Pilot Study of Zinc Supplementation and Cardiovascular risk in HIV

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Tool Revision History

Version Date: 1/31/19

Summary of Revision Made:

- Changes made to reflect NIH protocol template
- Updated to include R33 phase

Précis:

Study Title:

"Pilot Study of Zinc Supplementation and Cardiovascular risk in HIV infection"

STUDY OBJECTIVES

PRIMARY:
- To replicate findings from R21 and examine whether zinc supplementation will change biomarkers (hs-CRP, sCD14, sTNF-RI, and microbial translocation LBP)

-To examine whether zinc is safe and effective at increasing zinc levels in HIV-infected subjects with zinc deficiency.

SECONDARY:
-- To assess the associations between the inflammatory markers (hs-CRP, sCD14, sTNF-R1 and LBP) and insulin resistance and endothelial function.

-To assess the safety and tolerability of zinc supplementation

-To assess the association between changes in zinc levels and insulin resistance and endothelial function
-To assess the association between changes in zinc levels and changes in gut markers of intestinal fatty acid binding protein (I-FABP), and zonulin.

EXPLORATORY:
-To assess the association between changes in zinc levels on advanced glycation end (AGE) products accumulated in the skin.
-To assess the association between changes in zinc levels and lipids

STUDY ENDPOINTS

Co-PRIMARY:

-Changes in markers of inflammation and immune activation: hs-CRP, sCD14, sTNF-RI and microbial translocation LBP

-Changes in zinc levels (before and after supplementation)

SECONDARY:

- Metabolic/CVD factors: endothelial function and insulin resistance

-Safety/tolerability:- Tolerance of zinc reported by patients and safety measures will also be assessed (percent of subjects with >Grade 2 adverse events possibly or probably due to study drugs).

--Markers of gut integrity: intestinal fatty acid binding protein (I-FABP), and zonulin.

EXPLORATORY:

-Advanced glycation end (AGE) products accumulated in the skin.

-Lipids

-To assess the association between changes in zinc levels and lipids

Design and Outcomes:

This is a double-blind randomized placebo-controlled trial assessing the effect of zinc supplementation on improving zinc deficiency and risk of HIV-related comorbidities linked to inflammation and assessing its safety and tolerability in HIV deficiency compared to placebo drug in HIV-infected subjects ≥ 18 years old, on stable ART (for at least 12 weeks), and with baseline zinc levels ≤0.75 mg/L in the last 60 days.

Subjects will be evaluated, and data will be collected according to the following schedule:

Evaluation (Weeks)	Screen I	Screen II	Entry	6	12	18	24
Family history of CVD and diabetes		X					X
Framingham and Reynolds Risk Scores			X		X		X
Targeted physical, blood pressure, weight		X	X	X	X	X	X
Anthropometric Measurements		X	X				X
Compliance, safety monitoring				X	X	X	
Smoking, illicit drugs and alcohol status		X			X		X
Dietary and Activity status		X			X		X
Height		X					
Hematology/chemistries		X	X	X	X	X	X
Urine β-HCG (women)		X	X	X	X	X	X
Lipoprotein profile			X		X		X
Insulin, glucose, HOMA-IR			X		X		X
Inflammation and immune activation markers			X		X		X
Gut integrity, microbial translocation, intestinal permeability			X		X		X
Oxidative markers			X		X		X
Zinc level	X		X		X		X
Copper level			X				X
EndoPat, AGE testing		X			X		X
Resting Energy Expenditure (REE)		X					X
Cognivue			X				X
Stored plasma, serum and urine		X	X	X	X	X	X
Stored PBMC			X		X		X
Stored Stool		X	X		X		X

Intervention and Duration:

The duration of the study will be 24 weeks. Patients will be given zinc gluconate capsules at a dose of 90 mg elemental zinc daily or matching placebo for 24 weeks. No additional follow up will occur after 24 weeks.

Sample size and population:

The total sample size is 95 patients, 63 patients will be randomized to two 45 mg (or 90 mg) capsules of zinc gluconate and 32 patients will receive two matching capsules of placebo drug. This study will be conducted at the University Hospitals Cleveland Medical Center. Recruitment will be done at the Special Immunology Unit (SIU) at the University Hospitals Main Campus.

BACKGROUND:

It has been estimated by the World Health Organization that nearly two billion subjects may be zinc deficient in the developing countries. This is due to the fact that most of these populations consume mainly bread made of whole wheat flour, which is high in phosphate compound that decreases the absorption of both iron and zinc. The phytate to zinc molar ratio >20 in a diet is unfavorable for zinc absorption and this may lead to zinc deficiency. In the developed countries, zinc deficiency is also prevalent in the elderly population or even in younger subjects with HIV, where up to 30% of non-malnourished subjects were found to be zinc-deficient. Low concentrations of zinc are prevalent in HIV-1–infected male and female drug users as well as other HIV-1–infected cohorts[1, 2]. Such low concentrations of plasma zinc have been linked with disease progression, independent of baseline CD4 cell count, lymphocyte concentrations and age- and calorie-adjusted dietary intake[3]. Of particular importance, low plasma zinc levels have been associated with a threefold increased risk of HIV-1–related death in HIV-1–seropositive drug users[4].

