed, verbal learning/memory, and executive function) during each treatment phase. The primary outcomes for Aim 2 are M/M and T cell activation (CD16, CD163, and CCR2 expression; plasma CCL2 (MCP-1) and sCD14; CD38/HLA-DR on CD8 cells). Aim 3 will test the interaction between smoking status and treatment (GAL vs. PLA) on Aim 1 and 2 outcomes. Aim 4 will evaluate monocyte gene expression patterns.

Recruitment & Retention Pilot. This pilot study will utilize a randomized controlled trial design to evaluate two components of behavioral economic strategies to improve recruitment and retention. To be eligible for the pilot study participants must meet all eligibility criteria for one of the four participating studies (828958, 824061, 824860, **828125**), have a phone capable of receiving SMS messages, and consent to receive SMS messages. ~576 participants will be enrolled across the four participating research studies. All subjects will receive standard text messages and will be randomized to one of four groups (blocked within each study to ensure balanced groups): (1) Standard recruitment (SR): subjects will

receive text messages ~2 days prior to their Intake visit with relevant information about the time, date, and location of the visit as well as contact information for study staff ("You have a study visit on [Date] at [Time]. Visit comp is $10. Reply Y to confirm. See http://j.mp/2222222 for reminders. Reply or appt may be canceled."); (2) SR + Information Provision (IP): Subjects will receive personalized messages designed to target injunctive norms regarding participating in research (e.g., "[Name], wondering why you should volunteer for research? Many find it a rewarding way to advance science and be a part of a community http://j.mp/2222222."); (3) SR + Contingency Management (CM): CM will be provided in the form of a randomization table with a high proportion of numbers that correspond to "chips" with little ($1) monetary value. Participants draw by choosing numbers between 1-500 upon completion of an objectively verified target behavior (e.g., attending an Intake visit), and bonuses are often provided for continued performance. A similar strategy has been successful in augmenting visit compliance in several treatment studies. All text reminders will be delivered using the Way 2 Health (W2H) software platform. Upon completion of all requirements for a given visit, participants will receive 5 lottery draws for that visit. Attendance at all visits earns participants bonus draws upon completion of the study. Failure to attend an in-person visit without prior approval or failure to complete all visit requirements results in no draws for that visit. The lottery contains 500 "chips" (numbers that correspond to monetary values within a table): 250 have a value of $0, 219 have a value of $1, 30 have a value of $5, and 1 has a value of $100. The study completion bonus will be 5 extra draws. Thus, at each visit, subjects will have the opportunity to make 5 draws, for maximum possible earnings of $120; (4) SR + IP + CM (IC): In this group, subjects will receive the targeted text messages and receive CM. The design of our study allows us to examine each strategy independently as well as combined to evaluate the optimal approach.

3. Study Duration

Length of Subject's Participation in Study

Subjects will participate in study related activities for approximately 7 months from initial eligibility assessment in the clinic through follow-up. A subject's length of participation may be affected by center or subject scheduling conflicts. Additionally, subjects may be contacted after study participation to clarify self-report data collected during their active participation, if necessary for data analysis. The Recruitment & Retention Pilot Study will conclude when all participants have completed the study.

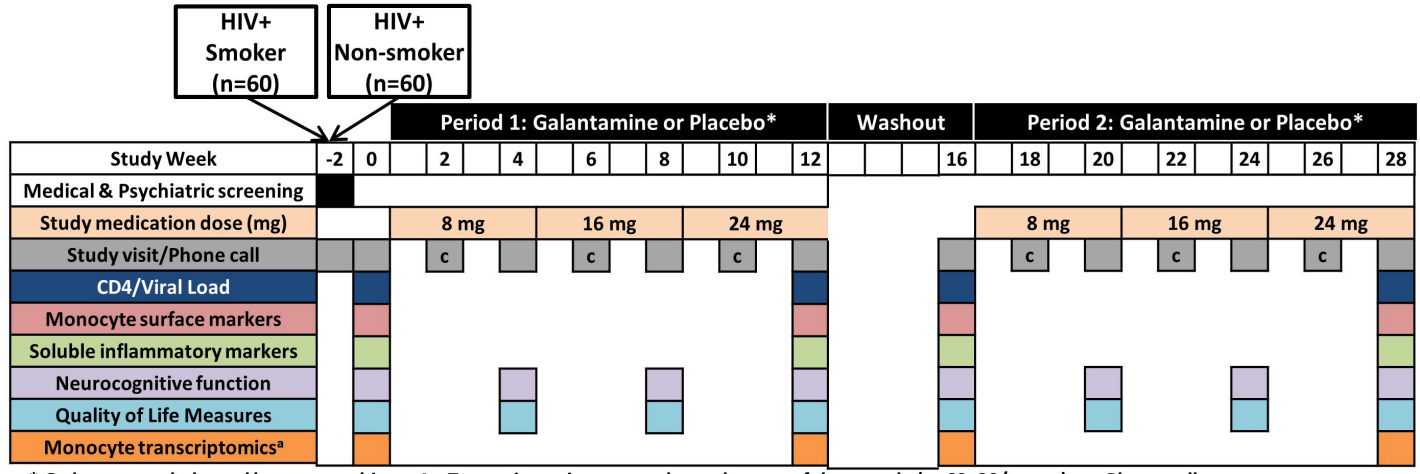
Projected date of completion of the proposed study

We expect to complete accrual in approximately 42 months, with all follow-up visits complete by January 2022. We expect to obtain our numbers in this timeframe by enrolling approximately 3-4 subjects per month.

DRUGS OR DEVICES

Galantamine

Figure 2. Study Timepoints and Outcomes



* Order counterbalanced between-subjects; [a] = Transcriptomics assessed on subgroup of the sample (n=60; 30/group); c = Phone call

The study will be performed using the 8mg, 16mg and 24mg doses of galantamine hydrobromide-ER, which is currently marketed for the treatment of Alzheimer's disease. The dosing regimen will be an initial 4 week of drug run-up at the lowest 8mg q.d. dose. Unless there are moderate to severe adverse events (see definitions below), the dose will be increased to 16mg q.d. for the following 4 weeks. If moderate to severe adverse events are reported, participants will be monitored and remain at the 8mg dose before titrating to 16mg. The dose will be increased for the last 4 weeks to 24mg followed by assessments. If moderate to severe adverse events are reported, participants will be monitored and remain at the 8mg or 16mg dose before titrating to the next highest dose. Side effects will be monitored and assessed at all study visits during the treatment phase as well as during monitoring phone calls scheduled throughout the medication run-up. Based on current study of galantamine in smokers (IRB#815789), the medication is well-tolerated and we expect few moderate and severe side effects (no severe side effects have been recorded to date in our current study).

The dosing regimen is documented to be safe and well tolerated in prior clinical studies (Aronson *et al*, 2009; Brodaty *et al*, 2005). Galantamine will be purchased and packaged into blister packs by the Investigational Drug Service at the University of Pennsylvania. Participants will be instructed to take one 8mg 16mg or 24mg pill (galantamine-ER or placebo) every morning, preferably with food, for a 12-week medication period prior to and then again following a 4 week washout. Participants will be blindly randomized to placebo at one of the two medication periods.

Impact of antiretrovirals on galantamine

Galantamine is metabolized by CYP3A4 and CYP2D6, so there is likely to be inhibition of the clearance of the drug by ritonavir and cobicistat, two cytochrome P450 isoenzyme inhibitors. There is no data describing the drug-drug interactions of galantamine and antiretrovirals. However, there are no recommendations to decrease the dose of galantamine when administered with other potent inhibitors of CYP3A4 or CYP2D6. (Razadyne ER prescribing information) Ketoconazole, a potent inhibitor of CYP3A4 and an inhibitor of CYP2D6, when administered at a dose of 200 mg twice daily for four days increased the AUC of galantamine by only 30%. Paroxetine, a strong inhibitor of CYP2D6, when administered at a dose of 20 mg daily for 16 days increased the bioavailability of galantamine by approximately 40%. In addition, a total of 356 patients with Alzheimer's disease enrolled in two Phase 3 studies were genotype with respect to CYP2D6 (n=210 hetero-extensive metabolizers, 126 homo-extensive metabolizers, and 20 poor metabolizers). Population pharmacokinetic analysis indicated that there was a 25% decrease in median clearance in poor metabolizers compared to extensive metabolizers. Dosage adjustment is not necessary in patients identified as poor metabolizers.

In this study we will monitor participants for adverse events. The most common adverse events associated with galantamine, including nausea, vomiting, dizziness, and anorexia, which occurred in 20.7%, 10.5%, 7.5%, and 7.4% of participants, respectively, in 8 placebo controlled, double-blind studies of galantamine. Subjects in our study who experience more than mild adverse events thought to be associated with galantamine will not be dose escalated, and subjects who experience more than mild adverse symptoms thought to be associated with galantamine will be dose reduced, if taking the highest dose of medication.

Impact of galantamine on pharmacokinetics of antiretrovirals

In vitro studies show that galantamine did not inhibit metabolic pathways catalyzed by CYP1A2, CYP2A6, CYP3A4, CYP4A, CYP2D6, or CYP2E1. This indicates that the inhibitory potential of galantamine towards the major forms of cytochrome P450 is very low. (Razadyne ER prescribing information). Therefore, the impact of galantamine on the activity of antiretroviral agents is likely minimal.

<u>Supply, Preparation, Storage, Packaging and Dispensing of Study Medication</u>

Galantamine-ER will be purchased and supplied via the University of Pennsylvania Investigational Drug Service (P1536). Matched placebo will be made in-house using lactulose filler in gel capsules. The IDS will store the medication as per the manufacturer guidelines. Specifically, medication will be stored at room temperature (20 – 25 degrees centigrade) and in airtight containers. Galantamine and the matching placebo will be packaged in blister packs. IDS will oversee the labeling of all study medication, and will assign each kit, which contains medication for one subject, a unique Pharmacy Randomization Number (PRN).

Once the Study Physician/Nurse Practitioner signs the prescription and a subject is assigned to treatment, medication kits will be ordered and picked up from IDS by a member of the research staff. IDS will assign a PRN to a subject, and provide the appropriate blister packs (8mg, 16mg, or 24mg). IDS' labeling will include the subject's study ID number and initials. In the event study staff are not able to pick up study medication, or a subject is unable to attend their visit in-person to collect their study medication, staff will work with IDS to determine the possibility of shipping the medication to the subject. Staff will confirm with the subject prior to shipment that they are able to receive deliveries and confirm their address.

