Effect of Interferon Alpha 2b Intensification on HIV-1 Residual Viremia in Individuals Suppressed on Antiretroviral Therapy

Short Title: Potential for Interferon Therapy in HIV-infected and ARV- suppressed Participants (PITHA)

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TABLE OF CONTENTS

TABLE OF ABBREVIATIONS

AIDS	Acquired immunodeficiency Syndrome
ART	Antiretroviral therapy
BDS	Beck Depression Inventory Score
DHHS	Department of Health and Human Services
FDA	Food and Drug Administration

G-CSF	Granulocyte colony stimulating factor
HCV	Hepatitis C virus
HIV-1	Human immunodeficiency virus type 1
HRPP	Human Research Protection Program
IRB	Institutional Review Board
INF	Interferon
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
PBMC	Peripheral blood mononuclear cells
PEG	Polyethylene glycol
PEG-INTRON	Pegylated interferon alpha 2b
PI	Principal investigator
PrI	Protease inhibitor
SCA	Single copy assay (viral RNA)

Précis

As a result of combination antiretroviral therapy (ART), morbidity and mortality from acquired immunodeficiency syndrome has declined significantly in the past 15 years, at least in developed countries. Human immunodeficiency virus type 1 (HIV-1) infected individuals now live longer, but must undergo continuous therapy that has substantial consequences on quality of life.

ART suppresses HIV-1 viremia below the limits of detection in current commercial assays (c. 50 copies/mL plasma)., but HIV viremia persists even after prolonged suppressive therapy. The origin of this residual viremia is yet not clear, but data suggest that production from long lived HIV infected cells may contribute to viremia. Antiretrovirals are extremely active against replicating cells, and can thus successfully stop viral replication, but have no effect on long-lived viral reservoirs of cells already infected with HIV-1 at the time antiretroviral therapy is initiated. As a result, new strategies are necessary to reduce or eradicate long-lived reservoirs. Interferon alpha is a natural cytokine with antiviral activity. Prior to the introduction of antiretroviral therapy, several studies demonstrated modest effect of interferon alpha in HIV-1 viremia in active cycles of infection in infected individuals. Interferon alpha was also effective *in vitro* in decreasing virus production from cells chronically infected with HIV-1. With the introduction of potent antiretroviral therapy, interferon was not developed as a direct anti-HIV drug. Interferon alpha is relatively effective in therapy of hepatitis C virus (HCV) infection, and has been used in HIV-1/HCV coinfected individuals. Kottilil and coworkers in the Laboratory of Immunoregulation NIAID have shown a decrease in HIV-1 RNA levels in HCV coinfected participants treated with pegylated interferon alpha and ribavirin. In stored samples from that study, we conducted a retrospective trial on samples from participants with HIV-1 RNA levels of <50 copies/mL, showing a further reduction in residual viremia using an ultrasensitive Single Copy Assay (SCA) developed in our laboratory. As such the effects of interferon on HIV viremia and cell associated HIV RNA are of growing interest.
In this protocol we will conduct a prospective, non-randomized, single arm, pilot study to investigate the effect of pegylated interferon alpha 2b on HIV-1 RNA levels as an additional drug in participants undergoing suppressive antiretroviral therapy with viral RNA levels suppressed to less than 50 copies/mL plasma. As patients may have levels of HIV RNA that are lower than our limit of detection, we will also investigate levels of HIV nucleic acid species in cells as well We will determine whether interferon alpha therapy will reduce residual viremia or cell associated HIV RNA in participants on suppressive ART, which will expand our understanding of persistent low-level viremia and the pathogenesis of HIV in infected individuals.

1 Introduction

The human immunodeficiency virus type 1 (HIV-1) is responsible for the development of acquired immunodeficiency syndrome (AIDS) that results in progressive immune system deterioration and death. Therapy treating HIV (antiretroviral therapy or ART) by inhibiting viral replication and allowing immune restoration, has dramatically reduced AIDS-related mortality, but is not curative (Palella, Delaney et al. 1998, Egger, May et al. 2002). After introduction of ART, viral RNA levels in the blood decline significantly. The decay of HIV-1 replication in the presence of an effective ARV follows a biphasic pathway (Shen and Siliciano 2008): a first rapid decay of the cells that produce >90% of plasma virus is followed by a second slower decline in viremia. Several studies have demonstrated that virus declines in plasma because new rounds of virus replication are interrupted by ART and because the cells producing virus die. As a result, the decline in viremia reflects the death rate of virus-infected cells. The first phase of decline in viremia involves mostly activated CD4+ T lymphocytes that have a very short life, surviving only about 1 day; the second phase involves mostly infected macrophages and partially activated CD4+ lymphocytes that have an half life of about 2 weeks.

Decline in viremia is measured by commercially available assays that measure HIV-1 RNA. The current limit of plasma HIV-1-RNA detection is 50 copies/mL, and this is the threshold that international guidelines use as the gold standard for therapy efficacy (Clumeck, Pozniak et al. 2008, DHHS 2008). Using ultrasensitive HIV-1 RNA assays, with sensitivity of HIV-1-RNA down to 1 copy/mL, it is possible to detect persistent HIV-1 viremia even in participants undergoing successful treatment for prolonged periods with ARV (Di Mascio, Dornadula et al. 2003, Maldarelli, Palmer et al. 2007). In participants who have undergone an effective ARV regimen for 7 years, we showed an additional third and a fourth phase of viral decay in these participants.

The set point of residual viremia is predicted by the pre-therapy HIV-1 RNA level; participants with pre-therapy HIV-1 RNA levels greater than 100,000 copies/mL had persistent detectable HIV-1 RNA levels over time (Palmer, Maldarelli et al. 2008). The origin of this residual viremia is uncertain. One possible source is an ongoing replication of viral strains spreading infection to uninfected cells (Zhang, Ramratnam et al. 1999, Havlir, Strain et al. 2003). Ongoing replication may occur if drug therapy does not completely inhibit virus replication, or if virus replication takes place in anatomic sanctuary sites in the body where antiretroviral drugs do not penetrate. Alternatively, HIV-1 may be produced from viral genomes integrated in cells from long-lived HIV-1 reservoirs (Palmer, Maldarelli et al. 2008, Shen and Siliciano 2008). Antiretroviral intensification studies revealed no decrease in HIV RNA levels after introduction of an additional potent antiretroviral(Dinoso, Kim et al. 2009, McMahon, Jones et al. 2010, Besson, McMahon et al. 2012), suggesting ongoing replication was not the source of persistent HIV present during therapy. Increases in 2LTR circles in some patients suggest that low level replication events may occur in some patients (Buzon, Massanella et al. 2010, Hatano, Hayes et al. 2011, Hatano, Strain et al. 2013). These data suggest that although replication events may occur, the predominant source of persistent viremia during therapy

is chronic viral production from long-lived cells, and strategies other than conventional antiretroviral therapy are needed to eradicate HIV-1 from infected individuals. In order to investigate the nature of the HIV-1 reservoir we plan to use the intensification strategy with novel antiretroviral therapy.

