Vitamin E for NASH Treatment in HIV Infected Individuals

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1.0 Background

The prevalence of nonalcoholic fatty liver disease (NAFLD) is not well defined in U.S. adults with HIV infection in the current era. Chronic liver disease is a major cause of morbidity and mortality among individuals with HIV infection (1, 2). With the availability of effective therapies for hepatitis B and C, NAFLD is predicted to become the leading cause of liver disease in the aging HIV population (3). In the non-HIV population, NAFLD is associated with a) increased overall and liver-related mortality (4); b) increased risk for cardiovascular disease and type 2 diabetes (5-9); and c) increased risk for liver cancer even in absence of cirrhosis or significant fibrosis (10-14). In HIV infected patients, the prevalence of NAFLD has not been well established. Reported prevalence has varied by study inclusion criteria and modality of NAFLD diagnosis, ranging from 15-54% when assessed by imaging modalities (15-17) and up to 73% in studies including liver biopsy (18). These studies suffered several limitations, including the inclusion of only a small number of HIV infected individuals (18, 19), inclusion of patients with concurrent hepatitis C infection (20-23) or limiting the HIV population assessed to only military personnel and their dependents or to men alone (15, 16).

The risk factors for NAFLD and NASH in HIV infected individuals have not been examined in depth. First, traditional risk factors for NAFLD in the general population may not apply to NAFLD in those with HIV infection. While most studies suggest an influence of insulin resistance, obesity and dyslipidemia on the risk of NAFLD in HIV infected individuals, this may not apply to male HIV patients who tend to be more physically fit, have similar or lower BMI and lower incidence of fatty liver compared to controls (16, 35, 40). Thus, it is essential to comprehensively evaluate NAFLD and NASH in both women and men with HIV infection and stratify by the metabolic syndrome and BMI to accurately determine prevalence in different risk groups.

The impact of novel antiretroviral agents on risk for NAFLD and NASH has not been comprehensively evaluated. Some antiretroviral agents, particularly nucleoside or nucleotide reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI), impair autophagy and mitochondrial function leading to lipodystrophy, insulin resistance, steatosis and steatohepatitis. (38, 41-46). There are, however, conflicting data from clinical studies on the contribution of ART to this risk and the impact of new antiretroviral agents with better metabolic profiles, such as integrase inhibitors, on the risk of NAFLD (15, 18, 20, 35, 47, 48).

Safe and effective therapies for NASH in HIV infected individuals are absent. Over the last decade, there have been several large randomized clinical trials (with more than a hundred ongoing Phase 2 and Phase 3 currently registered on Clinicaltrials.gov) to treat NASH in the general population, but the majority of them systematically excluded HIV infected individuals (74). Weight loss, exercise and therapy with vitamin E or pioglitazone have shown efficacy in improving NASH and are recommended for HIV uninfected patients with NASH (75). However, whether these measures will be effective in HIV infected individuals with NASH is unknown. A pilot study showed that in patients with HIV and

abdominal adiposity on stable ART, Tesamorelin, a growth hormone–releasing hormone analog, resulted in reduction of visceral and liver fat (76). Whether this expensive agent will be useful to treat NASH in HIV infected patients has not been established. Vitamin E is an attractive and inexpensive therapy for NASH in HIV infected individuals. Oxidative stress contributes to the progression of NASH (77-82). Based on its known function as an antioxidant (83), we and others have studied vitamin E used alone or with other compounds in multiple trials to treat NASH or NAFLD in the non-HIV population (84-93). Variable dosages ranging from 100-1200 IU/daily have been used with reported beneficial effects on liver enzymes, steatosis, inflammation, ballooning, and hepatic fibrosis. Duration of vitamin E monotherapy in these trials ranged from 4 to 96 weeks (92, 94). Vitamin E can decrease ALT levels in as early as 4 weeks (92) and improve histology in 6 months (87, 88). In the largest randomized trial, PIVENS (90), vitamin E was given at a dose of 800 IU/daily for 96 weeks and compared to pioglitazone or placebo. Vitamin E and pioglitazone improved ALT, steatosis, lobular inflammation, and ballooning and resulted in resolution of NASH in many patients. Vitamin E, however, was not associated with weight gain as was pioglitazone. Of note, low vitamin E levels were found in 7% of HIV infected men in one study (95), and certain ART regimens have been associated with lower vitamin E levels (96). Earlier small studies suggested potential reduction in oxidative stress and HIV viral loads and increased viability of circulating lymphocytes with vitamin E supplementation (97, 98). Thus, there is a strong rationale to investigate vitamin E as a therapy for NASH in the HIV infected individuals.

