

Immune Reconstitution By In Vivo Expansion Of Cd4 Lymphocytes By Human Recombinant Interleukin-2.

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A. Purpose Of The Study

History is replete with plagues, in modern history however, no infectious disease has been more devastating than AIDS. It is estimated that since its discovery in 1983 greater than 1-2 million people have been infected in the United States alone. To date, enormous progress has been made including: 1) the isolation and identification of a highly mutable retrovirus, the human immunodeficiency virus (HIV), 2) elaboration of the pathogenesis describing viral infection a distinct population of immune cells (CD4+) leading to destruction of the immune system thus allowing for susceptibility to unusual and aggressive opportunistic infections and ultimately death, 3) modulation of viral and immune cell growth by cytokines and chemokines, 4) rapid development of anti-retroviral agents, including but not limited to the following classes of drugs: 1) reverse transcriptase inhibitors (RT) e.g. AZT, DDI, DDC,; 2) nonnucleoside analogues (NA) e.g. nevirapine, and 3) protease inhibitors (PI) e.g. zidovudine; and 5) an armamentarium of antibiotics to prophylax/treat the most pervasive of the opportunistic infections 5.

Thus, there remain two treatment strategies for combating AIDS in the future: virus elimination and immune reconstitution.²

The peripheral T- lymphocyte pool or circulating T cells are significantly decreased in patients with late stage AIDS, as is their ability to respond to stimulation by antigen. ³ It is suggested that the population of CD4+ cells in AIDS patients are continuously produced but as disease becomes advanced the host is incapable of maintaining production in presence of overwhelming virus production⁴. Recently, it has been demonstrated that viral load or the abundance of HIV viral mRNA in both peripheral blood monocytes and in plasma of HIV infected patients can be reduced to undetectable levels with combination RT and PI therapy ^{6,7}.

Immune reconstitution theoretically could be accomplished in the following manner: 1) transplantation of immunocompetent cells via a) syn/allo/xeno geneic transplant of bone marrow, bone marrow stem cells, fetal thymus tissue; or 2) stimulation of T- lymphocytes by immunomodulating cytokines e.g. interleukins: 1,2,4,10.8 Interleukin-2, for example is a potent stimulator of cytotoxic T-lymphocytes and also activates CD4+ lymphocytes and causes CD4+ proliferation, as well as B-lymphocyte activation and proliferation ⁹.

Theoretically, expansion of CD4+ lymphocytes could be accomplished in the by the following methods: a) in vivo expansion by administration of human recombinant IL-2; b) ex vivo expansion of CD4+ cells by IL-2 following plasmapheresis and clonal expansion, then reintroduction.

Kovacs et al. reported in 1995 increases in CD4 lymphocytes in HIV+ patients (Please note that concentration of peripheral blood CD4 cells has been used as a marker of disease progression and those patients with less than 200 cells/cu mm to have advanced disease. Additionally, measurements of viral load or the abundance of viral particles is theorized to correlate more closely with disease progression, and to have substantial effect on treatment modification ¹⁰.) given intravenous recombinant IL-2. The maximum effect of IL-2 was seen in patients without severe immunodeficiency and low viral burden.

However, in patients with CD4<200 and high viral burden (100,000 copies HIV RNA/ml) had significant increases in viral load without increases in CD4 cell population. These patients were receiving RT monotherapy during the IL-2 administration ¹¹. Therefore, it appears theoretically possible to reduce viral load in HIV+ CD4<200 by administration of combination therapy with RT, PI, , then attempt immune reconstitution with IL-2.

The purpose of this study is to attempt immune reconstitution in vivo by exogenous administration of IL-2 in patients with fewer than 200 CD4 cells and viral load of less than 10,000 copies

HIV RNA/ml. This study will test ability of IL-2 to increase CD4 cells, evaluate changes in viral load, and assess clinical condition of patients receiving therapy.

B. Study Design: Clinical Trial

This is a randomized, placebo controlled study involving approximately 20 HIV+ patients who will be randomized into two groups by a computer based pseudo-random generator. Approximately ten patients will be assigned to the treatment arm and ten to the control arm through a masked allocation schedule.

Each patient will require 7 days of hospitalization with measurements of serologic, virologic, and immunologic markers, prior to infusion, immediately postinfusion, and to continue every eight weeks for one year. Infusions of IL2 will be given as continuous infusion through a peripheral line at dose of 18 million units in 5% dextrose in water containing 0.1% albumin over 5 days.

Statistical analysis of primary endpoints of CD4 cells and HIV RNA will be performed utilizing, one way analysis of variance of repeated measures after data has been collected and checked, data entered into computer and checked for descriptive statistics and data transformations. Additionally, secondary endpoints i.e. looking at associations of age, sex, clinical outcome, and previous antiretroviral experience will be analyzed.

C. Study Procedures

The study will require seven day hospitalization during which each patient in both arms of the trial will undergo phlebotomy (venipuncture) to obtain samples for peripheral blood CD4 cell count, HIV RNA, CBC with differential, SMAC, prior to infusion (day 1) and post- infusion (day 7). This will be repeated at eight week intervals over the course of one year. Then follow up samples without infusion every eight weeks for six months.