In mild to moderate deficiency of zinc, the clinical manifestations may include growth retardation in children, rough skin, poor appetite, mental lethargy, delayed wound healing, T cell-mediated immune dysfunction, and neurosensory disorders[5, 6]

Pertinent to this proposal, zinc has anti-inflammatory properties due to NFKB blockade, and is an anti-oxidant agent[7]. Zinc decrease NF-κB activation and its target genes such as TNF-α, IL-1β, and VCAM and increased the gene expression of A20 and PPAR-α, the two zinc finger proteins with anti-inflammatory properties. Thus, zinc decreased the expression of these cytokines and molecules by inhibition of NF-κB activation via A20 and PPAR-α pathways. As such, zinc supplementation has been investigated as immunomodulatory and anti-inflammatory agents[5, 8, 9]

In healthy volunteers who were supplemented with 45 mg elemental zinc daily, a significant decrease in TNF-α and IL-1β messenger RNAs and TNF-α induced NF-κB DNA binding were found in isolated peripheral blood mononuclear cells in comparison to placebo treated subjects[10]. In elderly, zinc supplementation with daily 45 mg elemental zinc as gluconate for 6 months was safe, and led to significant increase in plasma antioxidant power, decrease in

plasma oxidative stress marker, and decrease in the inflammation markers C-reactive protein, IL-6, MCP-1, and VCAM--1[5]. Even more, plasma zinc concentrations were inversely correlated with the changes in plasma levels of hsCRP, MCP-1, and VCAM-1 after the zinc supplementation.

The Recommended Dietary Allowance for zinc (intake of 15 mg zinc/d for men and 12 mg zinc/d women) was established in 1974 for the first time. We will use 90 mg elemental zinc/d as oral supplementation because it has been used safely in non-HIV studies including in elderly individuals for 1 year duration, and at this concentration, no serious adverse events were reported and copper status remained normal[5]. This is important because the only adverse effect of very high doses of oral zinc supplementation for a prolonged duration is copper deficiency.

Zinc sulfate is inexpensive, available over the counter, and has an excellent safety profile. If zinc positively influences the mechanisms postulated to play a role in HIV cardiovascular disease, this affordable treatment would become relevant to millions of people worldwide, not only for people living with HIV but also individuals suffering from other conditions of heightened inflammation such as rheumatoid arthritis, diabetes, or lupus.

RATIONALE:

Zinc is a dietary supplement with compelling preclinical evidence for potential health benefit that could be expanded not only to the entire HIV population, but also to other inflammatory conditions that share many facets of HIV infection, namely the persistent intestinal barrier dysfunction, monocyte activation and heightened inflammation state. Such diseases include inflammatory bowel diseases, rheumatoid arthritis, diabetes and even obesity in the general population. Our proposal incorporates assessment of defined signatures of biological effects and will provide important mechanistic insights to inform us on the mechanism of action through which zinc may produce clinical benefit, and as such will provide the information necessary to develop a competitive full-scale clinical trial.

This study will focus on subjects with documented zinc deficiency (levels <75 µg/dL) as group most likely to benefit from the zinc supplementation. We also acknowledge that zinc may be beneficial in all HIV subjects, regardless of the plasma zinc level; however initial studies should be done in subjects with low zinc levels as they are more likely to benefit. If we find promising results in these zinc-deficient subjects, future studies will explore other populations such as large scale trials in HIV-positive subjects regardless of zinc levels.

At the dose used, uncommon side effects include nausea, vomiting, and abdominal pain (gastritis). At higher doses and when used for a long periods (years), copper deficiency and related anemia may happen. This is very unlikely since we are only giving 90 mg daily and for up to 24 weeks. Such doses have been shown to be safe even when given for 12 months to elderly frail subjects. In addition, in diseases such as Wilson's disease, zinc is given at a dose of 50 mg three times daily for prolonged durations. We will closely monitor subjects who will receive blood draws for chemistries, hematology and liver enzymes every 6 weeks, and as needed between study visits

HYPOTHESIS:

In HIV-infected subjects on ART with documented zinc deficiency, short term zinc therapy will be safe, effective at increasing zinc levels, and will result in improvement in selected markers of inflammation and microbial translocation.

STUDY OBJECTIVES

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SECONDARY:
To assess the associations between the inflammatory markers (hs-CRP, sCD14, sTNF-R1 and LBP) and insulin resistance and endothelial function.

-To assess the safety and tolerability of zinc supplementation

-To assess the association between changes in zinc levels and insulin resistance and endothelial function
-To assess the association between changes in zinc levels and changes in gut markers of intestinal fatty acid binding protein (I-FABP), and zonulin.

EXPLORATORY:
-To assess the association between changes in zinc levels on advanced glycation end (AGE) products accumulated in the skin.
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STUDY ENDPOINTS

Co-PRIMARY:

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SECONDARY:

- Metabolic/CVD factors: endothelial function and insulin resistance

-Safety/tolerability:- Tolerance of zinc reported by patients and safety measures will also be assessed (percent of subject with >Grade 2 adverse events possibly or probably due to study drugs).

--Markers of gut integrity: intestinal fatty acid binding protein (I-FABP), and zonulin.

EXPLORATORY:

-Advanced glycation end (AGE) products accumulated in the skin.

-Lipids

INCLUSION CRITERIA:

-HIV-1 infection as documented by any licensed ELISA test kit and confirmed by Western blot at any time prior to study entry. HIV-1 culture, HIV-1 antigen, plasma HIV-1 RNA, or a second antibody test by a method other than ELISA is acceptable as an alternative confirmatory test

-Documentation of an HIV-1 RNA level of ≤400 copies/mL in the last 4 months prior to study entry

-Male or Female age ≥18 years

-Zinc level ≤0.75 mg/L *in the last 60 days*

-Receiving a stable antiretroviral regimen for at least the last 12 weeks prior to study entry

-Cumulative duration of antiretrovirals for at least 6 months at study entry

-Provides written informed consent and is capable of reading and comprehending the informed consent

Non-English-Speaking Subjects: Subjects who do not understand or speak English will also be eligible for this study. The consent form will be read to those non-English speaking study candidates in their primary language by a translator. A witness (who speaks English and the study subject's language) will be present to confirm the consent process has taken place. Both the witness and person conducting the consent process will sign and date the consent. The investigator obtaining consent will ask the study candidate via the translator to reiterate what will be required from him/her, risks and benefits, and his/her rights as a participant to ensure their full understanding of the study.