Vitamin E for NASH in the non-HIV infected population: The efficacy of vitamin E for NASH was best established with the PIVENS study, one of the largest randomized trials in NASH to date (90). Indiana University was a major participating center in this study. Vitamin E was given at dose of 800 IU/daily for 96 weeks and compared to pioglitazone or placebo. Vitamin E and pioglitazone improved ALT, steatosis, lobular inflammation, and ballooning and resulted in resolution of NASH in a significant number of patients (Figure.4). Vitamin E has not been tested previously as a therapy for NASH in HIV infected individuals.

2.0 Rationale and Specific Aims

The aim of this study is to conduct a proof-of-concept clinical trial to evaluate the efficacy of vitamin E for treatment of NASH in HIV infected individuals.

This project is a proof-of-concept, randomized controlled trial to test the hypothesis that vitamin E improves hepatic steatosis and markers of hepatic necroinflammation in HIV infected individuals with biopsy-proven NASH. In this placebo-controlled, double-blind, randomized pilot clinical trial we will randomize 56 eligible participants at two different sites to receive 800 IU/daily of vitamin E or matching placebo for 6 months. The primary endpoint is the change in hepatic fat content from baseline to end of treatment in the two groups. Subjects will undergo MR imaging to measure hepatic fat content using MR-PDFF at baseline and at the end of treatment. In the randomization visit, all participants will receive standard recommendations about lifestyle modifications including healthy

dieting, exercise, and weight loss, and use of alcohol and dietary and herbal supplements.

Vitamin E is a well-established therapy for NASH in the non-HIV population (see section B.5 in Significance). While variable dosages ranging from 100-1200 IU/daily have been used with reported beneficial effects on liver enzymes and histology, including steatosis, the dose chosen in this trial is the same used in PIVENS, the largest randomized clinical trial establishing the efficacy of vitamin E (90) and is the dose recommended by the multi-society guidelines for treatment of NASH in the non-HIV population (75). As vitamin E effects on ALT could be seen in as early as 4 weeks (92) and on histology in 6 months (87, 88), we believe the 6 month duration of the proposed proof-of-principle study is rational. Further, there is emerging data to suggest low vitamin E level may be seen is small subset (7%) of HIV infected men (95) and in association with use of certain ART (96). Benefits reported with vitamin E use in the HIV infected patient in earlier small studies include reduction in oxidative stress and HIV viral load and increased viability of circulating lymphocytes with (97, 98). Finally, vitamin E is relatively inexpensive and has no interactions with any ART drugs. Thus, there is a strong rationale to investigate vitamin E as a therapy for NASH in the HIV infected individuals.

A total of 56 subjects at two sites will be enrolled in this trial, with approximately 28 individuals included at IU and 28 at Massachusetts General Hospital. Subjects will be randomly assigned to either Group A or Group B.

Group A: Vitamin E (RRR alpha-tocopherol) 800 IU/daily for 24 weeks.
Group B: Matching placebo for 24 weeks.

3.0 Inclusion/Exclusion Criteria

Inclusion criteria:

1. males and females ≥21 years with biopsy-proven NASH within 6 months prior to enrollment
2. histological diagnosis of NASH will be confirmed by an experienced liver pathologist before study entry
3. HIV infection
4. stable dose of anti-diabetic agents and ART in the 3 months preceding enrollment and expected by the physician treating diabetes and HIV to remain on stable medications during the study
5. stable dose of tesamorelin in the 6 months preceding enrollment
6. willingness to participate in the study
7. ability to understand and give informed consent for participation
8. willing to stop use of Vitamin E and/or multivitamins containing Vitamin E at time of enrollment and for the duration of participation in the study

Exclusion Criteria:

1. Presence of other chronic liver diseases (hepatitis B or C, autoimmune hepatitis, cholestatic liver disease, Wilson disease, hemochromatosis, etc.)
2. average alcohol consumption >3 drinks/day for men or >2 drinks/day for women in the 6 months prior to enrollment.

