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Design of a Randomized Controlled Trial of Zinc Supplementation to Improve Markers of Mortality and HIV Disease Progression in HIV-positive Drinkers in St. Petersburg, Russia

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Abstract

Background—Russia continues to have an uncontrolled HIV epidemic and its per capita alcohol consumption is among the highest in the world. Alcohol use among HIV-positive individuals is common and is associated with worse clinical outcomes. Alcohol use and HIV each lead to microbial translocation, which in turn results in inflammation. Zinc supplementation holds potential for lowering levels of biomarkers of inflammation, possibly as a consequence of its impact on intestinal permeability. This paper describes the protocol of a double-blinded randomized placebo-controlled trial of zinc supplementation in St. Petersburg, Russia.

Methods—Participants (n=254) were recruited between October 2013 and June 2015 from HIV and addiction clinical care sites, and non-clinical sites in St. Petersburg, Russia. Participants were randomly assigned, to receive either zinc (15 mg for men; 12 mg for women) or placebo, daily for 18 months. The following outcomes were assessed at 6, 12, and 18 months: 1) mortality risk (primary outcome at 18 months); 2) HIV disease progression; 3) cardiovascular risk; and 4) microbial translocation and inflammation. Adherence was assessed using direct (riboflavin) and indirect (pill count, self-report) measures.

Conclusion—Given the limited effectiveness of current interventions to reduce alcohol use, zinc supplementation merits testing as a simple, low cost intervention to mitigate the consequences of alcohol use in HIV-positive persons despite ongoing drinking.

Trial Registration—NCT01934803

Keywords

Russia; HIV; alcohol use; zinc; inflammation; mortality risk; cardiovascular risk

Background

Russia has one of the fastest-growing HIV epidemics in the world and its per capita alcohol consumption is among the highest, particularly for men.^{1,2} Among hospitalized HIV-positive Russians, 48% met criteria for alcohol use disorder.³

HIV infection is associated with an increased risk of coronary heart disease.^{4–8} While the underlying mechanism is not known, HIV infection is linked to inflammation, which is a driver of atherosclerosis. Like HIV, alcohol consumption is also pro-inflammatory and causes microbial translocation, a process whereby bacterial products cross the gastrointestinal membrane into the portal circulation. High levels of these bacterial products lead to increased immune activation and can contribute to organ damage and death.^{9–12}

Some reports link zinc supplementation with lower levels of inflammatory biomarkers.^{13,14} However, nearly half of HIV-positive persons and over 1/3 of people with alcohol use disorders, have low zinc levels ($<0.75\text{mg/L}$).^{15–18} Observational studies demonstrate that among HIV-positive people on ART, low zinc levels are associated with an increase in HIV viral load and a nearly five-fold increased risk of total mortality.¹⁹ Low zinc levels are associated with increased levels of multiple inflammatory cytokines, which are also associated with alcohol related liver injury.^{20–22}

Zinc supplementation has known and potential benefits. One randomized controlled trial (RCT) among HIV-positive adults reported that supplemental zinc (12–15mg/day) reduced the likelihood of immunologic failure (CD4 count <200) fourfold as compared to placebo.²³ In animal models, zinc supplementation attenuates ethanol-induced increases in microbial translocation products via preservation of intestinal morphology and reduced permeability.^{24–27} These observations combined with a zinc anti-inflammatory effect demonstrate the potential for zinc supplementation to impact the health of HIV-positive heavy drinkers in a substantial way.

Methods/Design

We conducted a double-blinded randomized placebo-controlled trial of zinc supplementation (Zinc for INflammation and Chronic disease in HIV [ZINC]) among HIV-positive heavy drinkers in Russia to evaluate the efficacy of zinc to 1) improve markers of mortality, as measured by the VACS index (primary outcome at 18 months); 2) slow HIV disease progression, as measured by CD4 cell count; 3) improve markers of coronary heart disease (CHD) risk, as measured by the Reynolds risk score and; 4) decrease microbial translocation and inflammation, as measured by serum biomarkers.^{28–31} Participants received study medication over 18 months, with study visits occurring at 6, 12, and 18 months post enrollment, and shorter medication adherence visits at 6 weeks, 12 weeks, 9 months, and 15 months.