-Have no plans to alter antiretroviral therapy, diet or exercise or initiate structured/strategic antiretroviral treatment interruptions.

-Able to swallow pills.

-No diarrhea or nausea/vomiting for the last month.

-All women of child-bearing potential (WOCBP) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 72 hours prior to start of study medication. WOCBP is defined as any female who has experienced menarche and who

has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), who is not postmenopausal (defined as amenorrhea for 12 consecutive months), or is on hormone replacement therapy (HRT) with documented plasma follicle-stimulating hormone level 35mLU/mL. Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential.

-Female subjects who are not of reproductive potential (have reached menopause or undergone hysterectomy, bilateral oophorectomy or tubal ligation) or whose male partner has undergone successful vasectomy with resulting azoospermia or has azoospermia for any other reason, are eligible without requiring the use of contraception. Acceptable documentation of menopause, sterilization, and azoospermia is patient reported history.

-All subjects must not participate in a conception process (e.g. active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization), and if participating in sexual activity that could lead to pregnancy, the female subject/male partner must use condoms (male or female) in addition to one of the following forms of contraception while on study: either a spermicidal agent, diaphragm, cervical cap, IUD, or hormonal-based contraception.

EXCLUSION CRITERIA

All candidates meeting any of the exclusion criteria at baseline will be excluded from study participation.

-Pregnancy/lactation

-Presence of inflammatory condition (besides HIV) in the last month

-Regular use of agents that may affect inflammation in the last 3 months. The regular use of NSAIDS, aspirin, or statins will be allowed as long as dose has been stable for the last 3 months and is not expected to change during the study.

-Presence of active neoplastic diseases requiring chemotherapy and/or use of immunosuppressive drugs

-Consuming supplements containing more than the DRI level of nutrients known to affect the immune response, i.e. vitamins E, C, B6, selenium, zinc, or β -carotene and unwilling to stop.

-BMI <18 kg/m2.

-Known cardiovascular disease

-Uncontrolled diabetes

-Allergy or intolerance to zinc sulfate.

-Hospitalization within the previous 28 days.

-Alcohol or recreational drug use with poor history of compliance

-Inability or unwillingness of the individual to give written informed consent

-Any of the following laboratory findings obtained within 14 days prior to the screening evaluation including the following:

-AST and ALT > 2.5 x ULN

-Hemoglobin < 9.0 g/dL

-GFR <50 mL/min

STUDY DESIGN

This is a double-blind randomized placebo-controlled trial, studying zinc supplementation to prevent HIV comorbidities that are linked to inflammation in subjects ≥ 18 years old HIV-infected patients, on stable ART (for at least 12 weeks) and with zinc levels ≤0.75 mg/L in the last 60 days. The duration of the study will be 24 weeks. The total sample size is 95 patients (n=62 on active and 31 on placebo). Patients will be randomized 2:1 to zinc gluconate tablets at a dose of 90 mg elemental zinc daily or matching placebo. For this confirmatory phase of the randomize trial, we propose twice the number of subjects in the treatment arm compared to the placebo arm from the context of feasibility, resource allocation or logistics, ethical point of view, and risk-benefit ratio. We propose to measure a number of inflammatory and immune activation biomarkers. From our prior experiences with these biomarker distributions, we may find more variability (may include outliers) in some of the response variables (biomarkers) in the treatment arm than the variability in the response variables in the placebo arm. To reduce the impact of such variability in estimating the effect sizes, having more subjects in the treatment arm should lead to more precise estimates, and hence more powerful statistical inferences. Study drugs will be provided free of charge to study participants for the duration of their participation in the study. Antiretroviral therapy will not be provided by the study. The decision for ART changes or discontinuation will be left to the primary care provider, but these changes will be documented in the study chart and will be taken into account during the analyses.

Subjects will be evaluated and data will be collected according to the following schedule:

Evaluation (Weeks)	Screen I	Screen II	Entry	6	12	18	24
Family history of CVD and diabetes		X					X
Framingham and Reynolds Risk Scores			X		X		X
Targeted physical, blood pressure, weight		X	X	X	X	X	X
Anthropometric Measurements		X	X				X
Compliance, safety monitoring				X	X	X	
Smoking, illicit drugs and alcohol status		X			X		X
Dietary and Activity status		X			X		X
Height		X					
Hematology/chemistries		X	X	X	X	X	X
Urine β-HCG (women)		X	X	X	X	X	X
Lipoprotein profile			X		X		X

Insulin, glucose, HOMA-IR			X		X		X
Inflammation and immune activation markers			X		X		X
Gut integrity, microbial translocation, intestinal permeability			X		X		X
Oxidative markers			X		X		X
Zinc level	X		X		X		X
Copper level			X				X
EndoPat, AGE testing		X			X		X
Resting Energy Expenditure (REE)		X					X
Cognivue			X				X
Stored plasma, serum and urine		X	X	X	X	X	X
Stored PBMC			X		X		X
Stored Stool		X	X		X		X

*** Plasma, serum, urine and stool will be stored in the local laboratory in a -70 degrees freezer for potential future metabolic, cardiovascular, oxidative markers, inflammation markers, and nutrition markers. Urine will be used for possible oxidative and bone markers.

