Version 5.2
September 20, 2007

A RANDOMIZED PLACEBO CONTROLLED TRIAL OF ATORVASTATIN IN HIV POSITIVE PATIENTS NOT ON ANTIRETROVIRAL MEDICATIONS WITH THE SPECIFIC AIMS OF STUDYING THE EFFECTS OF ATORVASTATIN ON HIV VIRAL LOAD AND IMMUNE ACTIVATION MARKERS

Sponsored by: National Institute of Allergy and Infectious Diseases

NON-IND Protocol

Version 5.2

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SITES PARTICIPATING IN THE MAIN STUDY

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1.0 PRECIS

This protocol is a randomized, double blind, placebo controlled trial designed to study the effects of the lipid lowering statin, atorvastatin on HIV-1 viremia.

Untreated HIV-1 infection results in an incurable, progressive immunodeficiency and death, usually from opportunistic infections. Combination antiretroviral therapy (ARV) has been successful in suppressing HIV replication and reducing morbidity and mortality. [1] Long term ARV therapy is associated with the development of HIV-1 drug resistance, and significant adverse side effects including metabolic and cardiovascular complications. [2] Prolonged therapy with certain antiretrovirals is associated with increased risk of cardiovascular disease and a number of dyslipidemic syndromes, including increased levels of cholesterol, LDL, and triglycerides in peripheral blood. [3] New therapeutic strategies to suppress HIV-1 infection are essential.

Previously, in vitro studies suggested that exposure to cholesterol-lowering statins results in decreases in HIV-1 replication.[4, 5] The mechanisms of inhibition remain uncertain, but possibilities include disrupting membrane trafficking or cytoskeletal processes necessary for intracellular transport of viral proteins, or altering cellular activation state necessary for viral gene expression.[4-8] Initial in vivo studies of the effects of statins on HIV-1 have been largely anecdotal in nature and have yielded conflicting results. [9-12] Although statin therapy is commonly used in HIV-1 infection, adverse effects from the combination of antiretrovirals and statins are possible.[13] A more thorough understanding of the effects of statins on HIV-1 replication is essential to determine the potential therapeutic effect and to investigate the risks and benefits of this approach in vivo.

We plan to conduct a double blind randomized placebo controlled trial, with a cross over design, to study the effects of atorvastatin in 22 HIV-infected patients not currently taking antiretroviral therapy. Patients will be randomized to receive either placebo or atorvastatin 80mg for 8 weeks. After a 4 week wash out period patients on the atorvastatin arm will crossover to placebo and vice versa patients in the placebo arm will cross over to atorvastatin for an additional 8 weeks. Upon completion of study medications all patients will be followed for 4 weeks. Each arm will have a minimum of 11 patients each. The primary outcome measure in this study is the effect of lipid lowering agents on HIV-1 RNA levels; additional secondary outcome

measures include effects of lipid lowering agents on lipid profile, markers of inflammation and immune activation and investigations of host and viral genetic factors.

2.0 BACKGROUND

2.1 HIV structure and replication

HIV-1 is a member of the lentivirus family of retroviruses, a group of enveloped RNA viruses that replicate via a DNA intermediate. The HIV genome is composed of genes that code for structural components, enzymes necessary for viral replication, as well as regulatory and accessory proteins. Steps in HIV-1 replication depicted in figure 1 include viral binding and interaction with chemokine receptors **(1),** membrane fusion, **(2)** viral uncoating **(3)** and reverse transcription of viral RNA **(4).** The pre-integration complex is then transported to the nucleus where viral DNA integrates into the host cell chromosome **(5)** followed by viral RNA synthesis **(6)**. The later steps include translation of viral mRNA into viral proteins, virion processing, assembly, **(7)** maturation followed by budding and release **(8).**

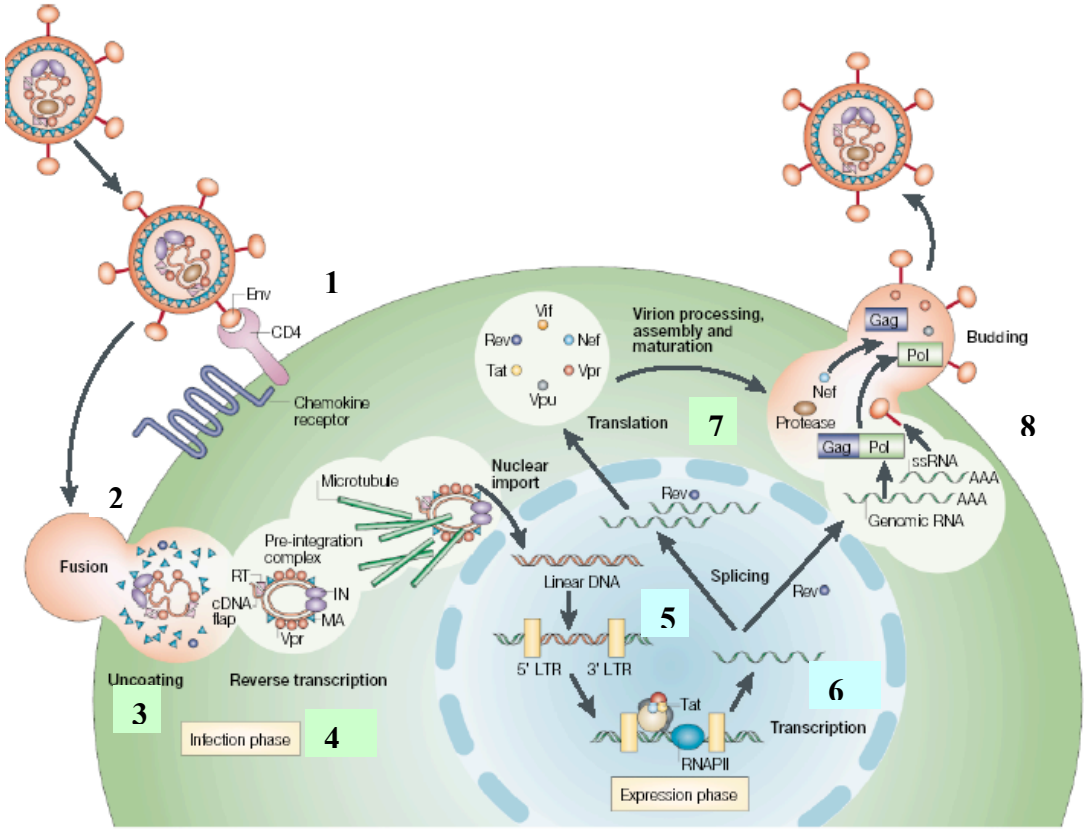

Figure 1 - Schematic representation of HIV-1 replication (adapted from the article by Peterlin and Trono [14])