Study setting

Participants were recruited between October 2013 and June 2015 from HIV and addiction clinical care sites and non-clinical sites in St. Petersburg, Russia. All study enrollment and assessment activities took place at the Laboratory of Clinical Pharmacology of Addictions at the First St. Petersburg Pavlov State Medical University. All Research Assessors (RAs) were trained medical personnel (MD, MD/PhD) with prior experience conducting research studies.

Participants and Recruitment

ZINC (N= 254) participants met the following inclusion criteria: 1) age 18–70 years old; 2) documented HIV infection; 3) heavy alcohol consumption (i.e., National Institute on Alcohol Abuse and Alcoholism risky drinking criteria: > 4 standard drinks in a day [or > 14 standard drinks/week] for men and $> 3/\text{day}$ [or $> 7/\text{week}$] for women) in the past 30 days;³² 4) willing to provide two contacts to assist with follow-up; 5) stable address within St. Petersburg or districts within 100 kilometers of St. Petersburg; 6) possession of a home or

mobile phone; 7) documentation of being antiretroviral therapy (ART)-naïve at the time of enrollment. Importantly, all participants were treatment naïve at the time of enrollment, but were not precluded from starting ART once they were enrolled. The following served as exclusion criteria for study enrollment: 1) not fluent in Russian; 2) cognitive impairment precluding informed consent; 3) breastfeeding or being pregnant. Pregnant women were excluded due to some reports suggesting possible adverse events with zinc supplementation.

ZINC was nested within the Russia ARCH cohort of the **Uganda Russia Boston Alcohol Network for Alcohol Research Collaboration on HIV/AIDS (URBAN ARCH)** Consortium. The URBAN ARCH Consortium aims to understand how alcohol use impacts people affected by HIV and develop interventions to address alcohol- and HIV-related consequences. Existing Russia ARCH participants were offered an opportunity to be screened for ZINC. Recruitment also occurred via information distributed to clinical HIV and addiction sites and non-clinical sites (e.g., NGOs, peer providers), and current study participants (i.e., snowball recruitment).

Once an interested participant reached out to the study team via phone or in-person at Pavlov University, the RA administered a verbal consent for the screening process, which included a brief description of the study. Following receipt of verbal consent the RA screened the participant to confirm eligibility. Screening for the study occurred in two steps: A (self-report) and B (with documentation). Once a participant was confirmed to meet study entry criteria (screening A), they were scheduled for their first study visit (screening B and baseline). When the participant arrived at Pavlov University for their baseline visit, the RA re-screened the participant to assess ART use, pregnancy and breastfeeding status, and confirmed HIV and ART status (screening B). As part of screening B, participants were required to provide documentation of HIV and ART-naïve status. This documentation took the form of letters from a medical provider, laboratory results, and excerpts from medical histories. Participants, who were found to be ineligible at either stage, (screening A or B) were thanked for their time and received a listing of local addiction and HIV care resources. In total, 427 screening A forms were completed, with 337 (78.9%) eligible to proceed to screening form B. Of those, 263 (78.0%) returned to complete screening form B and all 263 (100%) were eligible for the study. The main reasons for participant ineligibility (n=90) were not drinking at risky levels in the last 30 days (n=73); and past (n=26) or current (n=21) ART use. Reasons for ineligibility are displayed in Table 1.

After establishing study eligibility, the RA administered and documented informed consent, and enrolled participants into the study. Out of 263 eligible participants after screening form B, 258 (97.7%) were enrolled and 5 declined enrollment due to lack of timing or interest. Once consent was obtained, all female participants received a urine pregnancy test. No participants were found to be pregnant at this step. The RA then collected contact information for the participant and at least two alternative contacts. This information was entered directly into the secure, online study team tracking system and all phone numbers were verified as working and valid numbers.