Randomization:

The randomization schedule will be performed by a statistician using SAS software to create a list based on permuted variably sized block randomization with block sizes of 6 to generate 50 allocated numbers in two arms. The randomization schedule will be generated by the statisticians prior to the start of the trial by and provided to the investigational pharmacy. After being provided to the pharmacists, the randomization schedule will be maintained in a locked, secured location at the pharmacy. Only site pharmacists can access the randomization schedule at the time of the intervention assignment.

Blinding:

Patients, study staff, and the study statistician will be blinded to treatment allocation (zinc gluconate or placebo capsules).

The research staff as well as the principle investigator will remain blinded to treatment assignment. The research staff will be responsible to prepare DSMP reports with the principal investigator and will include enrollment and dropout rates, protocol deviations, subjects symptoms, review of clinical, laboratory results and percent adherence to treatment, while remaining blinded to treatment allocation.

The principle investigator, as well as the DSMB board will remain blinded throughout the conduct of the clinical trial unless unblinding is warranted to optimize management of an adverse event or for other safety reasons. This will occur in a private location to ensure the rest of the team remains blinded.

All statistical analyses will also be done in a blinded fashion. Data will be exported to the statistician as arm A versus arm B by the research nurse to preserve blinding. For analyzing adherence, they will also only have access to the percent as opposed to pill counts the research nurse uses to calculate this.

TIMING OF EVALUATIONS

Screening I/II: all screening evaluations to determine eligibility must be completed within 30 days prior to study entry.

Entry: evaluations must occur within 30 days after screening evaluations. If subject shows up ill with any acute symptom, the visit will be postponed until all symptoms resolved for at least 7 days.

On-Study Evaluations: study visits must be scheduled on the weeks indicated in the schedule of events +/- 14 days.

Premature study discontinuation: Subjects who stop study will undergo the premature study discontinuation evaluations as outlined in the Schedule of events under Week 24 visit. However if subjects stop study medication at any point during the study they will be asked to remain on study and continue to follow the procedures outlined.

DETAILED STUDY PROCEDURES BY VISITS

Screening I/II: Before they enter, patients will be asked to visit the clinic at least once to be screened and ensure that they meet the requirements for entry into the study. Screening may take place on the same day as a normally scheduled clinic appointment, as long as subject is fasting. Before any tests can be obtained as part of this study, patients would have to decide whether or not they would like to participate in this study.

- If they choose to enroll, the informed consent form will be signed. The site investigator or a member of the study team will conduct the consenting process and a checklist indicating date and time the consent was signed, who was present, version date and if a copy was provided to participant will be filed in the subjects chart
- A targeted physical examination will be performed including height, weight and blood pressure
- Recent blood work will be reviewed to be sure that they meet the inclusion criteria. The HIV-1 RNA, and HIV testing to confirm HIV status will be obtained from the clinical chart as these are a part of routine care.
- Blood will be drawn for hematology/chemistries and zinc
- Plasma, serum, urine and stool will be collected and stored for potential future testing.
- For woman of reproductive potential, a urine sample will be taken for a pregnancy test.

If a participant does not meet inclusion criteria it will be noted in the chart with the reason for ineligibility.

If a participant meets the inclusion criteria and chooses to enroll, randomization will occur at the entry visit. The study team will notify the pharmacist who will allocate them to one of the two interventions according to the randomization schedule. Participants should start the intervention within two days of randomization allocation.

Study Entry: For entry visit, this will be fasting, as defined by no food or drink, except for plain water and medications for at least 8 hours. The following procedures will be done at entry.

- A medical history including family history
- Targeted physical exam including weight and blood pressure
- Patients will fill out a dietary, physical activity, and a substance questionnaire.
- Blood, urine and stool will be obtained for markers of inflammation, microbial translocation, oxidative markers, glucose and insulin, chemistries, hematology, lipids and zinc. HIV-1 RNA, and CD4 count will be obtained from the clinical chart as these are part of routine care. Some of the plasma, serum urine and stool will be stored for potential future testing such as additional tests for inflammation, cardiovascular markers, endothelial function markers, oxidative markers. These blood samples will be batched and the tests will be done at the end of the study and are part of this study.
- For woman of reproductive potential, a urine sample will be taken for a pregnancy test.
- CVD Risk Score
- Endothelial function (EndoPAT): a non-invasive, user-independent technique using the FDA-approved EndoPAT2000 (Itamar Medical Ltd, Israel) provides a reliable and reproducible assessment of endothelial function that predicts atherosclerosis and CVD events. Using modified plethysmographic biosensors, the PAT signal is measured from the fingertip by measuring arterial pulsatile volume changes. A cuff is inflated around the upper arm to obstruct flow and released. The surge of blood flow causes endothelial dependent FMD, manifested as a reactive hyperemia. The EndoPAT® calculate the RHI, the ratio of digital pulse volume during reactive hyperemia and the baseline. In addition to the RHI, we will obtain peripheral Aix, adjusted for HR of 75. We have excellent track record in performing vascular studies, and carefully control for factors that could influence endoPAT; studies are performed at 24 C with the patient resting quietly. Subjects will be fasting, without smoking, caffeine, and exercise for >4 hours. Follow-up studies are completed at the same time of day as the initial study using the same equipment.
- Skin AGE levels will be evaluated by a Reader AGE machine. A small area (0.2 cm2) on the underside of the forearm will be shaved with a safety razor and shave cream if the participant presents with excess hair (this may cause irritation). The area will then be cleaned with an alcohol pad. The subject's arm will be placed in an arm rest for stabilization and the skin area will be scanned with the AGE Reader. The results of the scan will be documented on the study data collection sheets and/or subject research record. In order to measure skin AGEs the instrument used in the study is an investigational device that illuminates a small area of the forearm using skin autofluorescence with UV LEDs. The resulting skin fluorescence is measured over a 60 second period.