2.2- Potential antiretroviral targets

Initially, antiretroviral therapy was concentrated exclusively on viral targets and focused on available inhibitors of viral enzymes reverse transcriptase and protease (Steps 4, and 7, respectively) or the viral fusion reaction (Step 1). Newer approaches include targeting interactions between host and viral factors, including inhibiting virion binding to susceptible cells with HIV-1 coreceptor inhibitors.[15] HIV populations in vivo are genetically diverse, and one effect of drug therapy is selection of viral mutants that are resistant to antiretrovirals. Emergence of drug resistance is one of the most critical factors limiting long term suppression of HIV replication. New therapeutic strategies to limit HIV replication are essential. One such approach is to target cellular processes that are essential for virus replication in such a way that virus replication is inhibited without causing critical damage to cellular functions. In one potential example of this approach, several investigators have reported that statin therapy may inhibit HIV-1 replication in vitro, either by interfering with intracellular transport or cellular activation pathways, resulting in marked reductions in HIV replication without significant cellular injury.[4-8] In vivo data evaluating this approach are lacking, and we propose to investigate the effects of statin therapy on HIV-1 replication in chronically infected individuals.

2.3 Host and viral factors in HIV assembly, budding and release

HIV virion maturation is a complex process requiring interactions between the host and the virus. One critical step in virus replication, virion assembly, requires specific host virion interactions. Virion components are transported to the cell surface using specific lipid “rafts”, cholesterol-rich cellular micro domains and bud through cell membranes by co-opting the multivesicular body (MVB) process that normally functions to recycle plasma membrane components.[16] [17] Transport via rafts is a highly specialized process that requires specific protein protein interactions; for HIV-1 transport is thought to involve both cellular and viral gene products.

Host pathways involved in transport and budding include a series of membrane- protein complexes which bind target proteins and ensure targeting to specific intracellular locations. A number of cellular proteins have been identified that participate in intracellular trafficking, including TSG101, a lipid raft constituent, which binds specific protein domains ensuring transport.

HIV-1 viral genes involved in transport are products of the HIV-1 *gag* gene. Gag is synthesized as a polypeptide precursor molecule composed of several proteins including Matrix (p17), Nucleocapsid (p7), Capsid (p24), p6, p1, p2 and a spacer protein. Expression of gag proteins appears sufficient for the assembly and release of non-infectious virion like particles. [18-20] The gag precursor, referred to as pr55gag, is composed of several domains that play specific roles in viral assembly and release. These domains include the membrane binding domain that directs virus to the membrane (M), the multimerization domain that promotes gag - gag multimerization (I) and the late domain (L) that is involved in viral budding. [21] A specific HIV-1 Gag protein, denoted p6, may provide a critical link between cellular machinery and virion component. P6 has a specific proline-threonine-alanine-proline (PTAP) domain that is thought to define the L domain and interact directly with cellular protein TSG101. [22, 23] The P6-TSG101 interact may represent the docking mechanism by which virion components are loaded onto lipid rafts. Notably, in vitro investigations studying the effects of mutations in p6 resulted in marked decreases in HIV-1 production.[24] Similarly, depletion and overexpression of cellular TSG 101 inhibits viral budding.[25, 26] Recently Carrington et al investigated the hypothesis that TSG101 may have naturally occurring variants in the human population, and that these variants may influence circulating levels of HIV-1. Interestingly, TSG 101 polymorphisms in this study appeared to have a strong association with disease progression over time (Carrington M, in preparation). In addition, analysis of genetic polymorphisms in the Swiss Cohort, a large natural history study of HIV-1 infected patients in Europe revealed an association between TSG101 genotype and disease progression .[27] These data suggest that interactions between TSG 101 and HIV may have significant clinical consequences. The virion transport mechanism using lipid rafts may represent an attractive therapeutic target. In addition to proteins such as TSG101, lipid rafts contain large amounts of cholesterol. Disruption of lipid rafts by depleting cellular cholesterol using either methyl beta cyclodextrin or the statin simvastatin results in marked reduction in HIV virion production. Furthermore the virion particles produced are not as infectious as wild type virus.[4] This suggests that lipid rafts play a critical role in HIV-1 replication and may be potential therapeutic targets for control of HIV viral replication.

2.4- Statins and their putative effects on viral replication and T cell activation

Disruption of lipid rafts may not be the only mechanism by which statins might alter viral replication. Statin drugs are competitive inhibitors of the enzyme HMG CoA reductase. This enzyme catalyzes the conversion of HMG CoA (3-hydroxy 3 methylglutaryl-coenzymeA) to mevalonate, the rate limiting step in cholesterol synthesis. By limiting synthesis of mevalonate, statins inhibit the synthesis of not just cholesterol but also of non–steroidal isoprenoid derivatives. Non-steroidal isoprenoid compounds are involved in the post-translational modification of several cell proteins including the Rho and Rac GTPases.

The Rho family of GTPases, this family includes Rac GTPases, control actin cytoskeleton remodeling in response to stimuli.[28] HIV entry into cells is an actin dependent process.[6] It is believed that interactions between HIV *env*, CD4, and its co receptors activate Rac-1 GTPase. This stimulates actin filament reorganization, which is needed for HIV fusion.[7] By blocking mevalonate synthesis, statins prevent the functioning of Rho GTPases and actin cytoskeleton remodeling. [29] Recently published data suggest that by targeting the function of Rho GTPases statins inhibit HIV-infection of SCID mice grafted with adult human PBMCs.[11]

T cell activation plays an important role in HIV progression. Depletion of CD4 positive cells during HIV infection is not merely a consequence of direct infection of these cells. T cell activation leads to T cell proliferation T cell depletion and ensuing immunosupression. [30] Statins alter T cell activation and may affect disease progression in HIV infected patients. Statins may affect T cell activation via several mechanisms including altering MHC II expression and lipid rafts disruption. T cell receptors localize within lipid rafts and disruption of lipid rafts leads to altered T cell activation. [31] [32]