Determinations of lymphocyte subgroups and surface markers will be performed by one- color or two-color flow cytometry with monoclonal antibodies. Plasma HIV RNA levels will be determined by branched DNA signal amplification assay (Chiron) 12. One 20-gauge angiocath with heplock will be placed in peripheral vein and changed according to hospital protocol.

Human recombinant Interleukin-2 will be administered according to protocol established by Kovacs and Lane 13.

Placebo of 5% dextrose in water will be administered to control groups as a continuous infusion.

The duration of the study includes two parts. The first which will comprise one year of IL-2 infusion at 8 week intervals, followed by part two, which will assess status of immune response after IL-2 has been discontinued.

D. Study Drugs

1) Human recombinant interleukin-2 available through Chiron. IL-2 18million IU in 5% dextrose and water containing 0.1% albumin via a peripheral line each day for five days of hospitalization. With allowable dose reduction of 6 million units for adverse effects. Pre treatment and/or treatment with acetaminophen, ibuprofen, antiemetics, anxiolytics, are allowed for adverse reactions 14.

2) Side effects: Clinical: rash, fatigue, malaise, myalgias, arthralgias, nausea, capillary leak, fever, diarrhea, headache, aphthous ulcers, stomatitis, abdominal pain, altered perception, depression. Laboratory: Decreased calcium, albumin, magnesium, sodium, platelets, hemoglobin, increased alkaline phosphatase, increases in viral load. IL-2 has been shown to activate CD4 cells and when infected by HIV stimulate viral production, thus the potential exists to farther deteriorate immune function.

E. Study Subjects

Patients who are currently receiving care at Harkness 6 AIDS clinic at CPMC will be approached during scheduled clinic/office visit, this will be in agreement with the patient's respective clinic doctor.

a. Inclusion criteria

include: HIV+ determined by standardized serologic tests (institution standard- ELISA, western blot), CD4 lymphocytes of 200/ml or less, must be currently taking and compliant on regimen including at least 2 reverse transcriptase inhibitors, and a protease inhibitor. Optional anti-retroviral therapy with a non- nucleoside analog in addition to above regimen is permitted. Additionally, greater than two RT are also permitted in addition to above regimen. Viral load as HIV RNA must be less than or equal to 10,000 copies/ml. All candidates should be on approved antibiotic prophylaxis according to published standards and 01 history.

b. Exclusion criteria

concurrent active opportunistic infection, current chemotherapy.

F. Confidentiality

All subjects enrolled will be assigned a code number by which data will be obtained and tabulated and will be stored in a secure location. Only results will be published safeguarding anonymity of the study participants.

G. Location Of Study

CPMC

H. Risks And Benefits

a. Risks

The potential exists when utilizing an in vivo immune system activator and known viral activator that viral proliferation could predominate over the beneficial effects of CD4 cell expansion, possibly resulting in enhanced lysis of CD4 cells and further deterioration of immune function. However, in recent ex vivo expansion of HIV infected CD4 cells activated by IL2 in the presence of combination antiretroviral therapy, cells in culture were polyclonally expanded and did not lyse 15. The aforementioned constellation of influenza like symptoms is a real and likely outcome of IL2 therapy as demonstrated by Kovacs 16.

b. Benefits

The goal of this study is to ascertain whether immune reconstitution is a possibility in the most severely of affected HIV+ patients. Today it is possible to lower viral load to levels below 10,000 copies/ml even to levels undetectable by current assay methods, this may provide an opportunity to expand the severely depleted cells without adverse enhancement of viral replication, thus affording the participant of at least a temporarily more functional immune system.

I. Compensation And Costs

Costs will be covered by the insurance of the participant and by the investigator. No cost to the participant. Travel expenses to CPMC will be provided.

J. References

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5. Lange JMA. Triple Combinations: Present and Future. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology I O(Suppl. I)*S:77-88. 1995
6. Staszwski S. Zidovudine and Larnivudine: Results of Phase III Studies. *J of AIDS and Human Retrovirology I O(Suppl. 1)*: S57 1995.
7. Vella S. Rationale and Experience with Reverse Transcriptase Inhibitors and Protease Inhibitors *J of AIDS and HR I O(Suppl. 1)*S:5 8-61 1995.
8. Poli G., and Fauci A. Cytokine modulation of HIV Expression. *Immunology* 5:165-173, 1993.
9. *Immunology: A Short Course*. Eli Benjamini. Wiley-Liss Inc. N-Y. 1991
10. Harrigan R. Measuring Viral Load in the Clinical Setting. *J of AIDS and HR I O(Suppl. 1)* S:34-40. 1995
- 11,12,13,14,16. Kovacs JA., et al. Increases in CD4 T Lymphocytes with Intermittent Courses of Interleukin-2 in Patients with Human Immunodeficiency Virus Infection. *NEJM* 1995 332:9 567-575.
15. Wilson C., et al. Ex Vivo Expansion of CD4 Lymphocytes from Human Immunodeficiency Virus Type- I Infected Persons in the Presence of Combination Antiretroviral Agents. *The journal of Infectious Diseases* 1995; 172:88-96.