3. Alcohol Use Disorder Identification Test (AUDIT) score 8 or more
4. ongoing use of medications known to cause hepatic steatosis (e.g., corticosteroids, amiodarone, methotrexate, tetracycline, tamoxifen, estrogens at doses greater than those used for birth control, anabolic steroids, or valproic acid)
5. prior bariatric surgery
6. severe co-morbidities (e.g., advanced cardiac, renal, pulmonary, or psychiatric illness, as defined as at least one suicide attempt in the past 12 months)
7. allergy to vitamin E
8. use of drugs with potential effect on NASH such as ursodeoxycholic acid, S-adenosylmethionine (SAM-e), betaine, pentoxifylline, or milk thistle in the three months prior to enrollment.
9. changing doses of statins (simvastatin, pravastatin, atorvastatin, fluvastatin, lovastatin, rosuvastatin) or fibrates (clofibrate, fenofibrate) in the three months prior enrollment.
10. illicit substance abuse within the past twelve months, except for marijuana
11. breast feeding, pregnancy, inability or unwillingness to practice contraception for the duration of the study
12. contraindications for the MRI procedure (e.g., prostheses, severe claustrophobia).
13. poorly controlled diabetes with A1C >9 within in the last six months
14. use of total parenteral nutrition in the 6 months preceding liver biopsy or enrollment
15. has decompensated cirrhosis, as defined by the presence of ascites, variceal bleeding, and/or encephalopathy

4.0 Enrollment/Randomization

Participants found to have NASH will be reviewed for their eligibility to participate in this protocol. Eligible subjects will be contacted about this study and interested individuals will enter the screening phase of the protocol.

Screening period: Eligible subjects will be screened within six weeks prior to randomization. The following procedures will take place at the screening visit:

1. Complete explanation of the study, including all study visits and procedures and administration of the informed consent statement and HIPAA Authorization form.
2. An AUDIT questionnaire to determine alcohol consumption will be administered. Eligible subjects will be required to obtain a score of less than 8 on the questionnaire.
3. Current social and medical history will be thoroughly reviewed.
4. Inclusion and exclusion criteria will be verified during this visit.
5. Female subjects of child-bearing potential will have a urine pregnancy test and the pregnancy test must be negative to participate in the study
6. Information to be collected includes:
    a. age
    b. sex
    c. race
    d. ethnicity
    e. past medical and medicinal history
    f. BMI

g. waist and hip circumference measurements
h. vital signs, including blood pressure, respiratory rate, heart rate, temperature
i. historical laboratory values from medical records, including:
    i. serum electrolytes
    ii. liver profile
    iii. renal parameters
    iv. insulin
    v. glucose
    vi. A1C level
    vii. lipid profile
    viii. HIV viral load
    ix. CD4 count

Randomization-baseline evaluation visit

1. Subjects will return no more than six weeks after the completion of the screening visit for a baseline evaluation and randomization into the study. All eligible subjects who have provided informed consent and who have met all the inclusion criteria and none of the exclusion criteria will have multiple evaluations performed prior to randomization. This baseline evaluation will be conducted in a single study visit. Subjects will be asked to fast overnight and will undergo the following activities: review of medical history
2. detailed review of medications history
3. administration of the AUDIT questionnaire to capture alcohol use with a score less than 8 in order to participate in the study
4. collection of vital signs
    a. height
    b. weight
    c. blood pressure
    d. heart rate
    e. respiratory rate
    f. temperature
5. focused physical examination
6. measurement of waist and hip circumference
7. approximately 50mL of blood during a fasting state will be collected for the following tests:
    a. liver profile
    b. complete blood count
    c. lipid
    d. insulin
    e. glucose
    f. INR
    g. MDA
    h. K-18
    i. TNF-alpha
    j. IL-10
    k. alpha and gamma-tocopherols
8. female subjects of child-bearing potential will have pregnancy test and the pregnancy test must be negative to participate in the study
9. a positive urine pregnancy test will be confirmed with a serum pregnancy test