One possible candidate in this strategy is the antiviral immune modulator interferon alpha (INF). Interferon alphas (interferon-alpha 2a and 2b) are a class of compounds known for their antiviral, immune-activating and immune-suppressing properties (Tilg 1997, Patel and McHutchison 2001, Tilg and Kaser 2004, Stetson and Medzhitov 2006). The addition of polyethylene glycol (PEG) to interferon increases its half-life in the body. PEG-Interferon alpha 2b, administered with ribavirin, is Food and Drug Administration (FDA)-approved for the treatment of chronic hepatitis C. Several clinical trials have shown its efficacy (especially in association with ribavirin) against HCV (Lindsay, Trepo et al. 2001, Manns, McHutchison et al. 2001). In addition, interferon alpha 2b is FDA-approved for the treatment of: hairy cell leukemia, malignant melanoma, follicular lymphoma, condylomata acuminata, AIDS- related Kaposi sarcoma, and chronic hepatitis B and C. In vitro studies conducted with HCV-infected cell-culture systems showed that INF exerts its activity by inducing a non-virus-specific antiviral state within the cell by activating a number of INF-responsive genes (Sen 2001, Bekisz, Schmeisser et al. 2004, Jiang, Guo et al. 2008). Exogenous administration of INF has a similar inducing effect, leading to a rapid reduction of HCV-RNA levels (Guo, Bichko et al. 2001, Zhu, Zhao et al. 2003). Treatment with INF 2b or its pegylated form and ribavirin resulted in a successful clearance of hepatitis C in infected participants (Lindsay, Trepo et al. 2001, Manns, McHutchison et al. 2001), and the level of expression of genes related to INF response, immune cell signaling, defense response, and cell death has been associated with treatment outcome (Lempicki, Polis et al. 2006).

With respect to HIV, INF has shown antiviral activity both in vitro (Ho, Hartshorn et al. 1985) and in vivo (Lane, Kovacs et al. 1988, Hatzakis, Gargalianos et al. 2001). The major activity of INF against HIV is thought to be the inhibition of the release of progeny virions from host-infected cells (Poli, Orenstein et al. 1989, Gottlinger, Dorfman et al. 1991). This anti- HIV-1 activity is opposed by the viral protein Vpu (Gottlinger, Dorfman et al. 1993) and recently the cell protein that mediates viral retention was identified as an INF-induced protein, Bst-317 or tetherin (Neil, Zang et al. 2008). In addition it is known that retroviral restriction factors such as APOBEC3G and TRIM5α are INF INF-regulated genes and their induction might also play a role in INF antiviral activity (Goff 2004). A number of in vitro studies suggested that the effects of interferon were modest (2-8 fold reductions in viral RNA) but interestingly, the combination of interferon and early antiretrovirals, such as AZT, was markedly synergistic (Hartshorn, Vogt et al. 1987), including in vitro models of cells chronically infected with HIV-1. These results are particularly intriguing because in cultures of chronically infected cells, the majority of the cells produce HIV-1 from integrated proviruses, and there is little spreading infection; as a result, AZT has minimal or no direct antiviral effect, and interferon may have been the most potent antiviral in these experiments (Poli, Orenstein et al. 1989). Experiments with HIV-1 production from chronically infected cells may represent a

useful model for participants suppressed on antiretroviral therapy as experiments suggest the majority of the detectable virus is produced from cells with integrated proviruses. Although interferon has been studied in active spreading infections, little data are available from participants who are already suppressed on ARV.

In the pre-ART era, INF clinical trials showed a degree of efficacy in reducing viral replication, p24 production, and slowing progression to AIDS (de Wit, Schattenkerk et al. 1988, Lane, Davey et al. 1990, Berglund, Engman et al. 1991). After development of combination ARV, however, INF was not developed as an antiretroviral, due to lower antiviral efficacy, inconvenient route of administration, and adverse affects (Lane, Davey et al. 1990, Dusheiko 1997). Recently, several groups have shown a substantial HIV RNA reduction in participants treated with the pegylated form of interferon alpha (PEG-INF) as part of chronic hepatitis C therapy (Torriani, Ribeiro et al. 2003, Talal, Shata et al. 2004, Neumann, Polis et al. 2007). In addition, Asmuth and colleagues recently studied the effect of pegylated interferon alpha on HIV viral load in HBV/HCV seronegative HIV-1-infected participants who were not receiving antiretroviral therapy. HIV-1 viremia declined by a mean 0.77 $\log_{10}$ copies/mL over 12 weeks (Asmuth D 2009).

The mechanism of interferon effect on HIV viremia is uncertain. There is a substantial literature regarding the effects of interferon in retroviruses in vitro, and in HIV in particular(Pitha 2011). Interferon may induce expression of a number of innate immune responses, which have direct effects on a number of distinct steps in virus replication. Induction of the cellular gene APOBEC 3G and 3F can result in hypermutation of the HIV genome during reverse transcription(Goila-Gaur and Strebel 2008). Induction of the gene tetherin results in retention of HIV on the surface of infected cells and decreases in production of extracellular HIV virions(Perez-Caballero, Zang et al. 2009). The effects of interferon may be manifest as a decrease in peripheral viremia, and a corresponding accumulation of HIV RNA intracellularly

Much of the information available regarding the effects of interferon in HIV infection has been obtained in patients co-infected with HCV. We performed a retrospective analysis of HIV-1 RNA levels in plasma stored from HIV-1/HCV coinfected participants enrolled in NIH clinical trials for chronic hepatitis C treatment. We selected participants undergoing antiretroviral therapy with HIV-1 viral RNA suppressed to <50 copies/mL, and used only samples that were obtained within 4 months of initiating therapy. Plasma HIV-1 RNA from 5/6 participants undergoing interferon/ribavirin therapy was decreased in 5/6 participants. In this retrospective pilot study, we could not distinguish whether the decrease in viremia resulted from therapy with interferon or ribavirin, or the combination. Prior clinical studies have indicated no evidence of anti-HIV-1 activity of ribavirin, suggesting that interferon may represent the active agent responsible for this result. Although the number of participants is small, these findings suggest that INF might decrease HIV-1 RNA levels in participants undergoing a successful ARV regimen.

There are limited data on the in vivo effects of interferon alone in individuals undergoing antiretroviral therapy. Montaner et al., studied the effects of pegylated interferon on plasma HIV RNA in individuals undergoing antiretroviral therapy who underwent a structured ART interruption(Azzoni, Foulkes et al. 2013). Decreases in cell associated HIV DNA were detected in a subset of patients; no differences in

plasma HIV levels were detected (using an assay with a 4-5 copy limit of detection ,approximately 30 fold higher than the single copy assay. Differences in levels of cell associated DNA were detected, but no studies of intracellular HIV RNA were performed(Azzoni, Foulkes et al. 2013).Recently intracellular HIV RNA may be recognized by intracellular toll like receptors,which are themselves induced by interferon. As such the effects of interferon on cell associated HIV RNA are of growing interest. Recently we have developed digital droplet methods to quantify HIV RNA levels in infected cells, including levels of unspliced, spliced, and short (tat^-) transcripts, which may be synthesized in latently infected cells not producing full length HIV RNA. In combination with single genome sequencing, these approaches will be useful in investigating the effects of interferon alpha in HIV infected individuals, including determining overall levels of cell associated RNA, numbers of cells producing HIV RNA, and phylogenetic analysis of HIV RNA sequences in plasma and cells. This advance will permit us to investigate the effects of interferon in patients with and without detectable levels of HIV RNA in plasma; patients with levels of HIV RNA below levels of single copy detection are still be detectable by measurements of HIV RNA .