Each medication kit will consist of four weeks of 8mg galantamine (or placebo), four weeks of 16mg (or placebo), and four weeks of 24mg (or placebo). If no moderate or severe adverse events are reported at week 4 or week 8, we will proceed with the next highest dose (e.g., subjects will receive the 16 mg dose at week 4 and the 24 mg dose at week 8). If moderate or severe adverse events are reported, the study physician will be consulted and a decision will be made as to whether to proceed with the default or provide an alternative blister pack with a reduced dose of study medication. The PRN and study ID number must match for each blister pack a subject receives. Medication will be stored at CIRNA as per manufacturer's guidelines.

Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed by the research staff member who completed the reconciliation.

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated by the research staff. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

SUICIDAL IDEATION AND BEHAVIOR

The C-SSRS will be administered remotely (via phone or videoconferencing) as part of the Intake visit by a trained member of the staff. Individuals who endorse current (past month) suicidal ideation, suicidal behavior in the past two years, or multiple lifetime attempts will be ineligible to participate. Because these assessments may cause an adverse emotional reaction, staff will be trained to deal with such reactions and to provide additional referrals if needed. If necessary, referrals to appropriate psychological services will be provided.

STUDY PROCEDURES

1. Procedures

Initial Eligibility Screening.

Recruited subjects will complete an initial eligibility assessment in the HIV/AIDS clinic or over the telephone. This assessment reduces the likelihood that participants attend an Intake Session only to learn that they are ineligible or to allow us to ascertain physician's clearance should the participants have a medical condition that requires approval (e.g., mild hypertension). If a potential participant cannot be reached by phone, staff may send a text for the purposes of scheduling an initial eligibility screening phone call. This will help to reach potential participants who express interest in the study but do not answer phone calls from unrecognized numbers or do not regularly listen to voicemail messages. Texts will be sent from a central study account and not from the personal cellphones of any research staff. Participants who are initially eligible will be screened against our registration database to confirm that they are not currently participating in another research study at our Center and have not previously reported a condition or circumstance that would make them ineligible for the current study. Those participants who remain initially eligible will move forward with eligibility-determining tasks as part of the Intake session, which will be completed remotely and in-person. The Intake session must occur within 60 days of the initial eligibility screening or the participant will have to be re-screened.

Visit Reminders.
Participants will receive study visit reminders 24 – 48 hours prior to their scheduled study visits by text via the W2H software platform. Participants who cannot receive text reminders or who do not agree to receive text reminders will still be able to participate and will receive reminders via phone call or email.

Way to Health (W2H) is a software platform developed by the Penn Center for Health Incentives and Behavioral Economics (CHIBE), operated through a partnership between CHIBE and the Penn Medicine Center for Health Care Innovation and housed on Penn Medicine Academic Computing Services (PMACS) servers. W2H is an integrated, cloud-based platform that blends behavioral science with scalable digital technology to improve clinical outcomes. W2H automates many research functions necessary for conducting randomized controlled trials of healthy behavior interventions.

Remote Study Participation.
If needed, study sessions may be carried out remotely, including some Intake tasks. This enables us to continue collection of data in a rigorous way while maintaining the safety of subjects. Additionally, if a participant is unable to attend an in-person visit, either due to personal reasons or office closure, procedures will be modified to allow for remote collection of data. The procedure for visit reminders will remain the same. Participants will be contacted via call or text to remind them of the time of their session, as well as to remind them that the session will be conducted by phone. Staff will call participants to complete all measures that can be completed by phone. If collected on-site using session paperwork, these data will be stored in the participants' charts and locked in secure filing cabinets, as is standard procedure. In the event that research staff are off-site, paperwork will be completed via RedCap. If RedCap is unavailable, they will utilize Pulse Secure to enable a secure, remote connection to their desktops and the secure server. Data collected remotely will be stored on our secure server, and later transcribed to paper measures and stored in participant charts or entered into RedCap. Items that are typically dispensed to participants at certain visits may be shipped to the participant, including study medication and a ClinCard, for payment purposes.

Intake Session (Week -2): Consent, Eligibility Determination, Baseline Measures.
Subjects who pass the pre-screening will be asked to complete tasks to determine their eligibility for the study. Tasks 2-6 listed below may be completed remotely by phone or videoconference using BlueJeans (HIPAA-compliant), to reduce the length of in-person tasks. If remote, measures will be administered using RedCap surveys. The tasks may take up to 2.5-hours if completed entirely in-person. Subjects will be asked to:

1. Provide lab work to confirm recent viral load of less than or equal to 200 copies/mL and CD4+ counts of greater than or equal to 200 cells/mm^3. If a participant is unable to provide lab work, their clinic may be contacted to obtain the necessary documentation.
2. Complete the consenting process remotely (per procedure outlined in the Informed Consent section) or in-person. Staff will review the consent form and answer all questions. Staff will then administer a comprehension questionnaire and review incorrect answers as needed. If completed remotely, subjects who are unable to provide an electronic signature may be mailed a physical version of the consent that must be signed and returned to us prior to continuing with the Intake tasks.
3. Complete mental status examinations (Mini International Neuropsychiatric Interview, QIDS, and CSSRS and MADRS, YMRS, Bipolar Disorder Additional Screener, if necessary) with a trained research staff member.
4. Complete a medical history and concomitant medication review.
5. Complete the Shipley Institute of Living Scale.
6. Complete baseline measures to assess 1) background variables that may serve as covariates and will allow for the assessment of the study's external validity (e.g., smoking history, demographics, infection history), and 2) baseline smoking behavior.
7. Provide urine specimens for drug and (if applicable) pregnancy tests. Participants who test positive for study prohibited drugs (see Exclusion Criteria above and Measures below) may be ineligible. Participants believed to have a false-positive result on the drug screen may continue in the study, with investigator approval.

8. Complete a carbon monoxide (CO) breath assessment to confirm smoking or non-smoking status. The handheld device uses a disposable mouthpiece, reports CO in parts per million (ppm), and takes about 3 minutes.
9. Complete physiological measures such as blood pressure, height and weight.
10. Complete a brief physical examination (led by a medical professional). If the provider is off-site, this will be conducted via telehealth.
11. Provide a blood sample drawn for the assessment of liver function (LFTs and GGT enzyme levels) and creatinine levels. Participants will receive final confirmation of eligibility via phone call based on the blood test results.

Final Eligibility Phone Call.
When liver function tests have been reviewed and authorized by the Study Physician, a study staff member will call the subjects to inform them of their final eligibility.

Final Eligibility is defined as: Liver function test results and GGT liver enzyme levels that are no more than 20% outside the normal, clinical range and serum creatinine levels less than 1.5 X upper limit of normal. Albumin/Globulin ratios 20% outside of normal range will be evaluated for clinical significance by the Study Physician and eligibility will determined on a case-by-case basis.

Eligible subjects will confirm the study schedule and be randomized to medication group. Ineligible subjects may be referred to other studies at our center.

Laboratory Visits (Weeks 0, 12, 16 and 28).

At the beginning and end of each treatment period, subjects will be scheduled for 2-hour laboratory visit. The HIV+S group will be asked to smoke as usual and not change their smoking behavior. At each visit, subjects will:

1. Provide urine for drug screen and pregnancy test (if applicable)
2. Smoking status will be confirmed via breath CO and saliva cotinine. Cotinine will be stored and analyzed as a covariate at the end of the trial.
3. Subjects will complete self-report measures (see Table 1).*
4. Subjects will undergo examinations with the MADRS, YMRS, and Bipolar Additional Screener, if necessary.*
5. Subjects will complete neurocognitive tasks (see Measures and Outcomes)
6. Provide a total of 80 mL of blood for the following assays:
    a. FACS analysis of monocytes, T cell surface markers
    b. Plasma for soluble biomarkers
    c. PBMCs for monocyte transcriptomics
    d. HIV-1 RNA viral load monitoring

*Some tasks may be completed remotely to reduce the length of in-person visits.

Monitoring Phone Calls (Weeks 2, 6, 10, 18, 22 and 26).
During each treatment period, subjects will receive three brief (15-min) phone calls (after 2 weeks on each galantamine dose) to assess side effects and other self-report measures (see Table 1). Subjects who report moderate or severe side effects may be maintained at a lower dose of galantamine or may be withdrawn from the study. The calls will also serve to maintain contact throughout the trial and enhance retention.

Mid-treatment Visits (Weeks 4, 8, 20 and 24).

Twice during each treatment period (after 4 weeks on 8 mg and again after 4 weeks on 16 mg of galantamine), subjects will attend a 1 hour visit. Side effects of galantamine will be assessed by study staff and reviewed by the study physician prior to initiating the dose increase. The HIV+S group will not be asked to change their smoking behavior and will be instructed to follow their urges to smoke. Subjects will:

1. Provide a breath CO sample.
2. Complete self-report questionnaires.*
3. Undergo examinations with the MADRS, YMRS, and Bipolar Additional Screener, if necessary.*
4. Complete the cognitive task battery.

*Some tasks may be completed remotely to reduce the length of in-person visits.

Washout Period (Between Weeks 12-16).

After completing the first treatment period, subjects will undergo a 4-week washout period before the second period commences. During the washout, subjects will not take any study medication or have any study visits. The HIV+S will be instructed to smoke ad libitum.

End of Recruitment and Retention Pilot Debriefing Call.

After the participant has completed their final visit (due to completion, withdrawal or ineligibility), study staff will reach out to all participants enrolled in the recruitment and retention pilot to provide additional details about their participation in the pilot.

Post-Study Follow-up.

If necessary, subjects may be contacted after study participation to clarify, via phone, self-report data collected during their active study participation. Participants will be asked to provide verbal consent before any data is collected. No additional PHI will be collected during this follow-up. Although procedures are in place to ensure data accuracy during active participation, there are instances when it may be necessary to conduct a post-study follow-up to maintain data accuracy when analyzing study outcomes. If study staff are unable to contact a subject for follow-up, no further action will be taken.

Side Effect Monitoring.

Monitoring for adverse events will be conducted in real time by Dr. Ashare and Dr. Frank, the study physician. At each contact, participants will complete a side effect checklist, administered by trained staff. The SEC will assess the frequency and severity of common side-effects associated with galantamine (e.g., headache, nausea). These items will be rated by participants on a 0 (none) to 3 (severe) scale, and can be summed to provide an overall side effects index. Second, trained staff will ask participants a non-structured, open-ended question (SEC Open-ended) at each session to assess if participants are experiencing any additional symptoms or medical concerns that may be related to their participation in the study. Research staff are trained to inquire (time of onset, nature of issue reported, possible relation to galantamine treatment, review of previously reported side effects or concerns, etc.) about any notable side effects or medical concern reported by participants. Any severe (or a pattern of moderate) side effects or notable medical concerns will be reported to the PM/CRC, Study Physician, and Principal Investigators to determine a course of action (e.g. continue to monitor, reduce medication, stop medication). This consult, including all relevant information, will be documented.