Following collection of contact information, participants were accompanied by the RA to meet with the study nurse for phlebotomy. Inability to obtain blood after repeated attempts precluded randomization of four participants given its importance in the determination of study outcomes.

Upon completion of a successful blood draw, the RA administered the baseline assessment and randomized participants to the intervention or control group. In total, 254 participants were randomized, 126 participants to Group A and 128 participants to Group B (Figure 1). Of note, subsequent to enrollment and randomization, four participants were found to be HIV-negative following laboratory testing.³⁴ These participants were withdrawn from the study upon the team's learning of their baseline HIV status, but will remain in study analyses as per intention-to-treat protocol.

Randomization

Participants were randomized in a 1:1 ratio to zinc or placebo utilizing stratified block randomization. Stratification was based upon gender and heavy alcohol consumption during the past week. Randomization was conducted and monitored by the URBAN ARCH Biostatistics and Data Management (BDM) core. The software package SAS was used to generate randomization lists to assign participants as they were enrolled into the clinical trial.

As a double-blinded placebo-controlled study, ZINC participants, investigators including clinicians, RAs and the study nurse were unaware of participant group assignment.

Our strategy to pursue a double-blinded RCT to assess zinc's impact on study outcomes was to mitigate potential bias due to known and unknown confounders.

Intervention

Participants randomly assigned to the intervention (zinc) group received study medication at all but the final visit. Of the eight study visits, four included longer assessments with lab work and four were shorter medication adherence checks.

Zinc capsules were compounded using pharmacy-grade zinc gluconate and Riboflavin (adherence measure) at Bios Pharmaceuticals in St. Petersburg. Zinc capsules for men and women contained 15 and 12 mg of zinc gluconate, respectively, as this dose had been shown to be effective in previous trials and has minimal risk of adverse events.²³ The capsules were provided in bottles, each containing a 28-day supply of medication.

Participants received an extra 6-week of supply of study medication to accommodate for lost medication or missed visits. Participants were instructed to take one pill daily by mouth with a full glass of water and to not consume caffeine one to two hours before or after taking the medication. The latter is due to its potential interaction with zinc absorption.

In accordance with FDA guidelines, the study did not require an Investigational New Drug (IND) application, as it was conducted outside of the United States.

Control

The control group received all study procedures and instructions identical to the intervention group. Participants received a sucrose placebo identical to the zinc medication in appearance and taste. Riboflavin was added to both active and placebo medication as a biologic adherence measure.

Adherence

Medication adherence was assessed at each study visit using direct (Riboflavin) and indirect (pill count and self-report) measures.

Direct Adherence Measures—Riboflavin (50 mg), a vitamin yielding a change in urine color, was added to both zinc and placebo capsules. Participants were informed that the color change was harmless. At this dose, Riboflavin is expected to remain in the system at detectable levels for up to 24 hours.³⁵ At each study visit post-baseline, participants were asked to provide a urine sample, which was visually inspected for the presence or absence of Riboflavin in a room with low ambient light, using ultraviolet (UV) light at the long wave setting (33 mm).

Indirect Adherence Measures—Pill counts were used to supplement the Riboflavin adherence measure. Participants were instructed to return any unused medication at each study visit post-baseline. The assessor counted and recorded the number of remaining pills. The data management team extrapolated the amount of medication taken and provided a measure of adherence.

Medication adherence was also measured through self-report using modified Adult AIDS Clinical Trial Group (AACTG) adherence questions.³⁶ At each post-baseline study visit, participants were asked to draw a line on a paper ruler numbered from 0–100, to indicate the number best representative of how much of the study medication they had taken in the past 6 weeks. Participants were also asked questions about the longest period of time during which they took the study medication daily, if they stopped taking the medication, and if they took more than one pill of study medication on any day in the past 6 weeks.