In the present protocol, we will investigate the effects of interferon alpha 2b in reducing low level HIV viremia. We plan to conduct a non-randomized, open-label, single arm, pilot clinical trial of participants with viremia suppressed on antiretroviral therapy to investigate whether the addition of interferon alpha 2b therapy for 1 month (1.5 µg/kg interferon alpha 2b subcutaneously weekly for a total of 4 doses) results in changes in HIV-1 RNA levels in plasma or in peripheral blood lymphocytes. For the purposes of this pilot study, we will study 10 patients with suppressed viremia. Previously, we have obtained useful information regarding the effects of PI, NNRTI, and raltegravir intensification on HIV-1 viremia using this sample size. In the study planned here, we will perform gene expression studies to characterize the cellular response to interferon therapy in HIV infection. We will investigate potential correlations between expression of interferon-inducible genes and response. We will be particularly interested in the relationship between the interferon responsive gene, tetherin (Perez-Caballero, Zang et al. 2009), and the response to interferon.

2 Objectives

2.1 Primary Objective

This is a non-randomized, single arm pilot study of 10 participants to investigate the effect of interferon alpha on HIV-1 production in plasma and peripheral blood lymphocytes from HIV-infected individuals with HIV-1 RNA levels below the limit of detection in commercial assays.

2.2 Secondary Objectives

The secondary objectives of this study are the following:

1. Investigate HIV-1 genetic variation in individuals undergoing interferon therapy.

2. Investigate the safety and tolerability of PEG INF 2b in HIV-1 infected individuals taking FDA-approved antiretrovirals in combinations recommended by Department of Health and Human Services (DHHS) guidelines.

This study will allow us to elucidate the relationship between the viral decay in response to treatment intensification in HIV-infected individuals with residual viremia on antiretroviral therapy. This information will provide further clarifications on the origin of residual viremia and could provide clues to the eradication of HIV-1 infection.

This is a 48-week study to evaluate the effect of administering interferon alpha 2b to individuals in with viral RNA levels <50 c/ml undergoing antiretroviral therapy. Individuals entered into the study will viral RNA and PBMC sampling determinations prior to interferon intensification. During the second phase of the study, they will receive pegylated interferon alpha 2b (PEG-INTRON) at a dose of 1.5 μg/kg subcutaneously once weekly for 4 weeks starting at Study Entry, then at Visits 1, 2, and 3, for a total of 4 doses. During the interferon intensification phase, participants will continue to receive their standard of care HIV regimen. Following completion of the interferon dosing, participants will be monitored through 48 weeks and will remain on their standard HIV regimen. During all phases, participants will be assessed for viremia using standard and single copy viral load assays. In addition, CD4 and CD8 counts as well as interferon levels will be measured.

3 Study Overview

3.1 Specific Aims

The goal of this study is to investigate the ability of pegylated interferon alpha 2b to reduce residual viremia or cell associated HIV RNA in HIV-infected individuals who are receiving antiretroviral therapy. Viral RNA load will be measured using the sensitive single RNA copy per milliliter assay.

We will also delineate the safety and toxicity profiles of pegylated interferon alpha 2b when co-administered with antiretroviral therapy in HIV-1 infected individuals.

3.2 Study Design and Methods

3.2.1 Study Population

Ten evaluable HIV-1 chronically infected individuals with viremia suppressed to below the limit of detection, on antiretroviral therapy will be enrolled in this trial. Individuals with CD4 counts greater than or equal to 300 cells/μL at screening will be eligible to participate. We plan to conduct this study at the National Institute of Allergy and Infectious Diseases (NIAID)/CCMD Clinic at the National Institutes of Health (NIH) Clinical Center, and at the AIDS Clinical Trials Unit, Division of Infectious Diseases, University of Pittsburgh (active enrollment at Pittsburgh was completed in 2014).

3.2.2 End Points

Unless unacceptable drug-related toxicities emerge (see Appendix 2), clinical contraindications unrelated to drug therapy develop that compromise the health or safety of study participants, or one of the specific criteria for withdrawal from the study is met, all participants will be treated for 4 weeks. Participants will be followed through Week 48.

3.2.2.1 Primary End Points

We will compare viral RNA levels in blood and peripheral blood lymphocytes drawn before, during, and after intensification, including comparison of absolute values of viremia, as well as changes in viremia and area under the curve analyses to detect whether increases or decreases of viremia occurred as a function of interferon therapy. Standard analyses will be included.

3.2.2.2 Secondary End Points

In participants with detectable and amplifiable virus, HIV-1 genetic sequencing will be performed to determine whether interferon therapy results in differences in population structure during the 4-week intensification period. HIV RNA and DNA levels will be investigated using quantitative PCR approaches, including digital droplet PCR. The safety and tolerability of single agent interferon will be assessed through standard clinical management.

3.2.3 Sample Size

As described below (see Statistical Considerations) we expect that 10 participants completing the study will be necessary to yield useful clinical information. In order to enroll 10 participants for this study, we will be screening up to 65 individuals. Any dropouts and non-adherent participants will be replaced.

3.2.4 Safety Analyses

All safety data including adverse events, deaths, vital signs, physical examinations, discontinuations from study drug, and clinical laboratory measurements (hematology, serum chemistry, and urinalysis) will be listed and summarized, using the mean, standard deviations, and percentiles for continuous variables and frequencies and percentages for categorical variables. Any notable values will be flagged. Any comparisons with respect to safety parameters from baseline will be made using the same procedures described above for endpoint analyses.

4 Subject Selection

4.1 Inclusion Criteria

To be eligible for study participation, a volunteer must satisfy all of the following inclusion criteria:

1. Age $\geq$18 years.
2. Documentation of HIV-1 infection by any licensed ELISA test and confirmed by a Western Blot.
3. Receiving a DHHS-approved ARV regimen,.
4. Level of cell-associated HIV RNA ≥ 5 copies/million peripheral blood mononuclear cells (PBMC) done at screening visit 1
5. HIV-1 RNA levels less than detectable by current commercial means (e.g., Roche Amplicor, b-DNA test) for a minimum of 12 months prior to screening at all time points, and with at least 2 measurements in this 12 month window.

6. CD4 $\geq$300 cells/mm^3 at pre-entry visit within 14 days prior to enrollment.
7. Ability to sign informed consent and willingness to comply with the study requirements and clinic policies.
8. No evidence of viral hepatitis co-infection as assessed by Hepatitis C antibody, HCV RNA, and hepatitis B surface antigen; determinations at pre-entry visit within 28 days prior to enrollment.
9. No history of or evidence of autoimmune hepatitis or other autoimmune disorders at screening, or antinuclear antibody (ANA > 3x upper limit of normal.
10. Laboratory values at pre-entry visit within 14 days prior to enrollment:
    - Alkaline phosphatase <2.0 times upper limit of normal
    - Alanine transaminase (ALT) <2.0 times upper limit of normal
    - Total bilirubin <2.5 mg/dL (< 40 mg/dL if on Atazanavir)
    - Creatinine clearance $\geq$60 mL/min as estimated by the Cockcroft-Gault equation
    - Neutrophil count $\geq$1500 cells/mm^3
    - Platelets $\geq$150,000/ mm^3
    - Hemoglobin $\geq$12.0 mg/dL for men and >11.0 g/dL for women
    - Fasting glucose <126 mg/dL
11. No history or evidence of significant clinical depression at screening that in the opinion of the investigator would affect the ability of the patient to participate in the study, or which would constitute a threat for his/her health in case of relapse upon INF treatment. The Beck Depression Inventory score must be $\leq$13 at pre-entry visit.
12. No history of INF/PEG-INF therapy.
13. If capable of pregnancy: use of effective contraception during study: effective contraception methods include abstinence, surgical sterilization of either partner, barrier methods such as diaphragm, condom, cap or sponge, or use of hormonal contraception with an anti-HIV regimen that will not alter metabolism of hormonal contraception.
14. Participants must have primary medical care outside this protocol: participants will have to provide Primary Care Doctor's contact information.