Based on published reports and data from our current study using the doses of galantamine in the proposed study we expect few side effects and we expect these side effects to be mild and transient in nature. However, in the unlikely event of an adverse event (AE) the study physician will determine the severity of the AE, the relationship of the event to the study drug and decide the course of action for the study subject. The study physician will determine the relationship of toxicity of the study medication as not related, possibly related, probably related, or definitely related using standard

criteria. All adverse events occurring during each study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

Screening/Covariate Measures.

Demographics and Smoking History.
Standard questionnaires will be administered remotely or in-person, to collect demographic information (e.g., age, gender, race). For HIV+S, smoking history (e.g., age at initiation, prior quit attempts, current rate) and the Fagerström Test for Nicotine Dependence (FTND) will also be collected. The FTND is a 6-item self-report measure of nicotine dependence with satisfactory internal consistency and high test-retest reliability.

Physical Examination/Medical History.
A medical history and a physical examination will be conducted as part of the Intake visit to review for any contraindications listed previously. The medical history (including height and weight) will be completed by a research staff member and participants will undergo a brief physical examination led by a medical professional, remotely or in-person. If the NP/provider is off-site, staff will coordinate a telehealth visit for the participant while they are at the center, via phone, FaceTime or BlueJeans (both HIPAA-compliant). Current medical conditions and medications will be documented. Duration since HIV diagnosis, mode of transmission, viral load, and CD4 counts will be assessed.

Urine Drug Screen.
The urine drug screen will be administered at Intake and at all Laboratory Visits. The urine drug screen requires about 30ml of urine and indicates whether the subject has recently taken any of the following: cocaine, PCP, amphetamines, methamphetamines, tricyclic antidepressants, ecstasy, opiates, methadone, benzodiazepines, THC, and barbiturates). Participants with a positive urine drug screen for cocaine, PCP, methamphetamines, ecstasy, and barbiturates will be deemed ineligible. Participants prescribed other medications and screening positive will be considered on a case-by-case basis by the study physician and PIs. Participants believed to have a false-positive result on the drug screen may continue in the study, with investigator approval. In an effort to remain CLIA-compliant, results from urine drug screening will not be shared with participants and will not be placed in their electronic medical record. Participants will be informed that the testing is for research purposes only and that they will be informed of their eligibility status, but not of the specific testing results.

Urine Pregnancy Test.
At the Intake session and at all Laboratory Visits, participants will be supplied with a simple, CLIA-waived urine pregnancy screen and informed that pregnant women are not advised to participate in this research study. Participants will then be instructed to administer the pregnancy test independently and will inform study staff if they would like to continue participation after they have administered the pregnancy screen. Participants will be informed that there is no penalty for discontinuing participation at this point in the visit and that they will still receive travel reimbursement for the visit.

Blood Pressure.
At the Intake, participants presenting with elevated blood pressure (i.e., systolic blood pressure greater than 160 and/or diastolic blood pressure greater than 100) will have a second blood pressure reading taken after a ten minute period in which the participants will be instructed to sit comfortably. If, after the second reading, systolic blood pressure remains greater than 160 and/or diastolic remains greater than 100, the participant will be ineligible for the study.

Blood pressure will be measured at all subsequent in-person visits. If participants present with elevated blood pressure (i.e., systolic blood pressure greater than 160 and/or diastolic blood pressure greater than 100) at any subsequent visit, the staff will follow the same steps listed above. If, after the second reading, systolic blood pressure remains greater than

160 and/or diastolic remains greater than 100, the subject will be told to not take the next dose of study medication. The research staff will notify the Study Physician/Health Care Provider who will review the blood pressure reading and determine whether it is safe for the subject to continue. Research staff will follow up with the participant accordingly.

Shipley Institute of Living Scale (SILS).
At the Intake, subjects will complete the SILS remotely or in-person, which is a self-administered test to assess general intellectual functioning in adults (Zachary, 2000). The SILS correlates with the Wechsler Adult Intelligence Scale (WAIS-R) Estimated IQ Test and has good internal consistency (Cronbach's α=0.92). The SILS will be used to control for baseline cognitive function.

Liver Function, Gamma-glutamyl Transpepsidase, and Creatinine tests.
One 8.5ml blood sample will be drawn at the Intake visit. Blood will be collected and placed in a serum-separator tube (SST) and mixed thoroughly. Samples will be centrifuged at 3100 rpm for 10 minutes. An additional 5 minutes will be added, if necessary for complete separation. The following chemistry will be assessed:

1. Total protein.
2. Albumin.
3. Globulin.
4. Albumin:Globulin ratio.
5. Bilirubin (total, conjugated and unconjugated)
6. Aspartate amino transferase (AST).
7. Alanine amino transferase (ALT).
8. Alkaline phosphatase (AP).
9. Gamma glutamyl transferase (Gamma-GT)
10. Creatinine.

Psychiatric History.
The prevalence of depression, substance abuse, and suicidality will be determined by the MINI International Neuropsychiatric Interview (MINI) (Sheehan *et al*, 1998) and the Columbia Suicide Severity Rating Scale (C-SSRS) (Posner *et al*, 2011). The MINI and C-SSRS will be administered remotely or in-person at Intake. If a diagnosis of bipolar disorder is self-reported or revealed via the MINI at intake, the Montgomery-Asberg Depression Rating Scale (MADRS), Young Mania Rating Scale (YMRS), and a Bipolar Disorder Additional Screener will be completed at all study sessions to evaluate and monitor the presence and severity of bipolar disorder symptoms. An abbreviated version of the MINI may also be used to assess and monitor symptoms.

Adverse Childhood Experiences Questionnaire.
The occurrence of adverse experiences during childhood will be assessed using the Adverse Childhood Experiences Questionnaire (Felitti *et al*, 1998). Adverse childhood events are associated with health and social problems as an adult and may influence measures collected in this study.

Hep C Infection History.
At the Intake, participants will be asked to complete a measure to indicate any past diagnoses of Hepatitis C, as well as treatment history, if applicable. Some medical conditions, like Hepatitis C, may affect nicotine metabolism, and so, influence smoking behavior. This may be completed remotely or in-person.

HIV Related Health Outcomes.

HAART Adherence
HAART adherence will be assessed with a self-report measure, the Adult AIDS Clinical Trials Group Adherence to Antiretrovirals Instrument (Chesney et al., 2000) (collected at all in-person study visits).

Viral Load and CD4+ Assessment
A viral load assessment and CD4+ count will be collected for research purposes at Intake through medical record review and recorded. Participants may also be asked to sign a HIPAA authorization form to allow research staff to request lab results from their healthcare provider, if they are unable to obtain results. At each lab visit (weeks 0, 12, 16, and 28) one 10 mL blood sample will be drawn to assess HIV-1 RNA via quantitative real-time PCR. For the HIV-1 RNA sample, whole blood will be collected in an EDTA tube and 3 mL plasma will be separated and sent for analysis.

Quick Inventory of Depressive Symptomatology- Self Report (QIDS).
The QIDS is a 16-item self-report measure designed to assess the severity of depressive symptoms using the criterion symptoms designated by the DSM-IV.

Functional Impairment.
The Patient's Assessment of Own Functioning Inventory (PAOFI) will be used to assess cognitive difficulties in everyday life. The PAOFI assesses areas such as mental acuity, employment, social functioning, shopping, cooking, housekeeping, laundry, driving, use of public transportation, maintaining schedules, medication management, financial management, understanding media events, and child care (Chelune *et al*, 1986). The degree of functional impairment is used to distinguish between categories of HANDs (Antinori *et al*, 2007).

Quality of Life: HAT-QoL
The HIV/AIDS-Targeted Quality of Life scale (HAT-QoL; Holmes & Shea, 1998; 1999) measures overall functioning, which will be the primary measure of QOL for this study, but several subscales of QOL are also included such as life satisfaction, health worries, HIV mastery, financial worries, and disclosure worries. This measure will be administered at each visit after the Intake Session.

Subjective Cognitive Complaints:
The PROMIS (Patient-Reported Outcomes Measurement Information System) is a set of person-centered measures that evaluates and monitors physical, mental, and social health in adults and children. The PROMIS Short Form v2.0 Cognitive Function Abilities Subset 8a will be used to assess subjective cognitive complaints. Each of the 8 items are scored from 1 (Not at all) to 5 (Very much). This measure has been validated in medical outpatient samples (Saffer et al., 2015) and among those living with HIV (Solorio et al., 2016).

Fatigue:
The FSS (Fatigue Severity Scale) is a set of statements that explore severity of fatigue symptoms.

Treatment Measures.

Concomitant Medication Review.
Subjects will be asked about their use of medications (over the counter and prescription) and substances that may alter subjects' response to the study medication. The Study Physician/Health Care Provider will advise as to whether other medications being taken are contraindicated and prescribe appropriate action from there (i.e., discontinuation of the study medication). The concomitant medication review will be completed at every study visit following the Intake.

Adherence to Study Medication.
Medication adherence will be assessed, remotely or in-person, by pill count at each session that follows the first Lab Visit of each treatment period (Ray *et al*, 2009).

Side Effects.
A checklist of side effects based on the product insert will be administered remotely or in-person to participants at all sessions after the Intake, including at monitoring calls. The frequency and severity of common side effects of galantamine will be rated on a 0 (none) to 3 (severe) scale. An open-ended side effects question will also be included. Furthermore, participants will receive written instructions to call the Health Care Provider/Study Physician should they experience any severe side

effects or adverse events between study visits. There are no documented HAART/GAL interactions (see Drugs or Devices above).

Smoking Related Measures

Smoking Rate:
We will assess cigarettes smoked during the first laboratory session (dating back to the Intake visit), and throughout study participation. A standard timeline follow-back (TLFB) method will be used (Brown *et al*, 1998), as we have done previously (Lerman *et al*, 2004a) to assess self-reported smoking rate. This will only be administered to self-reported smokers and will be done remotely or in-person.

Biochemical Verification:
CO breath samples and saliva cotinine will be collected to biochemically verify self-report as in prior work (Lerman *et al*, 2004b; Lerman *et al*, 2015; Schnoll *et al*, 2015; Schnoll *et al*, 2010). As per convention, cotinine levels greater than 50ng/ml indicate smoking within the past 7 days.

Nicotine Withdrawal:
The Revised Minnesota Nicotine Withdrawal Scale (MNWS-R) (Hughes and Hatsukami, 2007) is a 15-item self-report measure. Subjects rate withdrawal symptoms on a scale of 0 (none) to 4 (severe). This will only be administered to self-reported smokers and will be done remotely or in-person.