2.5 Statin effects on HIV-1 RNA levels *in vivo*

In vivo data on statin use in HIV infection is limited. In a study of 6 early stage (CDC stage A1), ARV naïve patients with stable viral loads, treated with lovastatin, plasma viral load decreased with statin use. The reductions in viral load in this group of patients were between 0.2 and 1.3 $\log_{10}$ copies. Viral loads done 12 weeks after Lovastatin discontinuation demonstrated a viral rebound, with 5 of the 6 patients demonstrating a change in their set point. This study was non-randomized and did not study viral RNA levels in patients not treated with statins[11]. In a recent report, we reported analysis of patients treated with 40 mg Pravastatin as part of a study of

endothelial function. Seven patients in this study had detectable viremia allowing the authors to analyze the effects of Pravastatin 40 mg on viral load. 3/7 patients had a decrease in viral load while 4/7 had increases. We concluded that there appeared to be no significant effect of statin therapy on HIV-1 viral RNA levels in this group. Interestingly, however, 3/7 patients had rebound in viral load after discontinuation of Pravastatin.[12] Two additional studies have also failed to show any effect of statin therapy on HIV-1 viral RNA levels. In a retrospective study patients who started "first line HAART' were compared with those who started 'first line HAART" and were at some point thereafter started on statins. In this study the 2 groups did not differ in the number of patients who had viral rebound after initial suppression to less than 500 copies/ml or blips (defined in this study as a decline in viral load to <500 copies followed by a rebound to greater than 500 copies/ml and then a fall to <500 copies).[10] Finally, chronically infected patients who had discontinued antiviral therapy for 12 weeks were studied by examining the effects of Simvastatin (80mg) on HIV-1 RNA. No decreases in viral RNA were detected after 4, 8 and 12 weeks.[9] The results of this study are difficult to interpret given the missing data points.

There may be numerous reasons for the inconsistent effect of statin exposure on HIV-1 viral RNA levels; statins may be effective with certain strains of HIV-1 or with specific combinations of TSG101/p6 genotypes. Effects may not be manifest or may be blunted in patients with elevated cholesterol levels at baseline, where statin induced decreases may not sufficiently decrease intracellular cholesterol levels sufficiently to affect HIV replication. The lack of a rigorous study design for most of these studies may introduce sufficient bias to prevent clear cut results.

The 3 statins tested thus far in vivo models are pravastatin, lovastatin and simvastatin. Pravastatin is the only statin that does not have significant drug- drug interactions in HIV positive patients on antiretrovirals. However, pravastatin use is associated with modest decreases in serum cholesterol, as the decreases in serum cholesterol seen with pravastatin use may not be sufficient to result in lipid raft disruptions necessary to demonstrate any significant antiviral effects. Both simvastatin and lovastatin use is associated with significant decreases in serum cholesterol but both drugs are contraindicated with other antiretrovirals. Atorvastatin use is associated with significant reductions in serum cholesterol, and several reports suggest that the

use of atorvastatin while theoretically contraindicated with antiretrovirals is safe and well tolerated. Given this we chose to study atorvastatin for its antiviral effects.

To further define the effects of statins on HIV viremia and to study virus host interactions in the context of statin use we have designed a randomized placebo controlled cross over study of atorvastatin use in HIV positive patients. In this study the effects of cholesterol reduction on HIV-1 viremia, T cell activation, and metabolic parameters in HIV positive patients will be explored. Furthermore viral and host genetic factors as it relates to response or lack of response to statins will also be explored.

3.0 OBJECTIVES

3.1 Primary objective

The primary objective of this study is to compare the changes in HIV-1 viral load during the atorvastatin phase with the changes in viral load in the placebo phase.

3.2 Secondary objectives

- To compare the changes in the expression of immune activation markers between the placebo phase and the atorvastatin phase.
- To investigate HIV-1 and TSG 101 phenotypes among enrollees
- To investigate the changes in the metabolic profile between the placebo and the atorvastatin phase
- To investigate the safety profile of atorvastatin in HIV positive patients not on antiretroviral therapy
- To compare the subjective and objective evaluation of fatigue during the atorvastatin and placebo phase

4.0 STUDY DESIGN

Study design

This study utilizes a case cross over design to investigate the effects of atorvastatin on HIV viral load, immune activation markers and metabolic profile. Figure 2 is a diagrammatic representation of the study design. A double blind strategy was chosen for the design of this trial. In general statin therapy is associated with a number of side effects, some of which, including

fatigue and muscle aches may be highly subjective or multifactorial in origin. If statins do show an antiviral effect, then their use will be balanced by well described adverse effects. We wish to evaluate such adverse effects in detail in patients enrolled in the trial, and feel double blind approach is the most rigorous and objective.

HIV positive patients, who have been off anti-retroviral medications for a minimum of 3 months and with no documented evidence of resistance, will be recruited for this study. Each subject will have 3 baseline viral loads and will be randomized to receive either 80 mg of atorvastatin or placebo for 8 weeks. Following this, there will be a 4 week wash out phase. Upon completion of the wash out phase patients who were initially on the atorvastatin arm will switch to the placebo arm, while patients who started in the placebo arm will switch to atorvastatin to complete an additional 8 weeks. All patients will be followed for 4 more weeks upon completion of study medications. The study design is schematically represented in Figure 2.

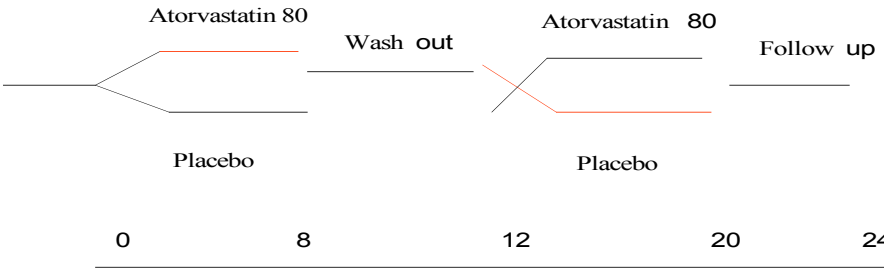

Figure 2 - Graphical representation of the study design (time represented in weeks)

5.0 SELECTION AND ENROLLMENT OF SUBJECTS

5.1 Inclusion Criteria

Establish with patient prior to Informed Consent:

- Adults 18 years of age or older
- HIV-1 infection, as documented by a licensed ELISA test kit and confirmed by a Western blot assay at any time point prior to study entry or at study entry (May do after informed consent if no test results are available).

Off all ARV for ≥ three months prior to study entry, no documented evidence of viral resistance, and no evidence of acute HIV infection. For the purposes of this study acute HIV infection will be defined as presence of a detectable HIV-1 viral RNA in the presence of a non reactive HIV-1 or HIV-2 antibody assay or an indeterminate western blot. For the purposes of this study viral resistance is being defined as having a genotypic or phenotypic evidence of resistance or in the absence of formal resistance testing clinical evidence of resistance for e.g. patients with persistent viremia in the face of adequate adherence

- Willingness to use a method of contraception during the study period. Adequate methods of birth control include: condoms, male or female, with or without a spermicide; diaphragm or cervical cap with spermicide; intrauterine device; any of the methods that require a prescription (such as contraceptive pills or patch, Norplant, Depo-Provera, and others) or a male partner who has previously undergone a vasectomy,

- Willingness to have blood drawn

- Non known allergy or contraindication to atorvastatin use
- Ability to understand and willingness to sign the informed consent
- Willingness to have blood stored for future phenotyping and genotyping

After Informed Consent:

- CD4 cell count greater than 350 cells/ml

- 3 viral loads that average greater than 1000 copies/ml within a 4 week period.