Adherence Aids—During each study visit at which medication was provided, strategies for adherence were discussed with the participant. Adherence plans were individually tailored, depending on the reason for non-adherence. To further increase medication adherence, an automated text message was sent twice per week, reminding participants to take their study medication. Participants were able to reduce the frequency or opt out of text message reminders entirely at any time throughout the study.³⁷

Assessments

Participants (n=254) were assessed at baseline, 6, 12, and 18 months post enrollment, along with shorter medication visits at 6 and 12 weeks, as well as 9 and 15 months. All study assessments took place at First St. Petersburg Pavlov State Medical University. On occasions when a study participant was unable to come to the study site for a face-to-face interview and was in danger of falling out of their assessment window, RAs conducted study

assessments over the telephone. Participants received 1250 rubles (approximately US \$22) in cash for their participation at each of the baseline, 6-, 12-, and 18-month visits and 500 rubles (approximately US \$9) for each shorter medication visit.

Questionnaires—The components of baseline and follow-up assessments are listed in Tables 2 and 3. Most sections of the study questionnaire were interviewer-administered, with sections on depressive symptoms and emotional health self-administered by the participant, as they contained potentially sensitive questions. Assessment data were entered electronically into study computers by the RAs or the participant for the self-administered sections. In addition to study questionnaires, at each study visit RAs measured and recorded participants' height, weight, and blood pressure.

Laboratory Procedures and Testing—This study required the collection of 22 mL of blood at baseline, 6-, 12-, and 18-month visits. Blood was tested in St. Petersburg for hemoglobin, platelets, CD4 cell count, hepatitis C virus (HCV) antibody and qualitative viral load (baseline and 18-months only), HIV viral load, high sensitivity C-reactive protein (HS CRP), total and high-density lipoprotein (HDL) cholesterol (baseline and 18-months only), creatinine, aspartate aminotransferase/alanine aminotransferase (AST/ALT), and zinc levels (baseline and 18-months only) (Table 4). Plasma samples were stored for biomarker testing (sCD14, D-dimer, IL-6, 16sRDNA). Dried blood spot cards were spotted using 60µL of blood per spot (or 300µL per card) and saved to be used for phosphatidylethanol (PEth) alcohol biomarker testing. The incorporation of PEth will provide complement to self-report of alcohol consumption.³⁸

Liver Status—As many of the biomarkers used to assess inflammation are synthesized in the liver, an inability to account for liver health makes interpretation of the biomarkers of inflammation (e.g., CRP) and their association with zinc and HIV disease progression, as well as acute myocardial infarction (AMI) and mortality risks, difficult. Therefore, to minimize confounding due to liver disease two baseline measures of liver health (fibroscan and FIB-4 score) were obtained.³⁹ Fibroscan is a non-invasive ultrasonic imaging technique used selectively, so as to obviate the need for liver biopsy. FIB-4 score is an inexpensive and accurate biomarker of liver fibrosis in HIV- and HCV-positive patients and those with alcohol use disorders.^{40,41} The study team calculated FIB-4 scores using baseline laboratory values (ALT, AST, platelet count) and age for all study participants and created an algorithm to place participants in one of three categories: high, low, and unclear possibility of liver fibrosis. Participants that fell into the “unclear” category (FIB-4 values ranging from 1.4–3.25) received a fibroscan at their next study visit to determine their liver disease status. Fibroscans were conducted at the First St. Petersburg Pavlov State Medical University.

Adverse Events

To minimize medication risks, participants were monitored for adverse effects at each study visit. Symptoms were assessed at baseline and any chronic conditions or symptoms that existed prior to introduction of study medication were documented. During each subsequent study visit, the RA asked the participant how he or she felt and reviewed the list of symptoms of concern, beginning with any symptoms recorded at the previous visit and the

four most frequent side effects of zinc: abdominal pain, diarrhea, nausea, and vomiting. The RA asked about any new symptoms experienced by the participant since the last study visit. Any event that met the criteria for an adverse event (AE), serious adverse event (SAE), or unanticipated problem was recorded. The research team reviewed the results of all blood work performed on study participants. Any abnormal lab results deemed clinically significant were recorded as an AE and/or SAE, and the participant was referred to their local medical provider for further evaluation and treatment. All events were presented to and reviewed by the Data and Safety Monitoring Board (DSMB).