4.2 Exclusion Criteria

A volunteer will be ineligible to participate in this study if any of the following criteria are met:

1. History of neoplastic disease requiring cytotoxic therapy including hydroxyurea.
2. Use of long-term systemic corticosteroids, immunosuppressive agents, or cytotoxic agents within 60 days prior to enrollment.
3. Any vaccination within 30 days prior to enrollment. Individuals interested in participating who require vaccination will delay screening until 30-day period is completed.
4. Concurrent therapy with investigational cytokines including IL-2 or IL-12 during the course of the study. Prior administration of cytokines is not an exclusion criterion; at least 4 months from most recent cycle of IL-2 or IL-12 is required.
5. Any febrile illness (>38^{o}C) in the 3 weeks prior to enrollment or any acute therapy for a serious infection completed within 30 days prior to enrollment.
6. Current pregnancy or lactation, history of pregnancy in the last 4 months.
7. Preexisting autoimmune disorders including inflammatory bowel diseases, psoriasis, idiopathic thrombocytopenic purpura, lupus erythematous, autoimmune hemolytic anemia, scleroderma, severe psoriasis, rheumatoid arthritis, and optic neuritis.
8. History of severe retinopathy or evidence of severe retinopathy judged by pre-entry ophthalmologic examination.
9. Known allergy/sensitivity to study drug or its formulation.
10. History of seizure disorders or current anticonvulsant use.
11. Any history of medical conditions associated with chronic liver disease (genetic hemochromatosis, alcoholic liver disease, toxin exposures, and autoimmune hepatitis) or documented cirrhosis due to any cause.
12. History of pulmonary disease associated with functional limitation.
13. Documented history of thyroid disease.
14. Active drug or alcohol use or dependence, which in the opinion of the investigator, would interfere with complying with the study requirements.
15. Known hypersensitivity to *Escherichia coli*-derived products such as filgrastim.
16. Any systemic illness that will make it unlikely that the subject will be able to return for the required study visits.
17. History of, or any condition that in the opinion of the investigator would interfere with the conduct of the study, or it would not be in the best interest of the subject to enroll in this study.

5 Schedule of Visits and Procedures

Participants will be seen in the NIAID/CCM Clinic at the NIH Clinical Center, NIH (PI: Frank Maldarelli, MD) OR at the AIDS Clinical Trials Unit, Division of Infectious Diseases, University of Pittsburgh (PI: Deborah McMahon, MD; active enrollment at Pittsburgh was completed in 2014). We plan to enroll a total of 10 completed

participants for this study. Any dropouts and non-adherent participants will be replaced.

5.1 Screening (Days -58 to -28)

Screening will consist of HIV-1 RNA level and if < 50 copies/mL by standard assay and have detectable PBMC-associated HIV RNA ≥ 5 copies/million cells will continue with screening. If > 50 copies/mL, the subject will not undergo further screening assessments.

Screening for the protocol will be completed within 4 weeks prior to the study enrollment. During screening visit the protocol design will be reviewed, and informed consent will be obtained prior to any study-related screening procedures. The subject will then be given the consent document to read. Consent forms may be sent to the subject for review prior to the screening visit. The site investigator will review the study with the subject and answer any questions the subject may have about the study design, interferon, or study procedures. After the subject has read this information, there will be further opportunity for discussion with one of the investigators. Potential participants will sign consent prior to any study-related procedures. Once consented, eligibility will be assessed.

5.2 Pre-entry Visit 1 (Day-27 to Day -1)

When eligibility evaluation is complete, the subject will return for further assessments and the standard consent will be administered. The subject's medical history will be reviewed and a physical examination will be performed, including weight and vital signs. Concomitant medications will be documented. The assessments are summarized in Appendix 1, and include:

- Complete physical examination, including vital signs
- Beck Depression Inventory Scoring (BDS)
- Complete blood count with differential, platelet count
- Serum chemistries (sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, alkaline phosphatase)
- Creatinine clearance (Cockcroft-Gault method)
- Fasting blood glucose
- TSH, free T4
- Urine pregnancy test (women of childbearing age)
- Antinuclear antibody (ANA)
- Urinalysis
- Amylase
- ALT, bilirubin
- Hepatitis B surface antigen, hepatitis B surface antibody, hepatitis C antibody and hepatitis C viral RNA level (HCV PCR)
- CD4 count
- Ophthalmology examination (dilated funduscopic examination)
- Single copy assay for HIV-1 RNA
- HIV-1 RNA standard assay

- HIV-1 ELISA/Western Blot (if documentation not available)

5.3 Pre-Entry Visit 2: Leukapheresis Visits (Day -14 to Day -1, Day 28)

Once the subject has been found to meet the eligibility criteria, the subject will return for leukapheresis for the purposes of peripheral blood lymphocyte (PBL) storage for use in future virologic and immunologic studies. Leukapheresis, will be scheduled to occur at Pre-Entry 2 and during Visit 4 (Day 28).

5.4 Entry (Day 0)

Participants will have laboratory safety assessments performed and receive their first dose of PEG-IFN. Administration of pegylated interferon will be performed by trained personnel. Vital signs will be obtained, concomitant medications documented, and a targeted physical examination will be performed based upon any signs or symptoms that the subject has experienced since the last visit. Assessments are detailed in Appendix 1 and include:

- Beck Depression Inventory score
- Complete blood count with differential, platelet count
- Serum chemistries (sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, alkaline phosphatase)
- Urine pregnancy test (women of childbearing age) prior to interferon administration
- ALT, Bilirubin
- Creatinine clearance (Cockcroft-Gault method)
- CD4 count
- Single copy assay for HIV-1 RNA
- HIV-1 RNA standard assay
- Interferon level (baseline)
- PBMCs for storage

5.5 Intensification Phase: Visit 1 (Day 7), Visit 2 (Day 14), Visit 3 (Day 21)

Participants will have laboratory safety assessments performed and receive their subsequent doses of PEG-IFN. Vital signs will be obtained and concomitant medications documented. Administration of interferon will be performed by study personnel. Dosing will be based on body weight obtained during initial evaluation. The last PEG-IFN administration will be on Day 21 (Visit 3). A targeted physical examination will be performed based upon any signs or symptoms that the subject has experienced since the last visit. Assessments are detailed in Appendix 1 and include:

- Beck Depression Inventory score
- Complete blood count with differential, platelet count (STAT, prior to interferon administration)
- Serum chemistries (sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, alkaline phosphatase)
- Creatinine clearance (Cockcroft-Gault method)

- Urine pregnancy test (women of childbearing age) prior to drug administration
- ALT, Bilirubin
- Single copy assay of HIV-1 RNA
- HIV-1 RNA standard assay
- Interferon level