Smoking Urges: The 10-item Questionnaire on Smoking Urges–Brief (QSU-B) (Cox *et al*, 2001) will be used to assess cravings to smoke. It contains 2 subscales (anticipation of reward, relief from negative affect). This will only be administered to self-reported smokers and will be done remotely or in-person.

Neurocognitive Outcomes
Neuropsychological tests will be administered in a quiet laboratory testing room on a Dell® desktop computer running on the most recent and compatible version of Windows at the CIRNA. Unless otherwise noted, all tasks will be administered via E-Prime 2.0 (Psychology Software Tools, Inc.). The computerized battery is administered in a random order using clickable icons. Total administration time is about 30 minutes. The outcomes and associated tasks are:

Working Memory
In the traditional N-back task, sequences of letters or numbers are displayed, and subjects respond with a button press to a single target using the following rules. During the 1-back condition, subjects respond if the image is identical to the one preceding it. In the 2-back condition, they respond if the stimulus is identical to the one two trials before. In the 3-back condition they respond if the image is identical to the one three trials before. The active baseline condition (0-back) is a simple target detection task. The version of the N-back used in this study utilizes fractal images in place of letters or numbers. The primary outcomes will be total correct and correct reaction time (task duration: ~18 minutes).

Executive Function
Color Shape Task: The Color Shape Task is a measure of flexibility. In each trial of this task (Miyake, Emerson, Padilla, & Ahn, 2004), a cue letter (C or S) appears above a colored rectangle with a shape in it (outline of a circle or triangle). Participants are instructed to indicate whether the color is red or green when the cue is C, and whether the shape was a circle or triangle when the cue is S. The colored rectangles are approximately 1.7” wide and 1.4” high, the circles were approximately 1.1” in diameter, and the triangles were 1.25” on each side. The color-shape figure appears in the center of the screen and the cue letters are centered 3/8” above its top edge.

Verbal Learning and Memory
The Hopkins Verbal Learning Test – Revised (HVLT-R) assesses verbal learning and memory (immediate and delayed recall, delayed recognition) (Brandt, 1991). The task has been validated in individuals 16 to 92 years old and within cognitively

impaired populations (Benedict *et al*, 1998). The 6 alternate forms (to reduce practice effects) consist of a list of 12 nouns (targets) with 4 words drawn from each of 3 semantic categories. The HVLT-R has high test-retest reliability, and its construct, concurrent, and discriminant validity have been well-established (Shapiro *et al*, 1999). Raw scores are derived for Total Recall, Delayed Recall, Retention (% retained), and a Recognition Discrimination Index. Verbal memory has been shown to be a cognitive domain negatively affected by HIV infection (Antinori *et al*, 2007; Robertson *et al*, 2014; Woods *et al*, 2004). Task duration is 10 min with a 25-min delay. The primary outcomes are Total and Delayed Recall.

Processing Speed and Response Inhibition

The Stop Signal Task (SST) is a measure of response inhibition used in previous work with smokers (Ashare and Hawk, 2012; Tsaur *et al*, 2015). Subjects are instructed to respond to left and right-facing arrows (go-trial) on the computer screen. Following practice trials, subjects complete three 64-trial blocks with stop signals (800-Hz, 100-ms, 70-dB tone) presented on 25% of trials. The initial stop delay in each block is 250ms and adjusts ±50ms depending on whether the subject successfully inhibits (Logan *et al*, 1997). Trials consist of a 500-ms warning stimulus, a 1,000-ms go-trial, and 1,000-ms blank screen inter-trial interval. Task duration is 10 min. The primary outcome is stop signal reaction time (SSRT), calculated as the mean RT on go-trials minus mean stop delay.

Inflammation Outcomes

Cellular Markers of Immune Activation:

Activation markers will be analyzed at the beginning and end of each treatment period by staining in whole blood. PBMC will be stained with flow panels for CCR2 (the receptor for CCL2/MCP-1, a key factor in monocyte migration in HAND (Ragin *et al*, 2006; Williams *et al*, 2014a; Williams *et al*, 2014b)), CD14, CD16, CD163, CX3CR1, CCR5 and CXCR4. We will also assess monocyte surface tissue factor (TF) given evidence that monocyte TF is activated by microbial products, one of the drivers of residual inflammation in HIV/ART, and upregulated in HIV infection (Brenchley *et al*, 2006; Deeks, 2009; Mudd and Brenchley, 2016; Wallet *et al*, 2010). Although monocyte activation is our primary focus, we will also stain cells in parallel for established T cell activation markers (CD3, CD4, CD8, CD38 and HLA-DR) (Ganesan *et al*, 2011; Liu *et al*, 1998). Cells are analyzed on a BD LSRII using FlowJo software. We have extensive experience with polychromatic FACS analysis and this panel is currently in place. Primary outcome measures are monocyte CD16, CD163 and CCR2; TF, CX3CR1 and other markers are secondary. Primary outcome measure for T cells will be CD38/HLA-DR on CD8 cells.

Soluble Markers of Immune Activation:

The following soluble markers will be measured in plasma or serum at the beginning and end of each treatment period: CCL2/MCP-1, sCD14, sCD163, TNF-α, sTF (by ELISA; R&D) (Cassol *et al*, 2010; Neuhaus *et al*, 2010). Primary outcomes are CCL2 (MCP-1) and sCD14. sCD163, sTF, and TNF-α are secondary. We will also carry out broader cytokine & chemokine analyses using Luminex Multiplex bead-based 25-plex human cytokine kit that measures 25 human cytokines/chemokines (Barqasho *et al*, 2009; Seoane *et al*, 2008). Luminex analysis will be carried out by the CFAR Immunology Core using the BioPlex 200 platform (Bio-Rad). This Luminex analysis is exploratory, but we expect it to provide both a broad pattern analysis of GAL effects, and valuable hypothesis-generating information. The Immunology Core just purchased a Simoa Digital ELISA machine (Quanterix), which offers Luminex-type multiplex bead-based analysis with several orders of magnitude greater sensitivity without loss of upper range detection (Rissin *et al*, 2010; Wilson *et al*, 2016); over the next few months, this machine will be operationalized and we will determine whether to migrate to this platform for this study. *We predict that GAL will reduce monocyte and T cell surface activation and soluble markers, including those linked to HAND (Aim 2), and that the magnitude of the effect may be enhanced in smokers (Aim 3).*

Monocyte Transcriptomics:

Gene expression analysis will be performed on a randomly-selected subset of the sample (n=60; 30/group). As in our prior work (Gekonge *et al*, 2012; Giri *et al*, 2009) and ongoing project analyzing atorvastatin modulation of immune activation (R01-MH061139), monocytes will be isolated by negative selection (Miltenyi), stored in RNAlater, and batched for RNA extraction and QC by Bioanalyzer. Gene expression analysis will be carried out at the UPenn Molecular Profiling Facility with the Clariom S Human platform (Affymetrix), which provides gene-level expression profiling for all known

well-annotated human genes, using the GeneTitan plate array system. Comparison of expression levels at baseline between two groups will be done using a two sample SAM test. We will use an FDR less than 0.2 significance threshold for general gene set enrichment analysis and FDR less than 0.1 significance with greater than 1.5 fold threshold to generate final gene sets. Differentially expressed gene lists will be analyzed for enrichment of genes sharing biological functions and pathways using Ingenuity Pathway Analysis (IPA) and DAVID. We will also carry Gene Set Enrichment Analysis (GSEA) on genes pre-ranked by SAM d-score and 1000 permutations to find significantly associated pathways (MSigDB sets C2cp and C5bp), using FDR less than 25% as a significance threshold. Where appropriate, enrichments of Gene Ontology (GO) terms, KEGG & BIOCARTA pathways in a gene list will be performed using DAVID software. We will analyze differences in monocyte gene expression between HIV+S vs NS analyzed at baseline (beginning of each treatment arm); and monocyte genes that are modulated by GAL in both HIV+S and NS, comparing GAL vs PLA. Gene expression patterns will also be correlated with soluble and cell surface markers of inflammation.

Additional analysis described in Sample Analysis Addendum, dated 06/22/2018.

Recruitment & Retention Pilot Outcomes

Meeting Eligibility Criteria:

The primary outcome is the percentage of subjects who meet final eligibility criteria (i.e., enrollment). We chose this as the outcome based on our data suggesting that subjects who meet these criteria are highly likely to reach ITT status.

Impressions of Research:

Attitudes towards research; motivations for participation; perceived risks/benefits of research; personal financial wellbeing and perceptions of influence or coercion will be assessed via text survey at baseline (following phone screen), mid-way through participation and at study completion. Study staff will attempt to contact subjects who withdraw or are lost-to-follow up to identify reasons for withdrawal. We will assess overall satisfaction with the study, acceptability of the frequency and content of messages (IP and IC groups only). For all models, a term will be included for individual study as well as other relevant covariates (e.g., sex, age, income).

Table 1. Study Time Points and Measures

Study Week: Period 1		**-2**	**0**	**2**	**4**	**6**	**8**	**10**	**12**	
Study Week: Period 2			**16**	**18**	**20**	**22**	**24**	**26**	**28**	
Activity	**Phone Screen**	**Intake**	**L1**	**C1**	**M1**	**C2**	**M2**	**C3**	**L2**	**Post-Study**
SCREENING/COVARIATES										
Demo/Smoking Hx/FTND[f]		X								
Physical Exam[g]/Medical History[f]		X								
Urine Drug/Pregnancy Screen		X	X						X	
Blood Pressure		X	X		X		X		X	
Shipley IQ (SILS) [f]		X								
LFTs & Creatinine		X								
Psychiatric Hx (MINI/C-SSSRS) [f]		X								
Bipolar Disorder Symptoms (MADRS, YMRS, additional screener)[af]		X	X		X		X		X	
Adverse Childhood Experiences[f]		X								
Hep C Infection History[f]		X								
Debriefing Phone Call										X
HIV-RELATED HEALTH OUTCOMES										
Adherence to Anti-retrovirals[f]			X		X		X		X	
Viral load/CD4+		X[b]	X						X	
Depression/Anxiety (QIDS) [f]			X		X		X		X	