Cognivue Test: At each visit you will be asked to answer a series of questions to measure your brain's ability to think, make decisions, detect differences between things, movement skills, remembering things and other skills using a computer monitor with a small wheel that you turn to indicate your answers. This test takes about 10 - 30 minutes to complete. Changes in how your brain works can be due to many things; some of these are being looked at in this study. The results of this test will not be provided to you because the test is not a routine clinical test and no change in your treatment will result.

Week 6 and 18:

- Questionnaires about symptoms or any change in health status
- A short targeted physical exam including weight and blood pressure
- Urine pregnancy test for women
- Assess compliance/safety monitoring
- Blood will be collected for hematology/chemistries. HIV-1 RNA and CD4 count will be obtained from the clinical chart, if available.
- Plasma, serum, urine will be collected and stored for potential future testing

Week 12 and 24:

- Targeted physical exam including weight and blood pressure
- Assess compliance/safety monitoring
- Questionnaire about symptoms or any change in health status, substance use and physical activity
- Dietary assessment
- Urine pregnancy test for women
- CVD Risk Score
- EndoPAT testing
- Skin AGE measure
- Blood, urine and stool will be collected for hematology/chemistries, lipids, insulin and zinc
- Plasma, serum, urine and stool will be stored for batch testing (similar to baseline visit) to check for inflammation markers, oxidative markers, and markers of gut epithelial destruction and microbial translocation. HIV-1 RNA and CD4 count will be obtained from the clinical chart.
- Cognivue assessment (week 24 only)

-At visits Entry, week 6, 12 and week 18 study medication will be dispensed by the study staff. Subjects will be asked to return all study medication at each visit and the remaining medication will be counted by the study team and returned to the investigational pharmacy. The study team will assess adherence and record in source document. Adherence will be calculated based on the number of pills dispensed and returned. Study staff will also note in the source document if patients report missed doses. Once a patient arrives at their scheduled visit, the study team will fax a prescription to the Investigational pharmacy who will receive the elemental zinc medication/placebo in bulk and dispense per patient when they arrive at their scheduled visit.

- The zinc will be purchased from a certified Compounding Pharmacy. It will be delivered directly to our investigational pharmacy at University Hospitals. The pharmacy will store the zinc according to their regular procedures, and will dispense study drugs at the time of each study follow up visits, and the number of dispensed pills will be based on the randomization arm.

TOXICITY MANAGEMENT

Only toxicities related to study drugs (zinc) will be considered. Toxicities felt to be unrelated to study drugs will be left to the discretion of the primary care provider of the study participants. AACTG grading system will be used for evaluation for Grading Adult Adverse Experiences.

-Subjects who develop a new Grade 1 adverse event or toxicity may continue study drug without alteration of the dosage. Subjects experiencing Grade 1 adverse event who choose to withdraw from the study should be encouraged to complete the study evaluations as outlined in the Schedule of events.

- For all toxicities of Grade 2 thought to be related to the study drug, the study medication can be held at the discretion of the local investigator. If unable to resume it within 3 weeks, then the subject will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events.

- For all toxicities Grade 3 thought to be related to study drug, study medication will be held until the toxicity grade returns to ≤ Grade 2 or to the entry value. If unable to resume study drugs for longer than 3 weeks, then the subject will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events.

- For any Grade 4 toxicity (confirmed X 2 values for laboratory abnormalities) regardless of the cause, subjects will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events. Exceptions are asymptomatic elevation of indirect bilirubin in subjects receiving indinavir or atazanavir therapy, asymptomatic elevation of CPK, or elevations in lipid levels.

CRITERIA FOR PERMANENT STUDY DISCONTINUATION:

-Request by the subject to withdraw

-At the discretion of the IRB

-Pregnancy or breast-feeding

-Request of the primary care physician if s/he thinks the study is no longer in the best interest of the subject.

STATISTICAL CONSIDERATIONS:

Primary goal of this study is to assess the benefit of zinc supplementation by increasing zinc levels and hence improving several important biomarkers. To assess the effect of supplementation and its' positive influence on markers of inflammation and immune activation known to be associated with HIV-comorbidities, 95 HIV+ subjects (see below for the sample

size justification) will be studied for 6 months from the available HIV+ subjects pool. During this period outcome variables (primarily, zinc levels and sCD14) and biomarkers will be measured at baseline (0), 6, 12, 18, and 24 weeks. The outcome variables will be analyzed, and predicted values will be portrayed longitudinally. The main outcome variables zinc levels and sCD14 are continuous variables. Therefore, linear mixed model and likelihood ratio test will be used in testing hypotheses (see details below).

The HIV+ subjects in the supplementation arm will take 90 mg elemental zinc daily for 24 weeks whereas the HIV+ subjects in control arm will take matching placebo. Both groups of subjects will be in the study for 24 weeks. Outcomes of the intervention will be measured longitudinally at baseline (0), 6, 12, 18 and 24 weeks as noted in the Schedule of Evaluations Table. These outcome variables are in continuous scale. Linear mixed-effects modeling will be used in analyzing these longitudinal measurements to understand their trajectories over the study period. We will collect information on the covariates demographics, lifestyle habits, dietary and socioeconomic factors, and HIV/ART factors. For reproducibility of the results, the data quality will be reviewed rigorously, and necessary transformations will be performed to satisfy assumptions related to residuals for parametric models. Basic descriptive statistics will be used to summarize outcomes and covariates within each group of subjects. We anticipate that the two groups will show some but not complete overlap in their distributions in terms of our baseline covariates.. To depict the impact of supplementation, summary statistics as well as predicted values of longitudinally measured outcome variables will be presented graphically. Models for longitudinally measured outcome variables, analysis method, and computation of power are presented below. One-sided hypothesis tests are performed assuming a 0.10 level of significance (standard for phase II trial)[13]. The error rate in multiple comparisons will be controlled by the step-up Bonferroni correction procedure. The sample size estimation will be based to achieve power of 0.80.