- The viral loads will be done using the bDNA method in the NIH laboratory and must be within 20% (log10bDNA of each other).

- A fasting total cholesterol lower than 240mg/dl and a LDL cholesterol lower than 130mg/dl

- Liver function tests (AST or ALT) not greater than 1.5 times the upper limit of normal. Evidence of active hepatitis B or C will not be considered an exclusion criteria if the liver function tests are within normal limits.

- Creatine phosphokinase elevations (CPK) not greater than 3 times the upper limit of normal (ULN) on two sequential determinations and, in the opinion of the investigator, without clear association with exercise.

- Laboratory values:

  Absolute neutrophil count (ANC) ≥ 1000/mm^3.

  Hemoglobin ≥ 11.0 g/dL.

  Platelet count ≥ 100,000/mm^3

  Creatinine ≤ 2 x ULN

  Serum amylase and lipase ≤ 1.25 x ULN

- Negative serum pregnancy test at randomization

5.2 Exclusion Criteria

- Pregnancy or breast feeding
- Active drug use or alcohol abuse/dependence, which in the opinion of the investigators will interfere with the patient's ability to participate in the study
- Serious illness requiring systemic treatment and/or hospitalization within 30 days of entry
- Evidence of active opportunistic infections or neoplasms that require chemotherapy during the study period except cutaneous Kaposi Sarcoma
- Allergy or hypersensitivity to atorvastatin or any of its components
- History of myositis or rhabdomyolysis with use of any statins
- History of inflammatory muscle disease such as poly or dermatomyositis
- Concomitant use of fibric acid derivatives or other lipid lowering agents including patients on statins and Ezetimibe
- Concomitant use of drugs that have significant interactions with atorvastatin. Please see appendix II for a listing,

- Concomitant use of St.Johns wort

- Concomitant use of Valproic acid

- Patients who are on concurrent immunomodulatory agents, including systemic corticosteroids will be ineligible for 3 months after completion of therapy with the immunomodulating agents. Topical, nasal or inhaled corticosteroids use is not an exclusion criteria

- Serum LDL cholesterol less than 40 mg/dl

- Vaccinations within 6 weeks of study entry.

5.3 Study enrollment procedure overview

Prior to implementation of this protocol, sites must have the protocol and informed consent forms approved by their local institutional review board (IRB). Health care providers at participating institutions will identify eligible patients. At the National Naval Medical Centers all providers and research nurses at the Infectious Disease Clinic (IDSI) will identify eligible patients. Once a candidate for study entry is identified, patients will be approached by their providers or research nurses about the protocol. If the candidate is interested details of the protocol will be reviewed with the candidate by the study PI/designee. If the volunteer is willing to participate in the study, they will be provided with copies of the informed consent to review. After all questions, about the study and the informed consent, have been answered to the participant's satisfaction, they will be asked to sign the IRB approved consent form.

Volunteers enrolled at the NIH will be required to sign a screening consent to undergo screening laboratories for the study. Patients who satisfy inclusion criteria and are willing to participate in the study will be required to sign the study informed consent. Since study participants at NNMC receive their routine care at NNMC screening consents will not be used at the NNMC prior to study enrollment. Volunteers at NNMC will be required to sign the study consent only.

It is anticipated that most subjects will have their routine immunizations prior to signing informed consent. However, in the event routine vaccination is required after the subject has been randomized; for DOD sites (NNMC and NMCSD) we will attempt to vaccinate the individual after completion of the week 8 visit or at the end of the study phase of the protocol. For subjects vaccinated during the wash-out phase or at the end of the study the wash-out phase

or the last study visit will be extended by 2 weeks. For patients seen at the NIH site, the protocol will not administer routine vaccinations, with the exception of the influenza vaccination. It is anticipated for some patients, participation may overlap with influenza season and that influenza vaccination will be necessary in the event that protocol participation begins prior to but extends into, influenza season. For such patients, standard, inactivated influenza vaccination will be administered (if necessary) during the week 8 visit. For subjects vaccinated during the wash-out phase or at the end of the study the wash-out phase or the last study visit will be extended by 2 weeks. Extension of both these study phases is necessary because of the effects of vaccination on viral kinetics.[33]

Definitions for Schedule of Events – Timing of Evaluations

5.3.1 Prerandomization evaluations

After signing either the screening/study informed consent, patients will undergo screening tests. Screening will include a complete medical history and physical examination. Laboratory testing will include a fasting lipid profile, flow studies (FACS), routine safety labs which will include a liver function test and creatinine kinase assay, a hepatitis profile and viral load assay to establish baseline viral load. Due to the variability in the viral load assay, we will utilize a minimum of 3 viral load assays to establish a baseline. All testing will need to be completed within a 4 week period prior to randomization. During this period, patients may come in for testing at their own convenience, but testing will not be done more than once within a 24 hour period. All laboratory tests except standard safety labs will be done centrally at SAIC Frederick to maintain consistency. Volunteers at the NIH who satisfy the inclusion criteria and who continue to be interested in the protocol will be now required to sign the study consent.

5.3.2 Randomization scheme

Subjects will be randomized after the investigators have ascertained that the subject meets criteria for inclusion. Prior to randomization investigators will ensure that the patient understands the risks and benefits associated with the study and informed consent has been signed. Randomization will be done using a random number table generated by the NIH pharmacy department. 11 patients will be randomized to the placebo arm of the study and 11 to the drug arm of the study initially. The 11 patients randomized to placebo will then cross over after completion of 12 weeks on study (8 weeks on study treatment and 4 weeks wash out) to the

study arm and vice versa patients in the atorvastatin arm will cross over the placebo arm after 12 weeks on study

5.3.3 Study entry

On the day patients get randomized, patients will undergo routine safety labs including liver function tests (LFT), creatinine phosphokinase, hematological parameters, fasting lipids including apolipoprotein B, hsCRP, plasma HIV viral load and a full FACS. Patients will also have a sample of blood stored for future phenotyping and genotyping. Patients will be required to answer both the Piper Fatigue Scale and the Beck Depression Inventory.