IRB LAY SUMMARY

TITLE: IMMUNE RECONSTITUTION BY IN VIVO EXPANSION OF CD4 LYMPHOCYTES BY HUMAN RECOMBINANT INTERLEUKIN-2.

PRINCIPAL INVESTIGATOR: CM EDELMANN MD

DEPARTMENT: MEDICINE

STUDY PURPOSE: To assess the ability to reconstitute immune cells specifically CD4 T-Lymphocytes in patients afflicted with HIV disease and who have progressed to severe deterioration of immune function. This study will utilize an experimental agent (Interleukin-2) to activate and cause proliferation of CD4 lymphocytes.

STUDY DESIGN: A randomized, placebo controlled clinical trial involving approximately 20 HIV+ patients who will be randomized into two groups by computer based randomization program generator. Approximately ten will be allocated to the treatment arm and ten to the control arm through a masked allocation schedule. Statistical analysis will be performed on primary endpoints of CD4 cells and viral load by one way analysis of repeated measures after data has been collected and checked, entered into computer and screened for data transformations and descriptive statistics. Secondary endpoints including age, clinical improvement by karnofsky score, and previous anti retroviral experience will be analyzed.

STUDY SUBJECTS: Patients who are currently receiving care at Harkness 6 will be approached during scheduled office visits in agreement with their physician. Inclusion criteria include: HIV+, CD4<200, HIV RNA< 10,000 copies/ml, must be currently treated with combination therapy including 2RT, 1 PI, or greater. Exclusion criteria include current 01, current chemotherapy.

STUDY PROCEDURES: Study patients will require 7 days of hospitalization every 8 weeks for 1 year, then monthly office visits for blood work and follow up. While an inpatient the patient will submit to venipuncture to obtain the following specimens: CBC, SMAC, CD4, HIV RNA, on three occasions during admission. Heplock will be placed according to protocol. IL-2 infusion will be given in hospital as outlined in IRB protocol,

ISSUES: The patient may experience a variety of severe influenza like symptoms. Additionally, a theoretical risk of enhanced viral replication exists when stimulated by IL-2, with a possibility of causing immune system deterioration. However, it is the purpose of this study to augment the immune system in those it is most profoundly needed.

**IRB CONSENT FORM
COLUMBIA PREBYTERIAN MEDICAL CENTER
CONSENT TO PARTICIPATE IN A RESEARCH STUDY**

TITLE: IMMUNE RECONSTITUTION BY IN VIVO EXPANSION OF CD4 LYMPHOCYTES BY HUMAN RECOMBINANT INTERLEUKIN-2.

IRB STUDY NUMBER#

STUDY PURPOSE:

You are invited to participate in a research study that will attempt to reconstitute the immune system in HIV+ individuals. Specifically, repopulation of CD4 lymphocytes. You were selected as a possible participant in this study because you are HIV+ are currently on antiretroviral treatment with Rt and PI, have fewer than 200 CD4 cells/ml, viral load less than 10,000 copies/ml, have no current OI's, and are not currently receiving chemotherapy. This qualifies you to participate in a study to attempt reconstruction of your immune system.

STUDY PROCEDURES:

If you decide to participate you will be required to be admitted to the CPMC for a period of 7 days, every eight weeks for 1 year, and then to be followed as an outpatient monthly for six months. When admitted you will be required to submit to 3 venous blood draws of approx. 50cc or 20z. of blood each. You will be randomized to either the treatment group or the control. The participants will receive IL-2 intravenously, in a peripheral vein, over the course of 5 days.

STUDY RISKS:

Your participation includes the following risks: Influenza-like symptoms, including malaise, fatigue, nausea, diarrhea, headache, rash, muscle or joint pain, leakage at the IV site, aphthous ulcers, abdominal pain, depression., which will be treated if develops. Also, there is a risk of further deterioration of your immune system, and resultant infection, complications, for which you will be closely monitored.

STUDY BENEFITS:

You may or may not benefit personally from this study. Benefits to you may include: Increases in the number of your CD4 cells, maintenance of a low viral load, possible enhancement of your immune system and its function, and scientific benefits that may help others in the future.

COST AND COMPENSATION:

There is no direct cost to the participant. All study drug, procedures, and hospitalization will be paid for through the participants insurance and through the investigator. Compensation for travel to and from CPMC will be provided.

CONFIDENTIALITY:

Any information regarding the participant will remain confidential only data collected will be utilized for study and publication. Each participant will be assigned a code number for collection of data, to assure anonymity of the participant. Only the principal investigator, the FDA, and your doctor have access to this study.

PARTICIPATION IS VOLUNTARY: You can refuse to participate or withdraw at any time. This will not affect your care at CPMC.

QUESTIONS: Any questions regarding this study can be addressed by C.M. Edelmann MD.