10. transient elastography (FibroScan®) will be performed by a trained technician to measure steatosis by Controlled Attenuation Parameter (CAP) and fibrosis by liver stiffness (LSM)
11. MRI will be performed to measure hepatic fat content by PDFF according to the established protocol

Randomization

At the same visit, after the baseline evaluation is completed, subjects will be randomly assigned to either Group A: vitamin E in the form of all natural RRR-alpha tocopherol at 800 IU/d for 6 months (n = 28) or Group B: matching placebo for 6 months (n = 28). We will use a stratified randomization plan based on one factor, diabetes, similar to the recent FLINT trial (142). This will ensure two treatment groups having similar distribution of diabetes. A stratum specific computer-generated randomization list will be prepared by the study statistician to randomly assign study subjects in one of the two treatment groups. A block size of four will be used to ensure balance in number of subjects recruited in the two groups. Participants will be given sufficient drug for daily dosing and will be given detailed instructions about how to take the medicine.

5.0 Study Procedures

Subjects will take the study medicine daily. They will return for a study visit at weeks 4, 12, 24 and at end of study at week 26. The study visits for weeks 4 and 12 should last about four hours and study visit at week 24 will last about eight hours, and the end of the study visit at week 26 will last about two hours. Total amount of time in the study is about 32 weeks, with six weeks allowed between the screening and randomization visits.

The following study procedures will take place at Week 4:

1. study staff will review medical records with the subject
2. subjects will be asked about any problems they are having
3. subjects will bring back unused medicine to monitor medication compliance
4. subjects will be asked about any medications taken, including supplements
5. subjects will receive sufficient amount of medicine to last until the next study visit approximately eight weeks later
6. AUDIT questionnaire will be administered to detail alcohol use and subjects must score less than 8 to continue participating in the study
7. female subjects of childbearing potential will undergo a urine pregnancy test and the test must be negative to continue participating in the study
8. vital signs will be taken, including height and weight
9. waist and hip circumference will be measured
10. subjects will undergo a focused physical examination
11. transient elastography (FibroScan®) will be performed by a trained technician to measure steatosis by Controlled Attenuation Parameter (CAP) and fibrosis by liver stiffness (LSM)
12. historical laboratory values will be captured from the medical records, including
    a. HIV viral load
    b. CD4 count

The following study procedures will take place at Week 12:

1. study staff will review medical records with the subject
2. subjects will be asked about any problems they are having
3. subjects will bring back unused medicine to monitor medication compliance

4. subjects will be asked about any medications taken, including supplements.
5. subjects will receive sufficient amount of medicine to last until the next study visit approximately twelve weeks later.
6. AUDIT questionnaire will be administered and subjects must score less than 8 to continue participating in the study
7. female subjects of childbearing potential will undergo a urine pregnancy test and the test must be negative to continue participating in the study
8. vital signs will be taken, including height and weight
9. waist and hip circumference will be measured
10. subjects will undergo a focused physical examination
11. transient elastography (FibroScan®) will be performed by a trained technician to measure steatosis by Controlled Attenuation Parameter (CAP) and fibrosis by liver stiffness (LSM)
12. historical laboratory values will be captured from the medical records, including
    a. HIV viral load
    b. CD4 count
13. approximately 50 mL of blood will be drawn to measure serum alpha- and gamma-tocopherols with the remaining amount used for future research.

The following study procedures will take place at week 24 (end of study visit):

1. study staff will review medical records with the subject
2. subjects will be asked about any problems they are having
3. subjects will bring back unused medicine to monitor medication compliance
4. subjects will be asked about any medications taken, including supplements
5. AUDIT questionnaire will be administered to detail alcohol use
6. vital signs will be taken, including height and weight
7. waist and hip circumference will be measured
8. subjects will undergo a focused physical examination
9. transient elastography (FibroScan®) will be performed by a trained technician to measure steatosis by Controlled Attenuation Parameter (CAP) and fibrosis by liver stiffness (LS).
10. approximately 50mL of blood during a fasting state will be collected for the following tests:
    a. liver profile
    b. complete blood count
    c. lipid
    d. insulin
    e. glucose
    f. INR
    g. MDA
    h. K-18
    i. TNF-alpha
    j. IL-10
    k. alpha and gamma-tocopherols
11. An MRI will be performed to measure hepatic fat content by PDFF according to the established protocol.