Aim 1&2: we assess the benefit of the zinc supplementation in HIV+ patients. In these aims we will study the impact of zinc supplementation in increasing zinc levels and improving markers related to HIV-comorbidities. We will measure the outcome variables in two-groups at 5 times points during 6 months period. To delineate the outcomes over 6 months period, we will analyze the 6 months outcome data using a linear mixed-effects model[14]. Let Y_{ij} denote an outcome variable from the *i*th subject at the *j*th week, where *i*=1, 2, ..., 95 HIV+ subjects; *j*=0, 6, ..., 24 weeks. Define T_{ij} to be the week when the response measurements are collected from subject *i*. A linear mixed model for the outcome Y_{ij} can be written as

$$Y_{ij} = \alpha + \beta^T{}_0 X + \beta_1 I + \beta_2 T_{ij} + \beta_3 I \times T_{ij} + v_{0i} + v_{1i}\, T_{ij} + \varepsilon_{ij} \qquad (1),$$

where X=vector of covariates, *I*=zinc supplementation (yes/no), the vectors of random effects $v_i = (v_{0i}, v_{1i})'$ are assumed to be independent multivariate normal with mean vector 0 and covariance matrix $G(\theta) = G(\sigma_{v11}, \sigma_{v12}, \sigma_{v22})$. The error term ε_{ij} in model (1) is assumed to be distributed as normal (0, σ^2). The β's are fixed effects parameters, and *G* is assumed to be an unstructured covariance matrix. From the fitted model (1), we will delineate the predicted outcome variables, association between outcomes and zinc supplementation therapy. The model parameters will be estimated by the method of restricted maximum likelihood. Likelihood ratio tests will be used to compare overall mean curves for the two-group. Tests for inequality in 2-group at baseline (0), 6, 12, 18, and 24 weeks will be performed with multiple testing corrections using the Bonferroni correction procedure. Note that if there are large numbers of missing values in this longitudinal study, we will estimate the parameters by utilizing the "pattern

mixture model"[15] which assumes model (1). In the case of parametric model assumption violation, we will consider using the generalized estimating equation approach; but this will be avoided, if possible, because it will lead to decreased power.

Correlational analyses of the secondary and exploratory objectives: To assess the associations of concurrently measured biomarkers, we will present descriptive statistics at each time point, plot the individual/group trajectory of changes, and apply bivariate linear mixed effects models ([14]). Here we use biomarker as a general term to indicate that it is a continuous variable from a statistical point of view. We will compute summary statistics (mean, median, SD, coefficient of variation, Cohen's d, etc.) for each biomarker, at each time point. We will plot these summary statistics over the study period for better understanding the trajectory of these biomarkers. Specifically, we will be presenting two biomarker summary statistics in each graph for better understanding the trajectory of one biomarker and its relationship with the other relevant biomarker: HOMA-IR vs. Zinc. We expect that an increasing trend in zinc should lead to a decreasing trend in HOMA-IR level. We will also plot the biomarkers at each time point using box plots. The box plots will depict a biomarker distribution at every time point (baseline, week 12 and 24). The box plots will give us an overall direction of change in the study population. Since our focus is in the treated group, we will present biomarker trajectories using spaghetti plots for examining individual changes. The spaghetti plots will help in determining analysis models. We will apply the bivariate linear mixed effects (BLME) model for studying evolving relationship in two biomarkers. The BLME model is an extension of equation (1), which allows jointly analyzing two biomarkers together. The BLME model will include random effects and/or first-order auto-regressive process, and independent measurement error for both biomarkers. The joint analyses will provide estimates of correlation coefficients among the biomarkers[14]. Again, for the correlational analysis our focus will be the treated group. The BLME model for Gaussian longitudinal biomarker data will be implemented in SAS MIXED procedure. For faster computational benefit, we will apply the Newton-Raphson algorithm, instead of EM algorithm.

Sample Size and Power analysis: The following sample size estimation and power analysis is based on the significance level 0.10[13] and 80% power. We hypothesize that after the zinc supplementation the HIV+ subjects will have higher levels of zinc and lower levels of sCD14. Based on our data from SATURN-HIV study[16], we assume that, at baseline/before the supplementation, the average ± SD of log(sCD14) for all HIV+ subjects is 7.66 ± 0.32. Using Frison and Pocock's[17] change method, we estimate the sample size based on the difference in the two groups of the mean change of the post-intervention measurements at month six from the baseline measurement, assuming that correlation between repeated measurements is 0.85. A mean difference of 0.08 ng/ml log(sCD14) change between two groups, 27 HIV+ subjects in the control arm and 54 HIV+ subjects in the intervention arm, in a longitudinal measures design with 5 longitudinal measurements can be detected with 80% power using a t-test. In this prospective longitudinal study we anticipate 15% subjects missing/non-compliance because of variety of reasons. Therefore, our final sample size estimates for the control and intervention arms are 31 and 62, respectively. The sample size estimation was performed using STATA 14.0.