5.6 Early Post-Intensification Phase: Visit 4 (Day 28)

Participants will have laboratory safety assessments performed. Vital signs will be obtained and concomitant medications documented. A targeted physical examination will be performed based upon any signs or symptoms that the subject has experienced since the last visit. Assessments are detailed in Appendix 1 and include:

- Beck Depression Inventory score
- Complete blood count with differential, platelet count
- Serum chemistries (sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, alkaline phosphatase)
- Creatinine clearance (Cockcroft-Gault method)
- ALT, Bilirubin
- CD4 count
- Single copy assay of HIV-1 RNA
- HIV-1 RNA standard assay
- Interferon level
- PBMCs for storage
- Leukapheresis

5.7 Late Post-Intensification Phase: (Visits 5 to 9)

Participants will return for visits at Days 35, 42, 49, and 56 during the pegylated interferon post-intensification phase. The Assessments are detailed in Appendix 1 and include:

- Beck Depression Inventory score
- Complete blood count with differential, platelet count
- Serum chemistries (sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, alkaline phosphatase)
- Creatinine clearance (Cockcroft-Gault method)
- ALT, bilirubin
- Single copy assay for HIV-1 RNA
- HIV-1 RNA standard assay
- CD4 count (Visits 8 and 9)

5.8 Long-term Follow-up: Week 16 (Visit 10)

The following determinations will be assessed at the Week 16 visit:

- Beck Depression Inventory score
- Complete blood count with differential, platelet count

- Serum chemistries (sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, alkaline phosphatase)
- Creatinine clearance (Cockcroft-Gault method)
- ALT, bilirubin
- Single copy assay for HIV-1 RNA and assays for cell associated HIV
- HIV-1 RNA standard assay

5.9 Weeks 24 and 36 (Visits 11 and 12)

The following determinations will be assessed at the Week 24 and Week 36 visits:

- HIV-1 RNA standard assay
- Single copy assay for HIV-1 RNA and assays for cell associated HIV

5.10 Week 48 (Final Visit, Study Discontinuation Visit)

The following determinations will be assessed at the Week 48 or Study Discontinuation visit:

- Beck Depression Inventory score
- Complete blood count with differential, platelet count
- Serum chemistries (sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, alkaline phosphatase)
- Creatinine clearance (Cockcroft-Gault method)
- ALT, bilirubin
- Single copy assay for HIV-1 RNA and assays for cell associated HIV
- HIV-1 RNA standard assay
- CD4 count
- PBMC

5.11 Window Periods for Study Visits

The following variable scheduling is permitted for study visits:
Days -58 to 14: ±1 day
Days 21 to 49: ±2 days
Days 56 to 84: ±5 days
Weeks 16 to 48: ±14 days

6 Concomitant Medications

All concomitant medications will be documented at each study visit.

Concomitant administration of immunomodulatory treatments (including steroids, radiation, IL-2, and anti-neoplastic agents) is not allowed during the course of this trial. Other investigational drugs are excluded. No vaccinations are allowed during the study period from 30 days prior to the time of signing consent to the end of the four week interferon therapy period.
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7 Study Drug Administration

7.1 Drugs and Dosing

All participants will receive pegylated interferon alpha 2b at the dose of 1.5 μg/kg subcutaneously every week for 4 doses. Dosing will be determined at Entry, and Visits 1, 2, and 3 based on subject's body weight obtained at the time of enrollment. Administration of study medication will be performed by study personnel. Antiretroviral therapy will be continued throughout the study.

7.2 Monitoring Parameters

The Schedule of Events is found in Appendix 1. Participants will be closely monitored for clinical and laboratory evidence of adverse events associated with the study drug or medical complications.

7.3 Dose Modifications/Toxicities

A complete blood count with differential (done STAT) will be reviewed before PEG-INF administration at visits 0, 1, 2, and 3. Participants experiencing an adverse event Grade ≤2 may continue at their current dose. No interferon dose modifications will be made in the study but additional visits or monitoring will be permitted to evaluate participants. Toxicity will be evaluated using the Division of AIDS Table for Grading Adults Adverse Events: (http://rcc.tech-res.com/safetyandpharmacovigilance/).

8 Management of Toxicities to Study Drug

8.1 Grade 4 Toxicities

Participants with life threatening or Grade 4 adverse events thought to be possibly, probably, or definitely related to study treatment will discontinue study treatment and remain on study follow-up and undergo additional visits and therapy as needed for toxicity management. Grade 4 neutropenia determinations will be repeated with duplicate samples to document that neutropenia is present.

8.2 Toxicity Management

Specific expected toxicities lower than grade 4 will be managed as follows:

Adverse Event	Action
Neutropenia	Participants who experience Grade 3 neutropenia will receive filgrastim at 300 μg subcutaneously up to 3 times a week. Absolute neutrophil counts will be monitored until return to subject's baseline or <Grade 1. Patient will be asked to return in 1-3 days for evaluation. If grade 3 neutropenia has resolved, patient will return 2-3 days later for repeat evaluation. If grade 3 neutropenia has not resolved at the day 1-3 visit, additional G-CSF will be administered

	and the patient will return daily for evaluation and additional G-CSF as medically indicated until resolved. Filgrastim will be discontinued at this time. Interferon will be continued for Grade 3 neutropenia.
CD4 cell count	If the CD4 count declines to <14%, pneumocystis prophylaxis will be offered until the percentage of CD4 cells increases to ≥14%.
Depression	If a BDS score of 14-19 is reported, assessment by the site investigator will be scheduled before the next dose of study drug. If a BDS score of 20-28 is reported, a psychiatric evaluation is required before the next dose of study drug. If a BDS score is >29 or suicidal ideation is reported, participants will permanently discontinue study drug and undergo prompt psychiatric evaluation.

8.3 Criteria for Study Permanent Drug Discontinuation

In addition to the criteria for stopping the study drug, the following will constitute criteria for discontinuation of study drug:

1. Development of life-threatening infection or malignancy.
2. Grade 3 or higher neutropenia.
3. Development of clinical depression (see Section 8.5).
4. Acquisition of hepatitis A, B, or C.
5. Pregnancy.
6. If subject's CD4 cell percent remains <14% (see Section 8.2).
7. If it is felt by the site's principal investigator that further participation in the study is no longer in the best interest of the subject.
8. Development of a medical condition, that in the opinion of the investigator, it is in the subject's best interest to discontinue study treatment even if criteria requiring drug discontinuation have not been met.
9. In addition, participants may be withdrawn from the study for:
    a. Withdrawal of consent
    b. Non-compliance with study visits or study treatment; subject misses a dose of study drug for reasons other than toxicity
    c. Termination by sponsor

9 Laboratory Studies

9.1 Serum Chemistry and Hematology Profiles

Routine safety laboratory tests (Appendix 1) will be performed to monitor clinical status and detect potential drug-related toxicities. Safety tests will be performed at each site's laboratory. During the intensification phase of the study, results of safety tests will be reviewed by the site investigator at each visit before PEG-Interferon administration.

9.2 HIV RNA Levels

HIV-1 RNA levels will be measured by FDA-approved standard assay **(**b-DNA or HIV RT-PCR assay) and SCA assay will be measured at screening and at each study visit. Samples for b-DNA testing and samples for SCA assay will be processed the same day and plasma will be stored at -80°C. Analysis of HIV in plasma and cells will be analyzed through the Clinical Retrovirology Section of the HIV Drug Resistance Program and the HIV Molecular Monitoring Core (Leidos).