5.3.4 On study evaluations

Efficacy Assessments

Patients will undergo HIV BDNA assays on days 7, 14, 42, 56, 84, 91, 98, 126, 140 and 168. Patients will also undergo evaluation for immune activation markers levels specifically CD38 and HLA-DR expression on CD4 and CD8 cells and Ki67 will be done on days 7, 56, 84, 91, 140 and 168. Fasting lipids, apolipoprotein and ultrasensitive hsCRP will be done at randomization and on day 7, 56, 84, 91, 140 and 168. All testing other than standard safety testing will be done at SAIC Frederick. The Beck depression inventory and the Piper fatigue scale will be administered on days 56, 84, 140 and 168.

Safety assessments

Safety assessments will be done at specified study visits and will include clinical assessment, physical exam, medication assessment, routine chemistry and hematology, serum creatinine phosphokinase, liver function tests, and serum pregnancy tests. The Piper Fatigue Scale will be administered prior to and following each arm. Please see table 1 for details. All safety labs will be done at the individual institutions. All laboratories performing study related tests are CLIA certified laboratories

Genetic studies

TSG 101 genotyping and HLA typing will be performed. Additional genotyping of specific host genes will be performed as indicated. Additional blood will be obtained as indicated for storage and future use.

Adherence assessment

Pill counts will be performed at each visit to monitor adherence to the protocol, and patients will be asked about adherence.

5.3.5 Post treatment evaluations

4 weeks after treatment discontinuation (± 1 week), patients will have repeat viral load, lymphocyte sub set analysis, immune activation parameters, safety labs including LFTs and creatinine phosphokinase. All visits will be completed on the indicated days of the protocol, for unforeseeable circumstances that might render the patient unable to complete the study visit on the designated days the patient may reschedule within a 2-5 day window except for the end of study visit which may be completed within a 1 week window.

5.4 Subjects can be discontinued from the study for the following reasons:

- Failure to comply with the protocol
- If they develop a serious adverse event (SAE)
- Pregnancy, if a patient becomes pregnant on study; she will stop the study drug and be followed as part of the study through the pregnancy including the peri-partum period to ensure her safety.
- **Any subject with a creatinine phosphokinase (CPK) value greater than 10 times the upper limit of normal (i.e grade 3 or higher), will undergo repeat testing. Repeat testing will be conducted at protocol specified time points (i.e. within 1-3 days of the initial result and also at day 7). Persistently elevated laboratory values, i.e. CPK measurements that remain at the same level or are higher than the initial value, on three consecutive determinations, despite cessation of exercise will result in study drug discontinuation.**
- **In addition, all CPK elevations associated with grade 3 or higher symptoms or for significant symptoms or elevations in CPK that do not fall into either of the above mentioned categories; a conference call with representatives from all three participating sites (either the PI or their designee) will be initiated. Decisions regarding study drug discontinuation for patients meeting the above listed criteria will be made on an individual case by case basis. The medical monitor at the sites will be involved in all decisions regarding study drug discontinuation.**

- Patient develops liver function elevations 3 times the upper limit of normal
- If the serum LDL cholesterol falls to less than 40mg/dl
- Develops a grade 2 or greater allergic reaction to the medicine or develops signs and symptoms of an immediate hypersensitivity reaction to the medication
- Patient needs to start antiretroviral agents using current DHHS guidelines as recommendations.
- Patient develops any condition that the study investigators deem would be detrimental for the patient to continue on the study
- If the patient desires to leave the study
- Patient starts taking other lipid lowering drugs including fibric acid derivatives, bile acid sequestrants and niacin preparations
- Patients will be dropped from the study if their CD4 count drops by fifty percent on two successive determinations, or falls below 250 cells/µl in the absence of any documented ongoing infections/inflammatory processes while on study

If subjects are discontinued from the study secondary to any of the above given reasons they will be replaced according to the prior randomization.

CLINICAL AND LABORATORY EVALUATION

Evaluation*	Screening	Randomization	D7 W1	D14 W2	D42 W6	D56 W8	D84† W12	D91 W13	D98 W14	D126 W18	D140 † W20	D168 W24
Documentation of HIV	Pos ELISA and Confirmatory Blot, previous results will be allowed											
Medical /Medication history	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nadir CD4	✓											
Clinical Assessment	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Questionnaires		✓				✓	✓				✓	✓
Physical Exam	✓	✓				✓	✓				✓	✓
Hepatitis profile	✓											
Fasting lipid profile	✓	✓	✓			✓	✓	✓			✓	✓
Markers of inflammation		✓	✓			✓	✓	✓			✓	✓
Hematology	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓
Chemistry including CK	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓
Liver Function Tests	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓
Urinalysis	✓	✓				✓	✓				✓	
Pregnancy Testing	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Full FACs	✓	✓	✓			✓	✓	✓			✓	✓
HIV viral load (bdna)	✓ (3 viral load measures will be used to establish baseline)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Statin/placebo		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Stored Plasma/PBMC		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Stored Sera		✓				✓	✓				✓	

- * Study consent will be signed at NNMC prior to screening and at randomization at the NIH
- † In the event subject is vaccinated these visits will be extended by 2 weeks

Table 1- Schedule of events

5.5 Breaking the blind

In the event of a medical emergency or in the event an off study criterion is met where knowledge of the treatment will affect medical management, the investigators after consulting with the medical monitor can break the blind. To break the blind investigators will contact the pharmacy department by pager and via electronic mail, as they will hold the randomization code.

In the rare event of such an occurrence the process will be documented in writing. If the DSMB decides to terminate the study, the blind will be broken and study participants and their primary physicians will be informed of their drug assignments.

5.6 Criteria for Stopping Enrollment

Once patient enrollment is completed the study will be closed for further enrollment or if the DSMB decides to terminate the study prior to complete enrollment. The DSMB will meet after half the participants in the clinical trial have completed the study. Alternatively they will also convene if two study patients have grade III or higher adverse events that in the opinion of the investigator are definitely, possibly or probably associated with the drug. Please note: unlike other lab AE's that would necessitate convening a DSMB if 2 subjects have a grade 3 or higher adverse event, the DSMB would convene only if the grade 3 CPK elevations are persistent.

6.0 STUDY TREATMENT

STUDY DRUG

6.1 Atorvastatin

Metabolism and mechanism of action

Atorvastatin calcium belongs to a group of lipid lowering agents referred to as statins. Atorvastatin is a selective competitive inhibitor of the enzyme 3 hydroxy-3methylglutaryl coenzyme A (HMG CoA reductase). This enzyme catalyzes the conversion of HMG CoA to mevalonic acid, the rate- limiting step in cholesterol biosynthesis. This class of drugs have been utilized in both the primary and the secondary prevention of coronary artery disease (CAD) [34] [35] [36].