Urine pregnancy tests were administered to all women at each visit and women were instructed to discontinue the study medication should they become pregnant. Participants were also alerted to possible interactions between zinc and certain antibiotics, and instructed to discontinue the study medication and contact the study team should they initiate a course of antibiotics.

Data Management

The URBAN ARCH Biostatistics and Data Management (BDM) Core provided active statistical collaboration in the design and analysis of the ZINC trial. Assessment data were collected directly onto computers by research assessors. Electronic questionnaires included programmed skip patterns and range checks, minimizing errors at data capture, and allowing for timely electronic data transfer to the Boston team at regular intervals. Participant screening, tracking and randomization utilized web-based systems, in which data elements necessary to determine eligibility and stratification factors for randomization were entered into a web-based application, minimizing the possibility of enrolling an ineligible person, while allowing US-based collaborators real-time access to enrollment data.

Analytic Methods

Major Hypotheses and Analytic Plans—The study aimed to test the hypothesis that compared with placebo, participants receiving zinc supplementation will have significantly 1) lower VACS index scores; 2) higher CD4 cell counts; 3) lower Reynolds risk score and; 4) lower biomarker levels of microbial translocation and inflammation. The primary outcome was improved markers of mortality, as measured by change in VACS index score between baseline and 18 months. The secondary outcomes were slower HIV disease progression, as measured by change in CD4 cell count; improved markers of AMI risk, as measured by the Reynolds Risk Score; and lower biomarker levels of microbial translocation and inflammation, as measured through IL-6, D-dimer, sCD14, and 16sRDNA.

This study was conducted under the intention-to-treat principle and thus main analyses will include all participants according to their randomized assignment. Descriptive statistics will be calculated for variables at baseline and each follow-up time point. At baseline, participant characteristics will be presented by randomized arm to assess whether there are any differences between groups. Spearman correlation coefficients will be obtained to identify pairs of variables that may be collinear ($r > 0.4$) and would therefore not be included together in regression analyses.

The main analysis evaluating the impact of zinc on the primary study outcome (i.e., change in VACS index) will use multiple regression models that include randomization group (i.e., zinc vs. placebo) as the main independent variable. The regression analyses will control for the two block randomization stratification factors: heavy alcohol consumption during the past week and gender. In addition, the models will control for baseline characteristics that differ between groups in order to avoid confounding. Potential confounders of interest, measured at baseline, include demographics, past month alcohol use, age, gender, anti-inflammatory medication use, cardiovascular disease risk factors, HCV status, substance use (e.g., alcohol, smoking, cocaine), CD4 cell count, HIV-1 RNA, duration of awareness of HIV infection, and socioeconomic status. If the data are normally distributed, multiple linear regression models will be used. However, if the distribution is skewed, transformations of the data will be performed (e.g., log transformation). If an appropriate transformation is not identified, a median regression model will be used.^{42,43} The secondary outcomes, including HIV disease progression, as measured by change in CD4 cell count (Aim 2); the Reynolds risk score (Aim 3); and biomarkers of microbial translocation and inflammation (Aim 4) will be analyzed using the same approach described above. A secondary analysis will be conducted using a per protocol approach that includes only those participants who were adherent with their assigned intervention (i.e., taking zinc or placebo 80% of the time).