Effect size consideration: As above, we previously stated that we will perform significance tests for the shift of distributions of biomarkers after the zinc supplementation using Kolmogorov-Smironow (K-S) test. The amount of shift or effect size will be measured using Cohen's d value. For estimating the effect size, we used our statin study data as the preliminary

data (NCT01218802). In the statin study the baseline mean (SD) of hs-CRP, log(sCD14), log(sTNF-RI), and LBP are 4.95(10.45), 7.71 (0.35), 7.46 (0.37), and 21.11(10.91), respectively. For zinc supplementation (90 mg) we will compute the change in a biomarker by taking the difference of a biomarker measured before- and after- the zinc supplementation. For example, we will compute the difference in LBP measured at baseline and after the zinc supplementation. Similarly, differences will be computed for the other biomarkers. The effect size estimation is performed using nQuary Advisor 7.0 software[18].

All analysis will be by intent to treat. All subjects, regardless of whether they remain on study drug, will continue to be followed throughout the duration of the study. It is standard practice in the HIV studies where inflammation markers are measured, that every attempt would be made to schedule study visits during a time that subjects are not acutely ill. That would mean that a visit would be rescheduled if a patient shows up sick, as acute illnesses may affect inflammation markers. A period of >7 days ill-free would be required. This happens rarely overall but if it does in the setting of this study, participants would be asked to come back after illness resolves.

HUMAN SUBJECTS

Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the University Hospitals Cleveland Medical Center CWRU IRB. A signed informed consent form will be obtained from each study subject. The informed consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and this fact will be documented in the subject's record.

Subjects and Data Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done with coded numbers only by study staff. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, FDA, or OHRP.

Recruitment plans

Subjects will be primarily recruited from the practice of the investigator (the Special Immunology Unit at University Hospitals Case Medical Center). All subjects seen at the Special Immunology Unit who meet the inclusion/exclusion criteria will be approached for study participation. In addition, subjects may be referred to the study by their primary care provider or their HIV provider from other HIV practices.

Study Discontinuation

The study may be discontinued at any time by the IRB as part of its duties to ensure that research subjects are protected.

Post-study Follow-up and Transition of Care

During the study period, the study subject will be encouraged to continue their routine regular follow up with their primary HIV care provider, whether in the Special Immunology Unit or in an outside HIV practice that referred them to the study. The study is not meant to replace the routine HIV care visits. After the study subject completes the study, he/she will continue to be followed by his/her primary HIV provider. There will be no additional follow-up for this study after the 24-week of the pre-defined study period. In addition, after the study subject completes the study, he/she will not be provided any further zinc supplements. If the subject primary care provider decides that it is in the best interest of the patient to be on zinc, he/she would be prescribed such supplements. The decision related to any follow-up or treatment after the study period will be left to the subject and his/her primary care provider.

BIOHAZARD CONTAINMENT

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

All infectious specimens will be packaged and sent in accordance with requirements mandated by the International Air Transport Association Dangerous Goods Regulations- Packing Instruction 602.

DATA SAFETY AND MONITORING PLAN

This is a pilot study that will serve to collect preliminary data on the anti-inflammatory effects of zinc in HIV. However, an independent data and safety monitoring board will be established to include an independent HIV expert, a statistician, and a community advocate. The DSMB will meet every 6 months (or earlier as needed) to review safety data and adverse events. The DSMB will function to: review and approve plans for data and safety monitoring for this trial; to review data on a timely basis and to ensure proper conduct and progress of study; to review credentials of investigator and project staff; to make recommendations to project investigator and staff regarding issues of concern; to address adverse events. The DSMB will meet twice a year in Cleveland, Ohio or by phone conferences to review study protocols progress, and safety data. Meetings will be held in an open format, except if privileged data are discussed. At the time of the continuing review, the DSMB report will be prepared by Dr McComsey and her staff and will include enrollment and dropout rates, protocol deviations, subjects symptoms, review of clinical and laboratory results.

The DSMB will review interim data to detect evidence of adverse events to determine if the trial should continue as originally designed, should be changed or even stopped based on the data. The DSMB will evaluate the progress of the trial, including periodic assessments of the recruitment goals, protocol adherence, accrual and retention of participants. The DSMB will protect confidentiality of the study subjects.

The Principal Investigator will report all > Grade 2 adverse effects deemed to be possibly, probably or definitely related to study participation to the University Hospitals Cleveland Medical Center CWRU IRB within one week in writing. All >1 Grade 1 adverse effects deemed to be possibly, probably or definitely related to study participation will be collected. The Principal Investigator, investigators, and study nurse will prepare every year an update to renew the University Hospitals Cleveland Medical Center CWRU IRB approval of the clinical studies. In this renewal, investigators will inform the IRB about adverse effects Grade > 1 noted during the performance of the study. Accrual, retention, and data quality and timeliness will be monitored by the Principal Investigator.

Protocol Deviations: All deviations will be documented in the source document and the IRB will review all protocol deviations. Any deviation that may affect study conduct or safety will promptly be reported within 72 hours.

Quality Control Committee: Not applicable

Adverse Events

Definitions

The definitions of Adverse Events (AEs) and Serious Adverse Events (SAEs) are given below. It is of the utmost importance that all staff involved in the study be familiar with the content of this section. The principal investigator is responsible for ensuring this.

Adverse Event

An Adverse Event (AE) is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, ECG). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

Any detrimental change in a patient's condition subsequent to them entering the study and during the follow-up period should be considered an AE. When there is a deterioration in the condition for which the study treatment is being used, there may be uncertainty as to whether this is lack of efficacy or an AE. In such cases, unless the reporting physician considers that study treatment contributed to the deterioration or local regulations state to the contrary, the deterioration should be considered a lack of efficacy. Signs and symptoms of disease progression are therefore not considered AEs.

Serious Adverse Event

- A Serious Adverse Event (SAE) is an AE occurring during any study phase (eg, run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.

Any event or hospitalization that is unequivocally due to progression of disease, as determined by the investigator, must not be reported as an SAE.