Atorvastatin is orally administered and is well tolerated. Atorvastatin is metabolized by the cytochrome P450 3A4 system to ortho and para hydroxylated metabolites. Atorvastatin and its metabolites are primarily hepatically excreted [37]. The metabolism of atorvastatin may be influenced by CYP 3A4 inducers and inhibitors.

Adverse effects of Atorvastatin

The most common side effects associated with atorvastatin use are gastrointestinal. In clinical trials the following events were reported as per the package insert in greater than 2% of the cases: chest pain, nausea, bronchitis, rhinitis, arthritis, insomnia and dizziness, urinary tract infection and peripheral edema.

Atorvastatin and effects on liver enzymes

Persistent elevations in liver enzyme to 3 times the ULN have been reported in 0.2-2.3% of patients studied on clinical trials of atorvastatin. Upon discontinuation of the drug these elevations are reversible. Fatal liver injury has been reported with atorvastatin use [38]. Overall it appears that fatal liver injury is a rare event in patients using statins. Liver function abnormalities associated with atorvastatin use are seen at all doses. However, the incidence of these abnormalities is greater with higher doses of the drug.

In a recently reported study of secondary prevention of CAD, 10,001 patients were randomized to receive either10 or 80 mg of atorvastatin. In this study persistent elevation in liver enzymes were seen in less than 1% of all patients, however the incidence was significantly higher in the 80 mg subgroup (1.2% vs 0.2%). [39]

The package insert states that the use of atorvastatin is not recommended in patients with active liver disease. Serial monitoring of liver function tests prior to start of drug, 6 to 12 weeks after and semi annually thereafter is advised by the drug manufacturers. More stringent monitoring at shorter intervals will be done as part of this protocol.

Atorvastatin and myopathy

Myalgia and rhabdomyolysis have been reported with atorvastatin use. In the ASCOT study there was a single case of non-fatal rhabdomyolysis among 5168 patients randomized to atorvastatin. [40] In a study of secondary prevention of CAD, among 10,000 patients randomized to receive either 10 or 80 mg of atorvastatin there were 5 cases of rhabdomyolysis.[39] While rhabdomyolysis is a complication of atorvastatin it appears relatively rare.

The risk of myopathy is increased when atorvastatin is used in conjunction with azole antifungals, erythromycin, clarithromycin, niacin, fibric acid derivatives or cyclosporine [37]. Patients who use fibric acid derivatives, niacin and cyclosporine will not be enrolled into the

study. Patients who start azoles or erythromycin or clarithromycin while on study will be monitored closely for signs and symptoms of myopathy, patients on these drugs at the time of enrollment will not be eligible for this study until completion of therapy with these agents.

In addition the risk of myopathy is increased with the concomitant use of grape fruit juice and atorvastatin. These interactions are mediated via the cytochrome p 450 system; grape fruit juice is an inhibitor of this system [41] While on study patient will be advised to avoid grape fruit juice and grape fruit.

To reduce risk to our study patients we will monitor liver function (LFT) and creatinine phosphokinase (CPK) at baseline and as indicated in the schedule of events. Patients will also be questioned about myalgia or weakness at each visit. Presence of any of these symptoms will require complete evaluation as judged suitable by their health care provider. Patients who participate in this study will be asked to refrain from vigorous exercise regimens for the study period. Vigorous exercise is known to increase CPK levels and may cause patients to be unnecessarily excluded /withdrawn from the study.

- Pregnancy and breast feeding are contraindications for atorvastatin use; atorvastatin is classified as a pregnancy category X drug. Pregnant or nursing women will not be enrolled in this study and patients will be required to use reliable methods of contraception to be enrolled in this study. If a patient becomes pregnant on study, she will be asked to stop taking study drug and will be followed through her pregnancy and the peripartum period.

6.2 Product Supply, Distribution, and Pharmacy

Both drugs (i.e. atorvastatin and Placebo) will be formulated by the NIH PDS and will be supplied to both the sites. Both placebo and drug will be identical in appearance. They will be packaged and labeled with the study number, and will have a space where the subjects initial and randomization number will be entered.

6.3 Drug Storage

Both drugs and placebo can be stored at room temperature in a locked storage area.

6.4 Accountability of drugs

Some drug records will be maintained centrally by the NIH PDS such as shipping records. The individual sites will also maintain drug records locally such as drug dispensing and

accountability records. Drugs will be dispensed to the sites by NIH PDS. Patients will be required to pick up medications per visit schedule.

7.0 STATISTICAL CONSIDERATION

This is a double blind randomized crossover placebo controlled trial of statin use in HIV positive patients with the specific aim of studying the effect of statins on HIV viral load. 22 chronically infected HIV positive patients will be randomized to either atorvastatin 80 mg followed by placebo or placebo followed by atorvastatin (11 per sequence). After 8 weeks of treatment and another 4 weeks washout period, patients in the placebo arm will receive atorvastatin 80mg, whereas patients in the atorvastatin arm will be followed on placebo for another 8 weeks. Table 2 displays the treatment sequence for the two groups.

(Week 0-7)	Week 8-11	(Week 12-19)
Atorvastatin 80mg	Washout	Placebo
Placebo	Washout	Atorvastatin 80mg

Table 2

The effect of lipid lowering agents on HIV-1 RNA levels will be evaluated at the end of 7 weeks and at the end of 19 weeks. The primary goal of this study is to assess the treatment effect by assuming that the carry-over effect is negligible. The primary strength of this cross-over trial is increased efficiency because each patient serves as his or her own control, and the within-subject variability is usually less than the between-subject variability. As a result, the sample size for a cross-over trial will be lower than that for a comparable parallel group design. There is a possible drawback of this design, however, if the carry-over effect cannot be ignored.

Based on historical data we estimate the standard deviation of the log10 viral load as.74 and the correlation coefficient of log10 viral loads before and after treatment as 0.83. To be conservative, a correlation coefficient of 0.75 is used in our calculation. The paired t-test is used

for power calculation. A trial of 20 subjects has a power of 77% (with type I error 0.05) for detecting a 1/3 log10 drop in viral load with atorvastatin. Power to detect a change of 0.4 or 0.5 in log10 viral load based on 10 patients per sequence is 90% and 98% respectively. Thus we have excellent power to detect at least .4 log10 viral load difference by the treatment and reasonable power to detect a 1/3 log10 viral load difference. Due to the possible loss to follow-up (10%), 11 patients will be accrued in each sequence.

At the end of this study, the paired t-test will be used to test this hypothesis. If the underlying distribution is clearly not close to a normal, then the sign-rank Wilcoxon test will be used instead.