Functional Impairment (PAOFI) [f]			X		X		X		X	
Quality of Life (HAT-QoL) [f]			X		X		X		X	
Subjective Cognitive Complaints[f]			X		X		X		X	
Fatigue (FSS) [f]			X		X		X		X	
TREATMENT MEASURES										
Concomitant Medication Review[f]		X	X	X	X	X	X	X	X	
GAL Adherence[f]				X	X	X	X	X	X	
GAL Side Effects (SEC) [f]			X	X	X	X	X	X	X	
SMOKING-RELATED OUTCOMES										
Smoking Rate (TLFB)[cf]			X	X	X	X	X	X	X	
Carbon Monoxide (CO)		X	X		X		X		X	
Cotinine[c]			X						X	
Withdrawal (MNWS)[cf]			X		X		X		X	
Craving (QSU-B)[cf]			X		X		X		X	
NEUROCOGNITIVE OUTCOMES										
Working Memory (N-back)			X		X		X		X	
Executive Function (Color Shape Task)			X		X		X		X	
Verbal Learning/Memory (HVLT)			X		X		X		X	
Response Inhibition (Stop task)			X		X		X		X	
INFLAMMATION OUTCOMES										
Surface and Soluble Markers			X						X	
Monocyte Transcriptomics			X[d]						X[d]	
RECRTUITMENT PILOT OUTCOMES										
% Eligible at Intake		X								
Attitudes Toward Research[e]	X									X
Compared Riskiness Scale[f]	X	X								
Perceived Coercion Scale[f]		X								
Previous Experience with Research Studies	X									
Prior Persuasion Questions[f]		X								
Personal Financial Well-Being	X									

Note. L = Laboratory session; C = Phone call; M = Monitoring visit; MINI = Mini International Neuropsychiatric Interview; C-SSRS = Columbia Suicide Severity Rating Scale; DCCS = Dimensional Change Card Sort Test; HVLT = Hopkins Verbal Learning Test

[a] Only administered if a diagnosis of bipolar disorder is self-reported or revealed via MINI at intake

[b] Ascertained via EMR only

[c] Only administered to self-reported smokers

[d] Subset of sample (n=30 per group)

[e] Interim survey will also be administered in Week 1 of the Washout Period (25 days before Period 2 Lab Visit 1)

[f] Measures may be completed remotely via phone or videoconference to limit the length of in-person visits (if applicable)

[g] Physical examination may be conducted via telehealth

2. Statistical Analysis

Power Analysis and Sample Size.

The goal of the study is to evaluate the effects of nAChR modulation by GAL on neurocognition (Aim 1) and inflammation (Aim 2) and test whether smoking status modulates these effects (Aim 3). We will maintain power greater than or equal to 80% using a global 5% type-1 error, corrected for multiple testing within each aim. Power and sample size were calculated using Stata software (StataCorp, College Station, TX) for a repeated measures ANOVA with a 2x2 cross-over design. The effect size for medication effects (GAL vs. PLA) came from two sources: (1) GAL improves working memory accuracy and processing speed compared to PLA in smokers; and (2) GAL attenuates inflammation (Reale *et al*, 2005). For neurocognition (**Aim 1**), processing speed mean differences were 83 ms (SD=190) and -30.1 ms (SD=180) for the GAL and PLA groups, respectively. For accuracy, mean differences were 3 (SD=6.2) and 1.2 correct responses (SD=8.9) for the GAL and PLA groups, respectively. Thus, the estimated effect size required for neurocognition is d=0.5. For **Aim 2**, the effect

size of GAL on inflammation was derived from a study comparing patients with Alzheimer's disease (n=21) to age-matched controls (n=10) (Reale *et al*, 2005). Following 1 month of AChEI treatment, reductions were observed for IL-6 (mean = 1989.1 pg/ml/10^6, SD=440) and IL-1β (mean = 1093.7 pg/ml/10^6, SD=350.9) yielding effect sizes of d=4.5 and 3.1, respectively. For our linear regression evaluating change from baseline to week 12 for GAL vs. PLA, in order to have 90% power to detect a medium effect size of d=0.5, using a type-1 error of 0.01 to correct for multiple comparisons, we would require 120 subjects. For **Aim 3**, the effect size for smoking status (HIV+S vs. HIV+NS) modulating the effect of GAL came from literature comparing smokers to non-smokers in samples with and without HIV. Among 962 adults (548 non-smokers, 414 smokers), current smoking was associated with differences in inflammation markers including CCL17, CCL11, IL-1β, IL-1Ra, CRP, IL-16, sIL-6R, and SCF, with effect sizes ranging from d=0.45 to 0.78 (Shiels *et al*, 2014). In a study of HIV+S (n=170) and HIV+NS (n=172), effect sizes for differences in sCD14 and sCD163 were d=0.83 and 0.36, respectively (Kooij *et al*, 2016). Collectively, these studies yield an average effect size of d=0.6. In two studies comparing neurocognition in HIV+S (n=74 (Bryant *et al*, 2013) and 27 (Durazzo *et al*, 2007)) to HIV+NS (n=26 (Bryant *et al*, 2013) and 27 (Durazzo *et al*, 2007)), effect sizes were d=0.45 for global cognition (Bryant *et al*, 2013), d=0.8 for learning/memory (Durazzo *et al*, 2007), and d=1.1 for working memory and executive function (Durazzo *et al*, 2007). In order to have 80% power to detect a medium effect size of d=0.45, using a type-1 error of 0.01, we would require 120 subjects. We plan to recruit 150 subjects to account for a conservative attrition rate of 20%. For **Aim 4,** 30 subjects/group provides 80% power to identify differential gene expression between HIV+S and NS if fold change is 2 and FDR less than 0.2. This assumes 20,000 genes are tested and the top 200 are prognostic (Guo *et al*, 2014). **Thus, all aims are sufficiently powered.**

Recruitment & Retention Pilot:
Power is provided for our primary aim. The analysis will compare the enrollment rates between the four intervention arms, and examine main and interaction effects. For SR arm, we expect a 28% of subjects who schedule an Intake will be eligible and enroll (based on existing data across the four studies). With the proposed sample of 576, we have >80% power (α=.05) to detect a difference between the SR arm and the IP and CM arms of 12%, corresponding to an OR of 1.75. For the interaction term, we have 80% power (α=.05) to detect a departure from additivity of the main effects corresponding to a ratio of odds ratios (ORR) of 5.5.

Data Analysis.
Data Screening and Missing Data: Analyses will be conducted using Stata, SAS, or R-Language software. Preliminary analyses will determine whether data meet distributional assumptions, and use appropriate transformations where they do not. We will also determine whether subject characteristics (e.g., gender, education, CD4 nadir) are associated with smoking status or outcomes using either chi-square, *t*-tests, or correlations. Variables with potential to predict outcome (*p*=0.2) will be tested for entry as controlling variables. Items missing at random on survey measures will be imputed prior to calculating final scores using conditional means, estimated with an iterated version of Buck's method (Gleason and Staelin, 1975). Dropout and missed sessions present a more serious issue. However, our medication studies maintain greater than 80% compliance and retention rates, thanks to our monitoring of participants and use of incentives to offset costs for travel. Nevertheless, we will examine the characteristics of subjects who drop out, and test whether there is an association between dropout and drug sequence. These analyses will be descriptive, as we expect few dropouts. Thus, primary analyses will be as-randomized and include all subjects who complete at least one treatment phase.

Main Effect of GAL on Neurocognition (Aim 1) and Inflammation (Aim 2). We will conduct multiple linear regression using a Generalized Estimating Equations (GEE) framework to test our primary hypothesis that GAL will attenuate inflammation and improve neurocognitive performance. These models are similar to repeated measures ANOVA, but can handle the correlation due to multiple observations from the same subject as well as missing observations (Hardin and Hilbe, 2003; Liang and Zeger, 1986). The model is a 2 (drug: GAL vs. PLA) x 2 (time: baseline vs. week 12) crossover design and will include all subjects (HIV+S and NS). GAL effect hypotheses will be addressed by the within-subject contrast between the outcomes under GAL vs. PLA. A two-level variable for treatment order will be included and a treatment by order interaction would indicate carry-over effects. The primary outcomes will be change in neurocognition (composite score) and inflammation (CD16, CD163, and CCR2 expression; plasma CCL2/MCP-1 and sCD14; CD38/HLA-DR on CD8 cells) following 12 weeks of treatment. Secondary outcomes include monocyte CX3CR1 & TF, plasma TNF, sCD163 & sTF.

Although the groups will be matched on key variables, we may include potential covariates (e.g., history of substance abuse, duration since HIV diagnosis, Shipley IQ), admitted at p=0.1.

Smoking Status by Treatment Interaction (Aim 3). Analyses for **Aim 3** are similar to **Aims 1 and 2**, except that the smoking status (HIV+S vs HIV+NS) by treatment (GAL vs PLA) interaction term will be tested using the difference score (change from baseline to end of treatment) to represent responses to treatment. Aim 2 will use the same methods in Aim 1. Importantly, Aim 2 is still clinically relevant, even if GAL does not have significant main effects (Aims 1 and 2). Thus, Aim 2 is not dependent on Aim 1.

Modulation of Monocyte Gene Expression (Aim 4). See above for description of analytical and statistical tools for determining important genes in the monocyte transcriptomics. The two-sample SAM test will be used to determine candidate genes that differ in monocyte gene expression between HIV+S vs NS at baseline (beginning of each treatment arm). Testing for changes in these monocyte genes over time and potential modulation by GAL will be performed using GEEs, similar to **Aims 1 and 2**.

3. Confidentiality

All subject information will be kept in a secure filing cabinet that is accessible only to authorized study personnel. All databases containing subject information will be password protected, and again, accessible only to authorized study personnel. Each subject will have a unique study ID number for all data collected. In all data sets we will use ID numbers, only. A separate data set linking names with ID numbers will be accessible only by the senior project staff. All communications about subjects will use the ID number only and never include names or other personal information. All data will be stored until all analyses are completed. No data will be shared with any unauthorized party (i.e., aside from study personnel and regulatory officials). Any publication of data will not identify subjects by name or with an identifier that could be used to reveal identity.

Data collected in MS Access and REDCap databases will be stored on a secure server administered by the Penn Medicine Academic Computing Services (PMACS) organization and will be restricted to only those individuals who are authorized to work on the trial. Individual user accounts with passwords will be used to restrict access to the database. Specific privilege assignments will also be employed so a user has access only to the functions necessary to complete applicable operations appropriate for their role in the trial. Additional measures to prevent unauthorized external access to the database environment will be employed using network firewall technologies. The Data Manager will maintain the database in an appropriate manner for the retention period required by regulation. Database administration includes user account maintenance, database security, performance monitoring, and database change management. Daily backups are performed to protect data against accidental destruction or corruption.

Remote study sessions will be conducted via phone or via BlueJeans, which is a HIPAA-compliant platform with security features including a room lock to ensure that communications within the platform remain private.

Data will be accessible the study Principal Investigator, Co-Investigators, the Study Physician, other study staff and the UPenn IRB and Office of Human Research.

How will confidentiality of data be maintained? Check all that apply.

☒ Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study.

☒ Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords.