9.3 Pharmacokinetics of Pegylated Interferon

Serum levels of pegylated interferon alpha 2b will be measured at Entry and Visits 1, 2, 3, and 4.

9.4 Peripheral Blood Mononuclear Cells Storage

PBMCs will be collected and stored at Entry, at Visits 4, , and at the Final Visit (13), or in the event of early study discontinuation. Samples will be processed according to standard processing for cryopreservation.

9.5 CD4 Counts

CD4 counts (absolute number and percentage) will be measured at Pre-entry, Entry, Visits 1, 4, 8, 9 and the Final Study or Discontinuation Visit. Samples will be processed according to standard flow cytometry procedures.

10 Statistical Considerations

This pilot study will investigate whether pegylated interferon alpha2-b will reduce residual HIV-1 RNA in participants with HIV-1 viremia suppressed below detectable levels as measured by commercial assays. Two relevant questions are: 1) does intensification reduce viremia in a consistent way across different patients, and 2) are there *any* patients in whom intensification reduces viremia? The first question is of primary interest, and it pertains to the population. The second question concerns whether individual intensification responders can be identified. It is possible to find no consistent pattern across patients, yet identify one or more patients in whom the pattern of viremia lowering over time convincingly rules out a flat slope for that patient. Therefore, analyses will be directed at both population responses and individual responses, with the primary emphasis being on the former.

For both population and individual analyses, one complication is that because patients are selected on the basis of low baseline viral loads, regression to the mean would cause subsequent viral loads to be somewhat larger, in the absence of intensification. To account for this, we will use one baseline measurement to determine eligibility and another to assess changes over time.

The primary analysis (which is at the population level), compares the viral loads obtained from the pre-intensification control period to the week 4 value using a paired t-test. A sensitivity analysis will use the nonparametric Wicoxon signed rank test to analyze these baseline to week 4 changes. Another analysis computes the slope of viral load over time for each patient, and uses a 1-sample t-statistic and confidence interval to estimate and test whether the average slope is 0.

Secondary analyses will attempt to identify individual intensification responders. One definition of responder is whether the patient had at least a 1-log decrease from baseline to 4 weeks in viral load. Because patients with baseline viral load below 10 cannot achieve this, another method will compute the least squares slope of viral load versus time for each patient and declare patients with statistically significant slopes as responders. Because the goal for this analysis is to summarize the response of a given individual, we treat the data as independent observations from that patient. This is the same assumption made when using a mixed model, namely that conditioned on the random, patient-specific intercept and slope, the observations are independent. For both the 1 log method and significant slope method of identifying individual responders, we attempt to find differences in baseline characteristics between responders and non-responders, though very low power precludes finding any of these statistically significant unless they are huge. Of particular interest is whether baseline viral load predicts responder status. As described above, for this analysis we will use one baseline value to characterize baseline viral load and another for the calculation of slope to identify responder status.

All tests will be two-sided and an alpha level of less than 0.05 will be considered statistically significant. Analyses will be performed on both intent-to-treat and as per protocol participants. Any dropouts and non-adherent participants will be replaced. We do not feel that stopping criteria are necessary as this pilot study is not for prognosis or clinical management; there is no blinding of study treatment and we will be monitoring toxicities in real time. In this study, we will be studying participants on an ongoing basis, and no interim analysis is planned.

11 Sample Size Estimation

This is a pilot study of the effect of pegylated interferon on HIV-1 RNA levels in participants who are receiving antiretroviral therapy. To date, there is limited published data available, and the proportion of participants who will respond with decreases in HIV-1 viral RNA levels remains to be clearly defined. As described above, responses may be similar to those observed with PI, integrase inhibitor, or NNRTI intensification, where no participants have experienced a decrease in viral RNA levels. If so, previous sample size analyses estimating the frequency of suppressible participants are relevant. In the accompanying table, we estimate the 90% confidence intervals of the probability that a participant has a detectable decrease in viral RNA levels (participants suppressed in the table below) after studying increasing numbers of participants without observing episodes of drug suppression. As shown here, if we study 10 participants and observe no

suppression in plasma or cell-associated HIV RNA, we have 90% confidence that the frequency of suppressible participants is less than 21%. For this pilot study, we will request a sample size of 65 participants to achieve our goal of studying 10 participants who have ≥0.1 copies/mL HIV-1 RNA at screening.

90% exact confidence intervals (using the Clopper-Pearson method) for the probability of being suppressible.

Participants intensified	Participants suppressed	90% confidence interval
5	0	0,0.45
6	0	0,0.39
7	0	0.0.35
8	0	0,0.31
9	0	0,0.28
10	0	0,0.26
15	0	0,0.18
20	0	0,0.14

12 Hazards/Discomforts/Risks

12.1 Study Drugs and Procedures

12.1.1 Pegylated Interferon Alpha 2b

Interferon alpha is a cytokine with proven antiviral activity against many viruses, especially HCV. The exact mechanism of this antiviral activity is not known. The proposed mechanisms of actions for interferon alpha are:

1. Antiviral activity through the inhibition of viral replication in infected cells (Sen 2001, Bekisz, Schmeisser et al. 2004, Jiang, Guo et al. 2008). With respect to HIV-1 specifically, INF has shown antiviral activity both *in vitro* (Ho, Hartshorn et al. 1985) and *in vivo (Lane, Kovacs et al. 1988, Hatzakis, Gargalianos et al. 2001)* studies.
2. Immune regulatory actions increasing the ability of reticuloendothelial cells to clear virus through the following mechanisms (Missale, Bertoni et al. 1996, Gerlach, Diepolder et al. 1999, Patel and McHutchison 2001):
    a. Increase in class 1 MHC antigens and cellular adhesion molecules on effector cells
    b. Activation of effector cells (NK cell and macrophages)
    c. Upregulation of Th1-T helper subset

In participants with cirrhosis, exacerbation of cirrhosis-related neutropenia and thrombocytopenia is a recognized risk associated with interferon and pegylated interferon. Participants with documented cirrhosis will be excluded from

participating in this study. The other recognized side effects associated with the use of pegylated interferon include fatigue, headaches, myalgia, fever, nausea, upper abdominal discomfort, depression, diarrhea, insomnia, reduced appetite, and dermatitis. There are also rare occurrences of autoimmune, infections, ophthalmic, and ischemic disorders associated with pegylated interferon use. Ophthalmologic complications include retinopathy, which is usually transient and completely resolves with continued treatment. Other side effects associated with subcutaneous injection include pain and inflammation at the site of injection.

The specific adverse events listed below will be handled as specified. PEGINTRON will be administered in clinic by trained personnel. Merck Inc. will be providing study drug PEGINTRON; other study agents will be provided through respective pharmacies as indicated.

12.1.2 Granulocyte Colony Stimulating Factor

Endogenous granulocyte colony stimulating factor (G-CSF) is a lineage specific colony-stimulating factor, which is produced by monocytes, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation.