Secondary Objectives for Statistical Consideration

1- To study the difference in the expression of immune activation markers (i.e HLA DR CD38 and Ki67 expression both percentage and number on CD4+ and CD8+ cells) between the end of 7 weeks and the end of 19 weeks in the placebo and atorvastatin sequence. All comparisons of changes between the end of 7 weeks and the end of 19 weeks, i.e. the difference between months 19 and 7 or months 7 and 19 will be conducted by using the paired t-test if the measurements are continuous. McNemar statistic will be used for the changes of discrete markers.

Because of the randomization, the baseline measurements (at beginning of this trial) in placebo and atorvastatin sequences should have the same distribution. This information can be incorporated into an additional statistical analysis, called analysis of covariance ANCOVA. A linear mixed effects model can be used to analyze the difference between the change of viral loads evaluated at the beginning of this trial and at the end of the 7^{th} week, and at the end of 11^{th} week and 19^{th} week in atorvastatin and placebo sequence and placebo and atorvastatin sequence. The difference of viral load at the end of 11^{th} week in two treatment sequences can be used to assess whether there is a treatment carryover effect.

Additional analyses will be conducted to assess whether there is any effect of drug on change viral load, beyond that predicted by the change in LDL.

Finally the correlation between reductions in serum cholesterol and HIV viral load in treatment arm will be assessed by using Spearman's correlation coefficient statistics.

8.0 DATA COLLECTION AND MONITORING AND ADVERSE EXPERIENCE REPORTING

8.1 Adverse and Serious Adverse Experience (SAE) reporting

8.1.1 Definition

An adverse event is defined as "undesired and unintended, though not unexpected, result of therapy or intervention".

An adverse event may also be defined as any **"**unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure. These include both anticipated and unanticipated risk".A serious adverse event (SAE) is one "that results in death, is life threatening or results in inpatient hospitalization or prolongs the existing hospitalization, results in a persistent or significant disability of incapacity, or results in congenital anomaly / birth defect". A serious adverse event may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient/study participant or may require intervention to prevent one of the other outcomes listed above."

8.1.2 Reporting

All adverse events regardless of treatment group or relationship to the drug will need to be reported to the necessary IRB as per their requirements. As reporting requirements are somewhat different at NIAID and NNMC we will follow reporting requirements at both institutions.

All SAE will be reported within 24 hours of when the investigator learns of this event. to both the NIAID and the NNMC IRB. In addition to the above listed event any event that in the investigators judgment is serious even if it does not meet the above criteria will be reported as such.

Adverse events will be graded as per the attached toxicity table in Appendix 1. For toxicities not listed in the table, grading will be based on the schema provided below

- Grade 1: Transient or mild discomfort; no limitation in activity; no medical intervention/therapy required.

- Grade 2: Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required.
- Grade 3: Marked limitation in activity, some assistance usually required; medical intervention/therapy required hospitalization possible.
- Grade 4: Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required hospitalization or hospice care.

Adverse events will also be assessed for their relation to study medications and procedures.

Patients coming to the NIH clinical center often travel long distances and are hospitalized for procedures that are routinely done as outpatients at other hospitals. Such elective hospitalizations will not be reported as a SAE or an AE.

For all AEs, the clinician who examines and evaluates the subject will determine the adverse event's causality based upon the temporal relationship to administration of the study agent, the pharmacology of the study agent, and his/her clinical judgment. Terms to describe the degree of causality between the study agent and an event will be definitely, probably, possibly, unlikely related or not related. The best estimate at the time of reporting of the causal relationship between the experimental intervention and an adverse event and the degree of certainty about causality will be graded as follows:

Definitely Related: The adverse event and administration of study agent are related in time, and a direct association can be demonstrated (e.g., disappears or decreases with reduction in dose or cessation of drug/investigational product and recurs with re-exposure).

Probably Related: The adverse event and administration of study agent are reasonably related in time and/or follows a known pattern of response, and the adverse event is more likely explained by study agent than other causes.

Possibly Related: The adverse event and administration of study agent are reasonably related in time and/or follows a known pattern of response, and the adverse event can be explained equally well by causes other than study agent (e.g., could readily have been produced by the subject's clinical state or could have been due to environmental or other interventions).

Unlikely Related: A potential relationship between study agent and the adverse event could exist (i.e., the possibility cannot be excluded), but the adverse event is most likely

explained by causes other than the study agent (e.g., could readily have been produced by the subject's clinical state or could have been due to environmental or other interventions).

Not Related: Adverse event is clearly due to extraneous causes (e.g., underlying disease, environment) or exposure to the investigational product has not occurred. Such events MUST have an alternative, definitive etiology documented in the patient's medical record.

Therefore, a suspected adverse drug/investigational product reaction would be one that is categorized as definitely, probably, possibly, and unlikely related to a study drug/ investigational product".

Please see attached appendix for the toxicity table. All adverse event reporting will end 4 weeks after the end of the study treatment.Any new toxicities not previously reported will be submitted to Med-Watch.

As the study is being done at both the NIAID and at the NNMC reporting requirements for both sites will be followed these include reporting all SAE within 24 hours after becoming aware of a subject death or a potentially life threatening serious adverse event. This will meet the reporting requirements of both the NIAID and the NNMC IRB, as the NIAID requires reporting within 7 days and the NNMC requires reporting within 24 hours of knowledge of death or potentially life threatening SAE. A completed NIAID IRB serious adverse event report will be submitted to the NIAID IRB within 15 days after becoming aware of an inpatient hospitalization (other than elective), a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Investigators will report within 15 days any other event or condition regardless of grade, which in their judgment represents an event reportable to the IRB. All other serious adverse events will be reported to the NIAID IRB at the time of the annual review. In addition a summary of all adverse events will be reported to the NIAID IRB with submission of a request for continuing review.

8.2 Protocol monitoring and DSMB

Day to day protocol management will be provided by the PI at the NIH and by the sponsors of the study at NNMC. As this is a randomized placebo controlled trial, a DSMB will monitor the progress of the trial. The DSMB will be constituted through the NIAID. The DSMB will evaluate data after enrollment and completion of study by 50% of the study patients.

The trial will be conducted in compliance with this protocol, International Conference on Harmonization Good Clinical Practices (ICH/GCP) and all applicable regulatory requirements. Monitors under contract to the NIAID will visit the clinical research site(s) to monitor all aspects of the study in accordance with the appropriate regulations. The objectives of a monitoring visit will be: 1) to verify the prompt reporting of all data points, including reporting SAEs, checking availability of signed informed consents; 2) to compare individual subject records and the source documents (supporting data, laboratory specimen records and medical records to include physician progress notes, nurse' notes, subjects' hospital charts); 3) to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit findings. The investigator (and/or designee) will make study documents (e.g., consent forms) and pertinent hospital, CRFs or clinical records readily available for inspection by the local IRB, the site monitors, and the NIAID staff for confirmation of the study data

8.3 Data and Safety Monitoring Plan.

Day to day protocol management will be provided by the PI at the NIH and by the sponsors of the study at NNMC. As this is a randomized placebo controlled trial, a DSMB will monitor the progress of the trial. The DSMB will be constituted through NIAID. The DSMB will evaluate data after 50% of the study patients have completed study.