The causality of SAEs (their relationship to all study treatment) will be assessed by the investigator(s)

Reporting of Adverse Events

When recording/reporting AEs, the use of diagnoses is preferred (when possible) to recording/reporting a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded/reported separately.

Illiterate Subjects

Subjects with all levels of literacy will be eligible for this study. The consent document will be read to those volunteers and the process will be documented in the research file. Subsequently, the informed consent will be signed by the volunteer making their mark in the signature section in order to document their understanding. A witness will be present to confirm the consent process has taken place. Both the witness and person conducting the consent process will sign and date the consent. The investigator obtaining consent will ask each subject to reiterate what will be required from them, risks and benefits, and their rights as a participant in order to ensure their full understanding of the study.

Non-English Speaking Subjects

Subjects who do not understand or speak English will also be eligible for this study. The consent form will be read to those non-English speaking study candidates in their primary language by a translator from the University Hospitals of Cleveland translation/International Visitors Center. A witness (who speaks English and the study subject's language) will be present to confirm the consent process has taken place. Both the witness and person conducting the consent process will sign and date the consent. The investigator obtaining consent will ask the study candidate via the translator to reiterate what will be required from him/her, risks and benefits, and his/her rights as a participant in order to ensure their full understanding of the study.

BENEFITS

There may be no direct benefit to patients from participating in this study. Their participation in the study will allow collection of valuable information about the potential benefits of zinc in persons infected with the HIV virus. This information may be useful to them and other people with HIV disease.

ALTERNATIVES

Alternatives to participation in this study are not to participate and to receive the standard of care from the patient's primary care doctor.

COST TO PARTICIPANTS

There is no cost to participants for the study related clinic visits, examinations or laboratory test required by this study. Medical costs of other treatment or examinations outside of the study will be the responsibility of the patient or their insurance company. Patients will be provided zinc supplements free of charge for the entire duration of the study. The study drug will not be provided after they finish the study (whether they complete the study or prematurely stop study participation).

PAYMENT TO SUBJECTS

For completed study visits screening I subjects will receive a food voucher and either a RTA pass or parking validation. Screening II, week 6 and week 18, patients will receive $25.00 that day. Entry week 12 and week 24, patients will receive $50.00 that day (total of $225 for completing the entire study). This payment will help cover the expense of childcare, transportation and time off work that patients may incur as a result of being in this study. To help with the cost of gas, for participants traveling 20-40 miles one way for their appointments, they will also be given a $15.00 gas card to cover the cost of the transportation. Participants traveling > 40 miles one way for their appointments will also be given a $30.00 gas card to cover the cost of transportation. Subjects will be asked to fast (nothing to eat or drink for 8 hours prior to visit) at entry, week 6, week 12, week 18 and week 24, therefore a $5.00 meal voucher will be provided for subjects to use in the University Hospitals cafeteria. In addition either one all day RTA pass will be provided or a parking voucher to use in designated University Hospitals parking garages will be provided to cover the cost of transportation.

RISKS AND DISCOMFORTS: The risks for taking part in this study are:

Blood Draw: Risks associated with drawing blood include: pain, bleeding, and bruising at the site of the blood draw. Other rare risks include: lightheadedness and/or fainting or infection at the site.

EndoPAT: This is a painless imaging test that has no short or long-term risks. The endoPAT test may be mildly to moderately uncomfortable because of the blood pressure cuff that is applied to the patients' arm tightly.

Fasting: Some individuals find fasting to be bothersome. It may make some individuals feel anxious, irritable, or hungry. Patients who are required to take their morning medications with food should wait until after the visit has been completed to take their medications.

Pregnancy: Zinc may not be safe for unborn babies. If patients are having sex that could lead to pregnancy, they must agree not to become pregnant . Because of the risk involved, patients and their partner must use two methods of birth control that they discuss with the study staff. They must continue to use both methods until 6 weeks after stopping study drug. They may choose two of the birth control methods listed below:

- Birth control drugs that prevent pregnancy given by pills, shots, intra-vaginal ring or placed on or under the skin
- Male or female condoms with or without a cream or gel that kills sperm
- Diaphragm or cervical cap with a cream or gel that kills sperm
- Intrauterine device (IUD)

If women can become pregnant, they must have a pregnancy test before they enter this study. The pregnancy test must be negative. In addition, if study subjects think they may be pregnant at any time during the study, they are to tell the study staff right away. In the event that a patient becomes pregnant while on study, they will be taken off study, and no further evaluations or tests will be performed as part of the study.

Zinc Supplementation: At the doses used, uncommon side effects include nausea, vomiting, and abdominal pain (gastritis). At higher doses and when used for a long periods (years), copper deficiency and related anemia may happen. This is extremely unlikely since we are only giving 90 mg daily for up to 24 weeks. Such doses have been shown to be safe even when given for 12 months to elderly frail subjects. In addition, in diseases such as Wilson's disease, zinc is given at a dose of 50 mg three times daily for prolonged durations. We will closely monitor subjects who will receive blood draws for chemistries, hematology and liver enzymes.

Cognivue test: There are no physical risks associated with completing this computerized questionnaire. The test will take about 10 - 30 minutes to complete during each visit

OTHER RISKS: Patients are informed to tell their study doctor or study nurse about all other drugs they are currently taking including non-prescription medications, alcohol, recreational, and herbal products. These drugs, if taken with the study medication, can result in dangerous interactions. If patients have questions about the drug that will be used in this study and the potential for interaction with other drugs that they take, they are instructed to ask their study doctor to provide additional information.

In addition to the risks and discomforts listed here, there may be others that are currently not known.

BIBLIOGRAPHY REFERENCED