☒ Prior to access to any study-related information, personnel will be required to sign statements agreeing to protect the security and confidentiality of identifiable information.

☒ Whenever feasible, identifiers will be removed from study-related information.

☐ A Certificate of Confidentiality will be obtained, because the research could place the subject at risk of criminal or civil liability or cause damage to the subject's financial standing, employability, or liability.

☐ A waiver of documentation of consent is being requested, because the only link between the subject and the study would be the consent document and the primary risk is a breach of confidentiality. (This is not an option for FDA-regulated research.)

☐ Precautions are in place to ensure the data is secure by using passwords and encryption, because the research involves web-based surveys.
☒ Audio and/or video recordings will be transcribed and then destroyed to eliminate audible identification of subjects.
☐ Other (specify):

The PMACS will be the hub for the hardware and database infrastructure that will support the project and is where the W2H web portal is based. W2H uses a role-based access control (RBAC) approach to assure that participant confidentially and study integrity is preserved. The PMACS provides a secure computing environment for a large volume of highly sensitive data.

4. Subject Privacy/Protected Health Information

The following personal health information will be collected as part of this study:

- Name, address, telephone number, email address
- Date of birth
- Social Security Number (W-9 form)
- Some personal information that may be considered sensitive, such as medical history, psychological history, alcohol use history, etc.
- Results from physical examinations, tests or procedures
- Information on smoking, cognition, or HIV-related biomarkers from the blood samples provided at the Intake Session
- Medical Record Number
- Results from urine for drug screening/pregnancy test

Every possible precaution, as described above, will be taken to ensure that the privacy of subjects' personal health information will be maintained. Potential participants will be contacted over the phone after responding to recruitment advertisements. Participants will undergo an initial phone screening where preliminary eligibility for the research study will be determined. Only if a participant is initially eligible, will they complete Intake tasks, remotely or in-person, to confirm eligibility. All data collected over the phone and during in-person visits will be collected by research staff who have completed the CITI Protection of Human Subjects Research Training as well as HIPAA Compliance Training. Information will never be recorded with identifiers other than study ID. A separate list of names with ID numbers will be accessible only by authorized personnel. All records will be kept in locked filing cabinets to maintain confidentiality. Results will not be communicated to other personnel or to the subjects. All analyses will be conducted on de-identified data. Data will be accessible to the Study Investigators, Study Physician, study staff, UPenn IRB, Office of Clinical Research, authorized UPenn staff (e.g. accounting and billing matters, provide treatment, etc.).

<u>Future use of Data and/or Biospecimens</u>

Participants will be given the option to let us store their biospecimens and information for use in future research. This storage may be for an indefinite amount of time. The information and samples provided may be shared with other research institutions, e.g. The Abramson Cancer Center at Penn, or researchers working with research institutions who want to learn more about neurocognitive disease in people living with HIV. Whole genome sequencing will not be conducted on samples. We will protect participant confidentiality by first labeling their information and samples with an identification number only (not their name). We will restrict access to the databases that hold their personal information. Samples will be stored in a locked, private bank, accessible only by authorized personnel. Permission to store their information and samples for use in future research is optional and they can indicate their choice at the end of the consent form. Participants may withdraw their permission at any time by contacting study staff and letting us know they no longer want their information and samples to be stored for use in future research. Participant samples may be used to create products, including some that may be sold and/or make money for others. If this happens, there are no plans to tell the participant, or to pay them, or to give any compensation to them or their family. Additionally, we will not follow up with participants about the specific research that will be done, and individual research results obtained as part of future research will not be shared with them.

5. Tissue Specimens

Blood.

One 8.5 mL sample of blood will be drawn at the Intake visit to evaluate liver and renal function. At weeks 0, 12, 16, and 28, subjects will provide blood samples for FACS analysis of monocyte and T cell surface markers (done in real time on fresh whole blood), plasma for soluble biomarkers (stored and run batched) and monitoring of viral load (total of 80 mL per visit). All specimens are to be collected solely for research purposes. Whole blood will not be stored. Blood samples will be processed upon arrival to the Collman lab for PBMCs, monocytes and plasma and will be analyzed. Monocytes and plasma will be stored at -80 so that assays may be run at a later time. A record of all samples will be maintained using the LDMS database software

Urine.

A urine sample will be required at the Intake Session and each Laboratory visit for drug screenings. Subjects who test positive for study prohibited drugs may be deemed ineligible. Participants believed to have a false-positive result on the drug screen may continue in the study, with investigator approval. All female participants of child-bearing potential will complete urine pregnancy tests.

Saliva.

A saliva sample will be collected from self-reported smokers at each Laboratory visit to assess cotinine levels and biochemically verify smoking status.

6. Genetic Testing

N/A

RISK/BENEFIT ASSESSMENT

1. Potential Study Risks

A detailed description of the study will be given to all subjects, which will include the risks of participation, assurance of full confidentiality, and the knowledge that their freedom to refuse participation or withdraw from the project will not affect the availability of treatment at the University of Pennsylvania. Informed consent procedures will comply with current standards of the IRB at the University of Pennsylvania. Subjects can choose, as an alternative, to not enroll in this study. Adverse reactions will be assessed and reported as required by Federal law and the regulations of PENN.

Potential Risks of Galantamine. The following are the common side effects that have been reported with galantamine treatment: The most common were nausea, vomiting, diarrhea, dizziness, headache, and decreased appetite. All of these side effects have been mild and transient in nature.

In addition to the side effects mentioned above, other less common (less than 5% of patients) side effects include depression, stomach discomfort, stomach pain, heartburn, weight loss, extreme tiredness, tremor, slowed heartbeat, and muscle spasms. Rare, but potentially serious side effects occurring in less than 1% of patients include dysgeusia (change in sense of taste), burning or tingling sensation in hands, arms, legs, or feet, fainting, shortness of breath, difficulty falling asleep or staying asleep, and excessive sweating. All of these side effects shall be queried using a side effects checklist at the first laboratory visit of each treatment period (to obtain a baseline estimate of any side effects prior to starting medication) as well as all subsequent visits and phone calls (up to 14 times throughout the study).

Stringent exclusion criteria are in place to limit the chance of these side effects. Additionally, participants will be informed about these possible side effects and be made aware to watch for any of these symptoms and report them as soon as possible to the research staff. All side effects will be closely monitored and the study physician consulted should moderate or severe side effects be reported. In case any participant experiences severe side effects or an adverse event they will be encouraged to contact the Health Care Provider/Study Physician immediately for appropriate intervention. The Study Physician's emergency contact numbers shall be on the medication blister pack, the study consent form, as well on an emergency card all participants will be provided with once they enroll into the study.

Reproductive Risks. In a study in which pregnant rats were dosed from the beginning of organogenesis through day 21 post-partum pup weights were decreased at 8 and 16 mg/kg/day, but no adverse effects on other postnatal developmental parameters were seen. The doses causing the above effects in rats produced slight maternal toxicity. No major malformations were caused in rats given up to 16 mg/kg/day. No drug related teratogenic effects were observed in rabbits given up to 40 mg/kg/day (32 times the MRHD on a mg/m^2 basis) during the period of organogenesis. There are no adequate and well-controlled studies of galantamine hydrobromide ER in pregnant women. Thus, galantamine hydrobromide must not be used during pregnancy. All female participants of child bearing potential will be given a pregnancy test at the Intake session and the Baseline visit at the beginning of each treatment period; and must agree in writing to use an approved method of contraception through the study.

Risk from Blood Draw. Blood draws may result in bruising and/or slight bleeding at the needle site. This is rare and happens infrequently. Occasionally, blood drawing results in a feeling of faintness. This too is rare. A trained professional will draw blood, so the chances of these discomforts are minimal. Procedures are in place to ensure that PHI is not linked with the results of this research.

Potential Loss of Confidentiality. Every attempt will be made by the investigators to maintain all information collected in this study strictly confidential. We will store subject information in a secure room with limited access. Only people working on this research project can access subject information. We will control access to the computer files that hold this information. This information will not be released to anyone. When the results of the study are published, no names or identifying information will be used. The final risk relates to potential disclosure of HIV status through participating in this study.

2. Potential Study Benefits

Participants who enroll in this study will benefit from the knowledge that they are contributing in an important way to potentially furthering scientific knowledge concerning neurocognitive disease and ways to improve its treatment in HIV-infected individuals.

3. Alternatives to Participation

As an alternative to enrolling in this study, participants may choose to continue to smoke or to seek assistance with quitting smoking through other treatment programs located in their area, including contacting the national quit-line. At any point in this trial, subjects may decide not to continue in their participation.

4. Data and Safety Monitoring

Who will monitor this study? Check all that apply.

☒ Principal Investigator
☐ Sponsor or contract research organization
☐ NCI sponsored cooperative group
☒ Cancer Center (if mandated by CTSMRC)
☐ Medical monitor
☒ Safety monitoring committee
☐ Data and safety monitoring board

4.1 Data and Safety Monitoring Committee

Data and Safety Monitoring will be conducted by the Principal Investigators and the Study Physician. They will review all possible Adverse Events (AEs) and Serious Adverse Events (SAEs). They will ensure that this information is captured in a comprehensive manner and reported according to Good Clinical Practice (GCP). The Principal Investigators, Study Physician, Project Manager, and the research staff will oversee and complete the monitoring process. Monitoring will be performed on an ongoing basis in accordance with the University of Pennsylvania Sponsor-Investigator Standard Operating Procedure PM 004.

The Principal Investigators are responsible for:

1. Obtaining IRB review and approval of a clinical investigation before the investigation is initiated and ensuring continuing review of the study by the IRB in accordance with 21 CFR Part 56;
2. Obtaining informed consent in accordance with 21 CFR Part 50; and
3. Assuring that all staff and subjects understand and accept the obligations incurred in undertaking this double-blind placebo-controlled study in accordance with 21 CFR Parts 312, 511, 812, 813 and any other applicable regulations.

The research staff is responsible for collecting and recording all clinical data. This includes ensuring that all source documents exist for the data on the Case Report Forms (CRFs), ensuring all fields are completed appropriately, and all corrections are done according to GCPs. Any inconsistencies/deviations will be documented on the CRFs and such findings will be reviewed at the weekly study meetings.

The Project Manager will oversee staff training. Training will include a review of the study protocol, informed consent, telephone screen, CRFs and the procedures that are in place regarding session check-in, data collection, data entry and quality control. All applicable regulations will be reviewed and the roles/responsibilities of each staff member will be explained. All questions will be answered and the training will be documented in a training log, which will be initialed by those involved. The Project Manager will also confirm all appropriate documentation of informed consent and storage of consents in a separate consent binder, and will maintain the study regulatory binder.