Approximately two weeks after the Week 24 end of study visit, subjects will return for a follow-up visit. The following procedures will take place at this visit:

1. The study staff will review medical records with the subject.

2. Subjects will be asked about any problems they are having.
3. Subjects will be asked about any medications taken, including supplements.
4. Subjects will undergo a focused physical examination.

Upon completion of this visit, subjects will have completed their participation in the study.

		Treatment Phase (weeks)				
	Screening	**Randomize**	**4**	**12**	**24**	**Follow-up**
Consent & reaffirmation	**X**	**X**				
Baseline medical history	**X**					
Interval medical history and anthropometric measurements		**X**	**X**	**X**	**X**	
AUDIT questionnaire	**X**	**X**	**X**	**X**	**X**	
Assessment of compliance			**X**	**X**	**X**	
Review of concomitant medicines	**X**	**X**	**X**	**X**	**X**	
Review of adverse events			**X**	**X**	**X**	**X**
Physical examination	**X**	**X**	**X**	**X**	**X**	**X**
Dispensing study medicines		**X**	**X**	**X**		
Blood sample collection		**X**		**X**	**X**	
Urine pregnancy test	**X**	**X**	**X**	**X**	**X**	
Liver stiffness and CAP by TE		**X**	**X**	**X**	**X**	
MR-PDFF		**X**			**X**	
Biobanking		**X**	**X**	**X**	**X**	

6.0 Reporting of Adverse Events or Unanticipated Problems involving Risks to Participants or Others

Data will be captured systematically with regards to new medications, over-the-counter medications and supplements, alcohol intake (if any), dietary intake, and changes in weight or physical activity. Patients will be instructed not to take any new medication unless reviewed with and approved by the study PI (wherever possible). During the trial, subjects will be instructed to avoid medications or supplements that may affect the primary outcome (as detailed in the inclusion and exclusion criteria).

Risks of blood draw include pain and bruising at the site. Blood will be drawn by experienced personnel.

Risks of completing the questionnaires include embarrassment or being uncomfortable with the questions asked. Subjects can refuse to answer any questions to which they do not wish to respond.

Risks of transient elastography include being uncomfortable lying supine pain, soreness at the site and a small chance of allergic reaction to the ultrasound gel. These study procedures will be performed by experienced personnel.

Risks of MRI include being uncomfortable lying still for a period of time and experiencing claustrophobia (feeling closed in or trapped).

Risks of taking Vitamin E include allergic skin reactions (inflammation or itching), blurred vision, changes in cholesterol levels, changes in insulin resistance, diarrhea, dizziness, fatigue, flu-like symptoms, headache, heart conditions, increased risk of death, increased risk of fainting or falls, increased risk of heart failure, increased risk of high blood pressure in pregnancy, increased risk of stroke, increased risk of tuberculosis, kidney dysfunction, nausea, severe response to infection (in preterm babies), sexual dysfunction, stomach pain, vision loss, and weakness.

There is a risk of loss of confidentiality.

A Data Safety and Monitoring Board (DSMB) will be organized and instituted to review study and safety data as well as reporting adverse events/unanticipated problems and will meet as necessary for a thorough review of this study. The DSMB will consist of a hepatologist, an infectious disease specialist, and a biostatistician.

7.0 Study Withdrawal/Discontinuation

It is anticipated that this study will be completed within the time frame proposed and that it will establish that vitamin E is beneficial in HIV infected patients with NASH. If vitamin E is found to exert beneficial effects on hepatic fat content and markers of NASH, it will justify undertaking larger multicenter randomized trials with histological end points to evaluate the efficacy and safety of vitamin E in this population.