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by *Escherichia coli* bacteria into which has been inserted the human granulocyte colony-stimulating factor gene. Filgrastim is available as a sterile, clear, colorless, preservative-free liquid for parenteral administration at a specific activity of 1.0 ± 0.6 x 10 U/mg (as measured by a cell mitogenesis assay). The product is available in single-use vials and prefilled syringes. The single-use vials contain either 300 μg or 480 μg filgrastim at a fill volume of 1.0 mL or 1.6 mL, respectively. It has been shown to be safe and effective in accelerating the recovery of neutrophil counts following a variety of chemotherapy regimens. Filgrastim is contraindicated in patients with known hypersensitivity to *E. coli*-derived proteins, or any component of the product. Allergic-type reactions occurring on initial or subsequent treatment have been reported in <1 in 4000 patients treated with filgrastim. These have generally been characterized by systemic symptoms involving at least 2 body systems, most often skin (rash, urticaria, facial edema), respiratory (wheezing, dyspnea), and cardiovascular (hypotension, tachycardia). Some reactions occurred on initial exposure. Reactions tended to occur within the first 30 minutes after administration IV. Rapid resolution of symptoms occurred in most cases after administration of antihistamines, steroids, bronchodilators, and/or epinephrine. Symptoms recurred in more than half the patients who were rechallenged. Other rarely reported serious complications associated with filgrastim include splenic rupture, acute respiratory distress syndrome, alveolar hemorrhage and hemoptysis, and sickle cell crises. G-CSF will be administered in clinic b trained personnel.

12.1.3 Potential for Previously Unrecognized Drug Interactions

Interferon alpha 2b has been co-administered with antiretroviral therapy in HIV-infected individuals without any severe toxicity. Adverse events observed in HIV/HCV-coinfected participants receiving treatment with PEG INF and ribavirin were similar to those occurring in HCV-monoinfected participants. Drug interactions between antiretrovirals and treatment for hepatitis C concern mostly ribavirin and reverse transcriptase inhibitors (Mallolas and Laguno 2008).

12.1.4 Potential for Adverse Effects from Leukapheresis

Risks associated with leukapheresis are similar to those seen with whole blood transfusions. They rarely include infection (<1% of persons) and commonly include nausea, fainting, dizziness, bruising at the needle puncture site, blood loss, and a transient decrease in platelet or red blood cell counts in 10% to 25% of persons. Potential problems of a leukapheresis procedure when citrate is given intravenously as an anticoagulant rarely includes seizures in <1% of persons and commonly includes muscle cramping, numbness, chilling, tingling sensations, and feelings of anxiety. Citrate is approved by the Food and Drug Administration for this use. If citrate/heparin or heparin alone is used as the anticoagulant, there may be a transient increase in clotting time, which may lead to blood loss in very rare instances (<1%). There is also a small risk of technical problems with the apheresis machine (1:1000 phereses).

12.1.5 Risks of Phlebotomy

The primary risks of phlebotomy include occasional bleeding or bruising of the skin at the site of needle puncture, and the sensation of transient lightheadedness or rarely, fainting or infection. The amount of blood drawn for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period.

12.2 Human Subject Protections

Participants will be fully counseled prior to entry into the study as to the potential risks of the study. Participants who, in the opinion of the study team, do not fully comprehend these potential risks will not routinely be offered participation in the study. Participants will be monitored closely during their participation in the study, and informed of any new information regarding the study treatment that may influence their willingness to continue study participation.

13 Study Recruitment

The study will be advertised and posted on the NIH web site. The University of Pittsburgh AIDS Clinical Trials Unit will recruit participants from its Volunteer Registry. IRB-approved recruitment materials will be distributed among AIDS service organizations and other HIV providers in the region. Both the NIH and the University of Pittsburgh have recently completed drug intensification studies and have been successful in enrolling participants willing to complete the frequent visits.

In the most recent study, we used the current viral RNA enrollment criterion (≥0.38 copies/mL) to study raltegravir intensification; 36 participants were screened for the study and 10 were eligible; the remainder were excluded chiefly on the basis of viral RNA levels. Nevertheless, 9 of the 10 enrolled participants completed this study and had evaluable data that were useful in determining whether viremia decreased. To improve our statistical analysis, we have increased the sensitivity of the assay (lower limit of quantification = 0.1 copies/ml) and will use 0.1 copies/ml as cutoff.

13.1 Gender, Ethnicity, and Race Consideration

Participants will not be excluded based on race, gender, or ethnicity. It is hoped that the racial and ethnic character of the participants in this study will reflect, as closely as possible, the demographics of the HIV epidemic in the United States. The NIAID has an outreach program to medically underserved minority participants in order to improve their access to clinical trials. The University of Pittsburgh's AIDS Clinical Trials Unit makes a concerted effort to provide access to AIDS clinical trials for women and minorities.

13.2 Children and Pregnant Women

This study will be limited to adults 18 years of age and older. Insufficient data are available to evaluate the safety and efficacy of pegylated interferon in the pediatric population. In addition, this study is a no benefit study and would not be applicable to pediatric populations. The risks of pegylated interferon alpha 2b treatment during pregnancy have not been evaluated. Therefore, pregnant or nursing women will be excluded from this study. Any woman of childbearing potential must have a negative pregnancy test at screening, baseline, and prior to each dose of pegylated interferon and also agree to use two accepted methods of contraception throughout the study.

14 Benefits/Compensation/Alternatives

Financial compensation will be provided to study participants as outlined below. Participants will receive remuneration for the immediate costs associated with their study-related expenses like travel expenses, lodging, etc., as provided by the National Institutes of Health.

Participants will be financially compensated according to the NIH Normal Volunteer Guidelines proportionate to the inconvenience of the procedures necessary to obtain the samples. The compensation schedule is as follows:
Day 0 to Week 24: $40 at each visit
Leukapheresis: $100 each

The University of Pittsburgh AIDS Clinical Trials Unit will provide compensation for travel based on zip code and distance from the University.

This is a no benefit study; the alternative is not to participate. By participating in this study, participants may contribute to our understanding of persistent HIV viremia in individuals receiving potent antiretroviral therapy.

15 Evaluation of Safety

All enrolled participants who have received any study drugs will be evaluated for safety. Safety will be assessed by physical examination, vital signs, hematology, chemistry, urinalysis, and assessment of AEs. The severity of signs, symptoms, and AEs will be determined by using the Toxicity Severity Scale (Appendix 2).

Recording/Documentation

At each contact with the subject, information regarding adverse events will be elicited by appropriate questioning and examinations. All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents will include: progress notes, laboratory reports, consult notes, phone call summaries, survey tools and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable adverse events that are identified will be recorded in CRIMSON, or the appropriate case report form (CRF), or source document. The start date, the stop date, the severity of each reportable event, and the PI's judgment of the AEs relationship and expectedness to the study agent/intervention will also be recorded in CRIMSON, or on the appropriate CRF, or source document.

15.1 Protocol-specific Adverse Events

15.1.1 Definitions of Adverse Events

Adverse Event: Any untoward or unfavorable medical occurrence in a human subject, that includes any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Serious adverse event: Any adverse event that

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition. *(examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse)*

Protocol Deviation: Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur

Serious Protocol Deviation: A deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as

1. Serious: Non-compliance that
    a. Increases risks, or causes harm, to participants
    b. Decreases potential benefits to participants
    c. Compromises the integrity of the NIH-HRPP
    d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that, is neither serious nor continuing.