The Project Manager, PIs, Nurse Practitioner, and Study Physician will work together to confirm eligibility criteria. The Study Physician/Nurse Practitioner and Dr. Ashare, will review charts for each subject to confirm eligibility and will document this review by signing/initialing and dating each chart or providing the determination via email.

The research staff and CRC/Project Manager will ensure all medication is properly ordered and received from IDS, stored at the center, labeled, and distributed to subjects.

The Project Manager, Stephanie Josephson, LCSW, will review all completed MINIs on an ongoing basis. In the event the PM is unavailable, the Principal Investigator Rebecca Ashare, Ph.D., will complete all review.

The data managers will be responsible for creating all CRFs and ensuring that all data will be entered and stored in a manner consistent with the design of the approved CRFs. They will also be responsible for developing the data entry/quality control producers for this study.

Enrollment will be complete when 120 subjects complete all study requirements. On average, ~4 subjects will be enrolled per month. During the course of the study, safety and data quality monitoring will be performed on an ongoing basis in accordance with the University of Pennsylvania Sponsor-Investigator Standard Operating Procedure PM 004 and any findings will be reviewed on a regular basis with the Investigators at the regular study meeting. The monitoring will include a regular assessment of the number and type of serious adverse events. The first monitoring day will occur no more than two weeks after the first subject is entered.

4.2 Adverse Event Reporting and Monitoring

Adverse Event (AE)

An Adverse Event (AE) is a subcategory of the broader category of "Unanticipated Problems Posing Risk to Subject or Others." An adverse event is defined as:

- Any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease occurring at any stage of the study
- Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:
    - results in study withdrawal

  - is associated with a serious adverse event
  - is associated with clinical signs or symptoms leads to additional treatment or to further diagnostic tests
  - is considered by the investigator to be of clinical significance
- May include an exacerbation of a pre-existing condition, intercurrent illness or injury, drug interaction, drug overdose, failure of expected action or significant worsening of the disease under study
- An event that may compromise the rights, safety, or welfare of research subjects

Any event that could be characterized by the definitions above is an AE whether or not considered related to the study or product.

Serious Adverse Event (SAE)
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:
- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.
All adverse events that do not meet any of the criteria for serious will be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 7 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the investigators until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, a research team member will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery will ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Other kinds of events can be labeled “serious adverse events” at the discretion of the investigator.

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe," which are not synonymous, the following note of clarification is provided: The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Severity Grading Scale for Adverse Events
Many disease specific groups have developed toxicity grading scales. For example, most cancer clinical trials use the Common Terminology Criteria for Adverse Events (CTCAE) developed by the NCI. The CTCAE provides a descriptive terminology which is utilized for adverse event reporting. A grading (severity) scale is provided for each adverse event term (http://ctep.info.nih.gov). If no guidelines exist, then the following scale can be used:
• Mild: Noticeable to the subject, does not interfere with the subject’s daily activities, usually does not require additional therapy, dose reduction, or discontinuation of the study.
• Moderate: Interferes with the subject’s daily activities, possibly requires additional therapy, but does not require discontinuation of the study.
• Severe: Severely limits the subject’s daily activities and may require discontinuation of the study. This would include all adverse events defined as “serious adverse events”.

Attribution/Association with the Drug or Intervention:
An assessment of the relationship between the adverse event and the drug/intervention will be made for each occurrence by the Principal Investigator.

Adverse Event Attribution Categories:
• Unrelated- The AE is clearly NOT related to the intervention
• Possible- The AE may be related to the intervention
• Probably- The AE is likely related to the intervention
• Definitely- The AE is clearly related to the intervention

4.3 Recording of Adverse Events

At each contact with the subject after the Intake, the study research assistant will seek information on adverse events by specific questioning using a side effect checklist and, as appropriate, by examination. Side effects will be monitored through a two-pronged approach. First, participants will complete a side effects checklist (SEC) at each study visit after the Intake, administered by trained staff, reporting with a frame of reference since their last study visit. The SEC will assess the frequency and severity of common side-effects associated with galantamine-ER treatment (e.g., nausea, vomiting).

These items will be rated by participants on a 0 (none) to 3 (severe) scale, and can be summed to provide an overall side effects index.

Second, trained staff will ask participants a non-structured, open-ended question (SEC Open-ended) at each study visit with a one-week frame of reference to assess if participants are experiencing any additional symptoms or medical concerns that may be related to their participation in the study.

Research staff are trained to inquire (time of onset, nature of issue reported, possible relation to galantamine treatment, review of previously reported side effects or concerns, etc.) about any notable side effects or medical concern reported by participants. Based on published reports using the 8mg and 16mg galantamine-ER doses we expect some side effects with galantamine-ER treatment; however we expect these side effects to be mild and transient in nature. Any severe (or a pattern of moderate) side effects or notable medical concern will be treated as adverse events, and reported to the Study Physician to determine the severity, relationship of the event to the study drug and decide the course of action for the study subject. This consult, including all relevant information, will be documented via email. The Study Physician is knowledgeable of side effects related to galantamine and is qualified to manage possible side effects. Mild side effects will not be reported as adverse events, but will be recorded and monitored by study staff.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

4.4 Reporting of Serious Adverse Events and Unanticipated Problems

The following information about adverse events will be reported:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment
- Welfare of subjects.

4.5 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) and the CTSRMC DSMC require expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

AND

Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).
Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Reporting Deaths: more rapid reporting requirements

Concerning deaths that occur during the course of a research study, the following describes the more rapid reporting requirement of the Penn IRB for specific situations:

- Report the event within 24 hours when the death is unforeseen (unexpected) and indicates participants or others are at increased risk of harm.
- Report the event within 72 hours, for all other deaths, regardless of whether the death is related to study participation.

For reportable deaths, the initial submission to the Penn IRB may be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

Reporting SAEs to the DSMC

• Penn Subjects (including subjects at networks, affiliates or investigator-initiated sites);
All on-site grade 3 or higher AEs or ADRs regardless of attribution or expectedness will be submitted to the DSMC within 30 days. SAEs or SADRs for Penn subjects regardless of attribution or expectedness will be submitted to the DSMC within 10 days. Reports will continue to be sent to the DSMC for 90 days following the last date the subject received study treatment/therapy or was exposed to an investigational device. All unexpected deaths or deaths related to the study agent(s)/device(s) must be reported within 24 hours. All other deaths should be reported within 30 days.

• IND Safety Updates/Alerts
IND Safety Updates/Alerts (sent by sponsors), that are specifically for the protocol open in the ACC, with a grade 3 or higher, regardless of attribution or expectedness will be submitted to the DSMC within 30 days. Events for studies using a novel agent, on any protocol in the Cancer Center, not specifically the protocol open in the ACC, shall be sent within 30 days. All other IND Safety Updates/Alerts shall be sent within 60 days of receipt. Once the study closes to accrual at Penn, reports shall be sent to the DSMC for 30 days from the date the last Penn subject was treated. Events for studies using a novel agent or agents manufactured on campus will be sent until the protocol terminates.

Reporting Events

All events will be entered into the ACC Clinical Trials Management System (CTMS) AE/SAE form.

Other Reportable events:

- For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
- An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
- Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
- A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- **Exception**: A one time, intentional action or process that departs from the IRB and CTSRMC approved study protocol, intended for one occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, advance documented IRB and DSMC approval is required.
- For exceptions on Industry or Cooperative group sponsored protocols, written approval must be obtained from the Sponsor prior to submitting your exception request to the DSMC.
- For in-house studies with a Medical Monitor or Safety Monitoring Committee (not DSMB), approval must be obtained from the Medical Monitor or Safety Monitoring Committee prior to submitting your exception request to the DSMC.
- **Deviation:** A one time, unintentional action or process that departs from the IRB and DSMC approved study protocol, involving one incident and identified retrospectively, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the DSMC within 5 business days and the IRB within 10 business days.

Data, Safety and Monitoring Report. The PI will provide a summary of the DSM report on an annual basis as part of the progress report. The DSM report will include the expected versus actual recruitment rates, treatment retention rates, any quality assurance or regulatory issues that occurred during the past year, summary of AEs and SAEs, and any actions or changes with respect to the protocol.

Evidence of Training in Human Subject Research. All research personnel associated with this study have completed the CITI-Protection of Human Subjects Research Training as well as HIPAA Compliance Training.

5. Management of Information for Multi-center Research where a Penn Investigator is the Lead Investigator of a multi-center study, or Penn is the lead site in a multi-site study.

This is a single-site study.

6. Risk/Benefit Assessment

There is minimal risk for serious adverse events. The treatments and procedures used in this study have been shown to be relatively safe. Galantamine has also been studied in several clinical trials and shown to be safe and efficacious. Nevertheless, there are risks in participating in this trial, which are described above.

Participants who enroll in this study will benefit from the knowledge that they are contributing in an important way to potentially furthering scientific knowledge concerning neurocognitive disease and ways to improve its treatment in HIV-infected individuals.

SUBJECT COMPENSATION

Subjects will be compensated for participation up to $590 (Table 2). Subjects will also be reimbursed for transportation expenses related to their participation in the study with $10 for each in-person visit. In place of $10/session to cover travel expenses, subjects may elect to use a round-trip car ride service (i.e., Lyft) which will be arranged and paid for in full by the research study. If they choose to use the ride service, they will not receive $10 for travel reimbursement and their total visit compensation may be up to $500. Travel will not be reimbursed for sessions designated as "in-person", if they have to be completed by phone.

The "task completion" compensation will depend on participant compliance (arriving on time for sessions, returning medication blister packs for adherence verification etc.). If a subject does not follow study instructions, the task completion compensation may be withheld.

The Greenphire ClinCard will be the primary form of payment for this study. The ClinCard is a reloadable, pre-paid card for the purposes of compensation. Compensation will be loaded onto the ClinCard within 24 hours of completed visits. Staff may ask participants to provide a Social Security Number, or complete a W-9 for this purpose, after determining eligibility so that a ClinCard can be assigned. Clincards may be mailed to subjects following the eligibility determination for the study.

If a subject is found ineligible at any session due to any of the above-mentioned criteria, they will only be compensated $10 for travel, unless they have elected to use the ride service for that session.

Subjects who successfully refer others to the program (i.e., person referred to program completes initial phone screen) will be awarded $20 per referral, for a maximum of 3 referrals.

Upon completion of all requirements for a given visit, participants randomized to CM will receive 5 lottery draws for that visit. Attendance at all visits earns participants bonus draws upon completion of the study. Failure to attend a visit without prior approval or failure to complete all visit requirements results in no draws for that visit. The study completion bonus will be 5 extra draws. Thus, at each visit, subjects will have the opportunity to make 5 draws, for maximum possible earnings of $120 per visit (and a maximum of $145 at the final visit if participants have earned the additional 5 draws). Compensation distribution is shown in the table below.