Sample Size and Power Calculations—Power was calculated for the overall primary study endpoint (change in VACS index score). Power calculations assumed a two-sided hypothesis test, with a significance level of 0.05. It was expected that 250 participants would be enrolled into the study. We anticipated 20% loss to follow-up due to death and participant withdrawal. Based on the VACS study, the standard deviation of the change in VACS index score from initiation of ART to after one year was 25.²⁸ We expect the standard deviation will be similar in the proposed study. Given these assumptions and with 200 evaluable participants (assuming 20% loss to follow-up, and 100 participants in each arm), the study has 80% power to detect a difference between the placebo and zinc groups in their mean changes in VACS index score over the 18-month period as small as 10 (e.g. 20 vs. 10 for the placebo and zinc groups, respectively) using a two-sided t-test.

Protection of Study Participants and Their Data

The ZINC study was approved by the Institutional Review Boards of Boston University Medical Campus and First St. Petersburg Pavlov State Medical University. All study participants completed the informed consent process and provided written informed consent.

All study data were captured electronically on netbooks via a secure, web-based data capture system with the exception of data on alcohol use disorder, which were captured on paper. Access to the system was protected via secure logins and all data transmissions were encrypted using secure socket layering (SSL). The project website and study data were located on a secure server within the Boston University Medical Center (BUMC) domain. All web-forms were protected using SSL encryption technology and files were protected by electronic firewalls that restricted access to designated users. Identifiers needed to track participants were kept separate from research data.

Data Safety Monitoring Board—To ensure the safety of the participants and the validity and integrity of the data, a Data Safety Monitoring Board (DSMB) was established to assume oversight of the entire URBAN ARCH Consortium and any studies originating from the URBAN ARCH cohorts, including the ZINC study. The Board met every 6 months and was charged with evaluating the quality of trial administration, monitoring safety issues, and providing guidance on scientific, methodological, and ethical issues. Specifically, the Board reviewed investigators' plans and processes for identifying individual or patterns of adverse events and reviewed accumulating safety data.

Discussion and Impact

The ZINC study tests the efficacy of zinc supplementation compared to placebo to improve biomarkers of mortality (VACS index score) and coronary heart disease risk (Reynolds risk score), slow HIV disease progression (CD4 cell count), and decrease biomarkers of microbial translocation and inflammation among HIV-positive heavy drinkers in St. Petersburg, Russia. Given the limited effectiveness of current interventions to reduce alcohol use, zinc supplementation holds potential for being a simple, low cost intervention to mitigate the consequences of alcohol use in the presence of continued alcohol consumption. In consideration of practical implementation, baseline zinc levels were not used as eligibility criteria for ZINC study enrollment for two reasons. First, it is unlikely that clinicians will assess zinc levels to establish deficiency prior to treatment, particularly in resource-limited locations where low zinc levels are likely common among HIV-positive heavy drinkers. Second, a recent RCT involving zinc supplementation demonstrated reduced levels of inflammatory biomarkers among healthy elderly participants without low zinc levels.¹³ Hence, the benefit was present even without documented low zinc state. Heavy drinking was defined as per NIAAA criteria for risky drinking: > 4 standard drinks in a day (or > 14 standard drinks/week) for men and > 3 drinks/day (or > 7 drinks/week) for women.³² This definition has been used extensively in the alcohol literature, but we ultimately do not know the threshold of alcohol consumption at which increased microbial translocation and lowered gut permeability are observed. The team relied on previous work in selecting the primary study endpoint of 18-months to allow for evaluation of long-term effects of zinc supplementation.²³ However, additional analyses using generalized linear mixed effects models will be used to incorporate the repeated measures for each outcome (collected at 6-, 12-, and 18-months) in the same model and will test for possible zinc by time interactions (e.g. does the effect of zinc increase over time).

Russia, with high prevalence of HIV and alcohol consumption, provides a unique setting for the ZINC study. Russia's many HIV-positive persons and low ART coverage enable this study to examine the effects of zinc on inflammation, separate from ART.⁴⁴ As zinc is readily available, inexpensive, and can be used with ART, zinc supplementation therapy, if effective, could become an adjunct therapy for the treatment of HIV-positive people with heavy alcohol use.