Inability to meet target enrollment in a timely fashion is always a concern for any clinical trial. However, the sample size is realistic and enrollment is spread across several funding years and at two leading liver centers. People with cirrhosis have been excluded from this trial, as hepatic fat usually diminishes or disappears with progression to cirrhosis and will likely be difficult to measure at baseline in these patients. To minimize observer variability, MR-PDFF images will be centrally read by the study radiologist. Although concerns have emerged about increased risk for prostate cancer with vitamin E use in healthy men (144), this should be less of concern in HIV infected men who are at lower risk for prostate cancer than non-HIV infected men, possibly due to hypogonadism (145). Assuming 10% drop out rate with the clinical trial, about ten percent more subjects than needed to get the statistical power to evaluate the primary outcome will be recruited.

8.0 Statistical Considerations

Because host genetic factors contribute to the response to therapy, pre-therapy baseline liver expression profiles will be studied to identify expression signature that predict response to vitamin E therapy. This approach has been used in other liver disease such as hepatitis C. For example, in one study, pre-treatment peripheral blood monocytes gene expression signature predicted response to hepatitis C therapy with 94% accuracy (143).

Quantitative global gene expression analysis on fresh frozen liver tissue from subjects participating in the clinical trial will be performed. Based on prior experience, it is expected to have a portion of the liver biopsy snap frozen and available for expression analysis on a sizable proportion of the subjects (>50%) undergoing liver biopsies as part of Specific Aim 2 who are the primary source for the proposed clinical trial. As such, HumanHT-12 v4 Expression BeadChip will be used for this aim. This chip provides genome-wide transcriptional coverage of well-characterized genes and their splice variants, and targets more than 47,000 probes derived from the NCBI RefSeq Release 38 (November 7, 2009) and other sources.

After detectable transcripts are identified, expression data will be standardized. The t-test statistic will be utilized to measure the expression difference between the sample means (responders vs. non-responders) in units of the standard deviation for which the difference can be tested using certain p value. For this, a significance cut-point of $p<0.001$ will be used. Then hierarchical clustering and gene network and pathway analysis will be performed to identify those predicting response to vitamin E therapy. A logistic multivariate-based analysis will be used to build up a receiver operating characteristic (ROC) curve, and the area under the ROC curve (AUC) will be calculated to assess the average sensitivity of the biomarker in discriminating the responders and non-responders.

All endpoints are measured as the change from baseline to 24 weeks. The primary outcome measure is the change in hepatic fat content as measured by central reading of MR-PDFF performed at baseline and end of study. Secondary endpoints will be changes in inflammatory markers (liver enzymes, TNF-alpha, IL-10 levels), serum K-18, Liver stiffness and CAP measurement by TE, and MDA level as a marker of oxidative stress.

The primary outcome is the improvement in liver fat as assessed by MR-PDFF at end of treatment compared to baseline. To our knowledge, there are no data on studying the effect of vitamin E on MR-PDFF in NASH patients with HIV. Using an alpha of 5% and a two-sample independent t-test and equal variance assumption, a sample size of 25 per group will have 91% power to detect an effect size of 0.97 and will have 80% power to detect an effect size as small as 0.81. To ensure this number is achieved, 28 subjects per group will be recruited, allowing for 10% drop-out during the trial. The primary purpose of this pilot study is to generate preliminary data to estimate the mean and variability of improvement in liver fat with vitamin E in HIV infected patients with NASH.

Primary outcomes analysis: The primary analysis will be based on an analysis of covariance (ANCOVA) to compare the improvement in liver fat due to vitamin E relative to placebo. The model will include one dummy variable for treatment group indicator as well as baseline demographic characteristics and baseline liver fat. The 95% confidence intervals for the difference between the two treatment groups will be reported.

Secondary outcomes analysis: Secondary efficacy end points are continuous and as such the ANCOVA model will be used as described for analyzing the primary outcome except that the model will include the baseline value of the response variable.

9.0 Privacy/Confidentiality Issues

Data will be entered by authorized study personnel only into RedCap, which is a password protected and access restricted research data entry system.

All study visits will take place in a private room. Informed consent will be obtained only after the study is thoroughly explained to the prospective subject and all his questions have been answered.

10.0 Follow-up and Record Retention

The study will last approximately three years. Data will be stored in RedCap in accordance with NIH and Indiana University Guidelines or until such time as the records are no longer needed.