Compensation Schedule								
Week	**Period**	**Study Visit**	**Visit Compensation**	**Task Completion**	**Travel[3]**	**Bonus**	**Total**	**Lottery[4]**
-2	N/A	Intake	$20		$10		$30	5 draws
0	1	Lab Visit 1	$35	$15	$10		$60	5 draws
2	1	Monitoring Call 1	$10		$0		$10	
4	1	Mid-Tx Visit 1	$25	$15	$10		$50	5 draws
6	1	Monitoring Call 2	$10		$0		$10	
8	1	Mid-Tx Visit 2	$25	$15	$10		$50	5 draws
10	1	Monitoring Call 3	$10		$0		$10	

12	1	Lab Visit 2	$25	$15	$10	$40[1]	$90	5 draws
16	2	Lab Visit 1	$35	$15	$10		$60	5 draws
18	2	Monitoring Call 1	$10		$0		$10	
20	2	Mid-Tx Visit 1	$25	$15	$10		$50	5 draws
22	2	Monitoring Call 2	$10		$0		$10	
24	2	Mid-Tx Visit 2	$25	$15	$10		$50	5 draws
26	2	Monitoring Call 3	$10		$0		$10	
28	2	Lab Visit 2	$25	$15	$10	$40[1]	$90	Up to 10 draws
					Study Total:		**$590**	
N/A	2	Referral Bonus				$60[2]	$60	
					Total w/ Referrals:		**$650**	

[1] Bonus awarded to participants that complete every visit in a period, at the end of each period.
[2] Table shows total compensation for three successful referrals
[3] Only paid if you opt out of the round-trip car ride service; will not be paid if a visit has to be completed by phone.
[4] Participants may be given 5 draws for the chance to earn additional monetary incentives. As a bonus for completing the study, participants will get an additional 5 draws during their final visit.

Traveling via the Ride Service

Participants may elect to use "Roundtrip", which is a car ride service that partners with Lyft to coordinate roundtrip rides to study appointments. Participants will be asked for their preference when scheduling the Intake session via phone, and at each session preceding an in-person session once enrolled. Study staff will schedule each ride using participants' first name, last name, and phone number via Roundtrip's HIPAA compliant platform. Participants will receive a reminder call within 24-48 hours prior to their study visit to confirm their visit, interest in using the ride service, and preferred pickup/drop-off locations. If the study staff cannot reach participants by 5pm the day prior to their study visit, their ride will be cancelled. Participants will still be permitted to attend the visit and will receive $10 to cover travel expenses. If participants need to cancel a previously confirmed ride, they must do so by contacting the study staff immediately, preferably by 5pm the day before their appointment. If participants fail to notify study staff within this timeframe, they may no longer be permitted to use the ride service at future study visits.

INFORMED CONSENT

1. Consent Process

Informed consent will be obtained using the combined consent and HIPAA form approved by the PENN IRB. This process will take place before study data are collected and prior to any treatment. The consenting process may occur remotely in-person as part of the Intake session. If completed remotely, subjects will be contacted via Blue Jeans (HIPAA-compliant) for a videoconference or by phone. Reviewing the consent form will be completed using a RedCap survey and PowerPoint to visualize key points. Staff will email or text the survey link to subjects. The PowerPoint will either be sent to participants or shared using screen share via BlueJeans. Whether in person or remote, staff will review the study description, and all study procedures, potential risks, and information about the study medication will be addressed. Subjects will be given the opportunity to read the consent form in full. Following this, subject questions will be answered, and staff will administer comprehension questions to ensure subject understanding. Any incorrect answers will be addressed by the staff member completing consent. If remote, subjects will indicate within the RedCap survey if they wish to participate and will then be prompted to enter their First and Last name and sign the form using their finger or mouse. Subjects who are unable to provide an electronic signature may be mailed a physical version of the consent that must be mailed back to us prior to continuing with the Intake tasks. If needed, staff may also ask subjects to sign a physical version of the form at their first in-person visit for record keeping. Subjects will be able to download their signed version of the form from RedCap, and staff will also download a version to be saved to the electronic regulatory binder on our secure server, or, printed and placed in our physical binder. If in-person, subjects will receive a physical copy of the combined consent and HIPAA form for their records. Subjects will also be given the PI and Study Physician's contact information should they wish to speak to either of them during the course of the study regarding their consent or the study procedures. The consent process will take place in English, there will be no waiting period, no coercion to participate, and all subjects will be

considered competent to provide informed consent (i.e., they will be asked if they understand what they are consenting for).

2. Waiver of Authorization

Verbal consent will be attained from participants via phone for contact that occurs after their study participation has ended. This contact will only be made to collect data that they have already consented to provide as part of the study, and no additional PHI will be collected. Participants will have the option to not answer additional questions related to their participation in the study. If we are unable to get into contact with a participant, the data will be marked as missing.

RESOURCES NECESSARY FOR HUMAN RESEARCH PROTECTION

Training and Quality Assurance

Given ongoing trials (R01 DA033681), systems for training and QA are established to ensure accurate eligibility screening and recruitment, accurate data collection, entry, and management, and optimal delivery of the study protocol. A new Manual of Operations (MOP) will be devised for this study, given unique procedures and measures. Dr. Metzger, who coordinates behavioral research within the PENN CFAR, will assist with staff training to ensure that unique issues related to the population are integrated into the MOP. Training sessions will occur over the first 3 months and annually. Monthly team meetings will review progress, assess adherence, and determine the need for protocol changes or additional training/QA. A study-specific Data and Safety Monitoring Committee will provide oversight. The MOP will ensure that the trial is conducted in a uniform manner over time and across staff. The MOP will describe roles and responsibilities for personnel and provide a detailed description of procedures for each point of contact with participants. For each visit/week, a checklist of events (e.g., measures, counseling) will be created that will be completed by study personnel. CRFs will be created for each measure at each week, and every participant will have a study binder, with sections for every visit/week. Every visit will be “milestoned” (e.g., attended, missed, scheduled) to ensure proper tracking of participants through the trial. Lastly, a manual for data collection and entry is developed for the RAs and the PM/Dr. Ashare will train the RAs and provide supervision. Ms. Ware, who oversees this DMS, has already developed this DMS for use in other trials. QA focuses on protocol adherence and data validity. We conduct 100% QA checks on study data. This involves comparison of all hard copy CRFs to computer data.

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Sample Analysis Addendum
Version 1: 06/22/2018

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Background

All living cells assemble a diverse repertoire of glycan (carbohydrate) structures on their surface.[1] Recent advances in the emerging field of glycobiology show that this repertoire, the glycome, plays critical roles in defining immune responses[2] and in cell-cell[3] and cell-pathogen interactions.[4] The specific structure of a glycan allows it to bind to a specific type of glycan-binding proteins called lectins, leading to activation of downstream signaling pathways. For example, galactose binding to galectins (β-galactoside-binding lectins), promotes immune evasion by inducing T-cell exhaustion and apoptosis, expanding regulatory T cells, and inhibiting natural killer cells.[5-9] **Sialic acid binding to** siglecs (sialic acid-binding Ig-like lectins), plays an essential role in inflammation, differentiation of self and non-self, and cell death.[6,10-20] Fucosylated glycans have shown to be involved in cell-cell interactions, T-cell exhaustion, angiogenesis, malignancy, and metastasis.[21-24] In addition, within the host glycome, glycans on circulating glycoproteins and antibodies (immunoglobulins G; IgGs), are known to play an important role in regulating several immunological functions.[25] Glycans associated with IgGs are of particular interest because these regulate/control the binding of IgG to its various Fc receptors, which defines antibody function, e.g., antibody-dependent cell-mediated cytotoxicity, or anti-inflammatory activities.[26-29] Several studies revealed that altered glycosylation is closely linked to several diseases, including, rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, cancer, and diabetes.[30-45] Glycoimmunology is increasingly being translated into the clinical setting, revealing the mostly untapped potential of the glycome in novel therapeutics. Smoking is associated with several glycomic alterations in the general population, including induction of fucose metabolism. Increased level of cell-surface fucose is known to mediate and drive immune activation/inflammation by inducing TCR signaling in T cells and TLR4 signaling in monocyte/macrophages. Furthermore, increased fucose level on antibodies (immunoglobulin G; IgG) is known to reduce the antibody-dependent cell-mediated cytotoxicity (ADCC). We will be taking advantage of recent advances in the emerging field of glycomics to establish a relationship between glycomic profiles, smoking with HIV, and smoking without HIV. We will compare host glycomic profiles (cell-surface and cell-free) between smokers (HIV+ ART+, or HIV-) and non-smokers (HIV+ ART+, or HIV-).

Cell-surface Glycomic Analysis

The PBMCs will be separated into CD4+ T cells and CD8 T+ T cells using magnetic bead separation technology from Stem Cell. We will employ a lectin microarray platform to profile 96 different glycan structures on the cell-surface of unfractionated PBMCs and isolated CD4+ T cells and CD8+ T. The lectin microarray is an emerging comprehensive novel technology, enabling high-throughput, rapid, and sensitive analysis of carbohydrate structures on a variety of samples. The lectin array employs a representative panel of immobilized lectins with known glycan structures binding specificity, subsequent monitoring of a binding event in pre-labeled samples, resulting in a specific map, often termed "the glycan signature." [46-54] In the HIV field, lectin microarrays have been used thus far for detailed analysis of the glycome of intact HIV-1 virions. The results from that study showed strikingly similar glycan fingerprints between HIV virions and cell-derived microvesicles, suggesting that HIV-1 shares a common exocytic pathway with microvesicles.[55,56] Cell-surface molecules from 10^5-10^6 cells will be purified using ProteoExtract Subcellular Proteome Extraction Kit (Merck). 2 µg of fractionated cell-membrane proteins will be labeled with Cy5, and hybridized to a lectin microarray. The resulting lectin chip will be scanned for fluorescence intensity on each lectin-coated spot using an evanescent-field fluorescence scanner GlycoStation Reader 1200. Data will be normalized using the global normalization method. Samples will be run in triplicates and will be repeated when the coefficient of variance is > 10%. The lectin array can assess eight samples in triplicate.

Cell-free Glycomic Analysis

Total IgGs will be isolated from the plasma using Protein G. For screening N-glycans we will use UPLC. *N*-glycans will be released with PNGase F, labeled with 2-aminobenzamide, and excess reagents will be removed using hydrophilic interaction liquid chromatography solid phase extraction.[57,58] Fluorescently labeled and purified *N*-glycans will be separated by HILIC-UPLC using an Acquity UPLC instrument as described in our recent publication.[59]

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