Unanticipated Problem (UP): Any incident, experience, or outcome that meets all three of the following criteria would be considered a serious UP:

1. unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
    b. the characteristics of the subject population being studied
2. related or possibly related to participation in the research
3. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated problem that is not an Adverse Event (UPnonAE): An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. These events may involve a greater risk of social or economic harm to subjects or others rather than physical/psychological harm. Such events would be considered a non-serious UP. Examples of a UPnonAE include a breach of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

15.1.2 Adverse Event Reporting

Expected adverse events are those listed in the package inserts for Pegintron. Adverse events will be graded according to the Division of AIDS Table for Grading Adults Adverse Events (http://rcc.tech-res.com/safetyandpharmacovigilance/ , Appendix 2), assessed for severity (mild/moderate/severe), expectedness (expected/unexpected), and relatedness to study drug (definitely, probably, possibly, unlikely, or unrelated). All adverse events will be recorded in the study database. Serious adverse events will be reported to the participating IRBs and the FDA. Adverse events that do not meet guidelines for expedited reporting will be included with each continuing review.

15.1.2.1

Expedited Reporting to the NIAID IRB

Serious and non-serious Unanticipated Problems, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. Serious Adverse Events that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 calendar days of investigator's awareness, regardless of expectedness.

15.1.2.2 Waiver of Reporting Anticipated Protocol Deviations, Expected non-UP AEs and Deaths

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected adverse events will not be reported to the IRB unless they occur at a rate greater than that known to occur in HIV infection. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are unanticipated problems. Deaths related to the natural history of HIV infection will be reported at the time of continuing review.

15.1.2.2 Annual Reporting

The following items will be reported to the NIAID IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems
- Expected serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All adverse events, except expected AEs and deaths granted a waiver of reporting.
- Serious and Non-Serious Protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported
- Protocol specific reporting as applicable

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15.1.2.3 Reporting of Pregnancy

Pregnancy that occurs during the course of the protocol will be reported within 7 days of the investigator's awareness

15.1.2.4 Type and Duration of the Follow-up of Subjects after Adverse Events

Serious adverse events will be followed until resolution; Some adverse events resulting that do not resolve completely will be followed until stable. Pregnancy will be followed until 3 months after delivery, miscarriage, or termination.

16 Monitoring

This is an open-label treatment protocol. Monitoring will be performed by the site investigators. The NIAID and the University of Pittsburgh Institutional Review Boards will review the study at least annually. Because of the nature of the study, we plan to constitute a Data Safety Monitoring Board (DSMB) at the NIH to review the study progress and safety at least annually. This function will be provided through NIAID DSMB, although the group will be functioning as a safety monitoring committee.

The NIAID Intramural DSMB is constituted to review the safety data of Intramural NIAID clinical studies that require DSMB oversight, and consists of experts in infectious diseases, biostatistics, and clinical trials. The DSMB will meet at least once a year to review the completeness of the study data collected, the adherence to the protocol, and PI's review summaries to evaluate safety, study conduct, and scientific validity and integrity of the study. Other reviews may occur as needed if other safety issues arise.

The initial review will occur prior to opening the study to enrollment.

16.1 Stored Samples and Future Research

Extra blood samples will be stored using a code number that only the study team can trace back to the subject. These samples will be stored for other investigators who may want to pursue additional research using these stored samples. If so, the NIH study team may send these samples to them, along with the coded number label. Once the protocol is terminated and data analysis is completed remaining samples will be maintained for future research by appropriate transfer to other protocols for ongoing human subjects protection oversight; requesting the use of stored samples may be submitted to the IRB for approval if the intended use is not covered by original consent. Any loss or destruction of stored samples will require written IRB notification.

16.2 Data Management Plan

All research data and results will be carefully recorded using data collection forms that will be saved and allow for continuous access. Further, all collected data will be entered and saved to a password-protected computer spreadsheet (Microsoft

Excel™) for analysis and review. The investigator is responsible for assuring that the data collected is complete, accurate and recorded in a timely manner. Source documentation should support the data collected on the CRF and must be signed and dated by the person recording and/or reviewing the data. All data will be annotated and indexed, including all notebooks and computerized information, to facilitate detailed review of data. All data, even those of experiments not directly leading to publication, will be treated comparably. All research data will be made available to scientific collaborators and supervisors for immediate review, consistent with requirements of confidentiality. All research data, including the primary experimental results, will be retained for a minimum of 5 years to allow for analysis and repetition by others of published material resulting from the data.

Demographic information and laboratory results will be collected using case report forms and then transcribed into an electronic database on an ongoing basis as participants are enrolled. At the NIH site, data will be stored in the CRIMSON database. Other experimental data, including HIV viral load results and IFN drug levels, will be analyzed and reported in batches. Standard laboratory results will be accessed by the site investigators and the study coordinators. All results, whether recorded on paper or electronically, will be maintained using only the subject's numeric code for identification. Data collection and quality assurance will be performed primarily by the site investigators and study coordinators.

Data management, including the decision to publish, will be the responsibility of the Co-Principal Investigators. After publication, all research data that form the basis of that communication will be made available promptly and completely to responsible scientists seeking further information. Exceptions include those requests that would infringe on confidentiality of clinical data or if unique materials were obtained under agreements that preclude their dissemination.

17 Anonymity and Confidentiality

As each subject is consented and then enrolled, he or she will be allocated a unique study number. To ensure subject confidentiality, these numeric codes will substitute for personal identifiers on all paper documents, computer records, and blood sample vials. The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted is prohibited. The results of the research study may be published, but subject’s names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the Principal Investigators will keep records with personal identifiers in double-locked cabinets and the results of tests will be coded to prevent association with subject names. It is expected that this data will be reported in scientific journals and scientific meetings. Confidentiality of participants will be maintained in all forms of reporting. Participants will be informed in general terms of the results as soon as practical.

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Visit	Screen	Pre-Ent 1	Pre-Ent 2	Entry	1	2	3	4	5	6	7	8	9	10	11	12	Final Visit*
Day/Wk	D-58 to -28	D-27 to -1	D-14 to -1	D0	D7	D14	D21	D28	D35	D42	D49	D56	D84	W16	W24	W36	W48
Consent	x	x															
Physical		x		x													
Vital signs	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
Tar. Phys.				x	x	x	x										
Med Hx		x															
Con meds		x		x													
BDS		x		x	x	x	x	x	x	x	x	x	x	x			x
Psy Eval		**		**													
C/D/Pl		x		x	x	x	x	x	x	x	x	x	x	x			x
Chem*		x		x	x	x	x	x	x	x	x	x	x	x			x
FBS		X															
ALT,Bili		x		x	x	x	x	x	x	x	x	x	x	x			x
Amylase		x															
Creat Cl		x		x	x	x	x	x	x					x			x
TSH/FT4		x															
UA		x															
Preg		x		x	x	x	x										
ELISA/WB**		x															
ANA		x															
Hep B SA		x															
HepB Sab		x															
HepC Ab		x															
HepC VL		x															
CD4+		x		x				x				x	x				x
HIV RNA	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
SCA	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
Eye exam		x															
PBMC	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Apheresis			x					x									
IFN admin				x	x	x	x										
IFN level				x	x	x	x	x									

*Chem includes Creatinine, lytes, BUN, Alk phos

** * If not available

Appendix 2: Toxicity Severity Scale

The toxicity grading scale for this protocol is the "Table for Grading the Severity of Adult and Pediatric Adverse Events; Version 1.0 - December 2004 (Clarification dated August 2009)."

It can be found at:
http://rcc.tech-res.com/safetyandpharmacovigilance/