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Figure 1.
Consort Diagram. ZINC Study Screening and Enrollment

Table 1

Reasons for Ineligibility of Individuals (n=90) Undergoing Screening A of ZINC Trial

	n
Not meeting drinking criteria	73
Past ART use	26
Current ART use	21
Cannot verify HIV status	2
Out of catchment area	2
Currently pregnant	2
Does not have HIV	1

Table 2**ZINC Trial Baseline Assessment**

Assessment	Description
Demographics ⁴⁵	Participant demographics and socioeconomic status.
HIV Testing/HCV Diagnosis	Date of first positive HIV test and awareness of HCV diagnosis
Co-Morbidities ⁴⁶	Participant and family medical history
Russian Brief Pain Inventory (BPI-SF) ⁴⁷	Participant pain severity in the past 7 days
Medications	Prescription and non-prescription medication taken in the past 7 days
Zinc Intake ⁴⁸	Participant multivitamin and supplemental mineral use
HIV Symptom Index ⁴⁹	A 20-item HIV symptom index includes patient-reported symptoms
Depressive Symptoms: The Center for Epidemiologic Studies Depression Scale (CES-D)* ^{50,51}	A measure of depressive symptomology
Emotional Health: International Personality Item Pool (IPIP) & the Revised Life Orientation Test (LOT-R)* ^{52,53}	A measure of participant personality and optimism vs. pessimism
Tobacco Use: Modified Fagerström Nicotine Dependence Scale ^{54,55}	A measure of nicotine dependence
Alcohol Use: Alcohol Surrogates ⁵⁶	Participant's use of alcohol surrogates (substances not intended for drinking) in the past year
Alcohol Dependence: Mini International Neuropsychiatric Interview (MINI) Alcohol ⁵⁷	Alcohol dependence according to DSM-IV criteria
Alcohol Use: 30-day Timeline Follow-Back (TLFB) Alcohol ⁵⁸	Estimates of participant's daily drinking over the past 30 days
Drug Use: Modified Risk Behavior Survey (RBS) ^{59,60}	Participant's self-reported drug use, modified to adapt to drug practices in Russia
24 Hour Activities	Participant past 24-hour activities that may impact inflammatory biomarkers measured
Social Support Scale ⁶¹	Measures access to companionship, assistance, or other types of support
Veterans RAND 12 Item Health Survey (VR-12) & Medical Outcomes Study HIV Health Survey (MOS-HIV) ⁶²	Assessment of overall health and cognitive function

**
Self-administered section

Table 3ZINC Trial 6, 12, 18-Month Follow-Up Assessments^{*}

Assessment	Description
Medication Adherence ^{36,63}	Adherence to study medication in the past 6 weeks, as indicated by measurement on a ruler ranging from 0–100 and additional questions on adherence patterns
Medication Satisfaction TSQM (Version 1.4)	Participant satisfaction with study medication
ART Use and Adherence ³⁶	Questions on ART and 30 day Adherence
Opportunistic Infections ⁶⁴	Assesses any history of candida or yeast infection of the esophagus, TB, pneumonia, or toxoplasmosis

^{*} Includes all Baseline Assessment components except Alcohol Dependence: Mini International Neuropsychiatric Interview (MINI) Alcohol

Questions asked at shorter medication visits (6 & 12 week, 9 & 15 months)

Table 4

Sample Collection and Laboratory Testing in the ZINC Trial

Laboratory Testing*
HIV Viral Load (VL)
CD4 cell count
Hemoglobin and platelets
HCV Ab and HCV VL (qualitative)
HS CRP
Cholesterol (total and HDL)
AST/ALT and creatinine
Zinc levels
Plasma stored for future biomarker testing
Dried Blood Spot (DBS) stored for future PEth testing

* Conducted at baseline, 6, 12, 18 months unless indicated otherwise.

Conducted at baseline and 18 months