8.4 Data management plan

"Study data will be collected and maintained on standardized Case Report Forms (CRF). The study investigators will be responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of a piece of data) should support the data collected on the case report form, and be signed and dated by the person recording and/or reviewing the data. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents include, but are not limited to, the patient medical records, laboratory reports, ECG tracings, x-rays, radiologist reports, patient diaries, biopsy reports, ultrasound photographs, patient progress notes, pharmacy

records and any other similar reports or records of procedures performed during the subject's participation in the protocol. Data for CRFs will be collected during patient visits, phone calls with subjects and health care providers, patient diaries and abstracted from the medical record. It is not acceptable for the CRF to be the only record of the patient participation in the study. This is to ensure that anyone who would access the patient medical record has adequate knowledge that the patient is participating in a clinical trial."

9.0 HUMAN SUBJECTS

Institutional Review Board (IRB) Review and Informed Consent

The protocol and any subsequent changes and the informed consent will be reviewed by the IRB before initiation of the study. The informed consent will explain the study, the procedures and the risks and benefits associated with the study. The study participants will be provided copies of the informed consent for review, any questions that he/she may have about the study will be answered prior to the final signing of the informed consent. A copy of the consent form will be given to the subject and the informed consent process will be documented.

HIV infection in pediatric patients may represent a somewhat different disease; with greater contributions from lymphoid organs such as thymus. We do not know whether children and adults will represent a uniform group mechanistically, and we propose to study adults (≥ 18y). As we obtain additional information, we may consider a pediatric study, although children with HIV infection are no longer seen at NIH and such studies would be carried out in collaboration.

9.1 Benefit

There is no immediate benefit to the patient. However, a potential benefit may be a reduction in their viral load and temporary improvement in their lipid profile.

9.2 Risks

9.2.1 Medication associated risk

Atorvastatin

Per the package insert the following warnings are associated with atorvastatin use

Liver dysfunction-"persistent elevations (>3 times the upper limit of normal (ULN) occurring on 2 or more occasions) in serum transaminases occurred in 0.7% of patients who received

atorvastatin in clinical trials. The incidence of these abnormalities was 0.2%, 0.2%, 0.6% and 2.3% for 10, 20, 40 and 80 mg respectively." Dose reduction, interruption or discontinuation of atorvastatin resulted in reversal of these abnormalities.

Skeletal muscle- "rare cases of rhabdomyolysis with acute renal failure secondary to myoglobinuria have been reported with atorvastatin use and with drugs in this class"

The following events have also been reported among patients in clinical trial of atorvastatin at a frequency greater than 2% this include chest pain, nausea, bronchitis, rhinitis, insomnia, dizziness, arthritis, urinary tract infection, peripheral edema. [42]

For events that were noted in less than 2% of the cases please refer to package insert appendix 2

9.2.2 Risks Associated with Hypocholesterolemia

Animal studies

As per the package insert a single female dog treated for 3 months with atorvastatin at a dose of 120mg/kg/day, which would result in an exposure that is 16 times that seen in humans, resulted in brain hemorrhage in the dog. Another female dog treated with escalating doses of atorvastatin to a max of 280mg/kg/day suffered optic nerve vacuolation and brain hemorrhage.

In animal studies treatment with HMG CoA reductase inhibitors has resulted in neurotoxicity. In normocholesterolemic dogs given doses of 180 mg/kg/day of lovastatin, this dose is 180 times the normal therapeutic dose in humans, there was evidence of neurotoxicity in 37% of the animals. These dogs demonstrated a hemorrhagic encephalopathy. [43]

In a recent study designed to study the effects on coronary outcomes of lowering LDL cholesterol to less than 100 mg/dl in patients with established coronary artery disease, 10,001 patients with established CAD were randomized to receive either atorvastatin 10 or 80 mg. The median follow up in this study was 4.9 years. The mean LDL cholesterol achieved in the atorvastatin 80 mg sub group was 77 mg/dl in this study. The authors of this study concluded that there was no difference in reported adverse effects in those with LDL cholesterol less than 70 mg/dl.[39] The results of this study seemed to suggest that the reductions in LDL cholesterol achieved in this population were well tolerated. However, not much is known about the effects of temporary reductions in cholesterol and LDL in normo-cholesterolemic HIV positive patients. Given the above animal data we will follow all patients on study and four weeks after

discontinuing placebo/statin for possible CNS and optic toxicities by targeted history and physicals and laboratory testing as necessary.

There have been case reports of CNS side effects with statin use. These include reports of memory loss, irritability and polyneuropathy. [44-46] These associations have however, not been borne out in large clinical trials.

9.2.3 Risks Associated with Phlebotomy

Risks include bleeding, bruising, infection and syncope (fainting). Fainting and complications thereof are rare but well recognized complications of phlebotomy. To minimize risk, patients will undergo phlebotomy in the sitting position and will be monitored for 10 minutes after the procedure.

9.3. 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. The link to the patient will be maintained at the site in a locked cabinet. All computer entry will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NIAID, the OHRP, the pharmaceutical supporter(s), or the supporter's designee. At the completion of the study the link will be destroyed and the remaining samples will be stored without any link.

9.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NIAID, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected

9.5 Sample Storage

This section describes the storage and use of samples in this study.

Samples and data collected under this protocol may be used to study _HIV infection, immune disorders and interactions of HIV and host immune metabolic and genetic factors. Genetic testing will be performed. Access to research samples will be limited using a locked room or a locked freezer or both. Samples and data will be stored using codes assigned by the

investigators or their designees. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data. Samples obtained at NIH will be tracked in the Crimson system. Samples will be stored and tracked utilizing the NCI FCRF REPOSITORY operated by SAIC FREDERICK. In the future, other investigators (both at NIH and NNMC) may wish to study these samples and/or data. In that case, IRB approval must be sought prior to any sharing of samples. Any clinical information shared about the sample with or without patient identifiers would similarly require prior IRB approval. At the completion of the protocol (at termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol or a repository.

The NIH Intramural Protocol Violation definition related to loss of or destruction of samples (for example, due to freezer malfunction) will be followed in reporting to the IRB: The violation compromises the scientific integrity of the data collected for the study.

Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to the IRB.

Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to both the subject and to the IRB. This decision may not affect the subject's participation in this protocol or any other protocols at NIH.

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